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Evaluating larvicidal, ovicidal and growth inhibiting activity of five medicinal plant extracts on *Culex pipiens* (Diptera: Culicidae), the West Nile virus vector

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Mosquitoes, one of the deadliest animals on the planet, cause millions of fatalities each year by transmitting several human illnesses. Synthetic pesticides were previously used to prevent the spread of diseases by mosquitoes, which was effective in protecting humans but caused serious human health problems, environmental damage, and developed mosquito pesticide resistance. This research focuses on exploring new, more effective, safer, and environmentally friendly compounds to improve mosquito vector management. Phytochemicals are possible biological agents for controlling pests and many are target-specific, rapidly biodegradable, and eco-friendly. The potential of extracts of *Lantana camara*, *Melia azedarach*, *Nerium oleander*, *Ricinus communis*, and *Withania somnifera* against 3rd instar *Culex pipiens* (Common house mosquito) larvae was evaluated. Methanol extracts had more toxic effects against *Cx. pipiens* larvae (95–100%, 24 h post-treatment) than aqueous extracts (63–91%, 24 h post-treatment). The methanol extracts of *Nerium oleander* (LC_{50} = 158.92 ppm) and *Ricinus communis* (LC_{50} = 175.04 ppm) were very effective at killing mosquito larvae, 24 h after treatment. *N. oleander* (LC_{50} = 373.29 ppm) showed high efficacy in aqueous plant extracts. Among the different extracts of the five plants screened, the methanol extract of *R. communis* recorded the highest ovicidal activity of 5% at 800 ppm concentration. Total developmental duration and growth index were highly affected by *R. communis* and *M. azedarach* methanol extracts. In field tests it was clear that plant extracts decreased mosquito larval density, especially when mixed with mosquito Bti briquette, with stability up to seven days for *N. oleander*. GC–MS results showed that the methanol extract had a higher number of chemical compounds, particularly with more terpene compounds. A high-performance liquid chromatography (HPLC) technique was used to detect the existence of non-volatile polyphenols and flavonoids. All five methanol extracts showed high concentrations of active ingredients such as gallic acid, chlorogenic acid (more than 100 µg/ml) and the rosmarinic acid was also found in all the five extracts in addition to 17 active polyphenols and flavonoids presented at

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moderate to low concentrations. Molecular modeling of 18 active ingredients detected by the HPLC were performed to the vicinity of one of the fatty acid binding proteins of *lm*-FABP (PDB code: *2FLJ*). Rutin, Caffeic acid, coumaric acid and rosmarinic acid which presented densely in *R. communis* and *N. oleander* showed multiple and stable intermolecular hydrogen bonding and π–π stacking interactions. The inhibition ability of the fatty acid binding protein, FABP4, was evaluated with remarkable receptor inhibition evident, especially with *R. communis* and *N. oleander* having inhibitory concentrations of IC_{50} = 0.425 and 0.599 µg/mL, respectively. The active phytochemical compounds in the plants suggest promising larvicidal and ovicidal activity, and have potential as a safe and effective alternative to synthetic insecticides.

Keywords *Culex pipiens*, Larvicidal, Ovicidal, GC–MS, Docking study, Medicinal plant extracts

Mosquitoes spread many harmful diseases to humans and animals, including malaria, dengue fever, yellow fever, filariasis, Japanese encephalitis, chikungunya, and Streptococcus epidermidis in livestock[1]. Although no region of the world is free of vector-borne diseases, mosquito-borne diseases have a disproportionate impact including economic (loss of commercial production and employment), disease, death, and poverty (resulting from reduced productivity). It particularly affects poorer people (e.g. without healthcare, mosquito nets, drugs, or employment protection should they fall ill) and is also a particular problem in poor countries within tropical and subtropical climates[2].

Synthetic insecticides were developed to kill mosquitoes and control vector-borne disease, and are have been very effective. However, mosquitoes have adapted resistance to many of these insecticides, and some of these have shown significant risk to the environment and human health[3]. Plants produce secondary metabolites like alkaloids, carbohydrates, flavonoids, saponins, tannins, and terpenoids, that they use as natural defenses against insects and bacteria. These compounds can be extracted and consequently be used by humans for anti-microbial, insecticide and pharmacological uses. Pesticides derived from plants that do not harm the environment have recently received increased attention for industrial, medical, and agricultural use. Natural insecticides tend to be less deleterious in human health, can be biologically sourced, and tend to cause less harm to non-target species and the environment[4–8].

In addition to insecticides, a variety of extracts and chemicals from several plant groups have been studied as potential new larvicides[9]. Plant extracts or essential oils contain a variety of phytochemicals such as tannins[10], essential oils[11], isoflavonoids[12], and stimulants[13], which can kill mosquito larvae. The effects range from oviposition inhibition, developmental toxins, hatching inhibition, adulticides, ovicides, and emergence inhibitors[4,14]. Extracts from plants have traditionally been used throughout the world both to treat diseases and as insecticides. For example, the roots of *Lantana camara* has been used to treat skin rashes, rheumatism, and malaria. Extracts from its flowers have been used as a mosquito repellent, and its leaves have shown larvicidal activity[15] as well as being used as an antibacterial and antihypertensive drug[16].

Five plants were evaluated for larvicideal and ovicidal activity in this study: *Lantana camara, Nerium oleander, Ricinus communis, Melia azedarach,* and *Withania somnifera*. The effect of *L. camara* extract on the mortality and sub-lethal effects of the mosquito *Cx. pipiens* has previously been scientifically evaluated. This includes the effect of extract in an acetone solution on *Cx. pipiens* larvae[17], and the ability of essential oils extracted from *L. camara* leaves to kill *Cx. pipiens* larvae[18]. Comparative studies have also been done by Mondal, et al.[19], finding that the ethanolic leaf extract of *L. camara* was better at killing *Cx. quinquefasciatus* mosquito larvae than *Cx. pipiens* larvae.

N. oleander (*Apocynaceae*) is a low ornamental shrub of the Dogbane family that grows naturally in subtropical regions of the Mediterranean and is native to north-central Morocco. It has been used in medicine as an antibacterial, anti-inflammatory, antinociceptive, antioxidant, hepatoprotective, antitumor, and cytotoxic compound[20,21] and has been extensively studied for its benefits in health and cytotoxicity. Extracts of *N. oleander* have been tested on 3rd and 4th larval stages of *Cx. pipiens*, and methanol extracts of *N. oleander* has shown positive effects on destroying *Anopheles spp* larvae. *N. oleander* leaf extract was shown to kill both the eggs and adults of the mosquito *Aedes aegypti* (a dengue vector)[20,21]. Raveen, et al.[22] also evaluated acetone extracts from *N. oleander* flowers (pink, red, and white) against larvae of *Aedes aegypti, A. stephensi, and Cx. pentamer* mosquitoes.

R. communis L. (*Euphorbiaceae*) is a plant widely distributed throughout the tropics and warm temperate regions of the world. Researchers have written much about how *R. communis* can help with various health problems, including protecting the liver, reducing inflammation, increasing urine production, fighting cancer, killing

No	Botanical name	Family	Common name
1	*Lantana camara*	*Verbenaceae*	Largeleaf lantana
2	*Melia azedarach*	*Meliaceae*	Chinaberry tree
3	*Nerium oleander*	*Apocynaceae*	Oleander
4	*Ricinus communis*	*Euphorbiaceae*	Castor bean
5	*Withania somnifera*	*Solanaceae*	Withania

Table 1. List of plant species tested against *Culex pipiens* larvae. Leaf extracts were used in each case.

bacteria and viruses, lowering blood sugar, killing fungi and insects, healing wounds, and stopping the growth of asthma and alleviating asthmatic conditions[23].

Other activities of various phytochemical compounds include preventing cancer cell growth by interfering with DNA non-replication, and stimulating the activities of enzymes[24]. Some phytochemicals may also have antibacterial and antioxidant properties[25].

In this work, we hypothesize that extracts of *L. camara*, *M. azedarach, N. oleander, R. communis, and W. somnifera* contain bioactive phytochemical compounds with lethal effects against the *Cx. pipiens* mosquito; namely by inhibiting larvae growth and killing the mosquito eggs. *Cx. pipiens* is important as it is a major vector of the West Nile virus, which kills both humans and animals (especially horses), as well as infecting various animals which may act as hosts, particularly birds.

Therapeutic targets of macro-molecules such as proteins have been developed following the full sequencing of the human genome. This has been aided by the extensive development of molecular structure visualization tools, such as x-ray diffraction (XRD), proton and carbon nuclear magnetic resonance (NMR), Fourier transform infra-red (FTIR), and other structure-identifying tool kits that lead to more success in identifying both protein–ligand and protein complex structure[26]. Effective and rapid structure identification has been invaluable to computer-aided drug design, and consequently, molecular modeling. This has presented a theoretical-based simulation between drug and host protein, defining a specific area called the binding pocket. The interactions between the drug and the host protein can be described using classical and advanced calculation. Currently most research articles on drug-protein interactions detail the use of one or other artificial intelligence applications that can describe ligand–protein and protein–protein interactions[27]. Drug design, therapeutic chemistry, and synthetic chemistry are fields of research that now depend to a great extent on complex computer aided molecular modelling[28]. Molecular docking analysis has been used in the elucidating the structure and possible synthesis of structures such as: PI3k[29], carbonic anhydrase[30], EGFR analogues[31], acetylcholinesterase[32], topoisomerase[33], Fatty acid binding protein, and m-tor inhibitors[34]. Such studies are necessary to produce the most powerful candidate for a drug from a database of various candidates selected to satisfy the purpose.

Within this study activated polyphenols and poly flavonoids were extracted from the plants with methanol, and analyzed with molecular docking analyses. Docking of the polyphenols and flavonoids was examined on one of the most important insect proteins, 2FLJ. The expectation was that the molecular modelling provides a convenient rationalization about the mechanism of protein inhibition caused by the active ingredients, when they bind to the fatty acid binding protein active site; consequently causing severe perturbation to insect biochemistry or growth enzymes.

Materials and Methods

Plant materials and analysis

Plant collection

Leaves of the study plants, *L. camara, M. azedarach, and N. oleander, R. communis,* and *W. somnifera*, were collected from different locations in agricultural land around the villages of Qalyubiya Governorate, Egypt, between March and June 2023 (Table 1). These plants are local and widespread in the agricultural governorates of Egypt's Nile Delta. Identification of the plants was done by Dr. Ahmed Mubarak of the Department of Botany and Plant Taxonomy (Faculty of Science, Banha University, Egypt) according to the Egyptian flora reference[35]. The study plant specimens were deposited in an herbarium of the botany department, Faculty of Science, with respective voucher numbers for *L. camara* (B112), *M. azedarach* (B33), *N. oleander* (B89), *R. communis* (B22), and *W. somnifera* (B315).

Plant extraction

The plant materials were shade-air-dried at room temperature until all water content removed and the dry weight was contracted. The dried tissues were ground in a stainless-steel electric mixer and transferred into airtight containers to protect them from humidity. Exactly 25 gm of plant powder was placed in a Soxhlet apparatus for 4–7 h (methanol was used as solvent). After filtration, the insoluble fibers were removed and the filtrate reconcentrated using rotary evaporator (at low temperature between 38 and 40 °C) utile all solvents were disposed. The solid residue was collected cautiously and re-dissolved in a definite volumes and stored in dark bottles[32].

The aqueous plant-extract was prepared using the same protocol with distilled water instead of methanol. The extraction solutions were concentrated using a freeze-drying lyophilization and the residue was then stored in dark bottles[36].

Mosquito larvicidal assay

Rearing of Culex pipiens

The larvae of *Cx. pipiens* were cultivated in an insectary, where they were kept at a temperature of 27 ± 2 °C and a relative humidity of 75 ± 5%. The larvae were exposed to a consistent photoperiod of 12 h light, 12 h darkness. The were provided with a diet consisting of fish food (Tetramin) and ground bread at a ratio of 3:1. Subsequently, the pupae were transferred from the enamel pans to a container containing dechlorinated water and then placed in screened enclosures of 35 × 35 × 40 cm, where the adult individuals ultimately emerged. The female mosquitoes were provided with regular blood feeds from a hamster rat, and both male and female the adult mosquitoes were provided with a 10% sugar solution. Larvae and pupae, representing two distinct stages of development, were consistently accessible for experimentation and housed within the same laboratory facility[37].

Larvicidal activity

The plant extracts by methanol and water of *B. glabra, D. regia, L. camara,* and *P. orientalis* were evaluated for the action on the 3rd larval instar of *Cx. pipiens* under laboratory conditions. The 3rd larval instar was treated with the following concentrations of active compound: 62.5, 125, 250, 500, 1000, and 1500 ppm (1 g/1000 mL of distilled water). Twenty larvae per concentration were transferred to a glass beaker containing 250 mL of distilled water. Three replicates were used for each concentration. Mortalities were recorded 24 h and 48 h after the initial exposure i.e. post treatment (PT)[38].

Ovicidal test

The technique of Su and Mulla[39] was used to evaluate ovicidal activity. Mosquito larvae and egg rafts were obtained from the Medical Insect Research Lab, Faculty of Science, Benha University. Each of the 130 eggs (on the egg raft) was collected and placed in separate ovicidal cups containing varying concentrations of plant extracts (20, 40, 60, 80, and 100 ppm). In parallel, a control cup was maintained with regular water mixed with acetone. There were three iterations of the experiment. Following the treatment, eggs of each concentration were moved to water cups that were kept until their hatchability could be evaluated. The percent egg mortality was determined based on unhatched eggs 4 days (96 h) post-treatment[40]:

$$\% \text{ of egg mortality} = (\text{Number of hatched larvae} \div \text{total number of eggs}) \times 100$$

Effect of the sublethal concentrations on survival and larval longevity

In this test, 25 mosquito larvae in the 3rd instar were exposed to different concentrations of plant extracts in a 100-mL water solution, from all five plants (*L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera*) to determine LC_{50}. The mosquitoes were left for 48 h, with 15 groups (375 larvae) being treated and three groups (75 larvae) being applied with dechlorinated water as a control. Mortality was assessed after 48 h by counting the total number of moribund and dead larvae, according to the WHO[38]. Any live larvae at this time were removed with a pipette and transferred on a wire gauze to plastic cups containing 100 mL of distilled water. The larvae were then fed a small portion of dry bread until they reached the pupation stage and reached adulthood.

Field evaluation of larvicides

L. camara, M. azedarach, N. oleander, R. communis, and *W. somnifera* extracts were tested on larval and pupal mosquito populations in standing water pools (average 2.50 m × 1.25 m and 0.35 m deep) in a field evaluation. This was done at Kafer Saad village, Qalyubiya Governorate, Egypt, using LC_{95} X2 concentration, where the water level was relatively stable with a high mosquito density. Dechlorinated water was used at the control site only. Three replicates were used for each treatment. Mosquitoes for each site were sampled before treatment and post-treatment daily, for a week. Using a larval dipper (450 mL) at each larvicide pond site, we collected fourth instar larvae within water from the pond, for counting and sample examination.

