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Larvicidal potential, phytochemical composition, and molecular docking analysis of *Panicum repens* as a natural insecticide

[*Potencial larvicida, composição fitoquímica e análise de acoplamento molecular do* Panicum repens *como inseticida natural*]

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ABSTRACT

Mosquito-borne diseases, including malaria and dengue fever, remain a significant public health concern, especially in developing regions. The limitations of synthetic insecticides, such as resistance and environmental impact, have driven the search for sustainable alternatives. This study evaluated the larvicidal potential of *Panicum repens* extracts against three mosquito species namely *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. Sequential Soxhlet extractions using methanol, ethanol, chloroform, n-hexane, and water revealed that the methanol extract exhibited the highest efficacy, achieving LC_{50} and LC_{95} values of 87.04 ppm and 215.68 ppm, respectively, for *Cx. quinquefasciatus*. Mortality rates increased dose-dependently, with *Cx. quinquefasciatus* being the most susceptible species. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified key bioactive compounds, including stigmasterol, gamma-sitosterol, and campesterol, with sterols representing a significant proportion of the methanol extract. The docking analysis of 4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol with the target protein 5X61 demonstrated the highest binding affinity, with a docking score of −10.5 kcal/mol. The binding interaction involved both hydrogen bonding and hydrophobic interactions. The molecular docking results provide insights into the potential mechanisms of action of these bioactive compounds, supporting their application in the development of targeted, eco-friendly mosquito control strategies. *P. repens* methanol extract shows promise as a natural, eco-friendly larvicide for mosquito control.

Keyword: *Ae. aegypti*, *An. stephensi*, *Cx. Quinquefasciatus*, methanol extract, GC-MS analysis, natural insecticides

RESUMO

As doenças transmitidas por mosquitos, incluindo a malária e a dengue, continuam sendo uma preocupação significativa para a saúde pública, especialmente nas regiões em desenvolvimento. As limitações dos inseticidas sintéticos, como a resistência e o impacto ambiental, impulsionaram a busca por alternativas sustentáveis. Este estudo avaliou o potencial larvicida dos extratos de Panicum repens *contra três espécies de mosquitos:* Aedes aegypti, Anopheles stephensi *e* Culex quinquefasciatus. *Extrações sequenciais de Soxhlet usando metanol, etanol, clorofórmio, n-hexano e água revelaram que o extrato de metanol apresentou a maior eficácia, atingindo valores de LC50 e LC95 de 87,04 ppm e 215,68 ppm, respectivamente, para* Cx. quinquefasciatus. *As taxas de mortalidade aumentaram de forma dependente da dose, sendo o* Cx. quinquefasciatus *a espécie mais suscetível. A análise de cromatografia gasosa e espectrometria de massa (GC-MS) identificou os principais compostos bioativos, incluindo*

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estigmasterol, gama-sitosterol e campesterol, com esteróis representando uma proporção significativa do extrato de metanol. A análise de acoplamento de 4,4,6a,6b,8a,11,11,14b-Octametil-docosahidropicen-3-ol com a proteína alvo 5X61 demonstrou a maior afinidade de ligação, com uma pontuação de acoplamento de -10,5 kcal/mol. A interação de ligação envolveu tanto a ligação de hidrogênio quanto as interações hidrofóbicas. Os resultados do acoplamento molecular fornecem percepções sobre os possíveis mecanismos de ação desses compostos bioativos, apoiando sua aplicação no desenvolvimento de estratégias de controle de mosquitos direcionadas e ecologicamente corretas. O extrato de metanol de P. repens *é promissor como larvicida natural e ecológico para o controle de mosquitos.*

Palavra-chave: Ae. aegypti, An. stephensi, Cx. Quinquefasciatus, *extrato de metanol, análise GC-MS, inseticidas naturais*

INTRODUCTION

Mosquito-borne diseases continue to pose significant public health challenges in many developing countries, where millions of people remain at risk of infection (Zhang *et al*., 2024). The primary vectors responsible for transmitting these diseases include *Culex*, *Aedes*, and *Anopheles* mosquitoes (Elkhidr and Ahmed, 2022). However, mosquitoes are increasingly adapting to synthetic insecticides, leading to resistance issues. Along with growing concerns about the environmental impact of pesticide use (Sudo *et al*., 2018), these challenges have driven the search for more sustainable alternatives.

Phytochemicals, derived from plants, have shown promise as eco-friendly insecticides. These natural compounds offer multiple modes of action against mosquitoes, including oviposition deterrents, developmental toxins, and adulticidal properties (Bakkali *et al*., 2008). Despite the potential of medicinal plants as larvicidal agents or mosquito repellents, much of this area remains underexplored. Recently, there has been growing interest in developing plant-based insecticides due to their eco-friendly nature, bio-based origin, and minimal risks to ecosystems and non-target organisms (Suman *et al*., 2012; Radwan *et al*., 2023). Numerous extracts and compounds from various plant families, including saponins, steroids, isoflavonoids, essential oils, alkaloids, and tannins, have demonstrated significant efficacy against mosquito larvae (Baz *et al*., 2021, 2024).

