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MOLECULAR MODELLING STUDIES AND LARVICIDAL POTENTIAL OF PHE (POLYHERBAL EXTRACT) OF SELECTED INDIAN MEDICINAL HERBS

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ABSTRACT

The present study attempts to investigate the larvicidal activity of methanolic extract of PHP (Poly Herbal Powder) of selected Indian Medicinal herbs against blood-feeding mosquito larvae namely *Aedes aegypti*, *Anopheles stephensi,* and *Culex quinquefasciatus*. The major compounds from the methanolic extract of PHP were identified through GC-MC analysis. Molecular modeling studies were examined to predict the binding interactions of major compounds with a sterol carrier protein (SCP-2). The results obtained from larvicidal activity demonstrated that the methanolic extract of PHP exhibits appreciable LC_{50} against *A.stephensi* and *C.quinquefasciatus* (LC_{50}: 453.30 & 462.17 ppm). Furthermore, molecular docking studies revealed two bioactive compounds namely Geranyl isovalerate (-8.0 K.cal/mol) and Marmelosin (-7.6 K.cal/mol) with greater binding affinity with SCP-2 receptor as compared to positive control Temephos (-6.8 K.cal/mol).

Keywords: Poly Herbal Powder, Larvicidal, Molecular Modelling, Sterol Carrier Protein.

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INTRODUCTION

In recent decades, plant-based products have received greater attention in the medical field mainly because of their more acceptability and wider popularity.[1] During the past decade, traditional systems have gained importance in the field of medicine. The therapeutic value of plants is due to the presence of pharmacologically active metabolites which possess remarkable demand in drug discovery and development. Now a day most of the modern medicines available in the market incorporate active pharmacophores derived from plant and other natural sources mainly due to their synergistic efficacy with fewer side effects.[2] Ayurveda and other Indian literature have revealed the importance and utilization of medicinal plants in the management of various human ailments.[3-5] Presently most countries face a huge increase in the number of people suffering from diseases like inflammation, diabetes, rheumatism, cancer, hepatic obstruction, jaundice, diarrhea, etc. Research has been carried out throughout the world to validate the effectiveness of traditional medicine and some of the findings have led to the generation of important plant-based medicines. However, continuous efforts must be made to systematically identify, recognize, and place herbal medicines in the design and implementation of these strategies.[6,7] Modern pharmacopeia provides authentic information corresponding to the drugs derived from the herbal origin and their potential pharmacological effects on the human body.[8-10] The cost of drugs available in the modern world is too expensive for the majority of the population in the world countries.[11,12] Therefore the need to explore some cheap sources of anti-diabetic, antioxidant, antibacterial, anti-carcinogenic and anti-viral substances in nature become inevitable.[13-15] Mosquitoes are responsible for causing millions of deaths every year by spreading life-threatening dreadful diseases like Japanese encephalitis, filariasis, malaria, yellow fever, and dengue hemorrhagic fever.[16-18] Widespread utilization of synthetic organic insecticides to control mosquito-borne diseases has resulted in various problems which include environmental toxicity, increased operational cost, and resistance developed by vectors.[19] Mosquito control is an important public health concern around the globe, especially in the tropics. Controlling immature mosquitoes in their breeding sites is proven to be

effective in decreasing their overall prevalence. The continuous application of synthetic organic insecticides results in the development of resistance in vector species, biomagnification of toxic substances, and adverse environmental effects on non-target organisms including humans. These factors have resulted in a great deal of interest to explore different approaches and strategies which are cost-effective, easily degradable, and, environmentally friendly. In this sense, secondary metabolites present in botanicals represent a potential source for the control of various vector-borne diseases.[20-23] Therefore in the present work by considering the advantages of compounds obtained from the natural origin for the first time the larvicidal activity was evaluated for PHE (Poly Herbal Extract) prepared from seven folk Indian medicinal plants and the binding affinities of major compounds identified through GC-MS towards sterol carrier protein were reported.