We also tested strains of the bacterial larvicide (Bti Dunks, Summit, USA, 7000 ITU; International Toxic Unit/mg) in combination with plant extract on *Cx. pipiens* larvae in test pools. Half a bacterial briquette (equivalent to 6 g) was ground and mixed with each plant extract and added to a pool to examine the effect on mosquito larvae.

Phytochemical identification and in silico analysis

GC/MS analysis

For the biochemical analyses of the plant leaf methanol and aqueous extracts, Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS and TG-5MS fused silica capillary columns, 0.1 mm, 0.251 mm, and 30 m thick, were used. Analysis was done using an electronic ionizer with 70 eV ionization energy. A helium carrier gas was used (flow rate: 1 mL/min). The MS transmission line and injector were both set to 280 °C. The oven was pre-heated and adjusted to the temperature of 35 °C, then increased to 150 °C at a rate of 7 °C per min, then 270 °C at a rate of 5 °C per minute (pausing for two minutes), and lastly to 310 °C by increasing at a rate of 3.5 °C per minute (maintaining this temperature for 10 min). Relative peak area was employed to quantify all the different chemical components discovered. The presence of the detected compounds and their concentrations were checked by comparing the retention times and spectral data fragmentation with those in the NIST and Willy libraries on the GC/MS instrument. Identification was done using the aggregate spectrum of user-generated reference libraries. To evaluate peak homogeneity, single-ion chromatographic reconstructions were performed. To verify GC retention times, co-chromatographic analysis of reference substances was used whenever possible[41].

Molecular docking study

Source of the objective protein

Binding capabilities of the detected polyphenols in the methanolic extracts on the *lm-FABP* binding site were assessed. This was to determine the ability of the polyphenols to form stable and successive interactions with the residue of the target protein, and consequently to propose the mechanism of enzyme inhibition. The three-dimensional structure of the fatty acid binding protein (FABP) of *Cx. pipiens* did not exist in the protein data bank, so the well-known crystal structure of the fatty acid binding protein in locust flight muscle in complex with oleate *lm-FABP* was instead used. This structure has been used as an equivalent structure for *Cx. pipiens* in many research articles[42]. Thus the protein lm-*FABP* (PDB code: 2FLJ) was downloaded from the protein data bank (https://www.rcsb.org/structure/2FLJ) in PDB format, all water and hetero-molecules were removed where chain a, and b constrained.

Solvent	Plant extract	Time (h)	Concentration (ppm)						
			0	50	100	200	400	800	1600
Water	*L. camara*	24	0 ± 0^{aG}	4 ± 1.87^{bF}	10 ± 1.58^{bE}	24 ± 5.10^{bD}	47 ± 2.55^{bC}	71 ± 2.92^{bB}	87 ± 2.00^{bA}
		48	0 ± 0^{aG}	9 ± 2.45^{aF}	17 ± 3.39^{aE}	31 ± 2.92^{aD}	55 ± 3.87^{aC}	84 ± 2.45^{aB}	96 ± 1.87^{aA}
	M. azedarach	24	0 ± 0^{aG}	6 ± 1.00^{bF}	12 ± 1.22^{bE}	26 ± 2.92^{bD}	53 ± 4.06^{bC}	74 ± 2.92^{bB}	91 ± 1.00^{bA}
		48	0 ± 0^{aG}	13 ± 3.74^{aF}	21 ± 2.92^{aE}	33 ± 3.39^{aD}	59 ± 3.32^{aC}	83 ± 1.22^{aB}	99 ± 1.00^{aA}
	N. oleander	24	0 ± 0^{aG}	4 ± 1.87^{bF}	9 ± 1.00^{bE}	21 ± 1.87^{bD}	43 ± 4.06^{bC}	66 ± 2.92^{bB}	80 ± 2.74^{bA}
		48	0 ± 0^{aG}	8 ± 2.00^{aF}	14 ± 1.87^{aE}	31 ± 2.45^{aD}	51 ± 2.92^{aC}	76 ± 4.30^{aB}	90 ± 2.24^{aA}
	R. communis	24	0 ± 0^{aG}	4 ± 1.00^{bF}	11 ± 1.87^{bE}	23 ± 2.55^{bD}	43 ± 3.39^{bC}	68 ± 3.00^{bB}	85 ± 2.74^{bA}
		48	0 ± 0^{aG}	9 ± 1.87^{aF}	17 ± 1.22^{aE}	31 ± 2.45^{aD}	53 ± 4.64^{aC}	80 ± 2.74^{aB}	93 ± 2.55^{aA}
	W. somnifera	24	0 ± 0^{aG}	0 ± 0.00^{bF}	7 ± 1.22^{bE}	19 ± 2.45^{bD}	31 ± 2.92^{bC}	46 ± 3.32^{bB}	63 ± 5.61^{bA}
		48	0 ± 0^{aG}	6 ± 1.87^{aF}	11 ± 1.87^{aE}	26 ± 3.67^{aD}	47 ± 6.44^{aC}	64 ± 6.96^{aB}	76 ± 4.58^{aA}
Methanol	*L. camara*	24	0 ± 0^{aG}	11 ± 1.87^{bF}	25 ± 1.58^{bE}	43 ± 1.22^{bD}	66 ± 2.92^{bC}	84 ± 2.92^{bB}	100 ± 0.00^{aA}
		48	0 ± 0^{aF}	19 ± 1.87^{aE}	39 ± 1.87^{aD}	66 ± 5.10^{aC}	85 ± 3.16^{aB}	99 ± 1.00^{aA}	100 ± 0.00^{aA}
	M. azedarach	24	0 ± 0^{aG}	11 ± 1.87^{bF}	26 ± 2.92^{bE}	46 ± 2.92^{bD}	71 ± 3.32^{bC}	90 ± 1.58^{bB}	100 ± 0.00^{aA}
		48	0 ± 0^{aF}	19 ± 2.92^{aE}	42 ± 2.55^{aD}	71 ± 3.67^{aC}	87 ± 4.90^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	N. oleander	24	0 ± 0^{aG}	14 ± 1.87^{bF}	32 ± 2.55^{bE}	58 ± 6.82^{bD}	80 ± 3.54^{bC}	96 ± 2.45^{bB}	100 ± 0.00^{aA}
		48	0 ± 0^{aF}	23 ± 2.55^{aE}	48 ± 2.00^{aD}	80 ± 2.74^{aC}	94 ± 2.45^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	R. communis	24	0 ± 0^{aG}	13 ± 1.22^{bF}	29 ± 2.92^{bE}	54 ± 2.92^{bD}	75 ± 1.58^{bC}	94 ± 1.87^{bB}	100 ± 0.00^{aA}
		48	0 ± 0^{aF}	20 ± 1.58^{aE}	45 ± 4.18^{aD}	76 ± 3.67^{aC}	92 ± 3.00^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	W. somnifera	24	0 ± 0^{aG}	8 ± 1.22^{bF}	16 ± 1.00^{bE}	30 ± 1.58^{bD}	50 ± 3.54^{bC}	76 ± 3.32^{bB}	95 ± 2.74^{bA}
		48	0 ± 0^{aG}	11 ± 1.00^{aF}	22 ± 2.55^{aE}	38 ± 3.74^{aD}	62 ± 4.36^{aC}	91 ± 2.92^{aB}	100 ± 0.00^{aA}

Table 2. Efficacy of *Lantana camara, Melia azedarach, Nerium oleander, Ricinus communis,* and *Withania somnifera* extracts on *Culex pipiens* larval mortality, 24 and 48 h post-treatment (mean ± SE). a, b & c…. etc.: There is no significant difference ($p > 0.05$) between any two means within the same column have the same superscript letter, and A, B & C… etc.: There is no significant difference ($p > 0.05$) between any two means for the same solvent within the same row have the same superscript letter. Five replicates were used for each concentration (20 larvae/ replicate were used).

Solvent	Plant extract	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	Chi (Sig.)	R
Water	*L. camara*	432.96 (376.39–501.54)	1928.62 (1511.58–2630.22)	2945.53 (2207.14–4273.30)	1.975 ± 0.140	0.374 (0.984)	0.9990
	M. azedarach	373.29 (325.09–999.13)	1643.47 (1302.43–2204.92)	2501.73 (1898.51–3562.10)	1.990 ± 0.139	1.791 (0.774)	0.9960
	N. oleander	515.34 (442.81–607.59)	2618.48 (1970.87–3789.40)	4151.16 (2964.16–6463.86)	1.815 ± 0.136	0.833 (0.933)	0.9980
	R. communis	467.02 (403.57–545.67)	2242.96 (1722.40–3150.84)	3499.47 (2558.73–5260.76)	1.880 ± 0.137	0.505 (0.973)	0.9990
	W. somnifera	898.56 (738.33–1141.40)	5882.60 (3890.26–10,431.76)	10,020.52 (6158.86–19,761.63)	1.570 ± 0.139	3.863 (0.424)	0.9477
Methanol	*L. camara*	225.64 (196.38–258.43)	955.84 (777.38–1236.57)	1439.18 (1124.59–1967.76)	2.044 ± 0.141	6.433 (0.169)	0.9488
	M. azedarach	203.35 (177.95–231.47)	778.35 (643.08–985.75)	1138.73 (907.45–1516.22)	2.198 ± 0.150	3.854 (0.963)	0.9677
	N. oleander	158.92 (139.15–179.98)	583.68 (495.34–710.62)	843.99 (694.74–1071.78)	2.268 ± 0.144	0.721 (0.948)	0.9871
	R. communis	175.04 (153.09–199.03)	648.99 (539.31–816.17)	940.95 (754.79–1243.25)	2.252 ± 0.156	2.935 (0.568)	0.9788
	W. somnifera	336.23 (292.25–387.90)	1526.49 (1209.63–2047.59)	2343.98 (1777.32–3340.58)	1.950 ± 0.136	5.530 (0.237)	0.9872

Table 3. Lethal concentrations (ppm) of *Lantana camara, Melia azedarach, Nerium oleander, Ricinus communis,* and *Withania somnifera* extracts against *Culex pipiens*, 24 h post-treatment.

Energy minimization

Phenolic active ingredients from the alcoholic extracts were automatically identified by the HPLC. A database set of 18 candidates of the polyphenolic active ingredients were selected for this study. The structure of the target compounds was drawn using CAMBRIDGESOFT CHEMOFFICE 2015 Professional 15.0.0 software after recalling their SMILES from the PubChem database. All the investigated compounds were saved as "Mol format" after fulfilling the "energy minimization" step using the default function "Amber12: EHT forcefield", until gradient convergence of 0.01 kcal/mol was achieved. The energy minimization step was assessed by Avogadro and the molecular simulations were done using Molecular Operating Environment MOE_2009, installed on a 64-bit operating computer [Intel (R) Core (TM) i5-2400 CPU @ 2.40 GHz, 8 GB RAM].

Solvent	Plant extract	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	Chi (Sig.)	R
Water	*L. camara*	295.53 (258.35–338.19)	1237.03 (1002.21–1607.32)	1856.25 (1445.80–2545.35)	2.061 ± 0.139	6.134 (0.189)	0.9889
	M. azedarach	262.24 (188.53–360.27)	1178.75 (875.65–2097.72)	1805.18 (1312.99–3562.57)	1.962 ± 0.130	10.275 (0.03)	0.9647
	N. oleander	353.29 (305.51–410.23)	1733.97 (1350.91–2383.10)	2722.06 (2021.44–3996.91)	1.854 ± 0.133	1.795 (0.773)	0.9959
	R. communis	317.96 (275.77–367.34)	1490.40 (1177.63–2007.26)	2309.37 (1744.00–3310.23)	1.910 ± 0.134	3.647 (0.455)	0.9930
	W. somnifera	505.79 (427.28–609.11)	3270.67 (2334.08–5123.51)	5551.87 (3712.82–9532.22)	1.580 ± 0.126	1.454 (0.126)	0.9962
Methanol	*L. camara*	128.03 (111.51–145.54)	445.22 (373.12–555.16)	633.88 (513.08–830.97)	2.367 ± 0.175	3.035 (0.551)	0.9915
	M. azedarach	119.01 (103.89–134.93)	391.88 (330.13–485.83)	549.37 (447.50–715.21)	2.476 ± 0.187	3.456 (0.484)	0.9738
	N. oleander	99.63 (86.58–112.91)	321.38 (278.28–381.43)	447.92 (377.75–552.45)	2.519 ± 0.171	0.626 (0.960)	0.9834
	R. communis	107.29 (93.84–121.31)	332.82 (281.49–411.55)	458.76 (375.30–595.84)	2.606 ± 0.206	1.531 (0.821)	0.9814
	W. somnifera	232.87 (168.50–317.69)	882.65 (669.45–1526.99)	1287.75 (963.40–2448.32)	2.214 ± 0.195	10.982 (0.026)	0.9589

Table 4. Lethal concentrations (ppm) of *Lantana camara, Melia azedarach, Nerium oleander, Ricinus communis,* and *Withania somnifera* extracts against *Culex pipiens*, 48 h post-treatment.

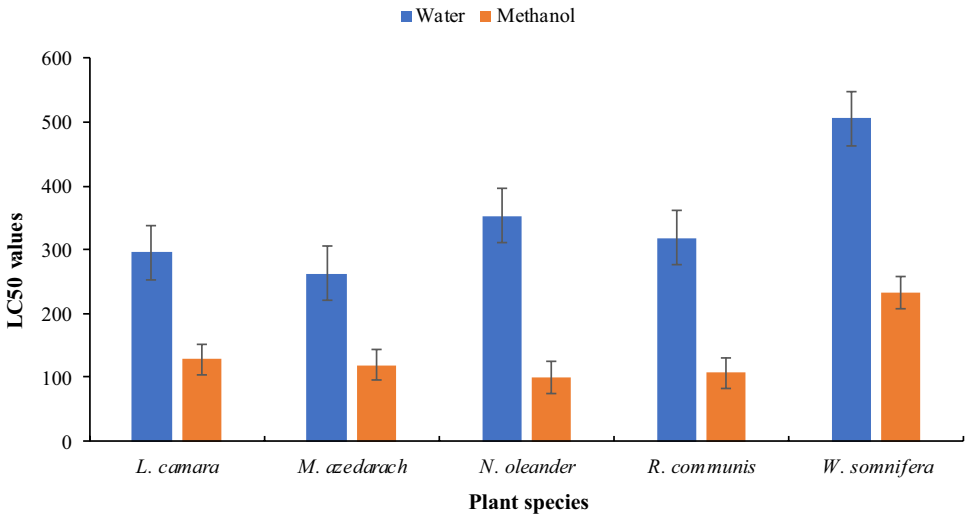

Figure 1. The mean number of larval mortalities induced by the effects of *Lantana camara, Melia azedarach, Nerium oleander, Ricinus communis,* and *Withania somnifera* methanol and aqueous extracts against the 3rd larval instars of *Culex pipiens*, 48 h post-exposure.

Docking procedure

The protein structure model of *lm-FABP* (PDB code: 2FLJ) was downloaded, and the candidate ligands and target protein were prepared as follows: (i) The reference drug, oleate, was colored green to be easily differentiated; (ii) The protein binding site was produced automatically from the "surfaces and maps" option and accordingly, the co-crystallized ligand's binding site; (iii) Similarly, the pocket site was created, separated, and saved in MDB format.