In addition to traditional phytochemical approaches, molecular docking has emerged as a pivotal computational technique in the discovery and development of insecticides. This method enables researchers to predict interactions between small molecules (potential insecticidal compounds) and target proteins or enzymes essential for insect survival. By providing insights into the binding affinity and specificity of compounds, molecular docking facilitates the identification of novel, effective insecticides (Gallinger *et al*., 2022). Integrating molecular docking with phytochemical analysis enhances the understanding of how plant-derived compounds exert their insecticidal effects at the molecular level.

Panicum repens L. (family Poaceae) is a perennial grass widely distributed across tropical and subtropical regions. It has thick, pointed rhizomes that spread through creeping along the ground or floating in water, forming dense mats (Belal and Khalafalla, 2011). Several species of Panicum have been reported to exhibit diverse biological activities, including hypolipidemic, diuretic, antidiabetic, anti-inflammatory, antibacterial, and antifungal properties (Zaki *et al*., 2017). Studies have also shown that *P. repens* releases volatile organic compounds (VOCs) that attract gravid *An. gambiae* mosquitoes, mimicking natural chemical cues used by mosquitoes to select oviposition sites (Bokore *et al*., 2021). However, its larvicidal potential remains unexplored.

This study aims to assess the larvicidal activity of *P. repens* extracts against three mosquito species: *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*. Additionally, GC-MS analysis was performed to identify bioactive compounds in the extract, and molecular docking studies were conducted to evaluate the interactions of major compounds with the 5X61 binding site. This integrated approach seeks to uncover the mechanisms through which *P. repens* exerts its insecticidal effects and to explore its potential as a natural, eco-friendly mosquito control agent.

MATERIALS AND METHODS

The plant *P. repens* (family Poaceae) was collected on August 10, 2016, from areas surrounding the Presidency College campus in Chennai, Tamil Nadu, India, located at 13°03'36.25" N latitude and 80°16'55.63" E longitude. The species was identified and documented by a Botanist at the Plant Anatomy Research Centre (PARC) in West Tambaram, Chennai. A voucher specimen (PARC/2016/3178) was prepared and deposited in the herbarium for future studies.

The aerial parts of *P. repens* were shade-dried and ground into a fine powder. The powder was stored in an airtight glass container. For extraction, 100 grams of the powdered plant material were processed using Soxhlet extraction for 24 hours with different solvents, including n-hexane, chloroform, ethanol, and methanol (purity grade: ≥99%, manufacturer: Sigma-Aldrich), applied sequentially. Separate plant powder samples were used for each solvent to avoid cross-contamination. Furthermore, 100 grams of the powder were immersed for 24 hours in 200 mL of double-distilled water and subsequently filtered using Whatman No. 1 filter paper to get an aqueous extract. The solvents were evaporated at 45°C under reduced pressure using a rotary evaporator (model: Buchi R-300, manufacturer: Buchi Labortechnik AG) to obtain concentrated extracts. These extracts were transferred into sealed glass vials and stored at -20°C for subsequent analysis.

The phytochemical analysis of the plant was performed according to the methods described by Nweze *et al*. (2004) and Senthilkumar and Reetha (2009). This analysis involved identifying and confirming the presence of various bioactive compounds within the plant extracts. Plants were first ground into a fine powder and then extracted using the solvents used in the study. The extracts were subjected to a series of specific tests designed to detect the presence of key phytochemicals, including alkaloids, flavonoids, tannins, saponins, phenols, steroids, terpenoids, and glycosides. These tests rely on the reaction of the plant extract with specific reagents, resulting in characteristic color changes or the formation of precipitates. These tests provided a preliminary profile of the bioactive compounds present in the extracts. The results were recorded using a plus/minus system: (+) indicating the presence and (−) indicating the absence of these compounds in the extracts.

Larvae of *Cx. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti* used in the larvicidal experiments were obtained from a laboratory colony maintained under controlled environmental conditions. The colonies were kept at a temperature of 28±1°C, with a 12-hour light and 12-hour dark cycle, and a relative humidity of approximately 70±5%. The larvae were provided with finely powdered fish feed daily to support their development until they reached the pupal stage, ensuring a steady supply for the larvicidal bioassays.

The larvicidal activity of *P. repens* was evaluated using five different extracts: aqueous, chloroform, ethanol, n-hexane, and methanol. Late third instar larvae of *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* were collected using a fine mesh strainer and carefully transferred into 250 mL glass containers filled with dechlorinated water. Stock solutions of each extract were directly prepared without the use of any co-solvent. Test solutions were prepared by dissolving the appropriate amount of each extract in 250mL of dechlorinated water to achieve final concentrations of 50, 100, 150, 200, and 250 ppm. Each concentration was tested in five replicates, with 20 larvae per replicate, totalling 100 larvae per concentration. Control groups included larvae exposed only to dechlorinated water. The larvae were maintained under controlled conditions without any food for a 24-hour exposure period. Mortality rates were recorded after 24 hours to assess the larvicidal potential of each extract.

