Trop J Phytochem Pharm Sci, September 2024; 3(6): 332 - 337 **ISSN 2955-1226 (Print)**
ISSN 2955-1234 (Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Effect of Phytochemical Components of The Leaf of *Moringa Oleifera* on the Development of *Anopheles Gambiae*

Balogun H. Adewale, Ayandiran T. Akinpelu*, Fawole O. Olubanjo, Ajala O. Olumuyiwa, Olanipekun A. Stephen, Adeyemi O. Olajumoke

Environmental Biology Unit, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

ABSRTACT

Female *Anopheles gambiae sensu stricto*, is a vector of *Plasmodium falciparum* accountable for malaria infection in man. The study investigated the use of phytochemicals from *Moringa oleifera* leaves, extracted with distilled and deionized water, to delay the vector's developmental stages. Extractions were applied to larvae and pupae of *A. gambiae*, with deionized water as a control. Observations of larvae and pupae development and mortality were recorded. Using ANOVA, the data was analysed to ascertain the significance ($p < 0.05$) of the larvae and pupae that remained active at various extracted phytochemical concentrations after the control stage. Extraction using deionized water was most effective at inhibiting development at 5×10^{-3} mg/ml, where 80% of the larvae were inhibited from developing to pupae, while extraction using distilled water was most effective at 5×10^{-2} mg/ml, where 70% were inhibited. Statistically, no significant difference was noted, between the extraction media ($p= 0.737$, 95% CI) after ninety-six hours, when control had developed to pupae. All inhibited larvae were still alive. The result of the larvicidal properties of *M. oleifera* leaf extracted with deionized water and distilled water revealed, 20 % mortality at the concentration of 5×10^{-3} mg/ml for deionized water while 30 % mortality was recorded at 5×10^{-2} mg/ml for distilled water. The activities of the extracted phytochemicals at inhibiting the development of larval and pupa stages of *A. gambiae* showed that the particles could be deployed for the control of mosquitoes.

Keywords: *Anopheles gambiae*, *Plasmodium falciparum*, Phytochemicals, *Moringa oleifera*, Larvicidal.

Received 26 April 2024
Revised 09 September 2024
Accepted 09 September 2024
Published online 01 October 2024

Introduction

Mosquito is the most important single group of arthropod of public health importance; therefore its control is equally necessary in order to successfully curtail the spread of mosquito borne diseases thereby improving ecological and public health.[1] Naturally, there are fishes that prey on mosquito larvae; such fishes include *Gambusia patruelis* and *Hemichromis sp*. These fishes feed greatly on diverse developing stages of mosquito and help decrease the occurrence of malaria diseases.[2] In the hunt for biological method of controlling malaria vector, eleven larvivorous fish species native to Ethiopia were identified and these include five species not previously reported in the country.[3] Verifiable evidences abound that under controlled laboratory conditions the fishes fed seriously on the larvae of *Anopheles gambiae*. The application of former synthetic insecticides in mosquito control programs has been restricted due to lack of original insecticide, elevated cost of man-made insecticides, and concern on ecological sustainability. Insecticide's detrimental effect on human health and other non-target populations, their non-biodegradable nature, high rate of biological magnification through ecosystem and increasing insecticidal resistance on a global scale are reasons why alternative sources of control was desirable.[4]

*Corresponding author. E mail: *taayandiran@lautech.edu.ng*
Tel: 08035327173

Citation: Adewale BH, Akinpelu AT, Olubanjo FO, Olumuyiwa AO, Stephenand OA, Olajumoke AO. Effect of Phytochemical Components of The Leaf of *Moringa Oleifera* on the Development of *Anopheles Gambiae*. Trop J Phytochem Pharm. Sci. 2024; 3(6):332 – 337
http://www.doi.org/10.26538/tjpps/v3i6.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The environmental protection act in 1969 included several rules and regulations to check the application of chemical control agent in nature[5]. This in turns prompt scientists to look for substitute methods ranging from the provision of or promoting the adoption of effective and transparent mosquito management tactics with focus on public education, monitoring and surveillance, source reduction and environmentally friendly least toxic larval control.[5] In view of these, the application of eco-friendly alternatives such as biological control of vectors has been the central focus of the control programme as a substitute to chemical insecticides.[5]