After the "energy minimization" step was done, all the investigated candidates were docked at the active site pocket using the "compute" option with the defaults of "rotate bonds" to produce flexible ligand with rigid receptor docking fulfillment. The scoring energy function was adjusted to be "London G" with a "triangle matcher" replacement set. The default "thirty conformers" was chosen as the total number of conformers and the best five scoring energy values were automatically selected. One of the best five conformers was chosen to represent ligand-docking and the results showed two- and three-dimensional receptor interactions. The docking results of all the tested compounds presented in the extract were listed in one table, regarding the predilection of the number of interactions, scoring energy (kcal/mol), RMSD (Å), and the bond length (Å). Three- and two-dimensional docking interactions were then determined. As with the co-crystallized ligand, all the tested ligands were marked in green color. Inter-molecular hydrogen bonding and π-π staking (aromatic) were labeled in magenta

Plant extract	Treatment	Concentration (ppm)					
		100	200	400	800	F value	P value
L. camara	Methanol	346.7 ± 3.3 (88.8)	316.7 ± 20.3 (81.1)	213.3 ± 6.7 (54.7)	113.3 ± 8.8 (29.0)	103.60	0.000*
	Water	400.0 ± 11.6 (96.8)	383.3 ± 8.8 (92.7)	298.3 ± 13.0 (72.2)	233.3 ± 27.3 (56.5)	25.26	0.000*
M. azedarach	Methanol	290.0 ± 37.8 (74.3)	243.3 ± 6.7 (62.3)	126.7 ± 6.7 (32.5)	25.0 ± 2.9 (6.4)	63.70	0.000*
	Water	373..3 ± 6.7 (90.3)	353.3 ± 17.0 (85.5)	253.3 ± 3.3 (61.3)	146.7 ± 3.3 (35.5)	134.81	0.000*
N. oleander	Methanol	333.3 ± 16.7 (85.4)	266.7 ± 16.7 (68.3)	170.0 ± 20.0 (43.6)	98.3 ± 25.8 (25.2)	41.76	0.000*
	Water	405.0 ± 13.2 (98.0)	395.0 ± 16.1 (95.6)	338.3 ± 7.3 (81.9)	266.7 ± 16.7 (64.5)	23.27	0.000*
R. communis	Methanol	273.6.7 ± (70 .0)	203.3 ± 3.3 (52.1)	101.7 ± 4.4 (26.0)	2.3 ± 1.2 (0.6)	825.57	0.000*
	Water	376.7 ± 12.0 (91.1)	356.7 ± 6.7 (86.3)	268.3 ± 9.3 (64.9)	163.3 ± 8.8 (39.5)	124.47	0.000*
W. somnifera	Methanol	333.3 ± 24.0 (85.4)	263.3 ± 8.8 (67.5)	166.7 ± 8.8 (42.7)	80.0 ± 5.7 (20.5)	94.65	0.000*
	Water	383.3 ± 8.8 (92.7)	370.0 ± 15.3 (89.5)	298.3 ± 15.9 (72.2)	190.0 ± 10.0 (46.0)	55.79	0.000*

Table 5. Effect of methanol and water extracts on egg hatchability of *Culex pipiens*. Core of table shows number of eggs ± Standard Error (with percentage egg mortality in brackets). * % embryo mortality.

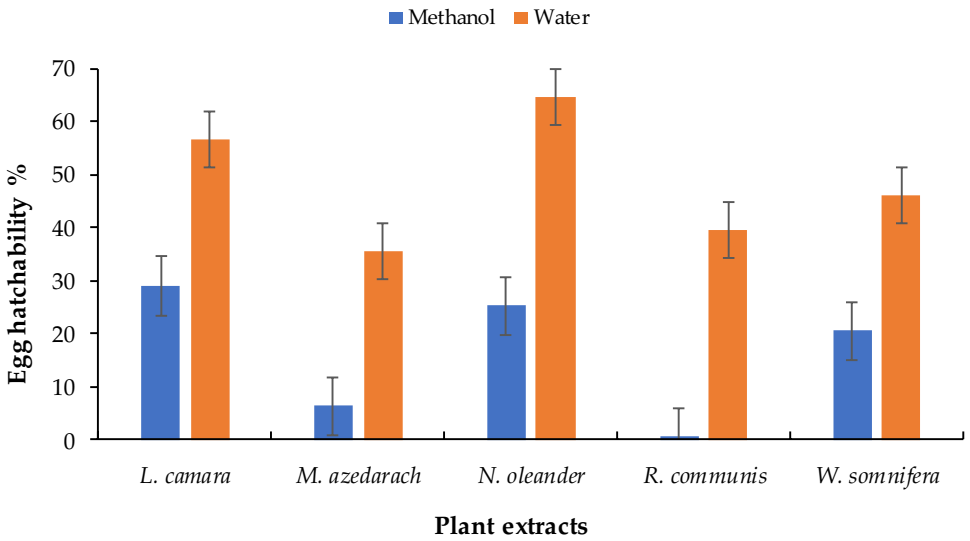

Figure 2. Ovicidal activity of the five different plant extracts against *Culex pipiens*.

and yellow color respectively, and loops, helical structure, etc. were colored automatically, with images rendered for better presentation.

Statistical analysis

SPSS V23 (IBM, USA) software was used for doing Probit analyses, to calculate the lethal concentration (LC) values, and for the one-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test). The significant levels were set at $P < 0.05$.

Results

Mosquito larvicidal activity

In the first part of the study, the larvicidal activity of *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* extracts on 3rd instar *Cx. pipiens* was evaluated. All the tested plant extracts in this study showed that methanol extracts had more toxic effects against *Cx. pipiens* larvae (95–100%, 24 h post-treatment) than aqueous extracts (63–91%, 24 h post-treatment). The mortality percent (MO%) reached 100% for *Lantana camara, Melia azedarach, Nerium oleander,* and *Ricinus communis*, and 95% (MO%) for *Withania somnifera*, 24 h post-treatment (PT) with 1600 ppm methanol extracts (Table 2) with LC_{50} (50%, median lethal concentration) = 225.64, 203.35, 158.92, 175.04, and 336.23 ppm, respectively (Table 3). With aqueous extracts, the mortality was 87, 91, 80, 85, and 63% for *L. camara, M. azedarach, N. oleander, R. communis*, and *W. somnifera*, respectively, with LC_{50} = 432.96, 373.29, 515.34, 467.02, and 898.56 ppm, respectively (Table 3). The results showed that methanol extracts of *N. oleander* (LC_{50} = 158.92 and 99.63 ppm) and *R. communis* (LC_{50} = 175.04 and 107.29 ppm) are very effective at killing mosquito larvae 24 and 48 h post-treatment, and *M. azedarach* (LC_{50} = 373.29 and 262.24 ppm) showed high efficacy within aqueous plant extracts (Table 4 and Fig. 1).

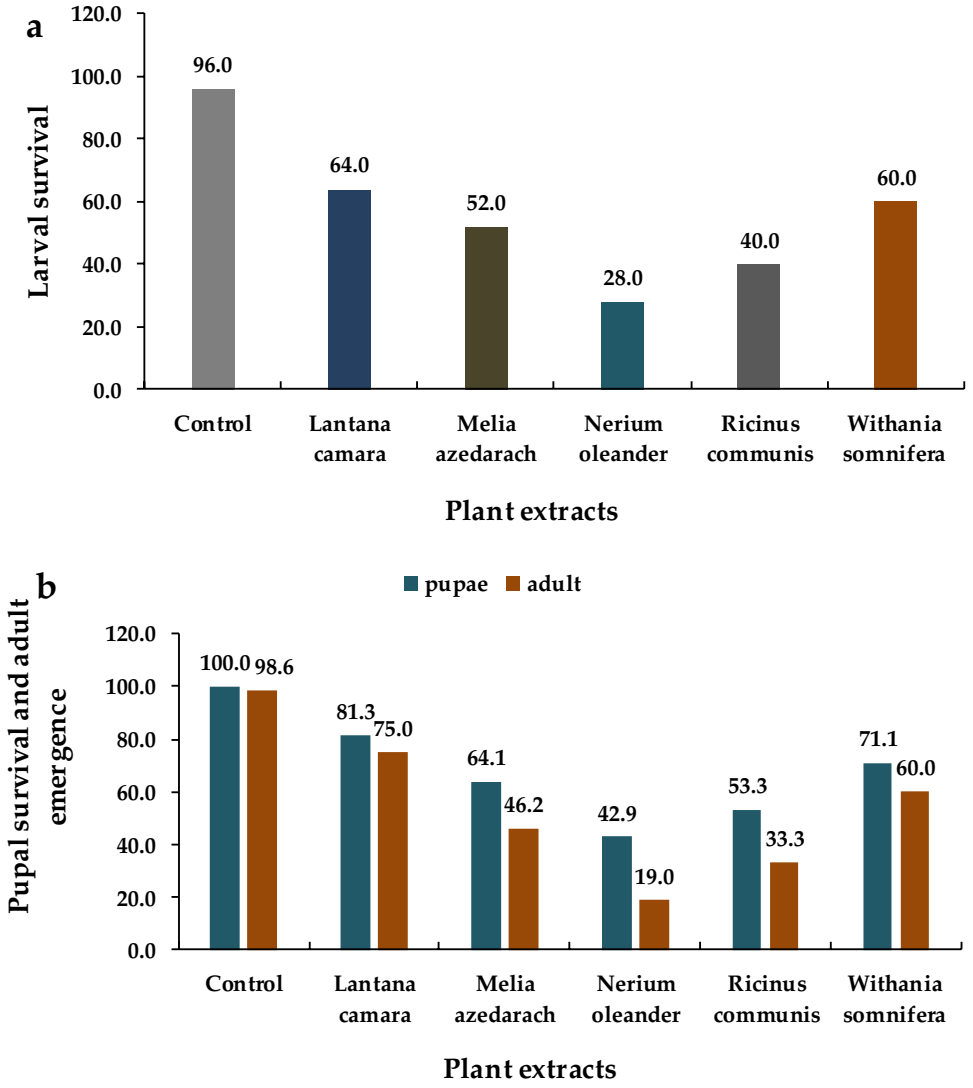

Figure 3. Percent 3rd instar larval survival (**a**) and percent pupal survival and adult emergence (**b**) of *Cx. pipiens* mosquitoes after 24 h exposure to LC_{50} concentrations of plant extracts. Percentages in a column followed by a different letter are significantly different (p = 0.05).

Ovicidal activity

The egg hatchability of the *Cx. pipiens* was tested with different concentrations of *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* extracts in both methanol and water (Table 5 and Fig. 2). Percent hatchability, as expected, was inversely proportional to the concentration of the extracts. Among the five plant extracts tested for ovicidal activity against *Cx. pipiens*, the methanol extracts of *R. communis* (0.6%) and *M. azedarach* (6.4%) had the highest ovicidal activity at 800 ppm, followed by *L. camara, N. oleander,* and *W. somnifera.*

Sublethal effect of plant extracts on mosquito larvae survival.

After exposure, the LC_{50} values of extracts in *L. camara* (225.64 ppm), *M. azedarach* (203.35 ppm), *N. oleander* (158.92 ppm), *R. communis* (175.04 ppm), and *W. somnifera* (336.23 ppm) were shown to significantly affect the survival percentage until adulthood in *Cx. pipiens* larvae. The control group did not experience any mortality. The percentage of mosquito larvae that survived and turned into pupae was much lower in all plant extracts after 48 h, with 64, 52, 28, 40, and 60% survival respectively, relative to the control group (Fig. 3a).

The rate of pupae that successfully transformed into adults was considerably lower after treatment with plant extracts compared to the control group (Fig. 3b). Overall, the survival rates of larvae and adult emergence after 48 h of exposure to LC_{50} concentrations of *N. oleander* (28% and 19%) and *R. communis* (40% and 33.3%) were significantly reduced ($F = 13.242$; df. = 2, 57; $P < 0.001$). These rates were much lower than the 96% survival rate seen in the control group.

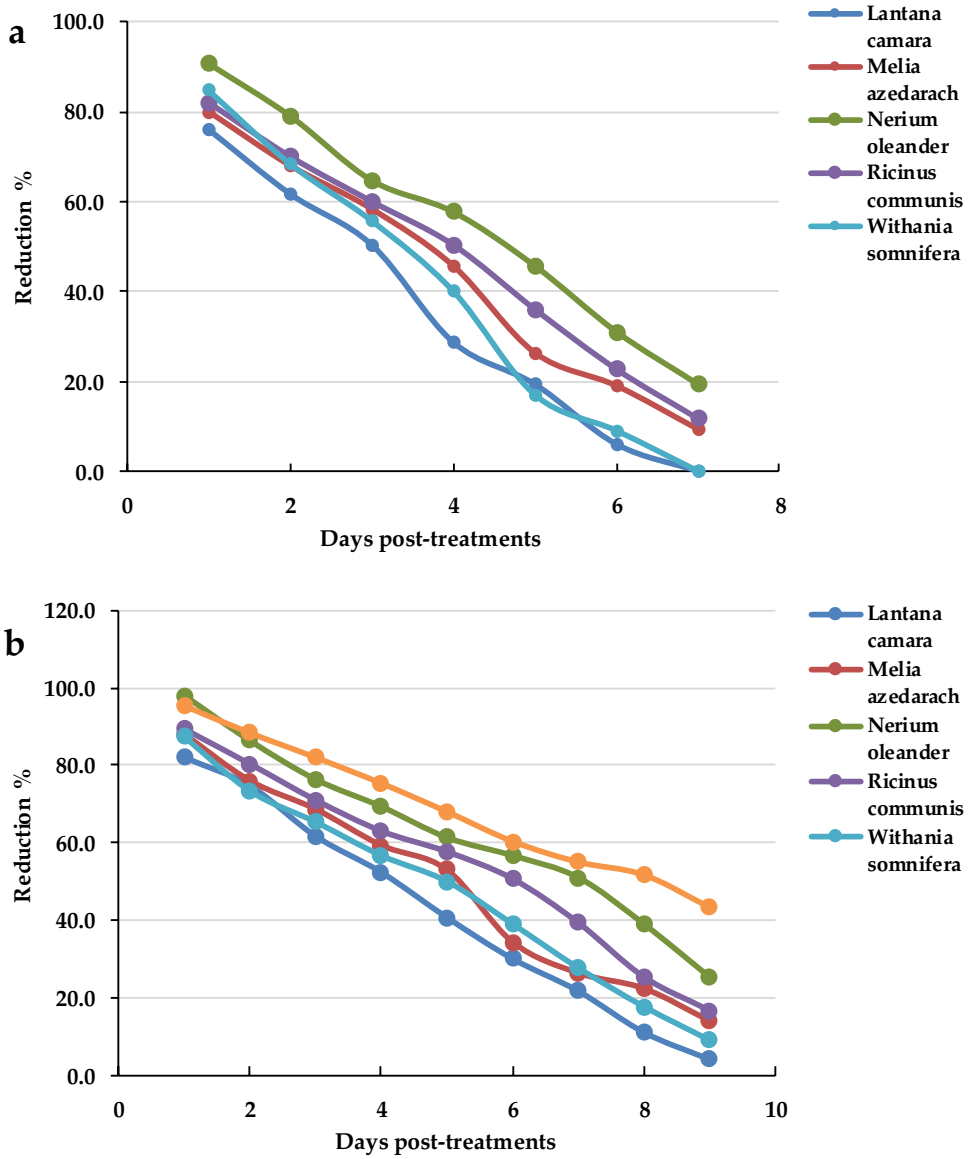

Figure 4. Field evaluation for larvicidal efficacy of *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* extracts (**a**) and plant extracts with Bti briquettes (**b**) treated at a dose of LC95 X2 (2878.4, 2277.2, 1688, 1881.9 and 4688 ppm) and half of a Bti briquette, respectively, in larval breeding sites.

