Using of lepidium sativum L. extract, as antimicrobial and anti-mosquito

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Abstract

Garden cress (Lepidium sativum L.), a member of the Cruciferae family, is widely planted across the world, especially in India, Europe, and the United States. It has been respected as a key medicinal plant from the time of the Vedic culture. Lepidium sativum Linn. seeds were tested for their efficacy as an antibacterial agent against pathogens found in food. The active components were extracted from the powdered dry seeds using chloroform, ethyl acetate, methanol, and dichloromethane. The antibacterial activity of various doses of the extracts was evaluated using agar well diffusion. We also estimated the MIC and MBC for the most effective extract using the tube dilution technique and the subculturing method, respectively. One of the most common mosquito species that carries Plasmodium falciparum is the Anopheles gambiae sensu lato, which may be combated by sprinkling fields with Lepidium sativum seeds. Scientists have begun to extract essential oils from Lepidium sativum and evaluate their bio-potential against juvenile and adult Anopheles gambiae as part of an attempt to identify ecologically viable vector control tactics. Based on the findings, it is clear that L. sativum essential oil effectively kills An. gambiae. Although field application on a wide scale is necessary for An. gambiae population control, more work has to be done in formulation and assessment.

1. Introduction

The fast-growing annual herb known as garden cress (Lepidium sativum L.) has its origins in Egypt and western Asia but is now planted in nearly every region of the world. Garden cress (GC), or Chandrasur as it is known in local languages, is widely cultivated in India for its therapeutic properties. The herbaceous annual grows to a height of 15–45 cm and has an upright, glabrous appearance. Small white flowers are carried on long racemes and the pods might be round or obovate in shape, elliptic without margins, notched at the tip, and wingless. The medicines found in nature are the best there is. Humans have used medicinal plants as a form of disease prevention ever since they developed civilization. (Painuli et al., 2021). Natural chemicals with therapeutic features in the herbs and medicinal plants that have been used for thousands of years is said to be the origin of herbal medicine and traditional healthcare systems like Ayurveda, Siddha, and Unani. 80% of the world's population, mostly in developing nations, rely on medicines made from plants for their health. and at present, one-fourth of all prescriptions are formulations based on chemicals derived from plants or plant-derived synthetic analogues. Indigenous wisdom that has been handed down from one generation to the next has had a significant impact on traditional medical practises around the globe. Likewise, the exploration of medicinal plants has been aided by this knowledge. (Imade et. al., 2020) The discovery of novel chemical entities has benefited greatly from this investigation of physiologically active natural compounds. This article summarises the research on Lepidium sativum Linn, including its traditional medical applications, isolated chemicals, and pharmacological studies.

Despite this, many medicinal plants are disappearing and native civilizations are being destroyed. We stand to lose a significant portion of our biodiversity, the variety of genes, species, ecosystems, and cultural practices if the current rate of extinction and destruction is not reversed. Native peoples have

been the guardians of 99 percent of the world's genetic resources, which are essential to the economic, agricultural, and health circumstances of people everywhere. Culture and biological variables are also intrinsically linked. (Ibraheem et.al., 2017)

Because so many people throughout the world are vulnerable to mosquito-borne illnesses, they represent a serious threat to human health. The Culex pipiens L. (Diptera: Culicidae) mosquito is a potential vector for a wide variety of diseases, including western equine encephalitis, West Nile virus, Rift Valley fever, St. Louis encephalitis, and bancroftian filariasis. With a flying distance of 1.15 to 2.48 km, Cx. pipiens may disperse their eggs over a vast area and contribute to the spread of illness. Mosquitoes thrive in areas with poor water drainage and improper waste management.(Getahun et.al., 2020) Constant and unchecked use of mosquitocidal chemicals has resulted in resistance in mosquitoes and has had negative consequences for humans, animals, and the environment.