EXPERIMENTAL

Collection of Plant Materials

Seven folk Indian medicinal plants namely Eugenia-jambolana (seeds), Trigonella-foenum-graecum (whole plant), Aegle-marmelos (leaves), Cassia-auriculata (flowers), Marsilea-quadrifolia (Whole Plant), Mangifera-indica (leaves) and Musa-paradisiaca (flower) were collected from Madurai district, Tamilnadu, India.

Preparation of Plant Extract

The shade-dried herbal powder of 100 g of each of all eight samples was taken and subjected to methanol extraction using the Soxhlet apparatus. After the completion, the extract was filtered off and further distilled in a vacuum under pressure. It was dried and stored in the refrigerator at 4 ºC until required for the determination of larvicidal activity Fig.-1. The Phytochemical screening and spectroscopic investigation of the polyherbal extract have been reported in our previous work.[24]

GC-MS Analysis of PHE

GC-MS analysis of the methanolic extract of PHE was carried out using the Agilent Technologies 7820A GC system. The GC–MS chromatogram recorded a total of 10 prominent peaks related to the phytoconstituents of the polyherbal formulation of the selected medicinal plants that were identified by comparing their peak area (%), peak retention time, height (%), and mass spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library.

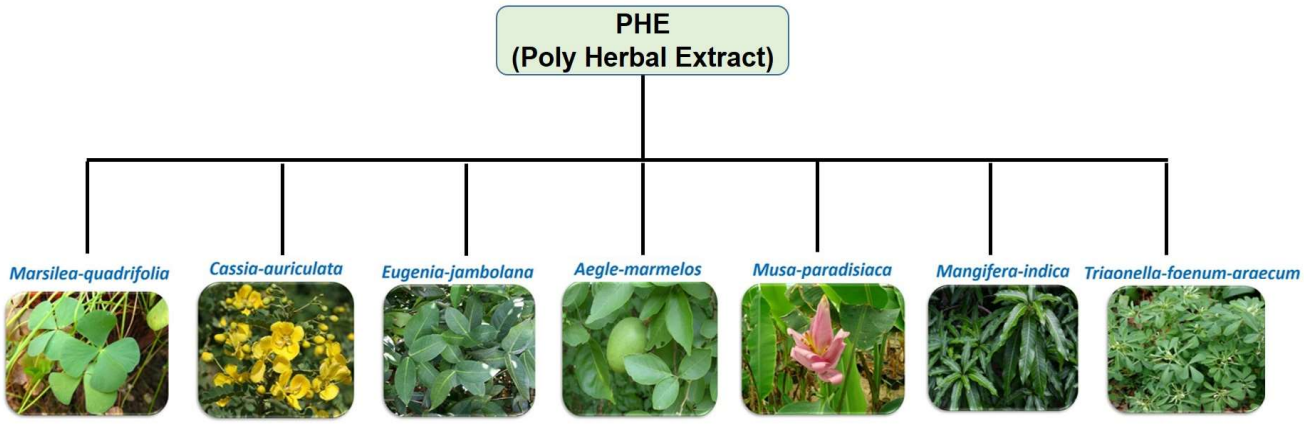

Fig.-1: Preparation of PHE (polyherbal extract) from Selected Medicinal Herbs

Mosquito Culture

The present study was conducted in the Vellore district (12.9165° N, 79.1325° E), Tamilnadu, India. Larvae of *A.stephensi*, *C.quinquefasciatus,* and *A.aegypti* were collected from rice fields, surrounding the area of Kaniyambadi, Vellore district, Tamilnadu, India, and identified by Zonal Entomological Research Centre. To start the colony the larvae were kept in different enamel containers in tap water. The larvae were fed with a diet of dog biscuits, Brewer's yeast, and algae collected from the surrounding ponds in the ratio of

4:2:1. They were maintained and all the experiments were carried out at a temperature of 28 ± 2°C and 85-90 percent relative humidity with 12 h each of day and night cycles.