Larvicidal Field Evaluation

Field evaluation of larvicides of *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* extracts was performed using LC_{95} X2 (2878.4, 2277.2, 1688, 1881.9 and 4688 ppm, respectively) in larval breeding site ponds at Kafer Saad village. Larval density was measured before and after adding the larvicides (or dechlorinated water in the control location). Lower larval densities were found 24 h after treatment with 76, 80, 90.7, 82, and 84.7% larval reduction for *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* respectively. With *N. oleander* extract this effect lasted four days (Fig. 4a). With larvicide extract mixed with the bacterial larvicide Bti briquette the % hile the larval reduction in the ponds 24 h after treatment reached 82, 88, 98, 87.3, and 95.3% respectively. *N. oleander* extract with the ti briquette lasted seven days post-treatment (Fig. 4b).

Biological characteristics of the plant extracts

GC–MS data analysis

The five extracts were subjected to metabolomic analysis, using GC–MS analysis to identify the range of compounds *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* leaves. The compounds include terpenes, fatty acids, esters, ketones, alkanes, steroids, aliphatic amines, and phenols. The analysis was conducted using only the methanol solvent.

L. camara extract contained 16 different compounds (Table 6), with the highest concentrations being 1-Dodecanamine, n,n-dimethyl- (32.98%), 1-Dodecanamine, n,n-dimethyl- (18.39%), and benzene,

No	RT	Compound name	Area %	M. F	M. W
Fatty acid and esters					
1	10.06	Dodecanal	1.27	$C_{12}H_{24}O$	184
2	12.01	1-Dodecanamine, n,n-dimethyl-	32.98	$C_{14}H_{31}N$	213
3	13.99	Oxirane, tetradecyl-	0.46	$C_{16}H_{32}O$	240
4	15.21	Cholestan-3-ol, 2-methylene-, (3á,5à)-	1.16	$C_{28}H_{48}O$	400
5	15.75	1-Dodecanamine, n,n-dimethyl-	18.38	$C_{14}H_{31}N$	331
6	17.39	1-Chlorooctadecane	1.09	$C_{18}H_{37}Cl$	288
7	22.33	9-Octadecenoic acid (z)-, methyl ester	8.49	$C_{19}H_{36}O_2$	296
Terpene (Monoterpene and Sesquiterpene)					
8	10.91	Humulene	4.34	$C_{15}H_{24}$	204
9	13.09	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (e)-	0.32	$C_{15}H_{26}O$	222
10	14.76	Eudesma-4(15),7-dien-1á -ol	0.99	$C_{15}H_{24}O$	220
Phenol					
11	3.93	Benzene, (chloromethyl)-	12.81	C_7H_7Cl	126
12	22.48	2-Methylenebrexane	4.88	$C_{10}H_{14}$	134
13	28.15	7-(Trifluoromethyl)naphthalen-1-ol	0.23	$C_{11}H_7F_3O$	212
Alkane					
14	11.25	1-Chloroundecane	3.82	$C_{11}H_{23}Cl$	190
15	22.52	Benzyl-(4-methylbenzyl)amine	9.35	$C_{13}H_{19}NO_2$	221
16	25.53	1-(n-Benzyl-n-methylamino)-4-methoxybutan-2-one	4.59	$C_{12}H_{20}N_2$	192

Table 6. The major chemical constituents of *Lantana camara* extracts.

No	RT	Compound nam	Area %	M. F	M. W
Fatty acid and esters			2.80	$C_{20}H_{38}$	278
1	24.11	Neophytadiene	0.55	$C_{15}H_{32}O$	228
2	24.31	1-Dodecanol, 3,7,11-trimethyl-	1.57	$C_{18}H_{32}O_2$	280
3	24.94	17-Octadecynoic acid	1.42	$C_{17}H_{34}O_2$	270
4	25.61	Pentadecanoic acid, 14-methyl-, methyl ester	6.55	$C_{16}H_{32}O_2$	256
5	26.42	Hexadecaoic acid	0.91	$C_{18}H_{36}O_2$	284
6	26.92	Hexadecanoic acid, ethyl ester	17.34	$C_{19}H_{36}O_2$	296
7	29.09	phytol	0.60	$C_{16}H_{28}O_2$	252
8	29.34	14-Pentadecynoic acid, methyl ester	4.77	$C_{12}H_{24}N_2O_3$	244
9	29.58	Pent-4-enoic acid, 2-(2-hydroxy-3-isobutoxypropyl)-, hydrazide	1.05	$C_{18}H_{34}O_2$	282
10	30.01	Oleic acid	6.70	$C_{20}H_{38}$	278
11	31.27	Tributyl acetylcitrate	37.39	$C_{20}H_{34}O_8$	402
12	35.77	Mono(2-ethylhexyl) phthalate	1.36	$C_{16}H_{22}O_4$	278
13	40.91	Hexadecadienoic acid, methyl ester	0.47	$C_{17}H_{30}O_2$	266
14	41.87	Linoleic acid, 2,3-bis-(O-TMS)-propyl ester	8.95	$C_{27}H_{54}O_4Si_2$	498
15	42.31	1-Heptatriacotanol	7.57	$C_{37}H_{76}O$	536

Table 7. The major chemical constituents of *Melia azedarach* extract.

(chloromethyl)- (12.81%). *M. azedarach* extract contained 15 compounds (Table 7), with the highest concentrations being tributyl acetylcitrate (37.39%), hexadecanoic acid, and ethyl ester (17.34%). The *N. oleander* extract contained 19 compounds (Table 8) with the highest concentrations being tributyl acetylcitrate (36.13%), mome inositol (12.11%), and squalene (9.98%). *R. communis* extract contained 14 compounds (Table 9), with the highest concentrations being bis(2-ethylhexyl) phthalate (48.29%) and hexadecanoic acid, methyl ester (13.33%). *W. somnifera* extract contained 17 different compounds (Table 10), with the highest concentration compounds being linoleic acid ethyl ester (20.14%), pentadecanoic acid, 14-methyl ester (14.10%), and isochiapin b (11.23%).

HPLC analysis and non-volatile constituents determination

One of the most important analyses to identify polyphenols and flavonoids is high-performance liquid chromatography (HPLC). The methanol extract of *N. oleander, R. communis, L. camara, M. azedarach,* and *W. somnifera* were analyzed, and 18 phenolic and flavonoid standards were used. The results of the HPLC peaks are presented in Figs. 5, 6, 7, 8 and 9 and the corresponding concentrations are listed in Table 11 and Fig. 10. All

No	RT	Compound name	Area %	M. F	M. W
Fatty acid and esters					
1	24.11	Neophytadiene	1.74	$C_{20}H_{38}$	278
2	25.59	Hexadecanoic acid, methyl ester	0.92	$C_{17}H_{34}O_2$	270
3	26.38	n-Hexadecanoic acid	4.28	$C_{16}H_{32}O_2$	256
4	28.77	9-Octadecenoic acid (z)-, methyl ester	5.07	$C_{19}H_{36}O_2$	296
5	29.57	9-Octadecenoic acid (z)-	5.41	$C_{18}H_{34}O_2$	282
6	31.27	Tributyl acetylcitrate	36.13	$C_{20}H_{34}O_8$	402
7	35.58	Hexadecanoic acid, 2,3-dihydroxypropyl ester	1.28	$C_{19}H_{38}O_4$	330
8	38.57	2-Hexadecanol	0.70	**$C_{16}H_{34}O$**	242
9	41.00	1-Heptatriacotanol	1.24	$C_{37}H_{76}O$	536
10	42.92	Glycidyl oleate	1.27	$C_{21}H_{38}O_3$	338
11	44.21	Androstan-17-one, 3-ethyl-3-hydroxy-, (5à)-	1.27	$C_{21}H_{34}O_2$	318
12	44.91	1-Heptatriacotanol	6.65	$C_{37}H_{76}O$	536
13		Methylglucoside			
14	19.82	Mome inositol	12.11	$C_7H_{14}O_6$	194
Keton					
15	35.76	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	0.87	$C_{28}H_{25}NO_7$	487
Terpene (Monoterpene and Sesquiterpene)					
16	40.12	Squalene	9.98	$C_{30}H_{50}$	410
17	40.23	Ethyl iso-allocholate	2.43	$C_{26}H_{44}O_5$	436
18	42.83	Cedran-diol, (8S,14)-	3.60	**$C_{15}H_{26}O_2$**	238
19	44.74	á-Sitosterol	5.09	$C_{29}H_{50}O$	414

Table 8. The major chemical constituents of *Nerium oleander* extract.

No	RT	Compound name	Area %	M. F	M. W
Fatty acid and esters			2.32	$C_{20}H_{38}$	287
1	24.11	Neophytadiene	0.74	$C_{20}H_{40}O_2$	312
2	24.94	Ethanol, 2-(9-octadecenyloxy)-, (z)-	1.98	$C_{17}H_{34}O_2$	270
3	25.60	Hexadecanoic acid, methyl ester	13.33	$C_{16}H_{32}O_2$	256
4	26.92	Hexadecanoic acid, ethyl ester	1.92	$C_{20}H_{36}O_2$	308
5	28.60	Ethyl (9z,12z)-9,12-octadecadienoate #	6.29	$C_{19}H_{36}O_2$	296
6	28.77	9-Octadecenoic acid (z)-, methyl ester	2.32	$C_{20}H_{38}$	287
7	29.51	9,12-Octadecadienoyl chloride, (z,z)-	9.66	$C_{18}H_{31}ClO$	298
8	29.98	8,11,14-Eicosatrienoic acid, (z,z,z)-	2.73	$C_{20}H_{34}O_2$	306
9	35.76	Bis(2-ethylhexyl) phthalate	48.29	$C_{24}H_{38}O_4$	390
10	41.30	1-Heptatriacotanol	1.05	$C_{37}H_{76}O$	536
11	44.21	Androstan-17-one, 3-ethyl-3-hydroxy-, (5à)-	2.47	$C_{21}H_{34}O_2$	318
12	44.73	1-Heptatriacotanol	6.59	$C_{37}H_{76}O$	536
Terpene (Monoterpene and Sesquiterpene)					
13	29.09	Phytol	2.64	$C_{20}H_{40}O$	296

Table 9. The major chemical constituents of *Ricinus communis* extract.

the five methanolic extracts had the polyphenols: gallic acid at concentrations of 114.75, 577.48, 168.4, 102.55 and 182 µg/mL respectively; chlorogenic acid at concentrations of 1007, 754.39, 115.7, 91.49 and 323.3 µg/mL respectively. Varied concentrations of catechin, methyl gallate caffeic acid methyl gallate, caffeic acid, syringic acid, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, rosmaricic acid, daidzein, quercetin, cinnamic acid, kaempferol and hesperetin were also found.

Fatty acid binding protein (FABP4)

The inhibition activity of the methanolic extracts of *Nerium oleander, Ricinus communis, Lantana camara, Melia azedarach,* and *Withania somnifera* were tested with FABP4 protein. The inhibition results showed that all tested extracts are capable of inhibiting the FABP4 enzyme with different IC_{50}s of 0.599, 0.425, 12.76, 1.47 and 4.55 µg/mL, respectively (Table 12 and Fig. 11).

No	RT	Compound name	Area %	M. F	M. W
Fatty acid and Esters					
1	4.84	Tetradecane, 1-chloro-	1.44	$C_{16}H_{30}O_2$	254
2	6.99	1,2–15,16-Diepoxyhexadecane	3.99	$C_{20}H_{38}$	278
3	25.61	Pentadecanoic acid, 14-methyl-, methyl ester	14.10	$C_{16}H_{32}O_2$	256
4	26.40	n-Hexadecanoic acid	1.82	$C_{18}H_{36}O_2$	284
5	26.92	Hexadecanoic acid, ethyl ester	2.93	$C_{20}H_{36}O_2$	308
6	28.60	Linoleic acid ethyl ester	20.14	$C_{19}H_{36}O_2$	296
7	31.27	Tributyl acetylcitrate	9.50	$C_{20}H_{34}O_8$	402
Phenol					
8	4.32	3-Trifluoroacetoxypentadecane	1.35	$C_{16}H_{28}O_3$	268
9	28.77	9-Octadecenoic acid (z)-, methyl ester	0.88	$C_{21}H_{22}O_{11}$	450
10	29.56	9,12-Octadecadienoic acid (z,z)-	7.33	$C_{18}H_{32}O_2$	280
11	31.08	Undec-10-ynoic acid, decyl ester	0.57	$C_{21}H_{38}O_2$	322
12	40.22	Hexadecadienoic acid, methyl ester	7.93	$C_{17}H_{30}O_2$	266
13	40.97	1-Heptatriacotanol	2.11	$C_{37}H_{76}O$	536
14	44.74	Linoleic acid, 2,3-bis-(O-TMS)-propyl ester	7.86	$C_{21}H_{38}O_2$	498
Terpene (monoterpene and sesquiterpene)					
15	29.09	Phytol	4.57	$C_{20}H_{40}O$	296
16	38.98	Isochiapin b	11.23	$C_{19}H_{22}O_6$	346
17	35.76	2-([(2-Ethylhexyl)oxy]carbonyl)benzoic acid #	2.25	$C_{16}H_{22}O_4$	278

Table 10. The major chemical constituents of *Withania somnifera* extract.

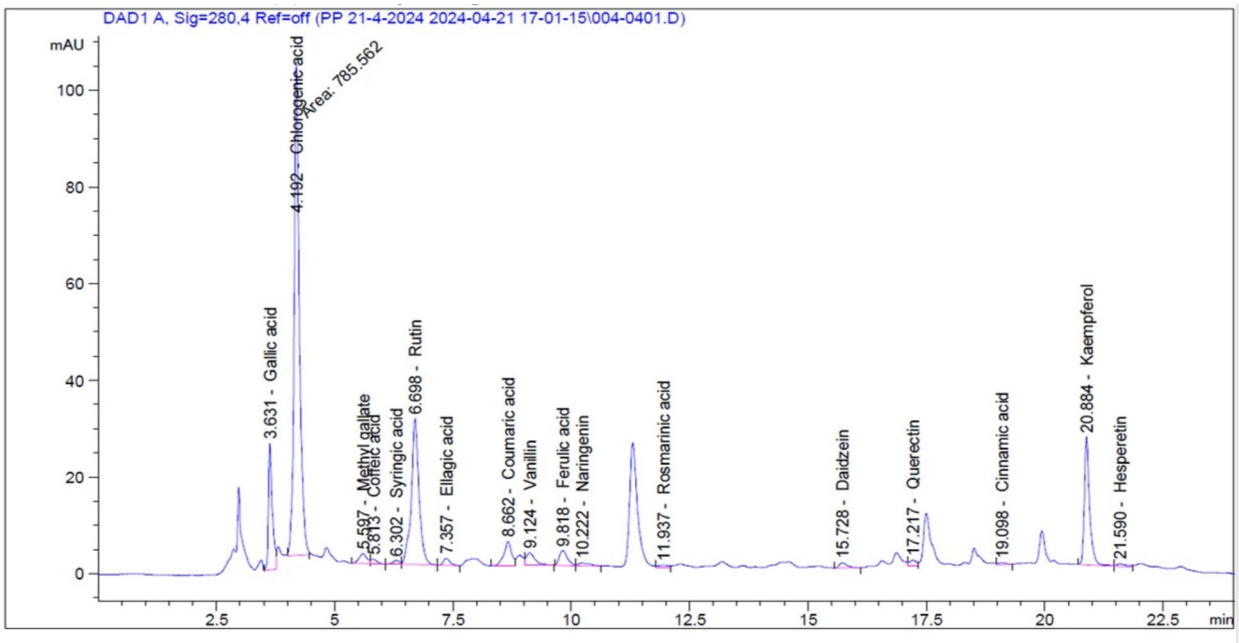

Figure 5. HPLC-Chromatogram of *N. oleander* methanolic extract.

