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IN-VITRO LARVICIDAL ACTIVITY OF *PEGNUM HARMALA* EXTRACTS AGAINST LARVAE OF MALARIA VECTOR ANOPHELES STEPHENSI

Shawana Nawaz[1], Shamim Khan[*2], Shehzad Zareen[*3], Bibi Ibtesam Shagufta[4], Momel Zohra[5], Qulbe Hassan[6], Arshad Mahmood[7], Waqas Khan[8], Sabir Ullah[9], Asad Zia[10], Rabail Farooq[11]

[1,*2,*3,4,5]*Department of Zoology, Kohat University of Science and Technology, Kohat*
[6]*Department of Zoology. Hazara University, Garden Campus Mansehra Pakistan*
[7,8]*Department of Zoology Government Postgraduate College Dargai 23060 Malakand Khyber Pakhtunkhwa Pakistan*
[9]*Nowshera Medical College Khyber Pakhtunkhwa, Pakistan*
[10]*Department of Zoology, University of Swabi, Pakistan*
[11]*Department of Microbiology, Kohat University of Science and Technology, Kohat*

[1]shawananawazkust@gmail.com, [*2]zo420211006@kust.edu.pk, [*3]shehzadzareen@kust.edu.pk, [4]ibteshagemi@gmail.com, [5]zahramomal170@gmail.com, [6]qalbehassan125@gmail.com, [7]arshadkhanpk3667@gmail.com, [8]zoologist.waqaskhan@gmail.com, [9]sabir3027116@gmail.com, [10]ziadir5020@gmail.com, [11]rabailyasir1420@gmail.com

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Corresponding Authors: * Shamim Khan, Shehzad Zareen

Abstract

Malaria is a mosquito-borne parasitic disease affecting over 300 million people globally each year, causing high child mortality. It is primarily spread by female Anopheles mosquitoes, especially An. culicifacies and An. stephensi in Pakistan.The disease is caused by five Plasmodium species, with P. falciparum and P. vivax being the most dangerous. Despite medical advances, malaria still leads to around one million deaths annually, particularly in regions like Pakistan. Peganum harmala, a medicinal plant rich in alkaloids like harmine and harmaline, shows antibacterial and anti-protozoal activity. In currunt study larvae of Anopheles stephensi were collected from water bodies in Kohat, KPK, Pakistan for experimental studies. Leaves of Peganum harmala were gathered from the same region and processed into methanolic extracts using a rotary evaporator. The leaves were shade-dried, ground, and soaked in methanol for 21 days to obtain crude plant extracts. Stock solutions were prepared from the extract and diluted for in-vitro bioassays. Different concentrations of the extract were tested against mosquito larvae to observe mortality rates over 30 minutes. Result show that Peganum harmala crude extract caused the highest larval mortality (15.33 ± 2.51) at a concentration of 8ml/10ml, with an LD_{50} value of 44.58, observed over a 5-hour period. The results indicate that the selected plant extracts, particularly in methanol, possess repellent and larvicidal properties, suggesting the presence of potent bioactive compounds. Although mortality was low in the first hour, it increased over time, peaking in the 4th hour at the highest concentration (8ml/10ml or 80%).

INTRODUCTION

Malaria is a parasitic disease from which more than 300 million people suffer yearly throughout the world. It is one of the main causes of infant and young child mortality [1]. Malaria is an infectious disease transmitted by mosquitoes which is acquired from the bite of the female nocturnal-feeding anopheles species of the mosquito [2]. It is caused by five different kinds of plasmodia, Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi. Plasmodium falciparum and Plasmodium vivax are more dangerous and common species [3]. In spite of the so many advances in medical sciences, approximately one million per annul deaths are caused by malaria [4]. Pakistan is one of the countries where malaria is one of the big problems for public health. Pakistan is an agricultural country having a vast irrigating network and monsoon rains.[5]. Two species of Anopheles, Anopheles stephensi and Anopheles culicifacies are considered to be mainly responsible for the transmission of malaria in Pakistan. Anopheles culicifacies is thought to be the primary vector, especially in rural areas, whereas, An. stephensi is considered to be of secondary importance in rural areas and only partially responsible for urban malaria transmission [6].