Larvicidal Activity

The larvicidal efficacy was assessed as per the procedure of the World Health Organization (WHO) with slight alterations considered for this analysis.[25] From stock solutions different concentrations of methanolic extract of PHP [250, 500, and 1000 ppm] were prepared. Batches of 25 third instar larvae were transferred using a dropper to 200 ml glass beakers each containing 100 ml water and one batch as a negative control for each concentration. Each test was carried out in triplicate. The larvae mortality rate was recorded after 24 h exposure in each concentration of test solution and the percentage mortality was calculated by using Abbott's formula as described in our earlier work by Angajala *et al.* 2018.

Molecular Modelling Studies

The effective binding affinities of major compounds identified through GC-MS analysis of PHE along with standard Temephos towards Sterol Carrier Protein (SCP-2) were analyzed using AutoDock.v.4.2 as per the procedure described in our recent report.[26]

Statistical Analysis

The percentage larval mortality data were subjected to one-way factorial ANOVA analysis. The results with $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

GC-MS Analysis

GC-MS chromatogram figured a total of 10 prominent peaks at various retention times (RT) that can be distinguished and different compounds identified corresponding to the various peaks are listed in Table-1.

Identification of Compounds

On comparison of mass spectral fragmentation patterns of PHP methanolic extract with the NIST library, 10 major bioactive compounds were identified and interpreted accordingly. The molecular weight (MW), Retention time (RT), and nature of the compounds were determined. The obtained results depict the identification of various phytoconstituents (Fig.-2).

Table-1: GC-MS Analysis of Methanolic Extract of Polyherbal Powder (PHP)

Peak No	RT [a]	Compound Name	Mol Wt
1	2.50	Toluene	92.14
2	8.49	Ethyl benzene	106.16
3	13.20	Phytol	296.00
4	19.78	6-Methyl-3,5-heptadiene-2-one	124.18
5	21.50	Geranyl isovalerate	238.37
6	23.03	4-(hydroxymethyl)phenol	124.13
7	25.79	Geraniol	154.24
8	26.39	Cyclo hexa-2,5- diene-1,4- dione	108.09
9	28.04	Limonene	136.24
10	30.44	Marmelosin	270.27

[a] Retention time.

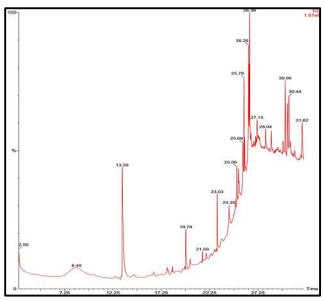

Fig.-2: GC-MS Chromatogram of PHE

The larval stage of mosquito is considered a more potential target to investigate the larvicidal potential of native herbal plants owing to its easiness to govern several processes and sluggish motility rate in their breeding habitats. There are several reports available in the literature regarding the larvicidal potential of indigenous individual plant extracts.[27-32] To the best of our knowledge no research has been carried out related to the larvicidal activity of PHP prepared by the combination of various parts of selected seven folk Indian medicinal plants and its molecular modeling simulations with SCP-2 protein. The results obtained demonstrated that PHP (methanolic extract) administered to the larva of *A.aegypti*, *A.stephensi* and *C.quinquefasciatus* generated LC_{50} at a concentration of 492.20, 453.30, and 462.17 ppm. The LC_{50} values of PHP herbal extract are slightly lower as matched to positive control Temephos (497.64, 467.14, 485.29 ppm) thereby showcasing its potential application in controlling several mosquitos-borne diseases (Table-2 and Fig.-3). Sterol is a very essential compound for the survival of most insects and mosquitoes to complete their life cycle. Conversely, they are unable to synthesize the sterol in their life cycle.