The chemical profiling of plant extracts was conducted using a JEOL GCMATE II GC-MS system (Agilent Technologies 6890 N) with an autosampler and a non-polar fused silica capillary column. Helium was used as the carrier gas at 1.0mL/min. The oven temperature was programmed from 50°C to 280°C, and the mass spectrometer operated in EI mode at 70 eV, scanning 50–500 m/z. A 1µL aliquot of a 1mg/mL extract solution was analyzed. Compounds were identified based on NIST and Wiley libraries (similarity index ≥90%) and validated using retention indices, as described by (Babu *et al.*, 2018).

In silico experiments involved the preparation, docking, and visualization of protein-ligand interactions. The three-dimensional structure of the target protein, Human Cyclin-dependent Kinase 2 (CDK2) (PDB ID: 5X61) was obtained from the RCSB Protein Data Bank and prepared using AutoDock Tools 1.5.7 by removing water molecules, adding polar hydrogens, assigning Kollman charges, and defining AD4 atom types. The processed file was saved in the .pdbqt format for docking studies. Thirteen major compounds were sourced from PubChem, converted to .pdb format using Open Babel, and further processed in AutoDock Tools to generate .pdbqt files. Molecular docking simulations were conducted using AutoDock Vina 1.1.2 to evaluate binding energies and inhibition constants. Visualization of protein-ligand complexes was performed using PyMOL and PLIP, enabling detailed analysis of hydrogen bonds, hydrophobic interactions, and 2D/3D representations of the complexes.

In this study, statistical analysis was conducted using a two-way analysis of variance (ANOVA) to evaluate the effects of extract type, concentration, and mosquito species on mortality rates. The analysis focused on determining the main effects of extract types (aqueous, chloroform, ethanol, methanol, and n-hexane) and concentration, as well as their interactions with mosquito species. F-values and p-values were calculated to assess the significance of these factors, with a significance level set at $p < 0.05$. This statistical approach allowed for a comprehensive understanding of the factors affecting mosquito mortality and the effectiveness of the different *P. repens* extracts.

RESULT

The phytochemical analysis of *P. repens* revealed the presence of various bioactive compounds across different solvent extracts. Significant metabolites detected include carbohydrates, saponins, alkaloids, anthocyanins, quinones, and phenols, which were present in all extracts (aqueous, chloroform, ethanol, methanol, and hexane). Tannins, terpenoids, and triterpenoids were also detected but were inconsistent across different solvents. Flavonoids, glycosides, coumarins, fatty acids, and steroids were variable in their presence depending on the extraction solvent. These findings indicate a diverse phytochemical profile in *P. repens*, supporting its potential use as a natural source for bioactive compounds with various therapeutic and insecticidal applications (Table 1).

Table 1. Phytochemical analysis of *P. repens*

S. No	Secondary metabolites	Aqueous	Chloroform	Ethanol	Methanol	Hexane
1	Steroids	+	-	+	+	-
2	Protein	-	-	+	-	-
3	Fatty acids	+	+	+	+	-
4	Phenols	+	-	+	+	+
5	Coumarins	-	+	-	-	-
6	Triterpenoids	-	-	+	-	-
7	Cardiac glycosides	+	-	-	-	-
8	Terpenoids	-	-	-	-	+
9	Glycosides	-	-	-	-	-
10	Quinones	+	+	-	+	-
11	Carbohydrate	+	+	+	+	+
12	Anthocyanin	+	+	+	+	+
13	Alkaloids	+	+	+	+	-
14	Flavonoids	+	+	-	-	+
15	Saponins	+	+	+	+	+
16	Tannins	-	+	-	+	-

+ bsent – Absent

The study evaluated the larvicidal effects of *P. repens* extracts against three mosquito species: *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, focusing on mortality rates and lethal concentrations (LC_{50} and LC_{95}). Among the extracts tested, the methanol extract emerged as the most potent, achieving the lowest LC_{50} and LC_{95} values across all species, particularly against *Cx. quinquefasciatus*, which demonstrated the highest sensitivity. The LC_{50} and LC_{95} values for *Cx. quinquefasciatus* with the methanol extract were 87.04 ppm (95% CI: 75.640 - 97.857) and 215.68 ppm (95% CI: 202.31 - 230.86), respectively, representing the lowest lethal concentrations observed in the study.

In contrast, the n-hexane extract was the least effective, recording the highest LC_{50} and LC_{95} values. For *An. stephensi*, the LC_{50} and LC_{95} with n-hexane were 303.84 ppm (95% CI: 273.343 - 344.528) and 599.39 (95% CI: 527.328 - 703.745) ppm, respectively, indicating that this extract required significantly higher concentrations to achieve lethal effects. Similarly, *Cx. quinquefasciatus* treated with n-hexane showed LC_{50} and LC_{95} values of 256.54 ppm (95% CI: 231.43–288.65) and 552.09 ppm (95% CI: 486.93–646.36), confirming the extract's reduced effectiveness compared to methanol (Table 2)

Table 2. Lethal concentration (LC_{50} and LC_{90}) of *P. repens* extracts against three mosquito species