Docking study

The target protein *Lm-FABP,* PDB:*2FLJ* was selected to perform a docking study using the detected polyphenols and flavonoids including gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, rutin, ellagic acid, coumaric acid, ferulic acid, naringenin rosmarinic acid, daidzein, quercetin, and cinnamic acid. These were all docked to the active site of the target protein with results presented in Table 13. Co-crystallized ligand, OLA, (Fig. 12) was used as a reference to use to compare and evaluate and compare the effectiveness of the active-ingredients presented in each extracts. Glutin, a low-molecular weight citrus flavonoid glycoside, had five electrostatic forces, a hydrogen bond, and multiple hydroxyl groups (Fig. 13) with arginine amino acid (Arg128), glutamine (Gln34), and aspartic acid (Asp75) in addition to one pi-pi stacking with the residue lysine (Lys60). Caffeic acid, coumaric acid, rosmarinic acid, and cinnamic acid had two dipole–dipole interactions and one dispersion force from Vander Waals forces (Figs. 14 and 15). Gallic acid, chlorogenic acid, methyl gallate,

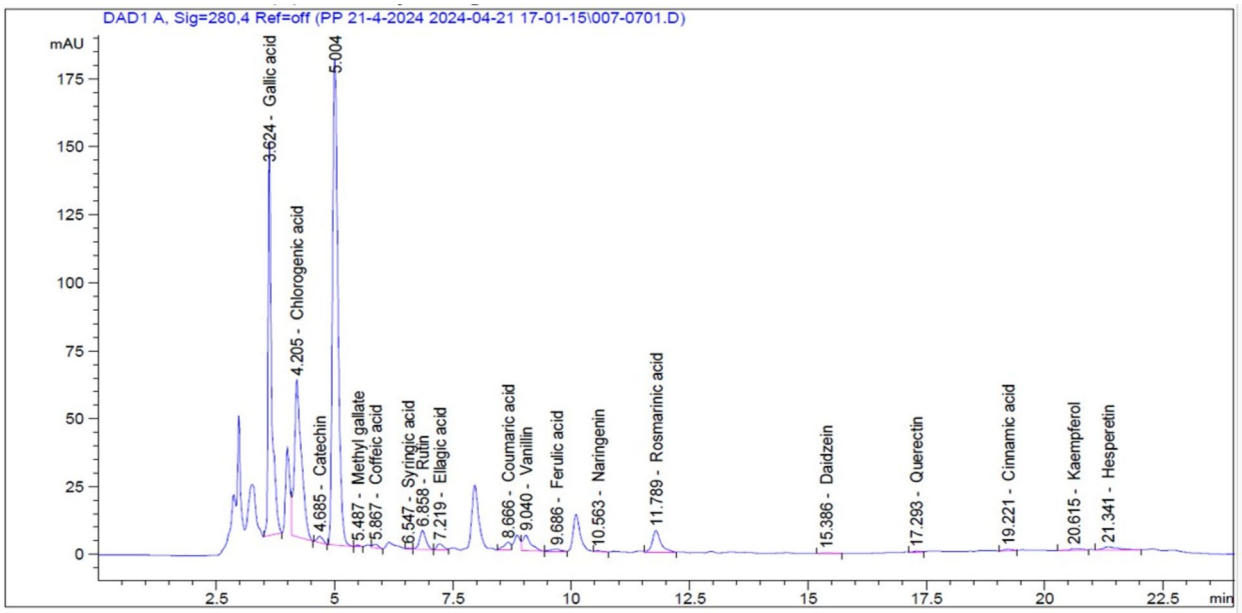

Figure 6. HPLC-Chromatogram of *R. communis* methanolic extract.

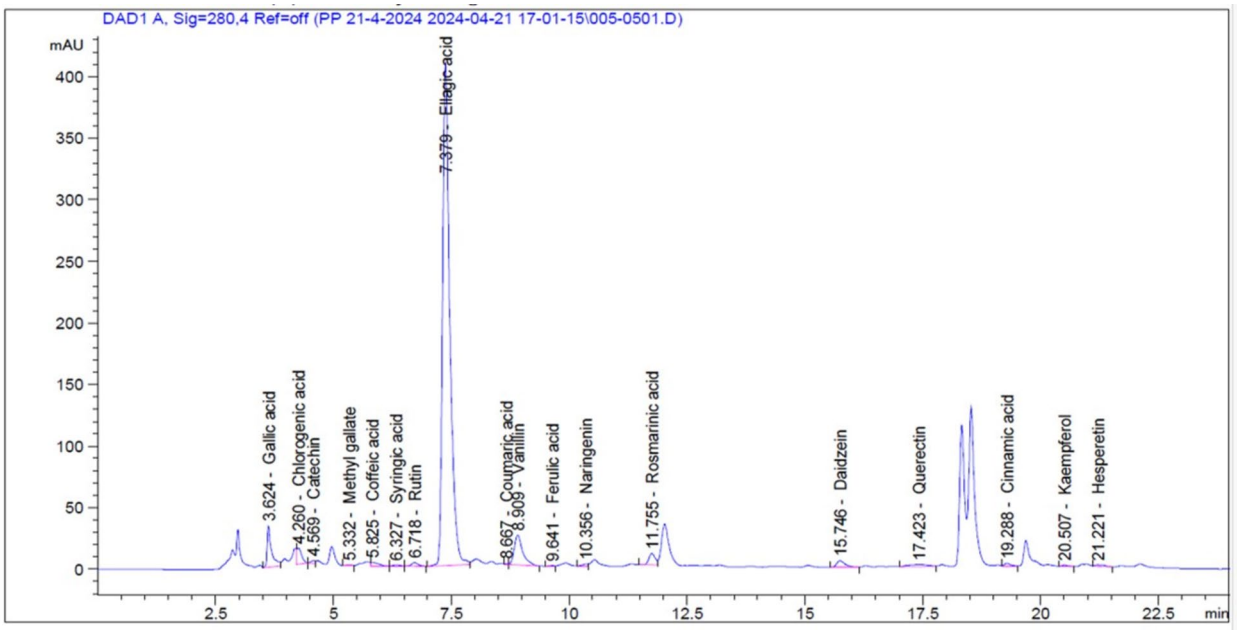

Figure 7. HPLC-Chromatogram of *L. camara* methanolic extract.

syringic acid, ferulic acid, naringenin, and daidzein had two intermolecular hydrogen bond between a hydroxyl group and a corresponding amino acid residue (Figs. 16 and 17). Catechin and quercetin had only one hydrogen bond (Fig. 18).

Discussion

Plant extracts and essential oils (EOs) contain several significant natural constituents that are effective in managing or eliminating pests and consequently associated diseases. They also undergo natural degradation processes[43]. Such biopesticides are being increasingly applied and are projected to surpass synthetic chemical pesticides soon, with an average yearly increase in usage of 9–20%[44]. The distinctive attributes of biopesticides, such as their low toxicity to non-target organisms and the environment, has contributed to their increased use in pest control.

All of the plant extracts we tested were very effective at killing mosquito larvae. According to our data, methanol extracts were more effective at killing larvae than aqueous extracts and produced high mortality. Methanol extracts of *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* achieved 95–100% mortality

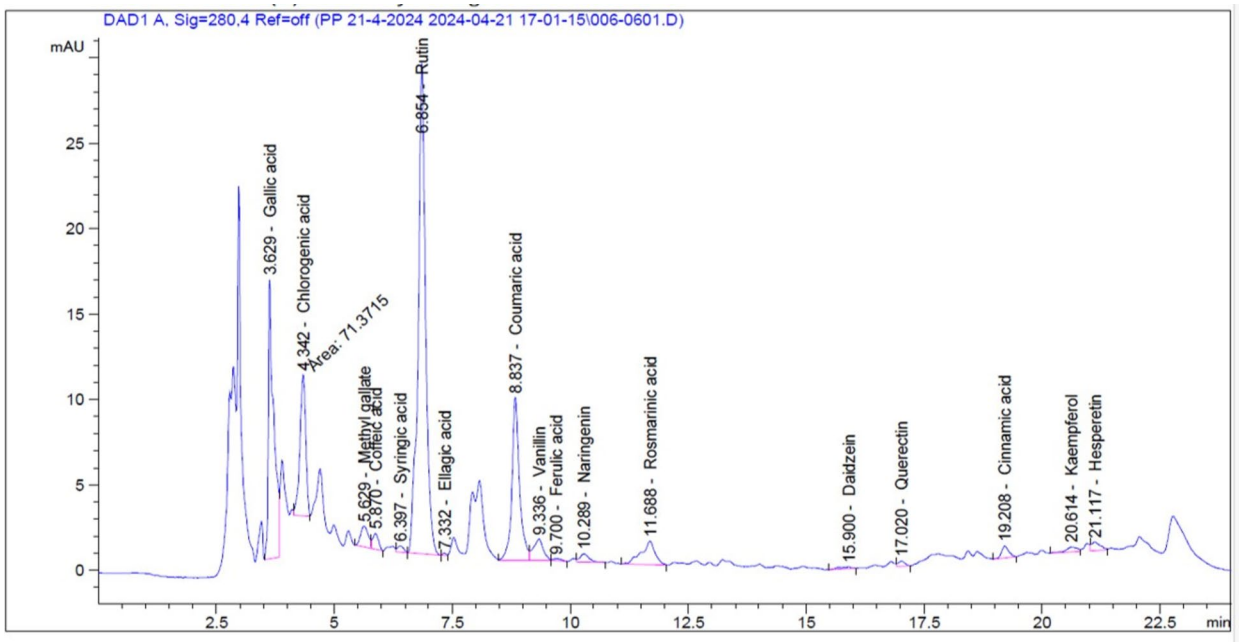

Figure 8. HPLC-Chromatogram of *M. azedarach* methanolic extract.

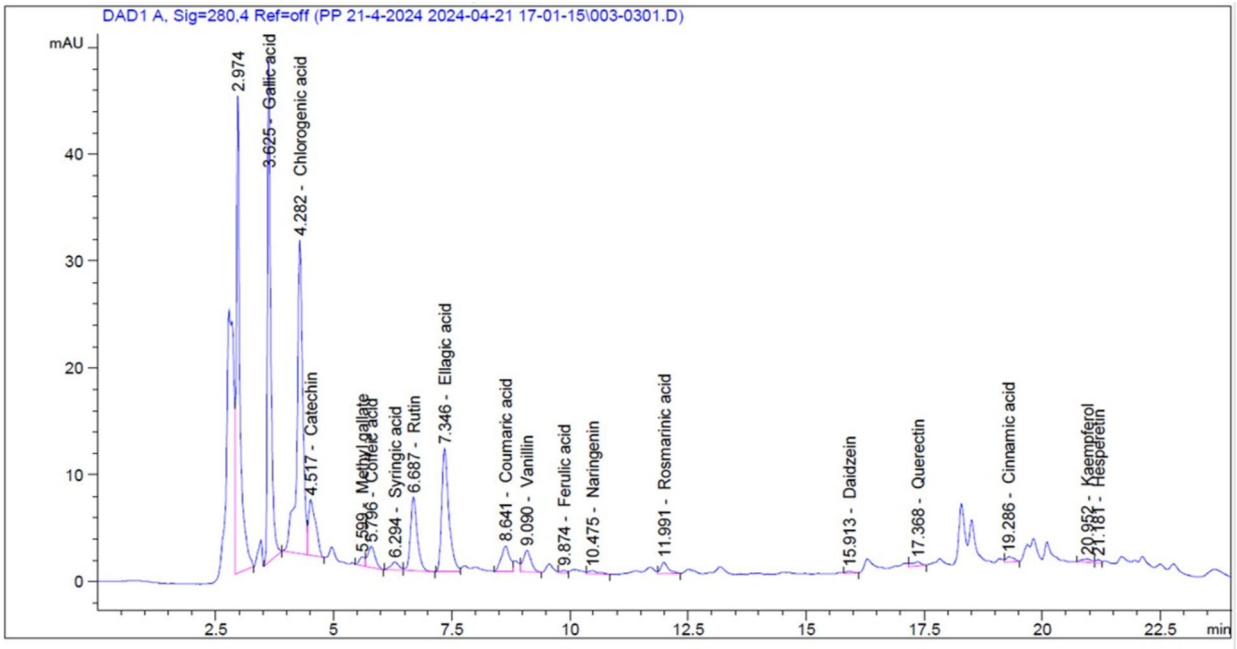

Figure 9. HPLC-Chromatogram of *W. somnifera* methanolic extract.

among mosquito larvae treated in the lab after 24 h, while the mortality rate with water extracts reached only 63–91%. Plant extracts of *N. oleander* and *R. communis* were indicated to be the most effective against mosquito larvae in this study, confirming results of El-Akhal, et al.[20], who tested extract effectiveness on various Culcidea mosquito larvae. El-Akhal, et al.[20] found that the extract of *N. oleander* influenced the 4th instar larvae of *Cx. pipiens*, with an LC_{50} value of 57.57 mg/mL and an LC_{90} value of 166.35 mg/mL after 24 h of exposure. Also, acetone extracts from *N. oleander* flower were evaluated on larvae of *Aedes aegypti*, *Anopheles stephensi,* and *Cx. quinquefasciatus* and had LC_{50} values of 94.60, 101.21, and 121.79 mg/L, respectively[22]. Despite using extracts from flowers (whereas we used leaves) and using a broader range of species, the patterns in results with our study shows a similar efficacy of *N. oleander.*

Some researchers have studied *N. oleander*, commonly known as oleander, for its insecticidal properties, including its effects on mosquito larvae. Studies have shown the insecticidal properties of several toxic compounds found in *N. oleander*, such as oleandrin and nerin[45]. Research on the effectiveness of *N. oleander* leaf