1.1 Lepidium sativum

Lepidium sativum is a tall, annual herb that can reach a height of 50 centimeters. White, little flowers are arranged in racemes, and the leaves can be either lobed or whole. The obovate pods contain two seeds and are around 5 mm in length. A plant's volatile oils can be found in its seeds and leaves. Natives of Saudi Arabia, Sudan, and other Arabic countries know all about the benefits of the Lepidium sativum plant and its seeds for facilitating the healing of bone fractures. Lepidium sativum seed extracts have been utilised historically to treat a broad range of medical conditions. as highlighted by several recent studies.(Alqahtani et. al., 2019)

1.2 Traditional uses

Asthma, a persistent cough, and hemorrhaging piles can all be helped by using the plant's aerial parts. The seeds are chewed for a variety of medical purposes, including the relief of a sore throat, cough, asthma, or headache. Moreover, they can be used topically as an effective insect repellent. The mild stimulant and diuretic properties of the leaves make them an excellent choice for treating scorbutic diseases and liver complaints. Syphilis can be treated with roots. This herb has many medicinal uses in Europe, including as a diuretic, an expectorant, and a treatment for cough and constipation. (Ullah, Abbasi et. al., 2019)

1.3 Applying Lepidium sativum L. Extract for Antimicrobial Purposes

A rising number of bacteria are becoming resistant to antibiotics, posing a serious health risk. Various biomolecules and metabolites with crucial biological functions are already present in plants. When it comes to combating germs that have developed resistance to multiple drugs, (Al-Snafi et. al., 2019) these organic compounds are a gold mine. Green synthesis, which employs botanical components, and which could yield new classes of antibiotics derived from plants. "Lepidium sativum" (L.) LS, also known as

garden cress, is a fast-reproducing annual plant. The antimicrobial, antioxidant, and anti-inflammatory properties of LS seed oil make it an intriguing therapeutic option.

1.4 Lepidium Sativum L. Extract as a Mosquito Repellent

Elimination of Vectors and Individual Safety Limiting your exposure to vectors is presently the most effective way to avoid contracting a disease spread by a vector. (Elspeiy et. al., 2021) It is normal practice to use chemically based intervention strategies for vector control and to reduce the transfer of human infections. Insecticides, repellents, and larvicides made from chemicals were once widely used, but their overuse led to the rise of insect-resistant strains, ecological disruption, and human and non-target organism toxicity. (Aslani et. al., 2014)

Insects are predators, and many plant species have evolved to have defense mechanisms that either eliminate or drive away these pests. These chemicals come in a wide variety of uses, including as repellants, feeding deterrents, poisons, and growth regulators. Phytophagous insect defence is the primary function of these chemicals, but many of them, especially volatile components released as a result of herbivores, are also effective against mosquitoes and other biting Diptera. And, as has been reported by The fact that many of these compounds are also effective against hematophagous insects may be a relic of an ancestral plant-eating lifestyle, given that they evolved as repellents/killers to phytophagous insects.

1.5 Phytochemical Constituents

Seeds

Seeds for instance, include 25% protein, 14%-24% lipids, 33–54% carbs, and 8% crude fibre. Non-starch polysaccharides make up the other 90% of the carbohydrates. The seed bran is high in fibre and may be able to absorb large amounts of liquid. GC bran is a great option for those looking to enhance their fibre consumption. Semilepidinosides A and B, sinapin 15, and sinapic acid are only some of the seven distinct imidazole alkaloids found in the seeds (Balgoon et al., 2019). These are in addition to the previously described monomeric alkaloids lepidine B, C, D, E, and F. Carotene, cellulose, calcium, phosphorus, iron, thiamine, riboflavin, niacin, uric acid, and uric acid are present; so are N, N'- dibenzyl urea, N, N'-dibenzylthiourea, Sinapic Acid and its choline esters (sinapin), and glucotropaeoline. As stated by several researchers (Raval et al., 2012).

Seeds Oil

Seed oil is primarily made up of alpha linolenic acid (32–40%), which gives it a yellowish appearance and makes it semidrying. In addition to natural antioxidants like tocopherols and carotenoids, its 46.8% polyunsaturated fatty acid (PUFA) content and 37.6% monounsaturated fatty acid (MUFA) content help keep it from going rancid. Benzyl isothiocyanate, benzyl cyanide, sterol, and sitosterol are present in addition to palmitic, stearic, oleic, linolenic, arachidic, behenic, and lignoceric acids.

Leaf

Protein, fat, carbohydrates, mineral, phosphorus, calcium, trace elements-iron, nickel, cobalt, iodine, and vitamin C; thiamine, riboflavin, niacin, ascorbic acid, and vitamin A are just some of the nutrients that can be found in leaf.