Botanicals are secondary metabolites which serve as a means of defense mechanism of the plant to withstand the continuous selection pressure from herbivores, predators and other environmental factors.[6] Several groups of phytochemicals such as steroids, alkaloids, terpenoids, essential oils and phenolics from several plants have been reported previously for their insecticidal activities. The insecticidal effect of different plants extract vary not only according to plant species, mosquito species, and parts used, but also as a result of the extraction methodology adopted and the polarity of the solvents used during extraction.[6] Different types of plant from herbs, shrubs, and large trees were used for extraction of mosquito toxins. The needs to control these mosquitoes led to the emergence of several control options ranging from plant extract, entomopathogenic fungi, and bacteria agents to some other biological control agents. A lot of work has been carried out on larvicidal, pupacidal and insecticidal properties of phytochemicals obtained from different parts of various plants using different extraction media.[6]

Moringa oleifera is the most widely cultivated plant species worldwide originating from western and sub-Himalayan region, Pakistan, India, Africa, Asia Minor and Arabia.[7&8] The seeds have been found to have health benefits in Cambodia, Philippines, Caribbean Islands, and Central North, including anti-inflammatory, anti-tumor, antimicrobial, antispasmodic, and diuretic properties. The plant extracts exhibit properties through larvicidal, repellents, deterrents and insect growth

regulators.[9] Extract of *M. oleifera* seed has showed a great potential as larvicidal and pupacidal agent against mosquitoes.[10]
Nigeria is at high risk of large epidemics because of overcrowded cities and unhygienic conditions. The misuse of insecticides on *A. gambiae*, in Agriculture and public health programs raised many health and environment related problems like insecticide resistance, resurgence of pest species, toxic hazards to humans and other non-target organisms and environmental pollution.[11] In order to overcome these challenges, scientists have shifted their attention to biological or plant-based products that can provide an effective alternative approach to synthetic insecticides. This research seeks to find a means of inhibiting the development of *Anopheles gambiae* sensu stricto at larva or pupa stages using phytochemical components of *M. oleifera* leaf, extracted using different solvents. This will reduce the population of adult *A. gambiae* in the environment; thereby leading to a reduction in the transmission of malaria among human population.

Materials and Methods

Collection of leaves of Moringa oleifera
The leaves of *M. oleifera* were collected from Apete Ibadan, Oyo state (Latitude 01° 13.165' N, Longitude 05°57.500' E) on August 12, 2016. The voucher number for the collected *Moringa oleifera* leaves is LHO 821. The leaves were washed to remove particles and they were air-dried for 18 days and seven hours, and then ground into a fine powder, sieved, and stored in a sealed glass container.
Qualitative phytochemical screening Test for Tannins
The plant extracts were stirred in distilled water and filtered. Ferric chlorine (0.1 % $FeCl_3$) reagent was added to the filtrate. A blue black or blue green precipitate was taken as preliminary evidence for presence of tannin[12].

Test for Alkaloids
Five millimeters (5 ml) of 10 % (v/v) HCL was added to 0.5 g of the extracts in test tube and was put in a water bath for 2 minutes, after which the mixture was filtered. To separate the parts, three drops of Dragendroff's reagent were added to 1 milliliter of the filtrate. The formation of a reddish brown coloring indicated the presence of alkaloids.[12]

Test for Steroids
After adding two milliliters (2 ml) of acetic anhydride to 0.5 g of the extract, 2 ml of H2SO4 was also added. When a color changed from violet to blue or green, steroids were present.[12]

Test for Saponins
The screening test for saponins was based on their capacity to cause foaming in an aqueous solution. In order to create a stable, long-lasting froth, 0.5 g of the extract was baled with distilled water in a water bath and forcefully shaken. Three drops of dreadful oil were added to the foaming, and after a vigorous shake, an emulsion was seen to develop.[13]

Test for Phlobatannins
Plant extract in aqueous form was baled with 1% HCL (aq). Red precipitate deposition was seen as initial proof of phalobatannin existence.[13]

Test for Anthraquinones
A five millimetre (5 ml) of benzene was added to the test tube containing 0.2 g of plant extract and was shaken. After adding 5 millilitres of a 10% ammonia solution to the filter, the mixture was shaken. The existence of free hydroxyl-anthraquinone was indicated by the presence of pink-red, crimson, or violet hue in the ammouniacal (lower) phase.[13]