Extracts	Mosquito Species	LC50 (ppm)	95% Confidence Interval (Lower - Upper)	LC90 (ppm)	95% Confidence Interval (Lower - Upper)
Aqueous	*Ae. aegypti*	203.46	185.93 – 222.96	443.45	403.60 – 495.90
	An. stephensi	220.22	201.88 – 241.14	460.21	418.60 – 515.03
	Cx. quinquefasciatus	228.01	209.27 – 249.63	467.99	425.57 – 523.95
Chloroform	*Ae. aegypti*	234.945	215.202 - 258.179	481.800	435.981 - 543.206
	An. stephensi	256.932	235.543 - 282.698	503.787	455.520 - 568.528
	Cx. quinquefasciatus	214.784	196.276 - 235.996	461.638	417.969 - 520.109
Ethanol	*Ae. aegypti*	141.682	131.349 - 151.922	278.757	263.560 - 296.419
	An. stephensi	130.867	120.258 - 141.304	267.942	252.894 - 285.375
	Cx. quinquefasciatus	103.902	92.590 - 114.763	240.977	226.626 - 257.433
n-Hexane	*Ae. aegypti*	284.235	256.040 - 321.355	579.780	510.490 - 680.107
	An. stephensi	303.841	273.343 - 344.528	599.386	527.328 - 703.745
	Cx. quinquefasciatus	256.540	231.430 - 288.651	552.086	486.927 - 646.356
Methanol	*Ae. aegypti*	118.331	108.021 - 128.367	246.974	233.106 - 262.955
	An. stephensi	140.513	130.678 - 150.262	269.156	254.823 - 285.790
	Cx. quinquefasciatus	87.036	75.640 - 97.857	215.678	202.309 - 230.861

Mortality rates varied across species and concentrations, showing a clear dose-response relationship. The methanol extract achieved the highest mortality rates, with 100% mortality in *Cx. quinquefasciatus* at higher concentrations (250 ppm). The lowest mortality rates were observed with the aqueous extract, which caused minimal larval deaths at concentrations below 200 ppm for all species. At 100 ppm, for example, the aqueous extract resulted in less than 20% mortality for *Ae. aegypti* (Fig. 1) and *An. stephensi* (Fig. 2), whereas the methanol extract achieved mortality rates exceeding 70% at the same concentration.

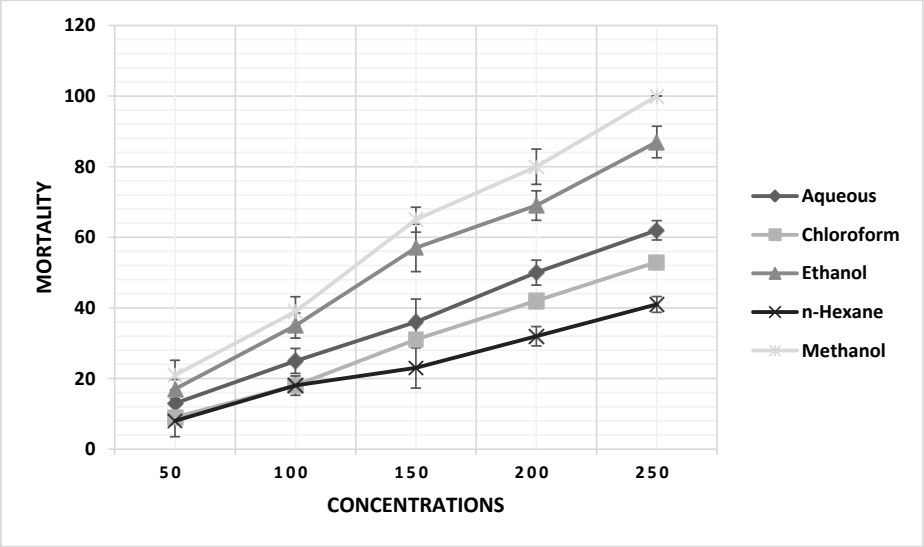

Figure 1. Mortality rates of *Ae. aegypti* at different concentrations of *P. repens* extracts.

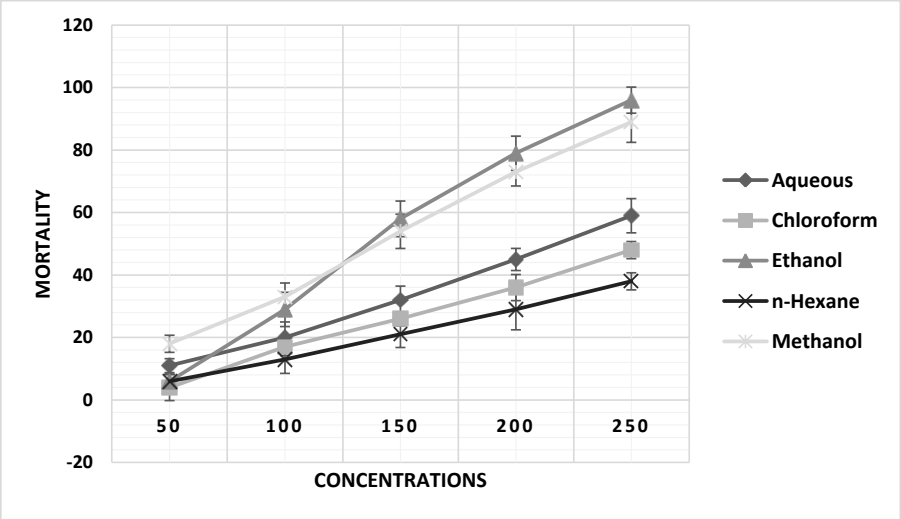

Figure 2. Mortality rates of *An. stephensi* at different concentrations of *P. repens* extracts.