Plant

The plant as a whole may include protein, minerals, vitamins, and other substances. Chemicals like glucotropoeolin, 4-methoxyglucobrassicin, caffeic acid esters, -sitosterol, benzylcyanide, calmodulin, sinapoyglucose, p-coumaric acid, ferulic acid, quinic acid, and many more have been identified. This chemical occurs in three different isomers, including 5 – 3'-dihydroxy-6,7,4'-tetramethoxyflavone and 5 – 4'-dihydroxy-7,8,3',5-tetramethoxyflavone.

1.6 Pharmacology

Antibacterial Activity

The effectiveness of the plant's extracts as antibiotics against Gram-negative and Gram-positive bacteria was investigated and the results were promising for both. The ethanolic extract was also found to be more effective against bacteria than the aqueous extracts. (Bigoniya et. al., 2011)

Anti-Microbial Activity: Seed extracts in petroleum ether, methanol, and water were tested against opportunistic pathogens like Staphylococcus aureus, Escherichia coli, Klebsiella pneumonae, Proteus vulgaris, Pseudomonas aeruginosa, and Candida albicans. In order to determine whether or not plant seed extracts might be used as an alternative to the antibiotics Gentamicin and Ketoconzol, they were put through a battery of tests. The findings also showed that petroleum ether was a more effective solvent than methanol and water for removing the plant's antibacterial compounds during extraction. To cite: (Mathewset al., 1993)

Antioxidant Activity

Leaves aqueous-methanolic extract N-butanol fraction was tested for antioxidant activity using DPPH and ABTS free radicals assay. Extracting the N-hexane fraction yielded three flavonol glycosides with anti-oxidant properties. The ethanolic seed extract has shown promising in vitro antioxidant activity in the DPPH radical scavenging assay, the ferric chloride radical scavenging assay, and the phosphor-molybdenum radical scavenging assay. According to the data, the Seeds ethanolic extract has strong potential in-vitro antioxidant activity.

Anti – inflammatory Activity

Albino rats developed pedal inflammation after suffering from carrageenan-induced paw edoema. The volume of each foot was determined using the displacement technique and a plethysmometer. Using oxyphenbutazone as a reference, we find that the ethanolic extract inhibits the activity of the drug. (Gokavi et al., 2004)

1.7 Seed chemistry and garden cress nutrition

Nayak 2012 report that the approximate composition (percent) of L. sativum seeds reveals sizable amounts of protein (24.2 0.5), lipids (23.2 0.2), carbohydrates (30.7 1.2), fibre (11.9 0.4), ash (7.1 0.1), and moisture (2.90.1). Varietal characteristics, agricultural methods, the time of year when seeds are harvested, and the local climate and geology all play a role in the final proximate composition of a crop. It is crucial for determining the nutritional value of plant and crop fruits and seeds, and it mandates more research into the more intriguing constituents. Higher ash contents suggest that the GCS are a healthy source of minerals. The stability, quality, and lengthened storage life of seeds can be gauged by their low moisture content. GCS have high food energy because their protein and lipid contents are higher.

2. Material And Methods

2.1 Plant material

May 2019 saw the purchase of Lepidium sativum seeds from a vendor in Ghaziabad, Uttar Pradesh, India. Using the available ethnobotanical data, a voucher specimen (ML- 1/2019) was deposited at the Microbiology Laboratory, Homoeopathic Pharmacopeia Laboratory in Ghaziabad, Uttar Pradesh, India.

2.2 Laboratory rearing of Anopheles gambiae

Due to high mortality rates during successive malaria epidemics over the past few years, immature stages of Anopheles gambiae were collected from breeding sites in the Ghaziabad district of uttar pradesh. The larval mosquitoes were gathered in the months of September and October of 2016 from hoof print, temporary flood accumulation areas. The larval stages were gathered with a kitchen strainer and placed in a wide-mouthed plastic container that was only about three-quarters full of water. We closed the container's hole with muslin so it could be taken to the General Entomology lab on the Maraki campus of the University of Gondar for examination. Larvae from instars I–IV were acclimatised in half-full trays of deionized water. The larvae's size and shape were compared to those described by Walker and Lynch to establish their instar. The adult and larval stages of Anophele gambiae s.l. are morphologically identical, as shown by the use of a shared key to identifying them. to members of other Anopheles species. Pupae were raised in the lab on a diet of dry yeast and dog biscuit crumbs. Adult emergence cages were set up, and the pupae were collected after the larvae had reached that stage. When mosquitoes matured into adults, they were kept in Bugdorm cages with unrestricted access to cotton soaked in a 10% sucrose solution (w/v). The ideal colony conditions included a constant temperature of 27.1°C, relative humidity of 65–70%, and a light-dark photoperiod of 12 hours daily. A adequate number of field-collected, laboratory-maintained, and bioassayed larvae were employed throughout the trial.