Standard			*N. oleander*		*R. communis*		*L. camara*		*M. azedarach*		*W. somnifera*	
Phenolic / flavonoid comp	Conc. (µg/mL)	Area	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)
Gallic acid	20	251.39	144.24	114.75	725.87	577.48	211.69	168.4	128.9	102.55	228.71	182
Chlorogenic acid	50	390.04	785.56	1007	588.49	754.39	90.25	115.7	71.37	91.49	252.21	323.3
Catechin	75	332.03	0	0	19.22	43.43	13.3	30.04	0	0	54.68	123.5
Methyl gallate	15	295.83	24.53	12.43	3.14	1.59	3.29	1.66	13.95	7.07	6.4	3.24
Caffeic acid	18	226.02	9.16	7.29	11.69	9.31	29.63	23.59	7.87	6.27	21.25	16.92
Syringic acid	17.2	258.23	8.29	5.52	3.85	2.56	10.02	6.67	3.34	2.22	9.15	6.09
Rutin	50	305.33	341.42	559.1	64.94	106.35	28.05	45.94	318.96	522.32	68.96	112.9
Ellagic acid	70	754.04	13.09	12.15	20.33	18.87	4322.28	4013	0.79	0.73	121.07	112.4
Coumaric acid	20	595.17	55.89	18.78	30.9	10.38	4.23	1.42	106.54	35.8	30.12	10.12
Vanillin	12.9	350.18	34.95	12.87	66.3	24.42	299.41	110.3	17.25	6.35	23.72	8.74
Ferulic acid	20	358.77	35.72	19.91	9.66	5.38	4.48	2.49	1.31	0.73	2.35	1.31
Naringenin	30	335.7	11.22	10.03	3.76	3.36	17.41	15.56	6.55	5.85	4.24	3.79
Rosmarinic acid	50	499.71	5.9	5.9	105.66	105.72	86.17	86.22	28.29	28.31	11.67	11.68
Daidzein	20	320.07	12.18	7.61	3.99	2.49	67.04	41.89	2.1	1.31	1.77	1.11
Quercetin	20	324.7	11.08	6.82	4.3	2.65	54.76	33.73	3.05	1.88	5.26	3.24
Cinnamic acid	10	581.21	4.03	0.69	7.44	1.28	21.16	3.64	7.1	1.22	5.68	0.97
Kaempferol	20	316.07	201.97	127.8	11.63	7.36	8.04	5.08	4.52	2.86	5.08	3.21
Hesperetin	20	463.54	8.29	3.57	27.62	11.91	14.18	6.11	6.63	2.86	2.23	0.96

Table 11. Concentration determination of the polyphenolic and flavonoid contents presented in *N. oleander, R. communis L. camara, M. azedarach* and *W. somnifera.*

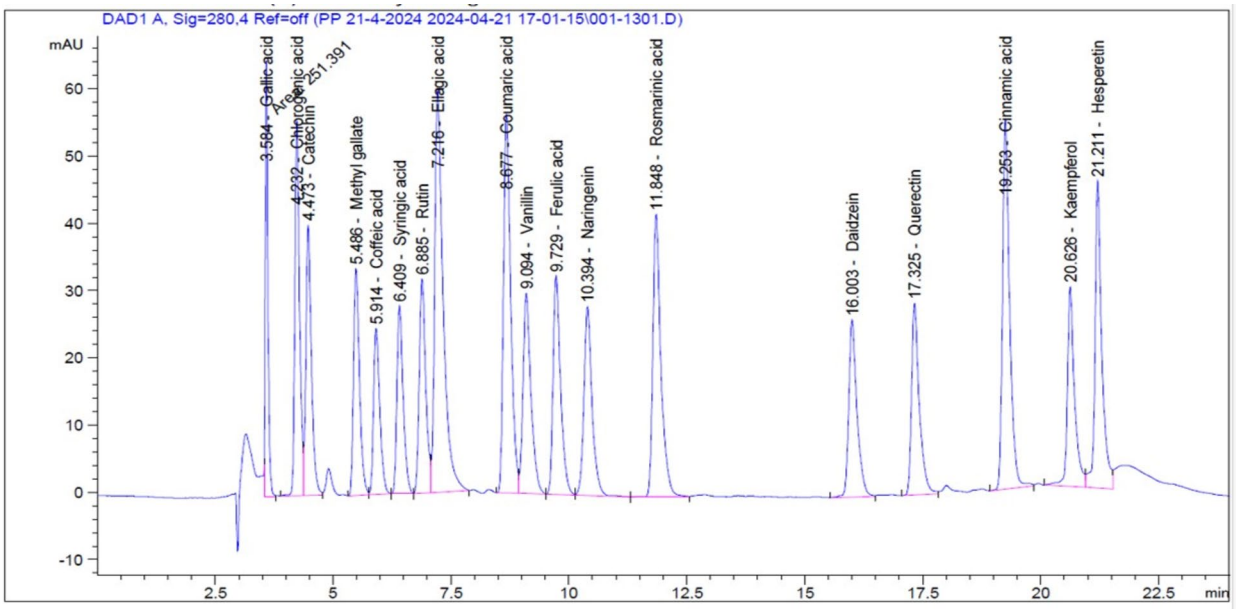

Figure 10. HPLC-Chromatogram of standard mixture.

extracts against mosquito larvae has shown promising results. Other studies have demonstrated that these extracts have larvicidal activity against several species of mosquitoes, including *Aedes aegypti* and *Culex quinquefasciatus*, which are important vectors of diseases such as dengue fever, Zika virus, and West Nile virus. Methanol extract from oleander leaves has also previously shown efficacy in destroying *Anopheles* spp. larvae in vitro with an LC_{50} of 4–85 ppm[21].

Oleander leaf extracts has been used for its larvicidal activity against Pine processionary moths, *Thaumetopoea pityocampa* with an LC_{50} value of 322.50 ppm and 190.00 ppm after 24 and 48 h post-treatment, respectively; using extract concentrations of 10, 25, 50, and 100 mg. *Trogoderma granarium* larvae also had a 10% mortality rate after 72 h at the 100 mg dose level[46]. Sotelo-Leyva, et al.[47] evaluated the insecticidal activity of *N. oleander* against sugarcane aphid (*Melanaphis sacchari*) showing a 96% mortality rate at 72 h, and when 40% concentration

Extract	FABP4 IC_{50} (µg/mL)
N. oleander	0.599
M. azedarach	1.47
R. communis	0.425
W. somnifera	4.55
L. camara	12.76
Cobimetinib	0.2354
Orlistat	0.529

Table 12. FABP4 enzyme inhibition assay.

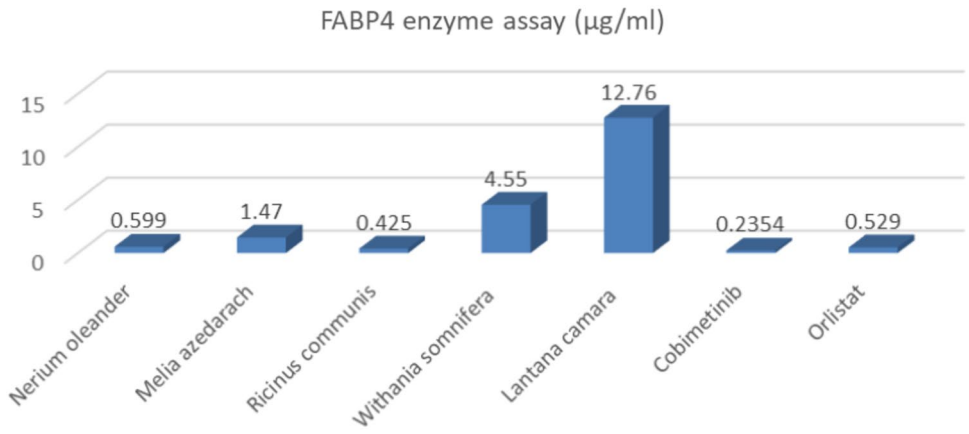

Figure 11. The inhibition assay of FABP4 against the five extracts of *N. oleander, R. communis, L. camara, M. azedarach,* and *W. somnifera.*

N. oleander leaf extracts were used on *Tribolium castaneum* adult beetles there was 100% mortality. Leaf and stem extracts of 70% hydroethanolic from *N. oleander* has also been shown to prolong the first instar larval period of *Pectinophora gossypiella*[48].

Our study showed that *R. communis* extract ranks second in its lethal effect on mosquito larvae after oleander extract, whether in methanol or aqueous extract. Many studies have confirmed the efficacy of *R. communis* extract in killing mosquito larvae in methanol, acetone, and aqueous extracts. The leaf extract of *N. oleander* has previously been tested[49] against 4th instar larvae of *Ae. aegypti* at concentrations of 50–250 ppm with a mortality rate of 16.7%–92.7% giving an LC_{50} of 108.17 ppm. Also, in a study with 5% aqueous *R. communis* leaf extract, the extract killed 50% of *Cx. pipiens* larvae in less than 6 h for the L2 stage, less than 12 h for the L4, and 100% of mosquito larvae after 4 h[50].

Waris et al.[51] and Al-Hakimi, et al.[49] tested both leaf and seed extracts of *R. communis* and found significant mortality against *Ae. aegypti* at concentrations of 31.25, 62.5, 125, 250, 500 ppm, and against *Anopheles culicifacies* (a malaria vector) larvae at 2, 4, 8, 16, 32, and 64 ppm. After 24-h exposure, larvicidal activities were higher for the methanol extract of seeds than of leaves, with LC_{50} of 9.37 for seeds and 15.52 ppm for leaves on *Ae. aegypti*, and LC_{50} of 31.1 ppm for seeds and of 45.24 ppm for leaves on *An. culicifacies*. This was compared to a positive control of synthetic Temephos larvicide, which had LC_{50} of 106.24 ppm and LC_{90} of 175.73 ppm against *Ae. aegypti*. Kombieni, et al.[44] has also found that that *R. communis* extract killed 75.8, 60.3 and 46.5% of *Spodoptera frugiperda* larvae at 250 g/L, 200 g/L, and 150 g/L of extract, respectively. Various solvents (aqueous, methanol, dichloromethane, and hexane) have been used to extract *R. communis* compounds from leaves and seeds to demonstrate larvicidal activity: severe toxicity on larval stages L2 and L4 of *Cx. pipiens* and the early IV instar larvae of *Aedes aegypti* and *Anopheles culicifacies*[23,52].

Results in this study on the efficacy of *Lantana camara* extracts are similar to those found in other studies. Sharma et al.[18] found leaf extract LC_{50} ranged from 47.47 to 52.06 ppm and LC_{90} ranged from 104.33 to 106.70 ppm on *Cx. quinquefasciatus*. Mondal, et al.[19], found *L. camara* ethanol extract had an LC_{50} at 234.43 ppm, 131.82 ppm, and 89.12 ppm at 24 h, 48 h, and 72 h post-treatment intervals respectively for larvae of the same species, *Cx. quinquefasciatus*. Against *Cx. pipiens*[53] (4th instar) it was extremely effective with an LC_{50} of only 29.3 ppm. Al-Solami[17] illustrated increased mortality of *Cx. pipiens* larvae over time with an *L. camara* acetone extract which they tested over two and ten days, showing an LC_{50} of 140.1 and 51.3 ppm, respectively, and finally resulting in 98% mortality.

Studies using *L. camara* ethanol extract against the house fly (*Musca domestica*) gave an LC_{50} of 1462.7 ppm for leaves and 2101.8 ppm for stems[54]. Also a methanol extract from *L. camara* showed the highest mortality

Name	No Inter	Residue	Type	Distance (Å)	Score (kcal/mol)	RMSD (Å)
Co-crystallized (Oleat)	3	Arg128 → (⁻OC = O)	H-bonding	1.70	–	–
		Arg108 → (O = CO⁻)	H-bonding	1.74		
		Try130 → (⁻OC = O)	H-bonding	1.62		
Gallic acid	2	Glu74 → OH	H-bonding	1.97	− 4.5841	1.22
		Glu74 → OH	H-bonding	2.04		
Chlorogenic acid	2	Arg128 → OH	H-bonding	2.12	− 5.9392	1.46
		Glu30 → OH	H-bonding	2.32		
Catechin	1	Arg128 → OH	H-bonding	2	− 4.4619	1.30
Methyl Gallate	2	Arg108 → O = C	H-bonding	1.92	− 4.6838	1.37
		Gln98 → OH	H-bonding	2.09		
Caffeic acid	3	Glu74 → OH	H-bonding	2.09	− 4.8039	1.12
		Gln98 → O = C	H-bonding	2.07		
		Tyr130 → O = C	H-bonding	1.88		
Syringic acid	2	Arg128 → O = C	H-bonding	2.01	− 4.9699	1.46
		Tyr130 → O = C	H-bonding	2.12		
Rutin	6	Arg128 → OH	H-bonding	1.83	− 4.8754	1.55
		Arg128 → OH	H-bonding	1.93		
		Arg128 → O	H-bonding	2.00		
		Gln34 → OH	H-bonding	2.01		
		Asp75 → OH	H-bonding	1.98		
		Lys60 → Pyran ring	π–π staking	–		
Ellagic acid	2	Asp76 → OH	H-bonding	1.90	− 5.1265	1.06
		Asp76 → OH	H-bonding	1.90		
Coumaric acid	3	Leu77 → O = C	H-bonding	2.40	− 4.5454	1.02
		Gln98 → O = C	H-bonding	2		
		Arg128 → benzene ring	π–π staking	–		
Ferulic acid	2	Leu77 → O = C	H-bonding	2.23	− 5.0227	1.00
		Gln98 → O = C	H-bonding	2.00		
Naringenin	2	Gln98 → O = C	H-bonding	2.05	− 5.5920	1.06
		Ser55 → benzene ring	π–π staking	–		
Rosmarinic acid	3	Arg128 → O = C	H-bonding	1.93	− 6.7813	1.7
		Tyr130 → O = C	H-bonding	2.39		
		Leu77 → benzene ring	π–π staking	–		
Daidzein	2	Arg128 → O = C	H-bonding	2.52	− 5.1146	1.08
		Gln98 → OH	H-bonding	2.39		
Quercetin	1	Arg108 → O = C	H-bonding	2.24	− 5.2240	1.34
Cinnamic Acid	3	Arg128 → O = C	H-bonding	2.10	− 4.4755	0.9165
		Tyr130 → O = C	H-bonding	1.97		
		Leu77 → benzene ring	π–π staking	–		
Kaempferol	1	Arg108 → O = C	H-bonding	2.23	− 5.1452	0.7974
Hesperetin	1	Gln98 → OH	H-bonding	1.94	− 5.8972	1.0237

Table 13. Docking results of the most abundant active ingredients in the five alcoholic extracts to the vicinity of *Lm-FABP,* PDB:*2FLJ* fatty acid binding protein.

(74%), whereas the lowest mortality was found in ethyl acetate extract (26%) at 2% (w/w) concentration against *Sitophilus zeamais*[54]. Aisha, et al.[55] showed that *L. camara* extract in essential oil against *T. castaneum* had an LC_{50} of 8.93 mg *L. camara* powder and LC_{90} of 13.54 mg/cm^3. At 48 h exposure the LC_{50} was 7.92 mg/cm^3 and LC_{90} was 10.47 mg/cm^3.