Test for Flavonoid
Plant extracts were cooked in a water bath for thirty minutes along with ten millilitres (10 ml) of ethylacetate. The mixture was filtered through four millilitres (4 ml) of a diluted ammonia solution in a conical flask, and each filtrate was agitated. Flavonoids were denoted by a yellow colouring.[14]
Phytochemicals are extracted from *M. oleifera* leaves using deionized and distilled water as solvents.
50 g of powder was added to one litre of solvent to extract the phytochemicals from the leaves of *Moringa oleifera*. To fully extract the plant elements, the mixture was agitated for 45 minutes using a magnetic stirrer and then left for three days. To extract all of the liquid, the material was strained, and the solid that remained was manually squeezed. The resulting liquid was filtered through Whatman number 1 filter paper to clarify it. The concentrate and extract were separated at 50 ^{0}C using a rotary evaporator. The parent stock solutions of the five solvents were obtained by following this process.

Preparation of Moringa oleifera solution
The concentration of Parent stock solution (PSS) was calculated to be:
Converting 50 g to mg
50g × 1000 = 50000mg
$\frac{50000mg}{1000ml}$ = 50mg/ml
50mg/ml × 1 % solution
50mg/ml × $\frac{1}{100}$
50mg × 0.01
0.5 mg/ml
Concentration of PSS = 0.5 mg/ml
This solution represented the parental stock solution (PSS) of the extraction, with a concentration of 5 × 10^{-1} mg/ml. Further concentrations were obtained by serial dilutions.

Serial dilution
By using Serial dilution law, which states that CsVs = CdVd Where Cs, indicate concentration of stock.
Vs indicate volume of stock.
Cd, indicate concentration of diluents.
Vd, indicate volume of diluents. First concentration
10 ml of 5 x 10-1 solution was measured by a measuring cylinder into a conical flask and 90 ml of deionized water was added to it, to give 5×10-2 mg/ml.
i. e. CsVs = CdVd
0.5 × 10 ml = Cd × 100 ml
$\frac{0.5mg/ml \times 10\ ml}{100\ ml}$ = C
C d = 5 × 10^{-2} mg/ml Second concentration
10 ml of 5 × 10^{-2} mg/ml was measured into another conical flask and 90 ml of deionized water was added to it, to give 5 × 10^{-3} mg/ml.
i.e. CsVs = Cd_2Vd
0.05 mg/ml × 10 ml = Cd2 × 100 ml
$\frac{0.05mg/ml \times 10\ ml}{100}$ = $\frac{C}{d_2}$
C_{d2} = 5 × 10^{-3} mg/ml
This procedure was repeated until 5 × 10^{-9} mg/ml concentration of each of the two solutions was obtained. The solutions were obtained using the solvents of distilled water and deionized water.
Treatment doses were determined after several trials, using different ranges of concentrations (from 5 × 10^{-1} mg/ml to 5 × 10^{-9} mg/ml) of the solutions of phytochemicals extracted from the leaves of *Moringa oleifera*. Effects of the extracts on the development of *A. gambiae* using the different solvents became noticeable between the concentrations of 5 × 10-1 mg/ml and 5 × 10-5 mg/ml. The five concentrations (5 × 10^{-1} mg/ml, 5 × 10^{-2} mg/ml, 5 × 10^{-3} mg/ml, 5 × 10^{-4} mg/ml and 5 × 10^{-5} mg/ml) were prepared from the stock solutions by a serial dilution method and 20 ml of each concentration was applied in the experiment.

Preparation of mosquito larvae and pupae
Isolation of A. gambiae eggs
Ten containers (34 cm – 21 cm – 5 cm) were used; each was filled with 1000 ml of deionized water. A little sand and leaf were spread on the

surface of the water to attract mosquito, they were placed in different corners of living premises, in 5 days there were presence of eggs in the water, and these were observed with naked eyes. The eggs of *A. gambiae* were identified by single appearance on the water surface and their canoe like shape with a pair of float on either side[16]. They were then isolated with fine brush.