2.3 Preparation of the extracts

Powder was made from the newly harvested seeds. The seeds were ground into a powder and then extracted using a Soxhlet apparatus with 150 mL of chloroform, ethyl acetate, methanol, and dichloromethane for 24 hours. We next concentrated the filtrates by evaporating them at 55°C in a rotating evaporator while maintaining a vacuum. The dry extracts were cooled after being reconstituted in their respective solvents to a final concentration of 100 mg /mL.

Lepidium sativum seeds freshly gathered in the Gondar region. The seeds were washed with distilled water and then dried in the shade of the lab. The dry seeds were ground into a powder using an electric blender. The powder utilised in the oil extraction process was obtained by passing the pulverised seeds through a fine mesh sieve. In a conical flask, we combined 200 grammes of seed powder from each plant with 1 litre of distilled water. The Clevenger equipment and the hydro-distillation procedure required three hours and a temperature of 100 degrees Celsius to extract the essential oil from the plant components. To prevent the oil from becoming rancid, it was filtered through cotton soaked in anhydrous sodium sulphate and then kept in dark, temperature-controlled glass bottles at 4 degrees Celsius. Different concentrations of the stock oil were used in a toxicity bioassay against An. gambiae.

2.4 Microorganisms used

The following species were utilized as tests in this study: The following strains were used: Escherichia coli (ATCC 8739), Salmonella typhi (ATCC 23564), Pseudomonas aeruginosa (ATCC 25668), Staphylococcus aureus (ATCC 9144), and Bacillus cereus (ATCC 11778), and Micrococcus luteus. Bacteria strains were cultured and kept on Nutrient agar at a temperature range of 5–8 degrees Celsius. Bacteria inocula were at a concentration of 10^6 CFU/mL, which is equivalent to a turbidity of 0.5 McFarland units.

2.5 Preparation of stock and experimental concentrations

A stock concentration of 10,000 ppm was achieved by diluting 1 mL of pure essential oil with 1 mL of acetone in a 250 mL conical flask with 100 mL of filtered water. After initially testing at 10, 100, and 1000 ppm, the researchers narrowed their range and did more focused experiments at 100, 75, 50, and 25 ppm. The impregnated filter paper bioassay method was used to test the effectiveness of essential oil in killing and repelling adult mosquitoes. Filter paper was impregnated with solutions at concentrations of 0.5%, 2.5%, 5%, and 10% ppm. In addition, quantities of acetone and distilled water equal to 1 mL were generated and utilised as a "control."

2.6 Antibacterial activity

Extracts' antibacterial efficacy was evaluated using the agar well diffusion technique. Eight-millimeter-diameter wells were drilled on a petri dish, and 100 µL of each extract was poured in, using strict aseptic procedures. The gentamicin antibiotic solution (10.0 g/mL) was used to make the positive control. The unadulterated version of the solvent was employed as a reference point (chloroform, ethyl acetate, methanol, and dichloromethane). The plates were allowed to sit at room temperature for one hour to allow the extract to diffuse into the agar. After that, the Petri plates spent 24 hours in an incubator set to

37 degrees Celsius. The inhibitory zones were measured in millimetres after the incubation durations. Three passes through the test ensured reliability.

2.7 Minimum Inhibitory concentration

Tube dilution was used to determine the MIC of methanol ex- tract. Diluting the methanol extract at various concentrations (0.097-50.0 mg/mL) allowed researchers to determine the MIC of the compound. In the course of 18–48 hours, all of the test tubes were kept in an incubator. All development in the tubes was tracked. The minimal inhibitory concentration (MIC) was calculated as the concentration of the extract below which no growth was detectable in comparison to the control tubes.

2.8 Minimum bactericidal concentration

Sub-culturing was used to establish the minimum bactericidal concentration. Subcultures were made on fresh nutrient agar plates from the test tubes that showed no turbidity or growth during the MIC assays. The bactericidal threshold was determined to be the lowest concentration at which no growth was seen after 24 hours of incubation.