Larval mortality occurred in all the pond studies when our plant extracts were added, both with and without Bti briquettes. Over 24 h, *N. oleander* was most effective at causing mortality, and was effective for up to 5 days. The next highest efficacy was with *R. communis* and *W. somnifera*. Combination of plant extracts with Bti briquettes (Mosquito unks) increased the larval reduction rate for all treatments, with up to nine days effect post-treatment for *N. oleander*. Previous studies we did with different natural extracts and essential oils showed no more than 5 days efficacy[56].

Methanol extracts of *L. camara, M. azedarach, N. oleander, R. communis,* and *W. somnifera* had a higher number of organic compounds than aqueous extracts, with both a higher number of fatty acid and terpene compounds. It is believed that a group of secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds, are the compounds in *R. communis* extracts that kill the mosquito larvae. These compounds

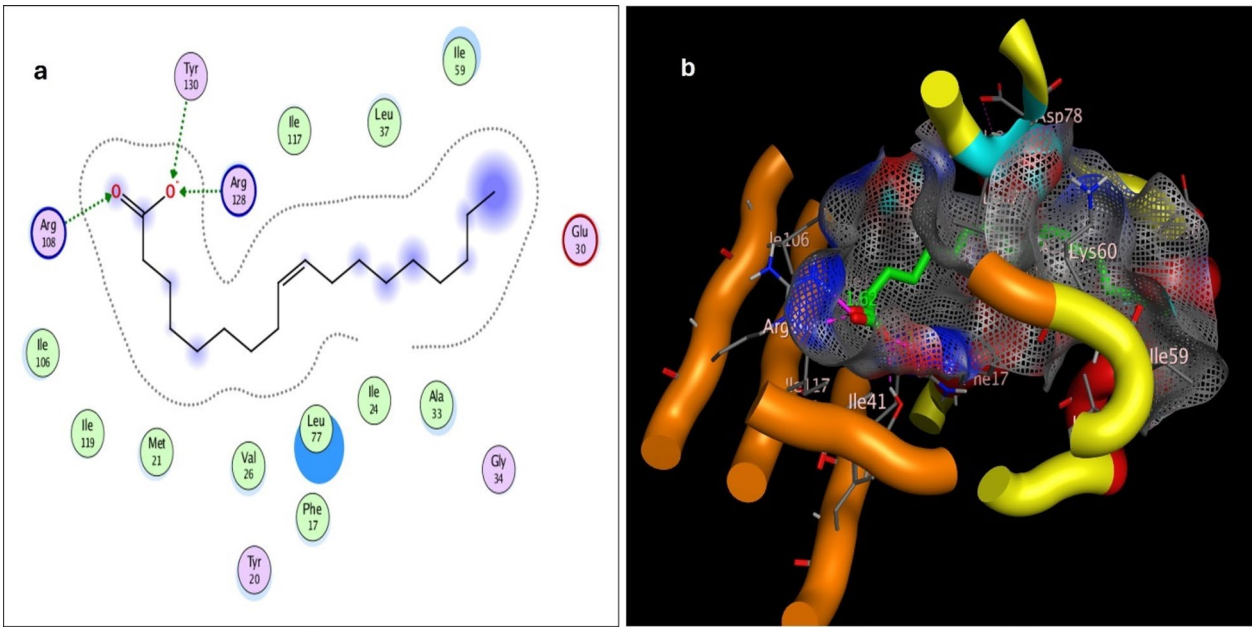

Figure 12. OLA co-crystallized ligand-docking of the two and three-dimensional interactions positioning interior *2FLJ* active site pocket.

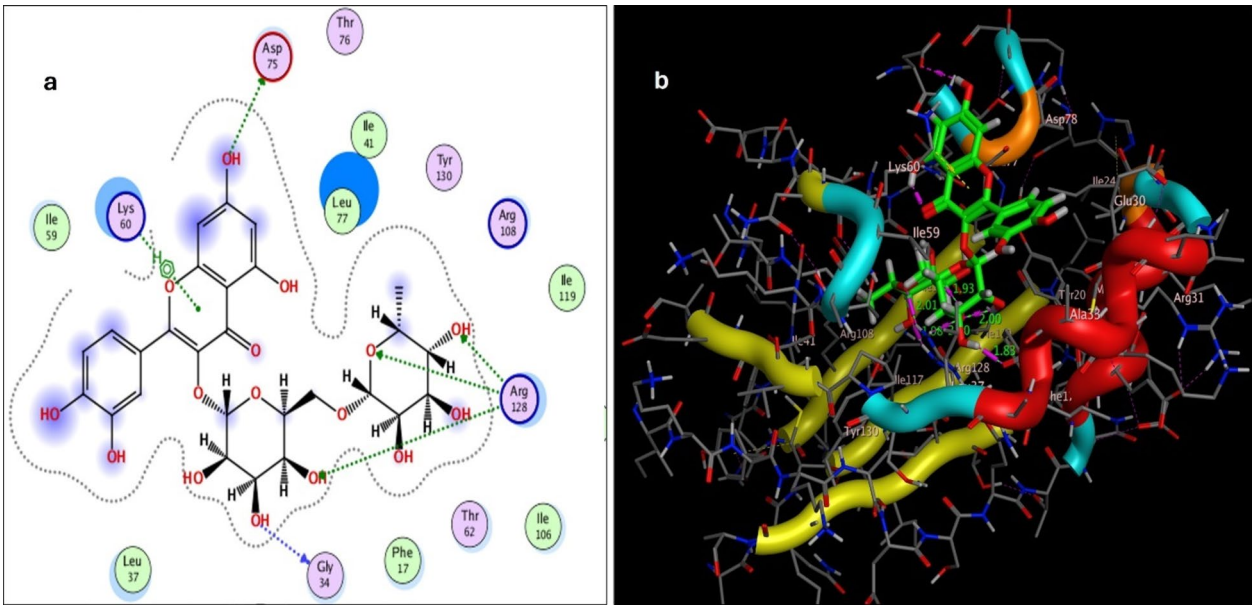

Figure 13. Two and three-dimensional interactions of rutin interior *2FLJ* active site pocket.

interfere with larval development or disrupt physiological processes, leading to death. The effectiveness of *R. communis* leaf extracts can vary depending on factors such as extraction method, extract concentration, mosquito species, and environmental conditions. More research is necessary to evaluate the human safety of these extracts and their potential environmental impacts, although they may provide a natural alternative to mosquito control.

Our findings align with prior research (Chengala et al.[57]) that endorses methanol as the preferred solvent for extracting useful metabolites from diverse medicinal and insecticidal plants. However, acetone is better at extracting polar phytocompounds like phenolics, being a polar solvent. This was shown with *L. camara* leaf[58] extracts. Such extracts have been shown to reduce inflammation, fight cancer, reduce the growth and kill bacteria, fungi, insects and nematodes[53].

The five plant leaf extracts that were analyzed had a high concentration of cedrol, caryophyllene, caryophyllene oxide, phytol, squalene, and caryophyllene; all of which are commonly found monoterpenes and sesquiterpenes. The observed insecticidal activity may be attributed to the main components, including caryophyllene (also known as isocaryophyllene), eucalyptol, and caryophyllene oxide. This finding aligns with the research conducted by Zoubiri and Baaliouamer[59], who also reported insecticidal activity in b-caryophyllene and caryophyllene

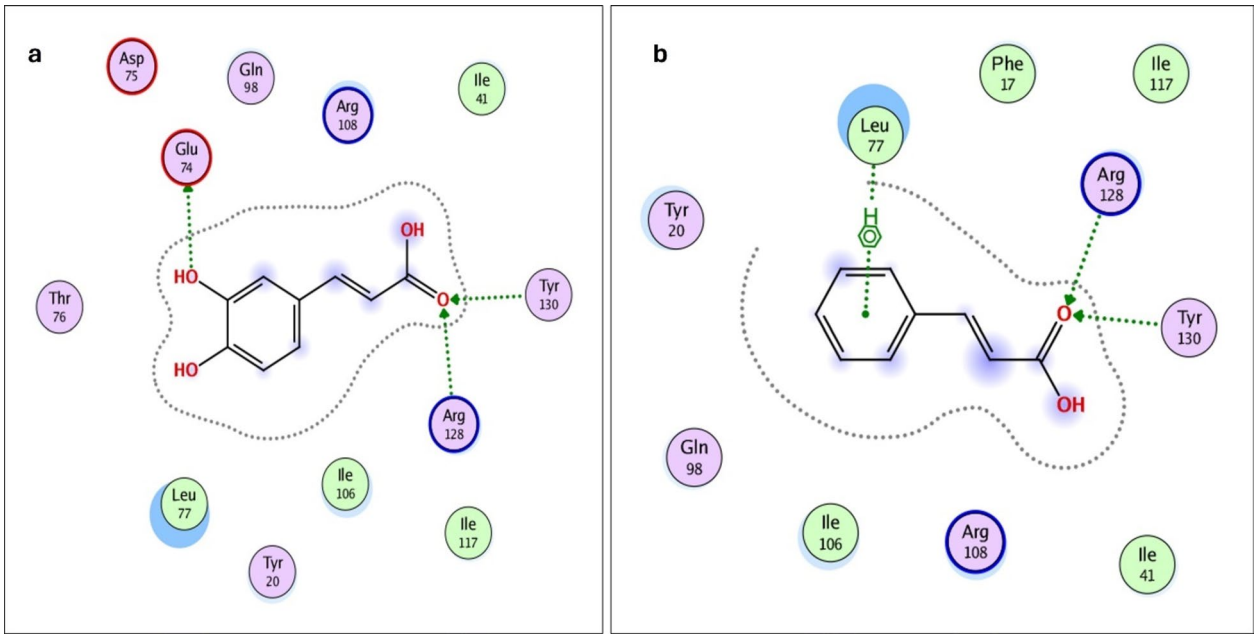

Figure 14. Two and three-dimensional interactions of (**a**) caffeic acid, and (**b**) cinnamic acid interior *2FLJ* active site pocket.

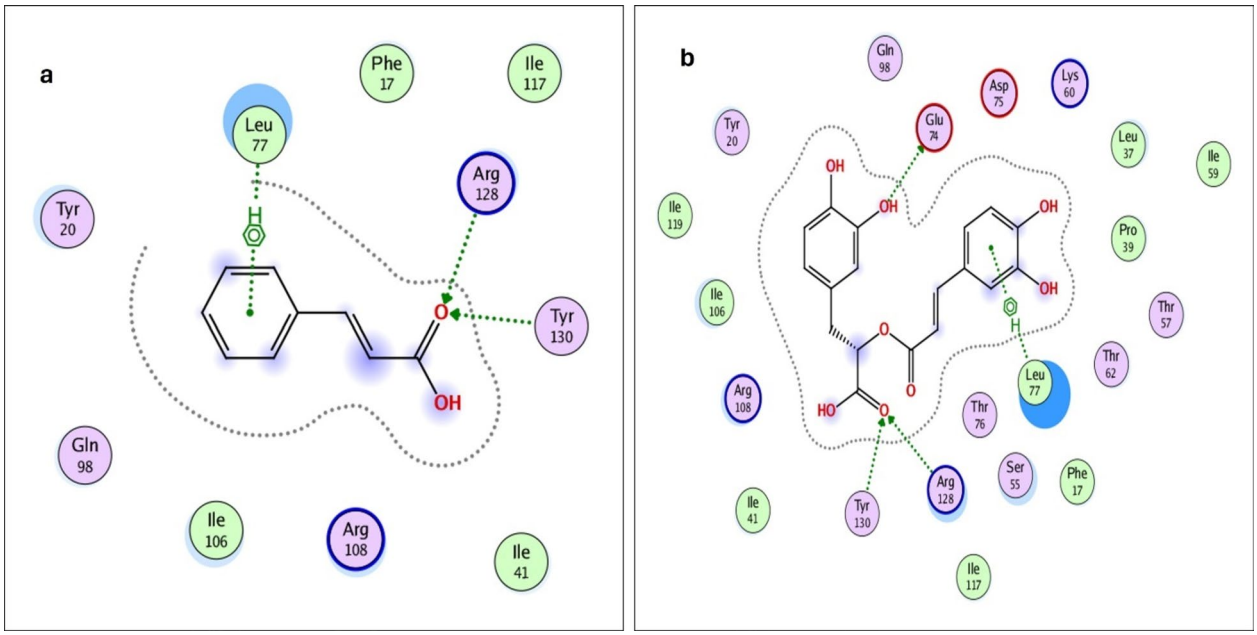

Figure 15. Two and three-dimensional interactions of (**a**) coumaric acid, and (**b**) rosmarinic acid interior *2FLJ* active site pocket.

oxide. Caryophyllene oxide, spathulenol, and germacrene-D have been identified as having anti-carcinogenic, anti-inflammatory, insecticide, pesticide, and antibacterial effects[59].

Plants also produce phenolic compounds, which are strong antioxidants[60,61] .Plants exposed to high metal concentrations have shown a significant increase in the accumulation of phenolic compounds and peroxide activity. The primary reason for the antioxidant activity of phosphonates is mostly attributed to their redox properties, which enable them to function as reducing agents, hydrogen donors, and quenchers of singlet oxygen[62]. Phenolic compounds are commonly referred to as polyphenols, and include flavonoids, phenolic acids, intricate flavonoids, and vibrant anthocyanins. Phenolic metabolites are crucial in various biological activities, including attractants for pollinating invertebrates, coloration for concealment and defense against herbivores, to inhibit consumption by invertebrates, and for antibacterial and antifungal purposes[63,64].

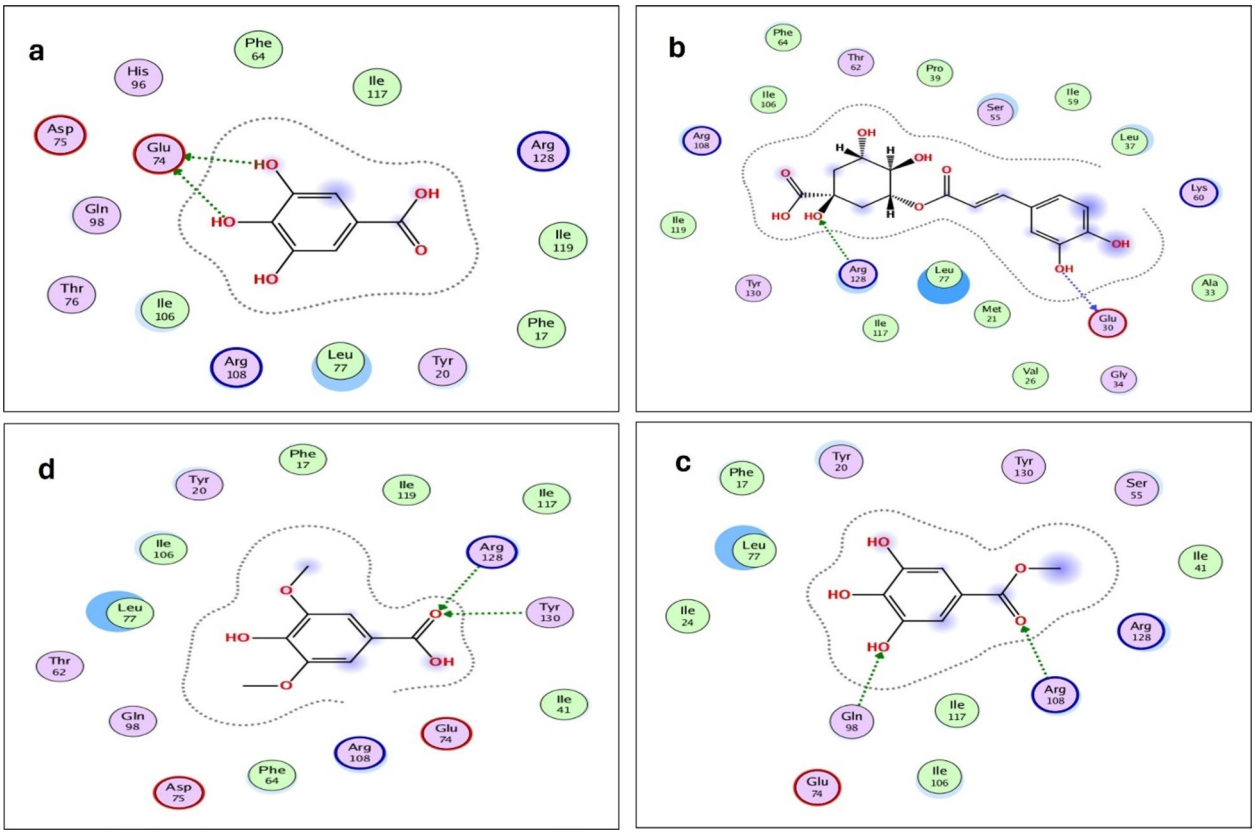

Figure 16. Two and three-dimensional interactions of (**a**) gallic acid, (**b**) chlorogenic acid, (**c**) methyl gallate, (**d**) syringic acid interior *2FLJ* active site pocket.