Preparation of mosquito larvae and pupae

The isolated eggs were put into ten containers of size (34 cm – 21 cm – 5 cm) filled with 1000 ml of deionized water each and allow to hatch into larvae. *A. gambiae* developed through four larval sizes of instars before pupating[17]. Larvae were very small in the first instar and increase in size until they reach 5 mm - 6 mm by the completion of the fourth instar because the larvae stage is a feeding period. They feed on organic matter and algae[18]. In this study all the instar stages were regarded as larva and were fed in the laboratory with a mixture of ground rat chow and brewer's yeast (1:1 w/w) daily under standard rearing conditions which were maintained at 28 °C, 80 % humidity, and photoperiod of 12 hours.

This procedure was repeated, and the larvae were left to develop into pupae. All had developed into pupae within 4-5 days.

Harvesting the Larvae and Pupae

The identification of *A. gambiae* larvae was done by the method of Foster and Walker.[17] The pupae were identified using the method of Huang.[19]

Larval and pupae bioassay

Introduction of larvae and pupae into the extracted phytochemicals

20 ml of each concentration of the extracts were put inside labeled test tubes, indicating the concentration and the extract, after which a plastic pipette was used to pick 10 larvae from the container and transferred them into the various labelled test tubes. The actions of each concentration on the larvae were observed at intervals of 12 h. Mosquito net was used to cover the exit of the test tube and held tight with paper tape to avoid escape of the adult mosquito into the environment if it eventually developed. This procedure was also employed for the introduction of the pupae.

Control experiment

A control experiment was set up using deionized water. Immediately after the introduction of the larvae or pupae into the various concentrations of the phytochemical extracts, the same numbers of larvae or pupae were also introduced into deionized water (control experiment) in two separate test tubes and the development of the larvae and pupae were monitored at interval of 12 hours.

Observations

The effect of each concentration of the phytochemical extracts was monitored on the larvae and pupae at 12 hours interval for three weeks, because the development of *Anopheles gambiae* from egg to adult is normally within 14 days.

Replication of the experiment

The experiments involved different concentrations of the extract using different media, for each developmental stage. Each experiment was replicated ten times. The concentration having the highest frequency out of the ten replicates, at which inhibition occurred while the control has developed to the next stage, was recorded as the effective concentration.

Percentage mortality

Percentage mortality was calculated by using Abbott's formula (II).[20]

Percentage mortality = 100 × number of dead larvae ÷ number of larvae introduced.

Statistical analyses

Analysis of variance was used to compare the means of the inhibited larvae in the different concentrations of the solution of the extracted phytochemicals to detect any significant difference at 95 % confidence interval. This was also repeated for pupae.

Lethal concentration (LD_{50}) of extracted solutions

Lethal dose that killed 50 % of the population of larvae or pupae at different concentrations of the extracts were determined by using Microsoft office Excel (2007).

Results and Discussions

Qualitative screening of aqueous extract of Moringa oleifera leaf

The qualitative screening of *Moringa oleifera* leaves identified three active compounds—tannins, saponins, and flavonoids—out of five investigated, with anthraquinones and phlobatannins absent. This finding aligns with previous studies that also reported the presence of these phytochemicals in *Moringa oleifera*, highlighting its rich bioactive profile. The presence of tannins, saponins, and flavonoids is consistent with findings from other research. For instance, a study by Malhotra and Mandal identified similar compounds in *Moringa oleifera* leaf extracts, confirming the plant's potential as a source of bioactive compounds with therapeutic properties.[21]

The absence of anthraquinones and phlobatannins in the current study is noteworthy. While some studies have reported the presence of various phytochemicals, including anthraquinones, in *Moringa oleifera*, the variability may be attributed to factors such as extraction methods, geographical locations, and plant maturity. For example, research by Sharifi-Rad *et al*.[22] showed a broader range of phytochemicals, including anthraquinones, suggesting that extraction conditions significantly impact the phytochemical profile.

The methods employed for phytochemical screening can also influence results. The current study utilized qualitative screening, which is effective for detecting the presence of specific compounds. In contrast, quantitative analyses in other studies, such as those by Younis *et al*.[23] provide a more comprehensive understanding of the concentration of these compounds, which can be crucial for assessing their potential health benefits.