2.9 Preparation of stock and experimental concentrations

One millilitre of pure essential oil was combined with one millilitre of acetone, and the resulting solution was diluted with distilled water to a final volume of one hundred millilitres in a two-hundred-fifty millilitre conical flask. This yielded a stock concentration of ten thousand parts per million (ppm) of the extracted oil. After conducting exploratory broad range tolerance tests at 10, 100, and 1000 ppm, the researchers settled on four doses for their experiments: 100, 75, 50, and 25 ppm. The impregnated filter paper bioassay method was used to test the effectiveness of essential oil in killing and repelling adult mosquitoes. Filter paper was impregnated with solutions at concentrations of 0.5%, 2.5%, 5%, and 10% ppm. Additionally, concentrations of acetone and distilled water, each at 1 mL, were prepared and used as a "control."

2.10 Testing the essential oil's effectiveness as a larvicidal

Essential oils isolated from L. sativum seeds were tested for their larvicidal efficacy using the World Health Organization's gold standard technique. Twenty-five larvae in the second and fourth instars were selected from the culture and placed into 250 mL plastic beakers. By changing the total volume of water in each beaker from 200 mL to 0.00 (control), 25, 50, 75, and 100 ppm, a constant concentration of essential oil of varying strengths was maintained. Temperature and humidity in the lab were controlled at 27 1°C (65–80% RH) for the duration of the experiment. Mosquito larvae were released in equal numbers after each concentration was replicated four times. In this study, we measured the mortality of exposed larvae at 24, 48, and 72 hours.

2.11 Analysis of the Essential Oil's Puppy Killing Capacity

We used the newly emerged pupae that had been collected from the culture to test the pupicidal activity of a few different essential oils. When using essential oils for their larvicidal efficacy, the concentrations

and methods were as indicated. To keep the adults from escaping, we released 25 pupae into the plastic beaker and covered it with nylon mesh. After 24 hours, 48 hours, and 72 hours of exposure, the total number of pupa that had died due to each concentration was recorded across all four replicates. By using Abbott's formula, we were able to accurately determine the pupal mortality rate and adjust for it.

2.12 Analysis of the Essential Oil's Adulticiding Capacity

Following World Health Organization guidelines, adult mosquitoes that had not been fed blood were used to test the knockdown action of the essential oil from a select group of plants. Following the preliminary screening, four concentrations (0.5, 2.5, 5.0, and 10.0% ppm) were created and impregnated with Whatman no. 1 filter paper. The 0.1% bendiocarb-treated Whatman filter paper served as the positive control, while acetone served as the negative control. All of the WHO toxicity and knockdown bioassays were performed on adult test tubes. Adult mosquitoes were placed in test tubes and fed sugar for three to five days before being exposed for one hour; during this time, the number of dead or recovering mosquitoes was recorded every five minutes. Mosquitoes in this experiment were exposed for 1 hour and then placed in recovery test tubes where they stayed for 24 hours while researchers watched for any signs of infection. During the restoration period, adults were fed cotton that had been soaked in a 10% sucrose solution and then placed on the mesh screen of the tubes. Both the temperature and the relative humidity were kept constant at 271°C and 65–70% respectively for the duration of the experiment. The proportion of death was computed after the data from four replicates was adjusted using Abbott's technique.

2.13 Statistical analysis

Ratio of mosquitoes killed in the control group to those killed in the experimental group was used to calculate the mortality rate. The error introduced by this fact has been corrected using Abbott's method, as the number of avoidable deaths is greater than 5%. A population's mortality rate after a course correction can be calculated as follows: The median percentage of deaths at different concentrations was compared using one-way ANOVA followed by the LSD post hoc test. For each concentration, we calculated its LC50, LC90, LCL, and UCL using Probit analysis. Statistical significance in the range of tested plant essential oil concentrations was established using a Chi-square (2) test. The 5% statistical significance threshold (P 0.05) was used for all analyses. For the statistical analysis, we utilised SPSS 20 on Windows 7.

3. Results

Table 1 displays the results of testing plant extracts for antibacterial activity. This study's findings demonstrate that plant extracts were effective against the organisms tested. The methanol extract significantly inhibited the growth of Staphylococcus aureus (22 millimeters), Bacillus cereus (16 millimeters), Escherichia coli (14 millimeters), Pseudomonas aeruginosa (14 millimeters), Micrococcus luteus (16 millimeters), and Salmonella typhi (13 millimeters), while the ethyl acetate and With the importance of the inhibitory zone in methanol extract, MIC and MBC analyses were performed.