Statistical analysis supported these findings, revealing a highly significant effect of extract type ($F = 45.46$, $p < 0.001$) and concentration ($F = 267.22$, $p < 0.001$) on mortality rates. The interaction between concentration, extract type, and mosquito species was also significant ($F = 6.43$, $p < 0.001$), emphasizing the variability in susceptibility among species and the differential effectiveness of the extracts. Among the species, *Cx. quinquefasciatus* was the most susceptible, requiring the lowest concentrations of methanol extract for significant mortality, while *An. stephensi* was the least sensitive, particularly to n-hexane and aqueous extracts.

In summary, the methanol extract of *P. repens* was the most effective larvicide, achieving the highest mortality rates and requiring the lowest LC_{50} and LC_{95} values, particularly against *Cx. quinquefasciatus* (Fig. 3). In contrast, the n-hexane extract was the least effective, with the highest lethal concentrations and lowest mortality rates. These findings highlight the potential of methanol-based formulations as a promising, natural alternative for mosquito control, especially targeting *Cx. quinquefasciatus*.

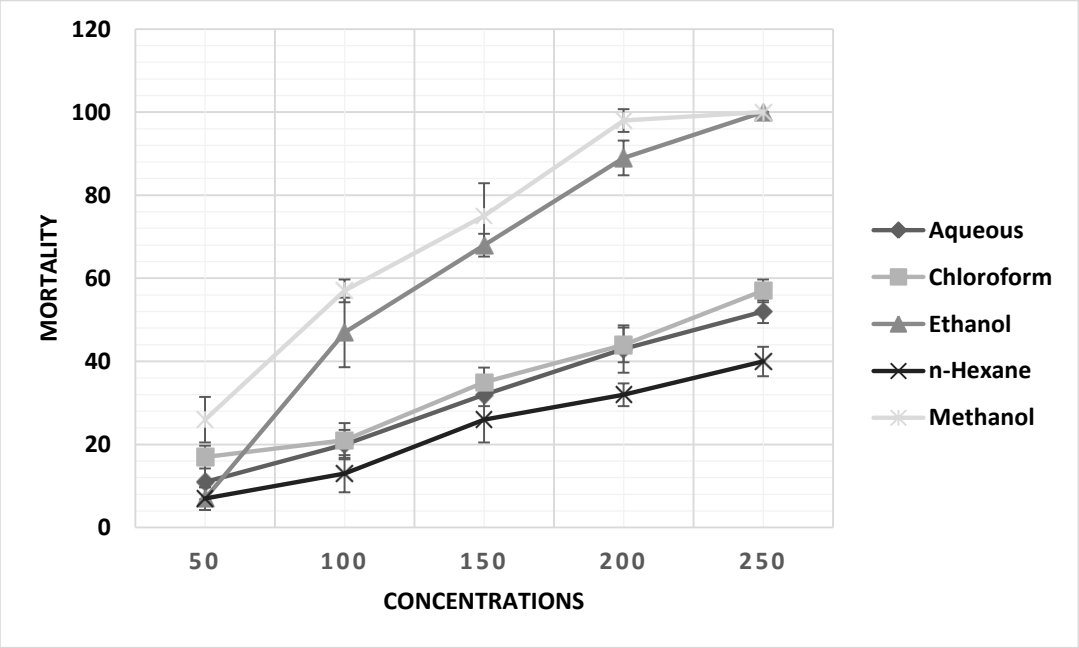

Figure 3. Mortality rates of *Cx. quinquefasciatus* at different concentrations of *P. repens* extracts.

The GC-MS analysis revealed a diverse array of compounds, including fatty acids such as hexadecanoic acid, methyl ester; n-hexadecanoic acid; methyl 13-octadecenoate; methyl 9-cis,11-trans-octadecadienoate; and methyl 8,11,14-heptadecatrienoate. Identified sterols included stigmasterol, campesterol, gamma-sitosterol, and 9,19-cycloergost-24(28)-en-3-ol. The terpenoids detected comprised lycopene, rhodoxanthin, rubixanthin acetate, and 8,12-di-O-acetylingol 7-(4'-methylpentanoyl). The most abundant compounds were stigmasterol (35.22%), campesterol (13.52%), and gamma-sitosterol (17.34%). Other notable compounds included 9,19-cycloergost-24(28)-en-3-ol (4.61%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.47%), methyl 9-cis,11-trans-octadecadienoate (1.71%), beta-sitosterol (1.67%), cholest-4-ene-3,6-dione (1.39%), and 17-(1,5-dimethylhexyl)-10,13-dimethyl-2,6,11,15-tetraoxacyclooctadeca-5,14-diene (1.05%) (Table 3).