The control experiment for metamorphosis from larvae to pupae

The results indicate that in a control experiment using deionized water, 80% of the larvae developed into pupae within 96 hours (Table 1). This finding establishes 96 hours as a critical timeframe for observing larval development in subsequent experiments with *Moringa oleifera* leaf extracts. The statistical analysis shows no significant difference in the means of larvae that survived or were still alive at 96 hours between the extracts using distilled water and deionized water, with a p-value of 0.737. Similarly, the analysis of dead larvae also revealed no significant difference, with a p-value of 0.557. These results suggest that the solvent used for extraction does not significantly affect larval survival or development in the concentrations tested.

Comparatively, previous studies have highlighted the variable effects of *Moringa oleifera* extracts on different biological systems. For instance, Bhalla *et al*.[24] found that *Moringa* extracts possess potent antioxidant properties, which could influence the overall health and development of organisms exposed to these extracts. However, the lack of significant differences in larval development in this study aligns with findings from other research, where variations in extraction methods and solvents did not yield substantial differences in biological activity.[25]

Moreover, the absence of significant effects on larval survival and development in this study may also reflect the inherent resilience of the larvae or the concentration levels of the *Moringa* extracts used. In a broader context, the findings contribute to understanding how *Moringa oleifera* can be utilized in biological applications, particularly in pest management, without adversely affecting non-target organisms.[26] Thus, while the extracts show potential, their effectiveness may depend on specific conditions and concentrations, warranting further investigation.

Table 2 shows no significant difference in larval outcomes at 96 hours between *Moringa oleifera* leaf extracts prepared with distilled water and deionized water. The choice of solvent did not impact larval mortality or development.

 ISSN 2955-1226 (Print)
ISSN 2955-1234 (Electronic)

Table 1: The percentage of surviving larvae at 96 hours against different concentrations of *Moringa oleifera* extract using deionized water and distilled water.

	(5×10^{-1})	(5×10^{-2})	(5×10^{-3})	(5×10^{-4})	(5×10^{-5})	LD_{50}
Hinderance of Larvae (mg/ml)						
Deionized	0	10	80	10	0	0.63
Distilled	0	70	50	0	0	0.714
Larvae mortality						
Deionized	100	90	20	70	100	
Distilled	100	30	40	100	100	

Table 2: Statistical table showing significant difference in the means of dead larvae at 96 hours between the leaf extracts of *Moringa oleifera* using distilled water and deionized water as solvent of extraction.

		ANOVA				
		Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
LARVAE THAT WERE ALIVE	Between Groups	1.633	1	1.633	.114	.737
	Within Groups	1697.833	118	14.388		
	Total	1699.467	119			
LARVAE THAT WERE DEAD	Between Groups	4.800	1	4.800	.346	.557
	Within Groups	1635.567	118	13.861		
	Total	1640.367	119			
LARVAE THAT DEVELOPED	Between Groups	.833	1	.833	.360	.549
	Within Groups	272.867	118	2.312		
	Total	273.700	119			

The results in Table 3 demonstrate that *Moringa oleifera* extract has a pronounced impact on pupae survival, particularly at higher concentrations. Notably, at the highest concentration tested (5×10^{-1} mg/ml), distilled water as a solvent resulted in 90% pupae mortality, whereas deionized water achieved 100% mortality. This suggests that deionized water may enhance the efficacy of the extract, possibly due to differences in solvent properties that affect the solubility or stability of active compounds in *M. oleifera*. As concentrations of *Moringa oleifera* extract decrease, the survival rates of pupae increase, indicating a dose-dependent response (Table 3). Both solvents show similar effects at lower concentrations, consistent with findings that solvent type influences the bioactivity of plant extracts. Alhazmi *et al*.[27] found that deionized water enhanced the larvicidal properties of plant extracts, supporting the idea that it may facilitate a more effective release of bioactive compounds in *M. oleifera*.

The LD_{50} values further illustrate this trend, with deionized water requiring a lower concentration (1.29 mg/ml) for 50% pupae lethality compared to distilled water (1.5 mg/ml). This difference aligns with Bagavan *et al*.[28] who reported that solvent choice can impact the lethal concentration of plant-based insecticides. The slightly higher LD_{50} in distilled water may be due to dissolved minerals that could reduce the extract's potency. These findings underscore the importance of solvent selection in optimizing the insecticidal properties of *Moringa oleifera* extracts and suggest its potential as a biological control agent in integrated pest management programs. Further research could explore the interactions between solvents and active compounds in *M. oleifera* to better understand the mechanisms behind these differences. Table 4 shows no significant difference in pupae survival at 144 hours between *Moringa oleifera* leaf extracts prepared with distilled and deionized water. The p-values (Sig. > 0.05) for all categories—alive, dead, or developed—indicate that the solvent type did not significantly impact pupae survival outcomes.