Staphylococcus aureus had an MIC of 1.56 mg/ml and a minimum inhibitory concentration (MBC) of 6.52 mg/ml, while Salmonella typhi had an MIC of 25 mg/ml.

Table 1
Extraction of Lepidium sativum seeds has antibacterial properties

S. no.	Microorganisms	Chloroform	Ethyl acetate	Zone of inhibition (mm)		
				Methanol	Dichloromethane	Gentamicin
1.	Micrococcus luteus	11	16	16	10	27
2.	Staphylococcus aureus	10	18	22	NZ	25
3.	Bacillus cereus	NZ	NZ	16	NZ	28
4.	Pseudomonas aeruginosa	NZ	NZ	14	NZ	21
5.	Escherichia coli	NZ	14	14	NZ	22
6.	Salmonella typhi	NZ	NZ	13	NZ	24
NZ = no zone of inhibition						

Table 2
Evaluation of Lepidium sativum seed methanol extract for minimum inhibitory and bactericidal concentrations

S. no.	Organism	MIC (mg/ mL)	MBC (mg/mL)
1.	Micrococcus luteus	12.5	ND
2.	Staphylococcus aureus	1.56	6.25
3.	Bacillus cereus	6.25	25.0
4.	Pseudomonas aeruginosa	6.25	25.0
5.	Escherichia coli	6.25	25.0
6.	Salmonella typhi	25	ND
ND = Not detected			

3.1 The effect of Lepidium sativum essential oil on the mortality of Anopheles gambiae larvae

An.gambiae pupae and larvae of the second and fourth instars exposed to essential oil from L. sativum for 24, 48, and 72 hours are shown in Table 3 with their average percentage of death. Second instar larvae exposed to 75 ppm, the highest exposure tested, had the highest mortality rate (96.0% after 48

hours). After 72 hours of exposure, 97 percent of those exposed to 100 ppm died. After testing for 24 hours, the LC50 and LC90 concentrations were calculated to be 317.8 and 437.1 ppm, respectively. For a period of 72 hours, the LC50 was calculated to be 136.6 ppm, while the 95% LCL and UCL were 132.7 ppm and 182.7 ppm, respectively. Results from a 2 test revealed statistically significant variations across time and concentration (2 = 122.57, P = 0.000 for 24 hours; 2 = 62.43, P = 0.000 for 48 hours; and 2 = 204.94, P = 0.000 for 72 hours). After 72 hours, 91.00% of fourth-instar larvae exposed to 100 ppm concentration had died. The half-life (LC50) was calculated to be 337.3 ppm after 72 hours of exposure, while the LC90 was calculated to be 466.4 ppm. Calculations were made for the 95% LCL and UCL of LC50 after 72 hours of exposure, and the results were 210.1 and 456.5 ppm. There was a statistically significant difference in each concentration over time, as measured by the 2 statistic: at the 24-hour mark, 2 = 44.93, P = 0.000; at the 48-hour mark, 2 = 100.19, P = 0.000; and at the 72-hour mark, 2 = 1372.59, P = 0.000. After 72 hours, the death rate for pups was 99.0% at 100 ppm and 98.00% at 75 ppm. After 72 hours of exposure, the half-life (LC50) and maximum tolerable concentration (LC90) were determined to be 245.3 and 344.2 ppm, respectively. After 72 hours of exposure, the 95% LCL and UCL of LC50 concentrations were calculated to be 186.8 and 297.3 ppm, respectively. Differences within each concentration were found to be statistically significant using the 2 test (2 = 133.53; P = 0.000 for 24 hours; 2 = 566.53; P = 0.000 for 48 hours; 2 = 792.39; P = 0.000 for 72 hours).