Pegnum harmala commonly known as Ispand or Espand is a widely used medicinal plant from the family Nitrariaceae [7]. Pegnum harmala is a perennial, glabrous plant which grows spontaneously in semi-arid conditions, steppe areas and sandy soils, native to eastern Mediterranean region [8]. Pegnum harmala, a flowering plant, is widely distributed in the Central Asia (India and Pakistan), North Africa and Middle East. It has also been introduced in America and Australia [9]. The pharmacologically active compounds of P. harmala are several alkaloids, which are found especially in the seeds and the roots. These include β- carbolines such as: harmine, harmaline, harmalol and harman and quinazoline derivatives: vasicine and vasicinone. The alkaloid content of the unripe seeds is less than the ripe ones [10]. Harmine, a compound present in P. harmala, fluoresces under ultraviolet light. P harmala has been shown to have antibacterial and anti-protozoa activity, including antibacterial activity against drug-resistant bacteria [83]. Keeping in view the importance of P. harmala and hazards of malaria vector the current study is designed to determine the in-vitro toxicological effects of P. harmala extracts against the Larvae of Malaria vector An. stephensi.

MATERIAL AND METHODS

Malarial Vector Collection

Collection of larvae of malarial vector Anopheles stephensi was made from District Kohat, KPK, Pakistan Collection team visited pools, streams, drains and ponds for experimental purpose. (Figure-1:A)

Plants Collection:

The fresh matured leaves of selected medicinal plants; Pegnum harmala was collected from Bilitung, District Kohat, KPK, Pakistan. (Figure-1:B)

Figure-1: A. Larvas of Anopheles stephensi , B. Pegnum harmala in its Natural Habitat

Extract Preparation

For the preparation of plant extracts, the amount of fresh washed leaves of Pegnum harmala were kept for shaded-drying for several days. Dried leaves of each selected plants were crushed in electric blender. Then 500gm powder of each selected plants leaves was mixed with 2.5litres methanol in two bottle flasks and allow it for 21 days. Mouth of each flask was closed with cotton plug aluminum foils. plant leaves extract was filtered with filter paper to obtain 100% filtrate. The dried remains were collected by evaporating the solvent with the aid of rotary vacuum evaporator and stored in a refrigerator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum to prepare the crude extract of selected medicinal plants in the laboratory of Chemistry Department, KUST (Figure-2). One gram of the plant residue was liquefied in 100ml of distilled water to make stock solution. The stock solution was further diluted.

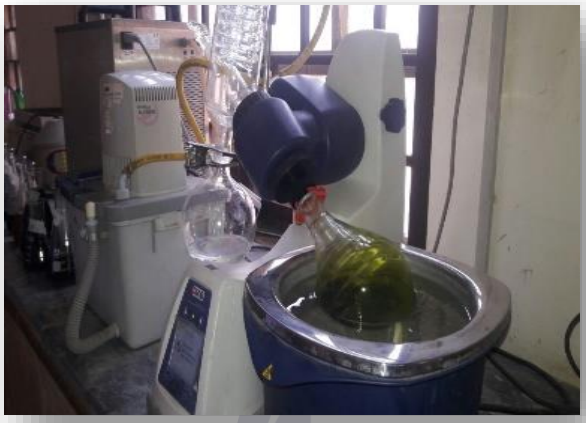

Figure-2: Extract Preparation in Rotary Evaporator

In-vitro Assay

To test the toxic effect of powder leaves of selected medicinal plant; Pegnum harmala extract at different doses with different exposure of time against the larvae of An. stephensi, larvae were placed in Petri dishes. A soaked cotton plug was placed in each Petri dish to maintain sample moisture content. First we labeled the Petri dishes with different concentrations (0.1mg, 0.2mg, 0.3mg, 0.4mg) then the required concentrations of methanolic leaves extract of each plant; P. harmala was 1000ml sprayed thoroughly on the filter papers placed in the Petri dishes by using micropipette. The Petri dishes were left exposed to open air for fifteen minutes to completely evaporate the solvent. Ten larvae samples were released into each Petri dishes and then Petri dishes were covered. Experiment was conducted in triplet for each sample concentration along with the set of control group and percent mortality was recorded after the equal time period of 30minutes

Figure-3: In-vitro Assay

Results

Effects of Pegnum harmala extract on larvae of Anopheles stephensi

Table I shows the percentage mortality due to Pegnum harmala at different concentrations of plant crude extract against the larvae of Anopheles stephensi with the time interval of one hour up to 5 hours at concentration of 8ml/10ml, there were high mortality recorded with the rest of concentration on larvae. The highest mortality was 15.33±2.51 as shown in the table: 3.6. **LD50** was 44.58. (Table-1/Figure-4)

Time interval	Concentrations (Mortality% ± SD)			
	2ml/10ml	4ml/10ml	6ml/10	8ml/10ml
Hour-1	0.33±0.57	1.33±0.57	2.00±1.00	4.66±1.52
Hour-2	1.00±1.00	2.66±0.57	3.33±1.15	6.66±1.52
Hour-3	2.66±1.52	4.33±0.57	5.00±1.00	7.66±1.52
Hour-4	4.00±1.00	4.33±0.57	6.66±0.57	11.66±2.51
Hour-5	7.33±1.52	5.66±1.15	10.33±1.52	15.33±2.51