Table 3: The percentage of surviving pupae at 144 hours in varying concentrations of *Moringa oleifera* extract using deionized water and distilled water

	(5×10^{-1})	(5×10^{-2})	(5×10^{-3})	(5×10^{-4})	(5×10^{-5})	LD_{50}
Hindrance of pupae (mg/ml)						
Deionized	0	90	90	0	10	1.29
Distilled	10	90	70	10	10	1.5
pupae mortality						
Deionized	10	10	10	100	90	
Distilled	90	10	30	90	90	

Table 4: Statistical result showing no significant difference in the means of survived pupae at 144 hours between the leaf extracts of *Moringa oleifera* using distilled water and deionized water as solvent of extraction

		Sum of Squares	Df	Mean Square	F	Sig.
PUPAE THAT WERE ALIVE	Between Groups	28.033	1	28.033	2.597	0.110
	Within Groups	1273.667	118	10.794		
	Total	1301.7	119			
PUPAE THAT WERE DEAD	Between Groups	28.205	1	28.205	2.580	0.111
	Within Groups	1279.257	117	10.934		
	Total	1307.462	118			
PUPAE THAT DEVELOPED	Between Groups	0	1	0.000	0.000	1.000
	Within Groups	1.967	118	0.017		
	Total	1.967	119			

Conclusion

The study finds that phytochemicals with insecticidal qualities found in *Moringa oleifera* leaf extracts include tannins, flavonoids, and saponins. These substances impede the growth and transformation of larvae, which may help manage mosquito populations. The effectiveness of these extracts varies depending on the concentration and extraction media; deionized water exhibits more inhibitory effects. The results emphasise how crucial naturally occurring plant chemicals are for controlling insects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Ritchie SA, Rapley LP, Williams C, Johnson PH, Larkman M, Silcock RM. A lethal ovitrap-based mass trapping scheme for dengue control in Australia: I. Public acceptability and performance of lethal ovitraps. Med Vet Entomol. 2009; 23(4):295–302.
2. Adesulu EA. The freshwater fishes and fisheries of Nigeria. Lagos, Nigeria: Macm Nig Pub Ltd. 2007; 397 p.
3. Fletcher M, Teklehaimanot A, Yemane G, Kassahun A, Kidane G, Beyene Y. Prospects for the use of larvivorous fish for malaria control in Ethiopia: search for indigenous species and evaluation of their feeding capacity for mosquito larvae. J Trop Med Hyg. 1993; 96(1):12–21.
4. Brown AW. Insecticide resistance in mosquitoes: a pragmatic review. J Am Mosq Control Assoc. 1986; 2(2):123–40.
5. Bhatt KC, Pandey A, Dhariwal OP, Panwar NS, Bhandari DC. "Tum-thang" (Crotalaria tetragona Roxb. ex Andr.): a little known wild edible species in the north-eastern hill region of India. Genet Resour Crop Evol. 2009; 56(5):729–33.
6. Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab H, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. Environ Int. 2005; 31(8):1149–66.
7. Santos AFS, Luz LA, Argolo ACC, Teixeira JA, Paiva PMG, Coelho LCBB. Isolation of a seed coagulant *Moringa oleifera* lectin. Pro Biochem. 2009; 44(4):504–8.
8. Mughal MH, Ali G, Srivastava PS, Iqbal M, Mughal MH, Al G, et al. Improvement of drumstick (*Moringa pterygosperma* Gaertn.) - a unique source of food and medicine through tissue culture. Hmd Med. 1999; 42(1):37–42.
9. Beula JM, Ravikumar S, Ali MS. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. Asn Pac J Tro Bio. 2011; (1):S143–6.
10. Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: *Culicidae*). Asian Pac J Trop Biomed. 2011; 1(2):124–9.
11. Zhu J, Zeng X, Yanma null, Liu T, Qian K, Han Y. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. J Am Mosq Control Assoc. 2006; 22(3):515–22.
12. Evans WC. Trease and Evans' Pharmacognosy. Els Hea Sci. 2009; 3322 p.
13. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books; 1993. 289 p.
14. Harborne AJ. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. Springer Science & Business Media; 1998. 330 p.
15. Evaluation and testing of insecticides. Report of the WHO Informal Consultation, 7−11 October 1996, WHO/HQ, Geneva [Internet]. [cited 2024 Feb 3]. Available from: https://www.who.int/publications-detail-redirect/ctd-whopes-ic-96.1
16. Impoinvil DE, Cardenas GA, Gihture JI, Mbogo CM, Beier JC. Constant Temperature and Time Period Effects on *Anopheles gambiae* Egg Hatching. J Am Mosq Control Assoc. 2007 Jun;23(2):124–30.
17. Foster WA, Walker ED. Chapter 15 - Mosquitoes (Culicidae). In: Mullen GR, Durden LA, editors. Medical and Veterinary Entomology (Third Edition) [Internet]. Academic Press; 2019; 261–325. Available from: https://www.sciencedirect.com/science/article/pii/B9780128140437000157
18. Garros C, Ngungi N, Githeko AE, Tuno N, Yan G. Gut Content Identification of Larvae of the *Anopheles gambiae* Complex in Western Kenya Using a Barcoding Approach. Mol Ecol Resour. 2008; 8(3):512–8.
19. Huang J, Walker ED, Vulule J, Miller JR. Daily temperature profiles in and around Western Kenyan larval habitats of *Anopheles gambiae* as related to egg mortality. Malaria Journal. 2006 Oct 12;5(1):87.
20. Abbott WS. A Method of Computing the Effectiveness of an Insecticide. J Econ Ento. 1925 Apr 1;18(2):265–7.
21. Malhotra S, Mandal G. Phytochemical screening and antioxidant activity of *Moringa oleifera* leaves and seed extracts. J Pharmacogn Phytochem. 2018; 7(5):1426-1430.
22. Sharifi-Rad J, Rodrigues CF, Sharopov F, Docea AO, Karaca AC, Sharifi-Rad M, Martins N. Diet, lifestyle and cardiovascular diseases: Linking pathophysiology to