Table 3
The median percentage of immature Anopheles gambiae that died after being exposed to essential oil from the plant Lepidium sativum

An.gambiae stages	Concentration in ppm	Duration of time of exposure		
		24 hr	48 hr	72 hr
IInd instar	Control	0.00 ± 0.00	1.00 ± 1.00	5.00 ± 1.91
	25 ppm	10.00 ± 2.58	19.00 ± 2.89	83.75 ± 2.01
	50 ppm	26.00 ± 2.17	58.50 ± 3.30	94.00 ± 2.58
	75 ppm	90.00 ± 2.00	96.00 ± 3.36	96.00 ± 1.63
	100 ppm	96.00 ± 1.62	97.00 ± 3.92	97.00 ± 1.91
	LC50 (LCL - UCL)	317.8 (297.8–327.0)	267.7 (253.7–291.3)	160.4 (136.6–182.7)
	LC90 (LCL - UCL)	437.1 (403.5–491.0)	389.9 (365.9–423.9)	261.7 (226.6–325.1)
	$\chi 2$	122.57	63.43	204.94
	P value	P = 0.000	P = 0.000	P = 0.000
IVth instar	Control	0.000 ± 0.00	1.00 ± 1.00	2.00 ± 1.15
	25 ppm	5.00 ± 1.91	7.00 ± 1.91	8.00 ± 1.63
	50 ppm	20.00 ± 4.22	29.5.50 ± 6.50	11.00 ± 1.91
	75 ppm	47.00 ± 5.18	60.00 ± 1.63	86.50 ± 5.50
	100 ppm	74.00 ± 2.58	88.00 ± 1.53	91.00 ± 3.26
	LC50 (LCL - UCL)	403.3 (385.5–423.5)	354.2 (331.0–378.7)	337.3 (210.1–456.5)
	LC90 (LCL – UCL)	653.7 (597.1–740.5)	564.0 (507.0–684.0)	466.4(379.0–2830.4)
	$\chi 2$	44.93	100.19	1371.59
	P value	P = 0.000	P = 0.000	P = 0.000
Pupae	Control	0.00 ± 0.00	2.00 ± 1.91	6.00 ± 2.58
	25 ppm	3.00 ± 1.91	6.00 ± 2.48	7.00 ± 1.91
	50 ppm	33.50 ± 2.98	46.50 ± 3.96	85.00 ± 1.81
	75 ppm	91.00 ± 2.51	96.00 ± 1.63	98.00 ± 1.15
	100 ppm	83.00 ± 4.43	94.00 ± 3.72	99.00 ± 1.00

An.gambiae stages	Concentration in ppm	Duration of time of exposure		
		24 hr	48 hr	72 hr
	LC50 (LCL – UCL)	321.5 (301.7–340.7)	292.9 (241.0–330.2)	245.3 (186.8–297.3)
	LC90 (LCL – UCL)	429.1 (398.0–481.5)	224.0 (350.6–635.1)	344.2 (286.5–600.1)
	$\chi 2$	133.53	566.53	792.39
	P value	P = 0.000	P = 0.000	P = 0.000

The median and standard deviation of fatality rates based on four experiments. The LC50 is the concentration at which 50% of mosquito larvae exposed to a certain substance die. Bounds of the confidence interval for the essential oil concentration, both lower and higher.

3.2 Knock downtime (KDT50) and Essential oils can be toxic to adults Gambiae

The median knock down time (KDT50) following 1 hour of exposure to L. sativum essential oils for adult, non-blood-fed female An. gambiae is shown in Table 4. At a concentration of 10% L. sativum, the half-life (KDT50) was determined to be 7.34 minutes. Conversely, the half-life of L. sativum oil was only 10.05 minutes when used at the same concentration. When compared to the L. sativum concentrations that resulted in the KDT50 (50% and 10%, respectively), the bendiocarb 0.1% impregnated filter paper performed poorly.

The X-axis shows the percentage of adult An. gambiae that died after being exposed to a 10% solution of L. sativum essential oils. According to the findings, bendiocarb, the positive control, showed a 100% death rate. Nonetheless, 95.66 2.4 and 82.66 3.48% L. sativum mortality rates were significantly higher than the negative control's (7.33 0.88).

Table 4
The essential oils of Lepidium sativum were tested for their ability to kill 50% of female adult Anopheles gambiae after exposing them to them for 1 hour (the KDT_{50})

Essential oil	Concentration in percentage			
	0.5%	2.5%	5%	10%
Lepidium sativum	31.13 ± 0.6	23.05 ± 0.2	13.25 ± 0.1	10.05 ± 0.5
Conrol (Bendocarb 0.1%)	22.75			

(KDT 50) is the length of time required to kill 50 percent of an exposed population. This data was derived via averaging four separate samples and adding their respective standard deviations.