In the molecular docking study with the target protein (PDB: 5X61), 4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol exhibited the highest binding affinity (-10.5 kcal/mol), supported by three hydrogen bonds involving CYS447, PHE490, and TYR494, and hydrophobic interactions with residues such as VAL235, TRP411, PHE449, and TYR493. Similarly, compounds such as gamma-Sitosterol (-9.4 kcal/mol) and beta-Sitosterol (-9.1 kcal/mol) formed hydrogen bonds with residues like CYS447 and GLY445, alongside hydrophobic interactions with ILE231, VAL235, TRP441, PHE449, and TYR494. Campesterol (-9.3 kcal/mol) displayed comparable affinity, forming hydrogen bonds with GLU448 and CYS447, and hydrophobic interactions with ILE231, TRP441, and PHE449. Dehydroergosterol 3,5-dinitrobenzoate (-9.5 kcal/mol) formed a single hydrogen bond with TYR489 and hydrophobic interactions, involving residues such as TYR282, TRP441, and TYR493. Sterols such as Lanosterol (-9.3 kcal/mol) demonstrated strong affinities, highlighting their binding potential. In contrast, non-sterol compounds like 9-cis,11-trans-Octadecadienoate exhibited weaker binding (-5.8 kcal/mol), showing significant variations in interaction strength based on structural characteristics. These findings underline the pivotal role of hydrogen bonding and hydrophobic interactions in governing binding efficiencies, with sterols displaying recurring interaction patterns with key protein residues, emphasizing their potential as lead compounds for further research (Table 4 and Figure 4).

Table 3. GC-MS analysis of methanol extract of *P. repens*

	RT	Peak Name	Area
1	29.883	Hexadecanoic acid, methyl es	0.58
2	30.189	2-Propenoic acid, 3-(4-hydro	0.533833647
3	31.157	n-Hexadecanoic acid	0.482514072
4	34.118	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	1.466666668
5	34.337	Methyl 13-octadecenoate	0.42115072
6	34.558	9-cis,11-trans-Octadecadienoate	1.714321453
7	35.038	Methyl 8,11,14-heptadecatrie	0.614596624
8	35.736	Oleic Acid	0.22
9	35.858	Ethyl 9,12-hexadecadienoate	0.145153221
10	38.759	Eicosanoic acid, methyl este	0.073602864
11	42.691	Methyl 20-methyl-heneicosano	0.08795157
12	46.344	Tetracosanoic acid, methyl e	0.142401501
13	48.199	psi.,.psi.-Carotene, 7,7',8	0.10736344
14	49.761	Hexacosanoic acid, methyl es	0.164828018
15	52.278	9(11)-Dehydroergosteryl benz	0.154233896
16	52.480	Lycopene	0.033057924
17	52.953	Pregn-4-ene-3,11,20-trione,	0.055574009
18	53.718	gamma.-Tocopherol	0.253158224
19	54.555	Stigmasta-5,22-diene, 3- methyl	0.323464666
20	54.602	Stigmast-22-en-3-one, 4-methyl-, (4.alpha.)-	0.146666667
21	54.949	Silanamine, N-[(17.beta.)-3,	0.526128831
22	55.134	17-(1,5-Dimethylhexyl)-10,13	1.04854284
23	55.273	Cholestano[7,8-a]cyclobutane	0.537961226
24	55.355	Rhodoxanthin	0.023015797
25	55.398	3-[18-(3-Hydroxy-propyl)-3,3	0.231144466
26	55.534	1,1':2',1''-Terphenyl, 3',4'	0.077883027
27	55.591	5H-Cyclopropa(3,4)benz(1,2-e	0.16647905
28	55.635	Rubixanthin acetate	0.028449068
29	55.664	Milbemycin B, 5-demethoxy-5-	0.312541439
30	56.171	Bis (eta.-5-cyclopentadienyl	0.095039725
31	56.410	Cholest-4-ene-3,6-dione	1.389618513
32	56.567	Dehydroergosterol 3,5-dinitrobenzoate	1.255197
33	56.739	1,4:5,8-Dimethanonaphthalene	0.700312696
34	56.908	Campesterol	13.5164478
35	57.345	Stigmasterol	35.2220138
36	57.874	25-Norisopropyl-9,19-cyclola	0.86596623
37	58.027	Cholesterol, 7-oxo-	0.532732959
38	58.064	(25S)-3Beta-acetoxy-5alpha,2	0.372307693
39	58.430	gamma.-Sitosterol	17.33583492
40	58.708	beta.-Sitosterol	1.670293936
41	58.904	8,12-Di-O-acetylingol 7-(4'-	0.22619137
42	58.995	Ursa-9(11),12-dien-28-oic ac	0.884540339
43	59.186	Cholest-5-en-3-ol, 24-propyl	1.75834897
44	59.442	9,19-Cycloergost-24(28)-en-3	4.607754852
45	59.728	4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol	1.128205129
46	59.932	Gorgostan-3-ol, 5,6-dichloro	0.827029394
47	60.089	Androst-5-en-3-one, 17,19-bi	0.941500939
48	60.251	17-(1,5-Dimethyl-3-phenylthiohex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopent(a)phenanthren-3-ol	3.447904944
49	60.621	Lanosterol	2.476547845
50	60.800	D-Homo-24-nor-17-oxachola-20	0.063066254
51	60.885	[(1H)-Pyrrole-3-propanoic ac	0.009434134