Table-2: Larvicidal Activity of Methanolic Extract of PHP

Species	Sample	Mortality rate (%) [Mean ± S.D]			LC_{50}	D_f	t value	P value
		250 ppm	500 ppm	1000 ppm				
A.aegypti	1	10 ± 0.58	16 ± 0.75	28 ± 0.10	1473.82	5	+2.62	< 0.05
	2	13 ± 0.17	18 ± 0.19	30 ± 0.84	1428.46	5	+2.60	< 0.05
	3	10 ± 0.76	18 ± 0.20	28 ± 0.90	1467.12	5	+2.62	< 0.05
	4	09 ± 0.24	14 ± 0.54	25 ± 0.21	1535.28	5	+2.64	< 0.05
	5	14 ± 0.25	21 ± 0.55	34 ± 0.68	1387.19	5	+2.59	< 0.05
	6	12 ± 0.15	18 ± 0.10	31 ± 0.62	1410.97	5	+2.60	< 0.05
	7	15 ± 0.18	24 ± 0.60	37 ± 0.85	1348.10	5	+2.58	< 0.05
	PHP	32 ± 0.15	54 ± 0.83	78 ± 0.53	492.20	5	+2.40	< 0.05
	9[c]	30 ± 0.95	52 ± 0.60	76 ± 0.10	497.64	5	+2.41	< 0.05
	10[d]	0	0	0	0	0	0	0
A.stephensi	1	14 ± 0.60	19 ± 0.25	31 ± 0.18	1395.24	5	+2.59	< 0.05
	2	16 ± 0.29	21 ± 0.40	32 ± 0.22	1382.69	5	+2.58	< 0.05
	3	15 ± 0.80	19 ± 0.34	31 ± 0.84	1386.22	5	+2.59	< 0.05
	4	12 ± 0.55	17 ± 0.23	29 ± 0.52	1428.64	5	+2.60	< 0.05
	5	18 ± 0.70	23 ± 0.15	33 ± 0.91	1374.28	5	+2.57	< 0.05
	6	15 ± 0.84	19 ± 0.76	31 ± 0.96	1385.29	5	+2.59	< 0.05
	7	19 ± 0.25	29 ± 0.55	42 ± 0.11	1284.70	5	+2.54	< 0.05
	PHP	35 ± 0.47	59 ± 0.72	80 ± 0.20	453.30	5	+2.34	< 0.05
	9[c]	32 ± 0.80	55 ± 0.19	78 ± 0.66	467.14	5	+2.38	< 0.05
	10[d]	0	0	0	0	0	0	0
C.quinquefasciatus	1	12 ± 0.19	19 ± 0.62	34 ± 0.79	1378.49	5	+2.57	< 0.05
	2	15 ± 0.26	23 ± 0.10	36 ± 0.55	1350.21	5	+2.55	< 0.05
	3	14 ± 0.92	21 ± 0.54	32 ± 0.16	1384.93	5	+2.59	< 0.05
	4	13 ± 0.18	17 ± 0.29	29 ± 0.30	1430.15	5	+2.60	< 0.05
	5	18 ± 0.79	25 ± 0.10	35 ± 0.26	1368.20	5	+2.57	< 0.05
	6	16 ± 0.55	22 ± 0.69	33 ± 0.38	1387.60	5	+2.59	< 0.05
	7	21 ± 0.24	28 ± 0.15	42 ± 0.73	1275.31	5	+2.54	< 0.05
	PHP	34 ± 0.92	55 ± 0.69	79 ± 0.26	462.17	5	+2.35	< 0.05
	9[c]	31 ± 0.25	53 ± 0.40	75 ± 0.32	485.29	5	+2.39	< 0.05
	10[d]	0	0	0	0	0	0	0

[a]Lethal Concentration that kills 50% of the exposed parasite
[b]Degrees of freedom,
[c]Temephos (Positive Control),
[d]Distilled water (Negative Control).