4. Discussion

As a result, it's reasonable to assume that the recovered antibacterial chemicals may kill bacteria via a mechanism distinct from that of conventional antibiotics, hence enhancing their potential therapeutic effectiveness. A literature review revealed no information on Lepidium sativum's potential antibacterial effects against various pathogens found in food. As a result, this study has the potential to be the first to show that extracts of Lepidium sativum seeds have antibacterial properties when tested against a broad spectrum of food-borne bacteria. Eco-friendly vector control measures are necessary now due to the need to minimise environmental contamination from synthetic pesticides, to mitigate the potential harm to non-target creatures, and to manage the spread of insecticide resistance in mosquito populations. One beneficial reason to focus on the mosquito's larval stage is that it decreases the number of adult mosquitoes that can develop into disease carriers. Small ponds, marshes, ditches, pools, drains, and water containers should all be emptied because they collect water that mosquitoes can use for breeding. Lab tests using essential oils extracted from L. sativum against An. gambiae larvae and adults provided valuable information for the development of future eco-products.

The present findings corroborated prior findings about the toxicity of L. sativum essential oils. Depending on the dosage of the plant extracts and the length of time the larvae and pupae were exposed to the extracts, the mortality rate changed dramatically between the second and fourth instars. Possibly due to the accumulation of secondary metabolites in seeds, L. sativum oil has a high mortality rate. These findings are consistent with those of Andemo et al., who discovered that treating adult A. arabiensis with a methanol extract of L. sativum seeds completely eradicated the pest population. This study suggests that phytochemicals and the solubility of individual phytochemicals in their respective solvents may be responsible for the high mortality rate observed among An. gambiae exposed to L. sativum essential oil. Producing pesticides with rotenoids dates back to 1848.

5. Conclusion

Overall, the current investigation confirmed that antibacterial chemicals are widely distributed across therapeutic plants. It is believed that these chemicals have a role in plant defence mechanisms against microbial infections. These results suggest that methanol extract is more effective against Gram-positive bacteria than Gram-negative bacteria. The presence of benzyl isothiocyanate in Lepidium sativum seeds may account for their potent antimicrobial action against a wide variety of pathogenic bacteria responsible for life-threatening diseases. It is notoriously difficult to use standard antibiotics to treat infections caused by these germs, in cases when the relevant microorganisms have become resistant to many medicines. L. essential oils from the sativum plant had a strong mosquito-killing effect on the An. mosquitoes of the genus Culex that are responsible for spreading malaria. In order to counter An in the field, widespread use of this tool is required. gambiae and thereby lessen the likelihood of malaria transmission in places like impoverished nations, however its efficacy relies on accurate formulation and field assessment.

Declarations

Ethical Approval

N/A

Competing interests

N/A

Authors' contributions

1- EH. provided pivotal input regarding the interpretation of the results, collect plant and extract

2- WM designed the study, conducted the field work and co-wrote the manuscript2

3- MA led the statistical analysis and co-wrote the manuscript.

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Availability of data and materials

The data used and analyzed during this project are available from the corresponding author.

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Figures

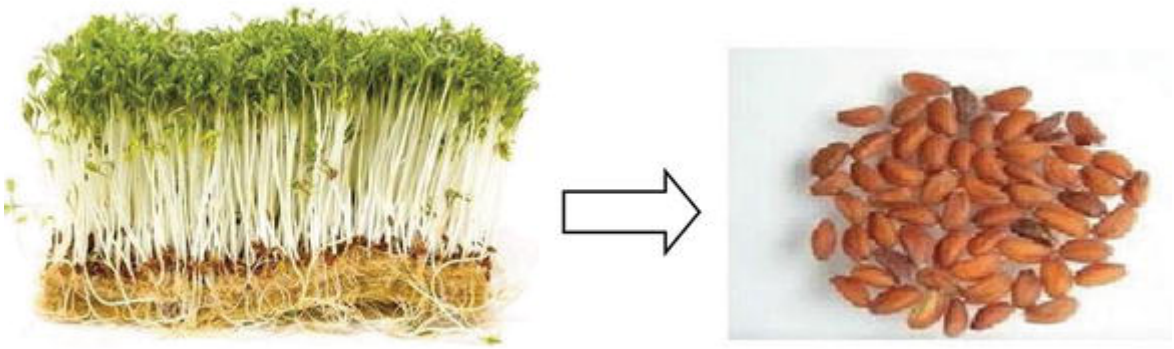

Figure 1

Seeds and plants for garden cress