23. cardioprotective effects of natural bioactive compounds. Int J Environ Res Public Health. 2020; 17(7):2326.
24. Younis W, Asif H, Sharif A, Riaz H, Bukhari IA, Assiri AM. *Moringa oleifera* Lam.: A review on its therapeutic and pharmacological potential. Saudi Pharm J. 2022; 30(1):10-18.
25. Bhalla N, Ingle N, Patri SV, Haranath D. Phytochemical analysis of *Moringa oleifera* leaves extracts by GC-MS and free radical scavenging potency for industrial applications. Saudi J Biol Sci. 2021 Dec; 28(12):6915-6928.
26. Ahmed M, Marrez DA, Abdelmoeen NM, Mahmoud EA, Abdel-Shakur Ali M, Decsi K, Tóth Z. Proximate Analysis of *Moringa oleifera* Leaves and the Antimicrobial Activities of Successive Leaf Ethanolic and Aqueous Extracts Compared with Green Chemically Synthesized Ag-NPs and Crude Aqueous Extract against Some Pathogens. Int J Mol Sci. 2023 Feb 9;24(4):3529. doi: 10.3390/ijms24043529. PMID: 36834941; PMCID: PMC9960608.
27. Baldisserotto A. Barbari R. Tupini C. Buzzi R. Durini E. Lampronti I. Manfredini S. Baldini E. Vertuani S. Multifunctional Profiling of *Moringa oleifera* Leaf Extracts for Topical Application: A Comparative Study of Different Collection Time. *Antioxidants* 2023, *12*, 411. https://doi.org/10.3390/antiox12020411
28. Alhazmi MI, Hasan TN, Shafi G, et al. Evaluation of the larvicidal potential of different plant extracts against *Aedes aegypti* larvae: Insights from solvents. Saudi Journal of Biological Sciences. 2020;27(1):52-58.
29. Bagavan A, Rahuman AA, Kamaraj C, Geetha K. Larvicidal activity of saponins from plants against *Aedes aegypti* and *Culex quinquefasciatus* (*Diptera: Culicidae*). Parasitology Research. 2009;104(6):1365-1372.