Mosquitoes attain sterols in the form of phytosterol during their larval stage from the plant decays. In a later stage, the phytosterol is converted to cholesterol which is essential for reproduction and growth. The complete mechanism of transformation is carried out with the help of sterol carrier protein-2 (SCP-2). Therefore, in the present work, the bioactive compounds present in PHP extract can effectively blocks the

uptake of cholesterol by restricting the function of SCP-2 which subsequently causes detrimental effects in mosquito larvae leading to death. It is evident from the molecular modeling analysis that two bioactive compounds from PHE namely Geranyl isovalerate (-8.0 K.cal/mol) and Marmelosin (-7.6 K.cal/mol) exhibit greater binding interactions with SCP-2 protein as compared to Temephos (-6.8 K.cal/mol). The most stable docking structure of Geranyl isovalerate and Marmelosin complexed with SCP-2 protein was shown in Fig.-4 and Table-3.

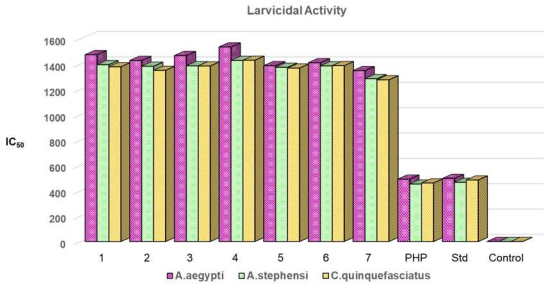

Fig.-3: Larvicidal Efficacy of PHP and Various Plant Extracts

Table-3: Binding Affinity Studies of Various Compounds Identified Through GC-MS with Sterol Carrier Protein (SCP-2)

S.No.	Compound	Binding Affinity Score (Kcal/mol)
1	Toluene	-2.4
2	Ethyl benzene	-3.2
3	Phytol	-5.8
4	6-Methyl-3,5-heptadiene-2-one	-6.2
5	Geranyl isovalerate	-8.0
6	4-(hydroxymethyl)phenol	-5.7
7	Geraniol	-6.6
8	Cyclo hexa-2,5-diene-1,4- dione	-5.4
9	Limonene	-4.4
10	Marmelosin	-7.6
11	Temephos[a]	-6.8

[a]Standard

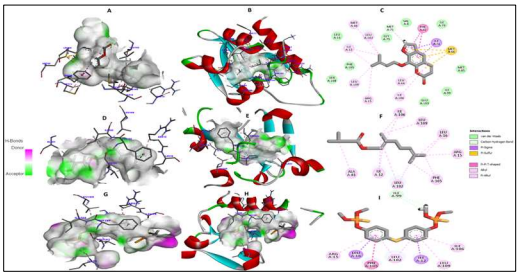

Fig.-4: Binding Interactions of Identified Potent Bioactive Compounds Towards Sterol Carrier Protein (SCP-2) [3A-3C] Binding Affinity of Marmelosin in Complex with SCP-2 [3D-3F] Binding Affinity of Geranyl Isovalerate in Complex with SCP-2 [3G-3I] Binding Affinity of Temephos (Std) in Complex with SCP-2

The synergistic effect of various bioactive compounds from PHP is mainly responsible for the higher larvicidal efficacy of PHP when compared to individual plant extracts. Out of the individual plant extracts Aegle-marmelos leaf extract [sample 7] showed slightly higher larvicidal efficacy against *A. aegypti* (IC_{50}:1348.10 ppm), *A. stephensi* (IC_{50}:1284.70 ppm) and *C. quinquefasciatus* (IC_{50}:1275.31 ppm) respectively. The experimental data obtained demonstrated that in overall comparison with positive control PHP extract exhibits appreciable larvicidal activity and further separation and characterization of potent bioactive compounds Geranyl isovalerate and Marmelosin is very essential to surpass synthetic insecticides in near future and also in controlling blood-feeding mosquito vector populations in disease-endemic areas if utilized through systemic and integrated approaches.

CONCLUSION

In the current scenario environmental sustainability is considered as a crucial concept that ultimately protects global ecosystems and natural resources. In the present work, the obtained results have opened doors for future investigations regarding the isolation and characterization of potent bioactive components and the amalgamation of prepared polyherbal formulation into the control of mosquito vectors, with an opinion to develop an emerging environmentally benign safer product.

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