Table 4. Molecular docking results for compounds with the target protein (PDB: 5X61), including binding affinities, hydrogen bond interactions, and hydrophobic interactions with key residues

No.	Compound Name	Binding Affinity (kcal/mol)	Target Protein	Hydrogen Bonds	Other Interactions
20	Stigmast-22-en-3-one, 4-methyl-, (4.alpha.)-	-8.6	5X61	CYS447	ILE231(2x), VAL232, VAL235, TYR282, TRP441(2x), TRP441, PHE449, PHE490A, TYR493(2x), TYR494A
22	17-(1,5-Dimethylhexyl)-10,13-diene	-7.8	5X61	CYS447 (2x)	ILE231, VAL235, TRP441, PHE490(2x), PHE490, TYR493(2x), TYR494
31	Cholest-4-ene-3,6-dione	-8.8	5X61	TYR282, CYS447	ILE231(2x), VAL235A, TYR493, PHE449
32	Dehydroergosterol 3,5-dinitrobenzoate	-9.5	5X61	TYR489	TYR282 - TRP441(2x), PHE490(2x) , TYR493 (4x)
34	Campesterol	-9.3	5X61	GLU448, CYS447	ILE231(2x), TRP441,PHE449, TYR494
35	Stigmasterol	-8.8	5X61	-	ILE231(2x), TRP441A, PHE449, ILE446, CYS447, PHE449,TYR489,PHE490 , TYR493 (2x)
39	gamma.-Sitosterol	-9.4	5X61	GLY445, CYS447	ILE231(2x) , VAL235, TRP441, PHE449,TYR494
40	beta.-Sitosterol	-9.1	5X61	CYS447	ILE231(2x), VAL235,TRP441, PHE449, TYR494
43	Cholest-5-en-3-ol, 24-propyl	-8.6	5X61	CYS447A	ILE231, VAL235, TRP441(4x), PHE449,TYR493, TYR494A
44	9,19-Cycloergost-24(28)-en-3	-8.8	5X61	CYS447	ILE231, PHE449, PHE490, TYR493 (2x), PHE449
45	4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol	-10.5	5X61	CYS447, PHE490, TYR494	VAL235, TRP411 (2x), PHE449, TYR493 (2x), TYR494
48	17-(1,5-Dimethyl-3-phenylthiohex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopent(a)phenanthren-3-ol	-9.4	5X61	-	ILE231, VAL235, VAL235 (3x) , TRP441 (4x), PHE449, PHE490, TYR493
49	Lanosterol	-9.3	5X61	-	ILE231, TRP441, LEU444, PHE449, PHE490, TYR494(2x)

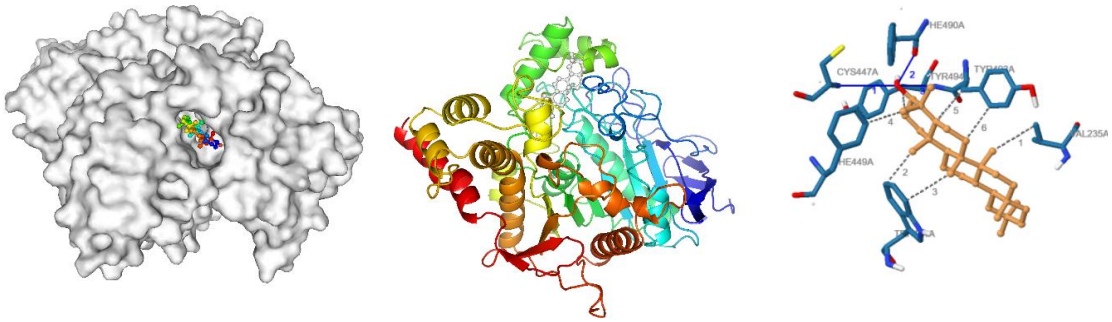

Figure 4. In silico molecular docking analysis of the binding interaction between the compound 4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol and acetylcholinesterase (PDB: 5X61) based on binding energy predictions using the AutoDock program. (A) Close-up view of the active site surface structure of 5X61. (B) The ribbon model represents the protein highlighting secondary structures, with the ligand depicted in 3D to illustrate its orientation and interactions within the binding site. (C) 2D structure of pinoresinol showing interactions with active site residues of 2BMF.