The efficacy of *L. camara, M. azedarach, N. oleander, R. communis and W. somnifera* leaf extracts can vary depending on factors such as the extraction method, concentration of the extract, mosquito species, and environmental conditions. Additionally, while these extracts may offer a natural alternative for mosquito control, further research is needed to assess their safety and potential ecological impacts. Biopesticides, despite their advantageous insecticidal properties, constitute only 5% of pesticides used globally[43].

HPLC analysis revealed that *N. oleander* and *R. communis* leaf extracts had the highest percentage of gallic acid and chlorogenic acid, at concentrations exceeding 500 µg/mL reaching 1000 µg/mL in the case of *N. oleander* extract. Gallic acid and chlorogenic acid, was also present in the other plant extracts.

Lahlou et al.[65] evaluated the in-vivo and in-vitro insecticidal and physiological effects of gallic acid on *Cx. pipiens* larvae under laboratory conditions. Gallic acid has been extensively in mosquito larvicides including in combination with the globally most used natural pesticide: the bacteria *B. thuringiensis* var. *israelensis*, to increase its potency as an anti-oxident in damaging the larvae central nervous system. Gallic acid also damages the central nervous and digestive systems of the cotton leaf worm, *Spodoptera littorales* at low concentrations[66]. Upon ingestion, the phenolic compound causes acute toxicity and paralysis to this economically important agricultural pest. Gallic acid was also found to display low genotoxicity potential in multiple assays and was successfully used as potential anti-malarial candidate[67].

Another phenolic product is chlorogenic acid; a member of hydroxycinnamic acids. It has been extensively studied and used in several applications, including food, medical, and pesticide formulations. Synthetic insecticidal analogs, based on the parent chlorogenic acid scaffold, are commercially available for broad spectrum insect control. So, they are applied in the fight against mosquitoes, as they do not constitute a substantial threat to human life. Chlorogenic acid is a strong inhibitor of acetylcholine esterase (AChE) activity[68]. AChE is responsible for the termination of excitatory transmission in the nerve synapse.

HPLC confirmed a high percentage of rutin (a flavanol glycoside) in all five extracts, but particularly in *N. oleander*, *R. communis* and *M. azedarach*. Rutin has shown fast and effective larvicidal effects, as well as a possible chemical for deterring egg-laying. Rutin showed larval mortality of 10.05–82.52%. It possesses a wide range of pharmacological activities including anti-inflammatory, anti-carcinogenic, antiviral, and anti-bacterial activities.

HPLC confirmed an abundance of caffeic acid, rosmarinic acid and coumaric acid, such plant-derived products are known for their eco-friendliness, biodegradability, and availability in nature[69] as well as for environmentally friendly mosquito control strategies[70]. Other secondary metabolites in the plants included catechin, methyl gallate, syringic acid, ellagic acid, ferulic acid, naringenin, daidzein and quercetin, which also enhance the pest control activity in the extracts. Indeed, the synergistic effects of the secondary plant metabolites as larvaecides

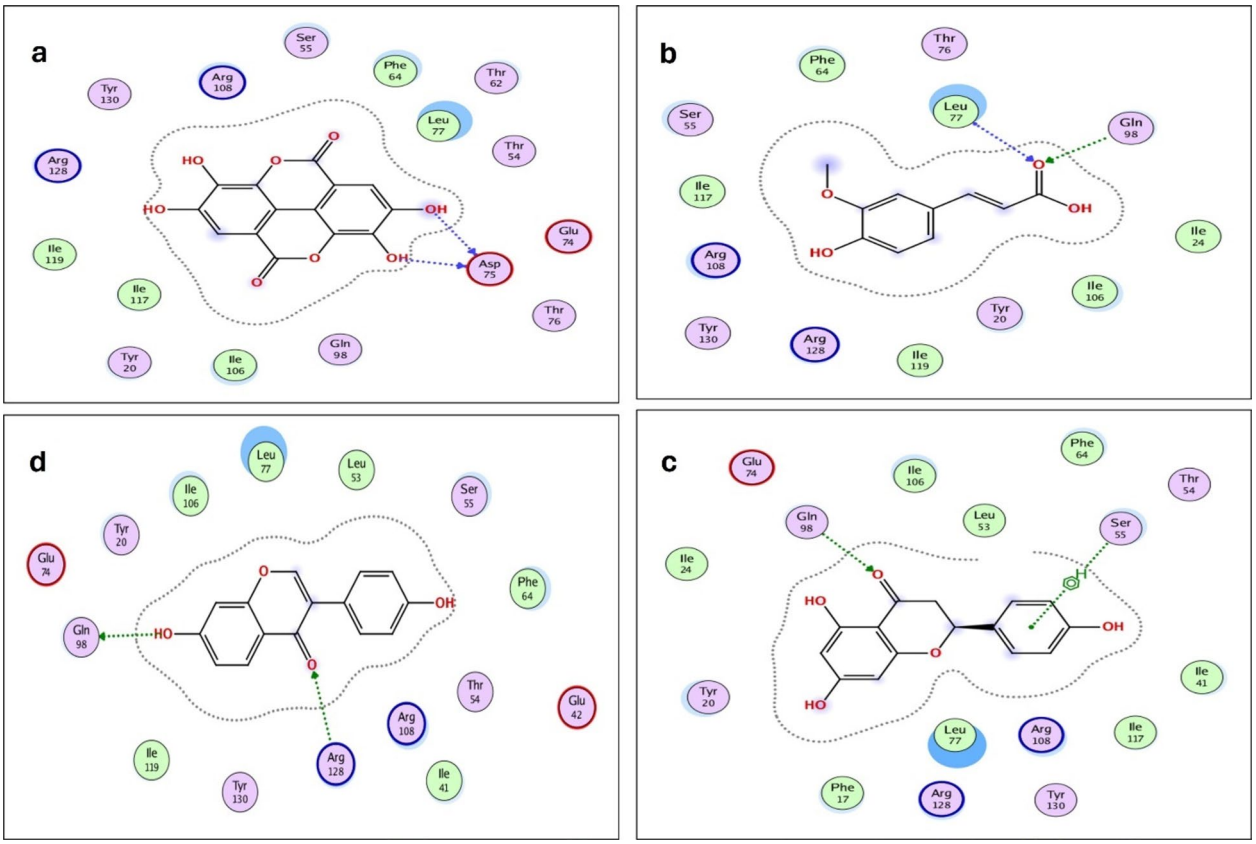

Figure 17. Two and three-dimensional interactions of (**a**) ellagic acid, (**b**) ferulic acid , (**c**) naringenin, (**d**) daidzein interior *2FLJ* active site pocket.

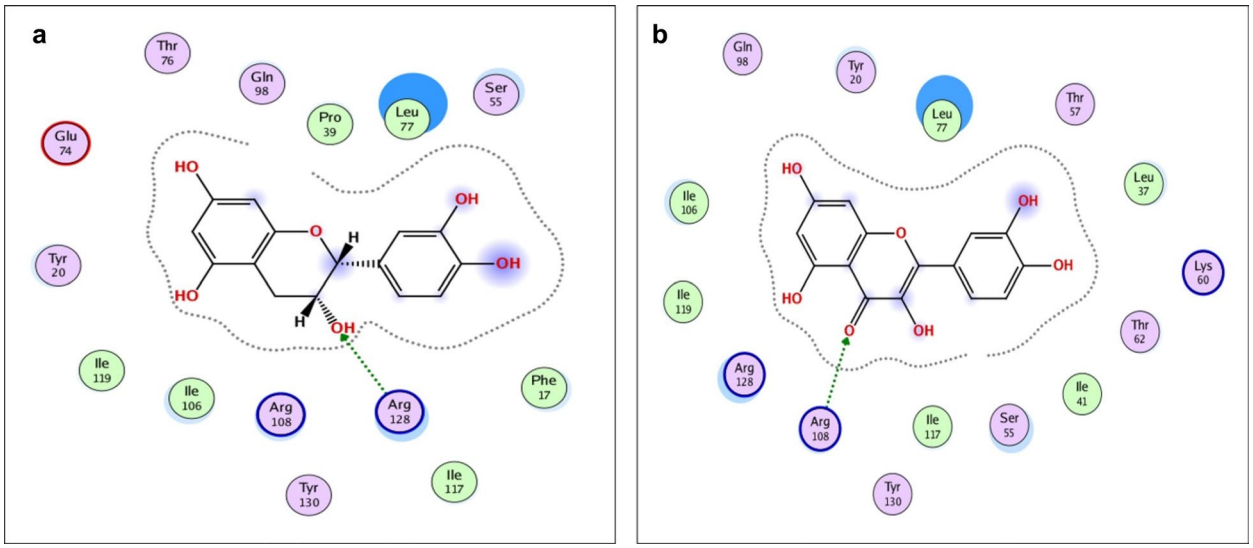

Figure 18. Two and three-dimensional interactions of (**a**) catechin, and (**b**) quercetin interior *2FLJ* active site pocket.

likely increases the potency of the extracts, whilst still being composed of biodegradable and environmentally friendly chemicals, suggesting them to be ideal substitutes for toxic synthetic chemical compounds.

Fatty acid binding proteins (FABPs) are a collection of intracellular binding proteins, which bond to the hydrophobic lipids and water-insoluble materials for many purposes such as synthesis of phospholipids, and lipid metabolism. In-vivo studies conducted on measuring the FABP4 levels in mice show that down-regulation of FABP4 are associated with many metabolic diseases[42,71]. In our study the enzyme inhibition activity of the methanolic plant extracts was assessed. The inhibitory concentration of *N. oleander* and *R. communis* was

IC_{50} = 0.599 µg/mL and 0.425 µg/mL respectively. This is very close to commonly used positive control reference drugs: IC_{50} = 0.599 µg/mL for Orlistat, and IC_{50} = 0.235 µg/mL for Cobimetinib. IC_{50} values of *L. camara*, *M. azedarach*, and *W. somnifera* extracts were higher i.e. less potent.

The high enzyme inhibition of *N. oleander* and *R. communis* may be due to the presence of both volatile and non-polar substances (detected by GC/MS) or non-volatile, polar substances (detected by HPLC). Extracts *N. oleander* and *R. communis* contain very high quantities of natural phenolic acids such as Gallic acid and chlorogenic acid, and the flavanols kaempferol and rutin. Earlier in-vitro study to evaluate the inhibition ability of methanolic and aqueous acacia extracts were done by our team showing that acacia methanolic extract had IC_{50} of 0.681 µg/mL, and aqueous extract had IC_{50} of 2.311 µg/mL, with a positive control of Orlistat with IC_{50} of 0.535 µg/mL[72].

Fatty acid binding proteins (FABPs) are low-molecular weight single chain polypeptides. Their biological function is to solubilize and shield sensitive hydrophobic and water-insoluble retinoids, fatty acids, and bile acids constituents transported into the cytosol or any organelles in the cell for purposes such as phospholipid synthesis, lipid metabolism and mitochondrial beta oxidation. FABP synthesis is extensive in both animal (vertebrate and invertebrate) and insect kingdoms[73,74]. Most FABPs share the same amino acid sequence such that they have a 70% similarity, and their three-dimensional stereo-structure are all restricted to the β-barrel structure with a ligand binding cavity[75,76]. The first insect FABP discovered was in the flight muscle of the desert locust *Schistocerca gregaria*[77]. FABPs from various insects affect the physiological metabolism through modifying intracellular fatty acid components, modulating sleep, long-term memory reinforcement, lipid accumulation, and a role in feeding and social caste divisions[78].

In this study, *lm*-FABP (PDB code: *2FLJ*) was used as the target protein, and the docking with the ligands of the 18 detected polyphenols and flavonoids modeled. Rutin (a flavanol) showed the highest number (five) of electrostatic forces and one additional dipole–dipole interaction between the target protein and rutin. Two such interactions were possible, with the same amino acid residue with a co-crystallized ligand. The root mean square deviation (RMSD = 1.55) of the interaction was less than 1.7, meaning a possibility of the co-crystallized ligand being replaced[78]. The *N. oleander* extract had high efficacy, may is probably due to having a high rutin concentration, with rutin being confirmed (in the docking model) to be an effective FABP inhibitor.

Caffeic acid, coumaric acid and rosmarinic acid all make two types of intermolecular hydrogen bonds with different amino acids, with bond lengths ranging from 1.88 to 2.40 Å and scoring energy ranging from (– 4.47 kcal/mol) to (– 6.78 kcal/mol) kcal/mol, and one dispersion force (from Van der Waal forces). Furthermore, gallic acid, chlorogenic acid, methyl gallate, syringic acid, ellagic acid, ferulic acid, naringenin and daidzein all have two interactions with at least one residue, similar to the co-crystallized ligand. Quercetin and daidzein each had a only one hydrogen bond, and thus a limited connection to the target protein.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization, methodology, investigation, data curation, formal analysis, resources, writing-original draft preparation, M.M.B., I.T.R, A.S., A.M.A., M.H.A., S.M.A., H.S.G., M.E.G.; editing and writing-review, M.M.B., I.T.R, A.S., M.H.A., A.M.A., S.M.A., H.S.G., M.E.G.; project administration, A.S.; funding achievement, M.M.B., I.T.R, A.S., A.M.A., S.M.A., M.H.A., H.S.G., M.E.G. All authors have read and approved the published version of the manuscript.

Ethical consideration

The study was carried out according to the guidelines of the declaration of Benha University and approved by the Ethics Committee of the Faculty of Science, Benha University (Code: BUFS-REC-2024-241Ent).

Competing interests

The authors declare no competing interests.

Additional information

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