Table-1: Effects of Pegnum harmala extract on larvae of Anopheles stephensi

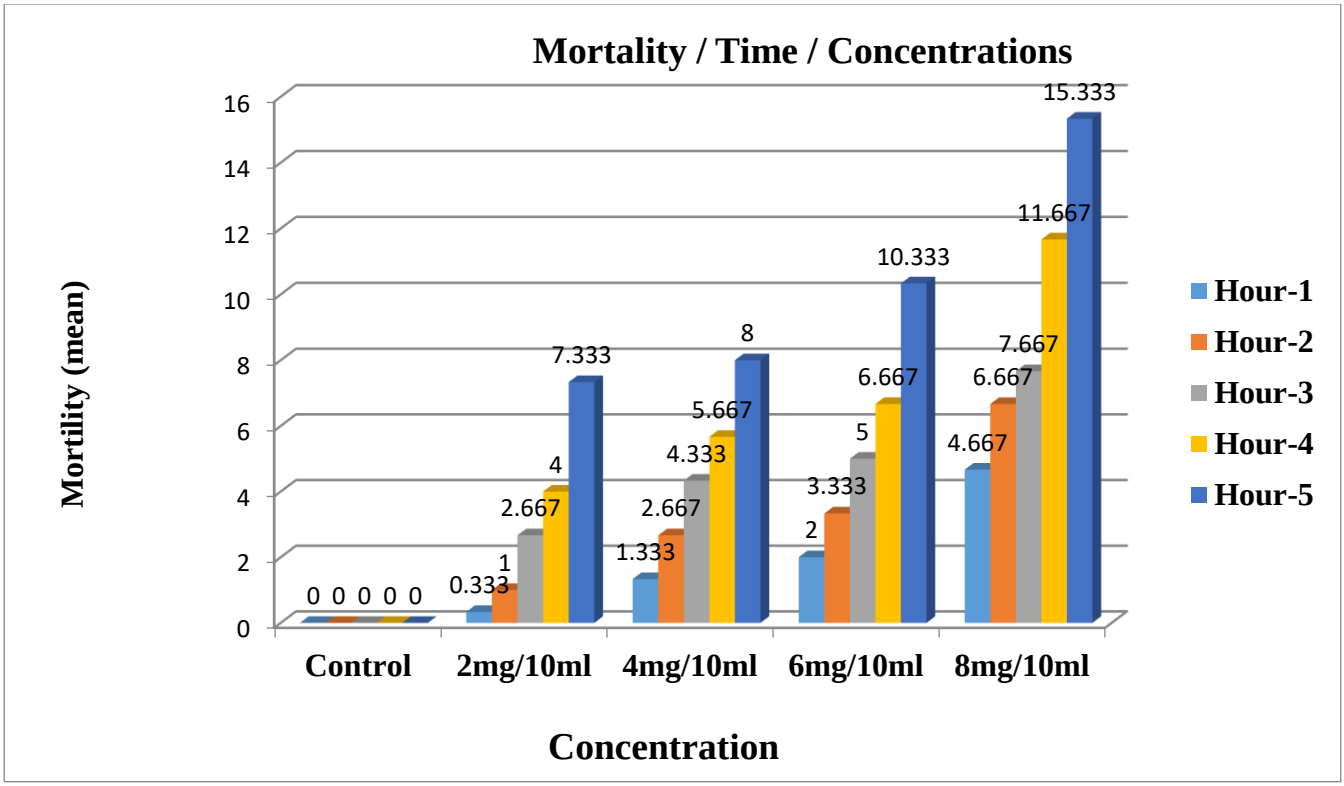

Figure-4: Effects of Pegnum harmala extract on larvae of Anopheles stephensi

Conclusion

The results of current investigations suggest that the selected plants have potential for repellent action. It also suggests that these plants contain such active compounds which were completely soluble with the high polarity solvent (methanol) used in preparation of the extracts and might be useful as potent vector controlling agent. Crude extract of these plants works slowly, in the first hour of treatment and lesser mortality was recorded. Then the mortality rate increases with the passage of time and gives maximum mortality in the 4th hour. On the concentration of

80% or 8ml/10ml, selected plant gives maximum mortality.

Recommendations

- Further work on the identification of active ingredients of P. harmala extract needed which are more effective with low cost environmental friendly.
- More indigenous plants should be explored to use against malaria vectors which have potential to envy.
- Further research should be done to study the impacts of these medicinal plants on human health, their mode of action and other aspects.

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