DISCUSSION

This study aimed to assess the larvicidal potential of *P. repens*, its phytochemical composition, and its interactions with target proteins through molecular docking. The larvicidal bioassay results demonstrated promising activity against mosquito larvae, highlighting the potential of *P. repens* as a bio-based insecticide. These findings align with previous research emphasizing the insecticidal properties of plants within the Poaceae family. For example, Nonviho *et al*. (2010) reported the insecticidal effects of *Cymbopogon schoanenthus*, *Cymbopogon citratus*, and *Cymbopogon giganteus*, achieving 100% mortality of *An. gambiae* at 1.1, 586.58, and 1549µg·cm², respectively. Similarly, *Paspalum conjugatum*, *Oplismenus compositus*, *Capillipedium huegelii*, and *Echinochloa colona* achieved complete larvicidal efficacy against second instar larvae of *Aedes* species, with 100% mortality at a concentration of 1 mg/mL (Kekuda *et al.*, 2020). Additionally, essential oil from *Vetiveria zizanioides* extracted via steam distillation demonstrated larvicidal and adult repellent activity against *An. stephensi*, with LC50 values for instars 1-4 ranging from 281 to 475mg/L after 24 hours (Aarthi *et al.*, 2014). These findings reinforce the potential of *P. repens* as a valuable candidate for integrated pest management strategies.

The phytochemical analysis of *P. repens* showed the presence of several bioactive compounds, including, steroids, fatty Acids, terpenoids, lipids and other organic compounds. These compounds are known to exert insecticidal, antimicrobial, and anti-inflammatory activities, which contribute to the overall potential of the plant (Hilal *et al.*, 2024, Kumari *et al.*, 2024, Boate and Abalis, 2020). Similar findings have been reported by Gade *et al.*, (2017) and De Geyter, (2012), who emphasized the role of phytocompounds in plant-based insecticides. They are known to inhibit enzymes involved in insect digestion and detoxification (Boate and Abalis, 2020), while terpenoids, such as saponins and essential oils, often act by disrupting the insect's nervous system or serving as repellents (Gade *et al.*, 2017, Sami *et al.*, 2018, Jankowska *et al.*, 2017). This aligns with the current study's findings, where the presence of these bioactive compounds is a strong indicator of *P. repens* potential as a natural insecticide.

The molecular docking study further elucidated the interactions between the identified compounds and insect-specific target proteins. Among the compounds tested, 4,4,6a,6b,8a,11,11,14b-Octamethyl-docosahydropicen-3-ol demonstrated the strongest binding affinity (-10.5 kcal/mol), suggesting its high potential to interact with target proteins critical for insect development. Moreover, the compounds gamma-Sitosterol and beta-Sitosterol showed promising binding scores (-9.4 kcal/mol and -9.1 kcal/mol, respectively), with key residues like CYS447, GLY445, and TYR494 involved in the interaction, which are critical in protein-ligand binding. These findings further reinforce the hypothesis that sterol

compounds, abundant in *P. repens*, could act as potent insecticidal agents. Our result aligns with Gade *et al.* (2017), who investigated the larvicidal potential of *Chromolaena odorata*, traditionally known for its insect-repellent properties, against *Cx. quinquefasciatus*. The plant extract exhibited significant larvicidal activity, with chromatography isolating stigmasterol and 1-hexacosanol as the key bioactive compounds. These compounds exert neurotoxic effects by inhibiting acetylcholinesterase (AChE) activity. The neurotoxic effects of stigmasterol and 1-hexacosanol resulted in the mortality of *Cx. quinquefasciatus*, *A. aegypti*, and *Chironomus riparius*.

Comparative studies also support these findings. Johnson *et al.* (2021) Vivekanandhan *et al.* (2024) reported that *Cymbopogon citratus* and *Acacia nilotica* displayed similar binding affinities with insecticidal target proteins, emphasizing the recurring trend of plant-based compounds interacting with insect proteins. These results underscore the relevance of *P. repens* in a broader context of plant-based insecticide research, suggesting that its bioactive compounds can effectively interact with insect proteins, contributing to their insecticidal potential. Moreover, the docking results provide valuable insights into the structural features responsible for strong binding interactions, such as the importance of hydrophobic interactions and hydrogen bonding, which may enhance the potency of these compounds in pest control applications.

However, despite the promising results observed in the laboratory setting, it is essential to recognize that these findings were obtained under controlled conditions, which may not fully capture the complexity of real-world environmental interactions. Moore *et al.* (2007) and Sarmah *et al.* (2024) highlighted the importance of conducting field trials to assess the practical efficacy of plant-based insecticides in diverse ecological settings. Additionally, while molecular docking provides valuable preliminary insights into protein-ligand interactions, it is critical to further perform in vitro and in vivo studies to validate these compounds' biological activity and safety profile. This notion is supported by Abutaha *et al.*, (2023), who recommended further experimental validation of docking results and exploring potential synergistic effects of plant compounds.

CONCLUSION

P. repens exhibits significant larvicidal potential, supported by its diverse phytochemical composition and promising molecular docking interactions. Identifying bioactive compounds such as steroids and terpenoids and their favourable binding affinities supports the plant's potential as a bio-based insecticide. Further research is necessary to optimize extraction methods, validate the results in field trials, and explore possible synergistic effects of the compounds identified in this study. This research paves the way for developing *P. repens* as a sustainable and eco-friendly alternative to synthetic insecticides.

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