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Mohamed M. Baz (✉ Mohamed.albaz@fsc.bu.edu.eg)

Benha University

Reham M. Mostafa

Benha University

Heba Talat Ebeed

Damietta University

Heba S. Essawy

Benha University

Ghada E. Dawwam

Benha University

Ahmed B. Darwish

Suez University

Nancy M. El-Shourbagy

Benha University

Article

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Evaluation of four ornamental plant extracts as insecticidal, antimicrobial, and antioxidant against the West Nile vector, *Culex pipiens* (Diptera: Culicidae) and metabolomics screening for potential therapeutics

Mohamed M. Baz^{1,*}, Reham M. Mostafa², Heba Talat Ebeed^{3,4}, Heba S. Essawy², Ghada E. Dawwam², Ahmed B. Darwish⁵, Nancy M. El-Shourbagy¹

¹ Entomology Department, Faculty of Science, Benha University, Benha 13518, Egypt

² Botany and Microbiology Department, Faculty of Science, Benha University, Benha, Qalyubiya 13518, Egypt

³ Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta, 34517, Egypt

⁴ National Biotechnology Network of Expertise (NBNE), Academy of Scientific Research and Technology (ASRT), Cairo, Egypt

⁵ Zoology Department, Faculty of Science, Suez University, Suez, 43518 Egypt

*Correspondence: Mohamed.albaz@fsc.bu.edu.eg

Abstract

Mosquitoes, one of the deadliest animals on the planet, cause millions of fatalities each year by transmitting several human illnesses. Phytochemicals are possible biological agents for controlling pests that are harmful to both human and animal health as well as crops. Plants contain a wide range of potential phytochemicals that are target-specific, rapidly biodegradable, and eco-friendly. The potential of leaf extracts of *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis* against *Culex pipiens* and microbial agents was evaluated. Acetone extracts had more toxic effects against *Cx. pipiens* larvae (99.0–100%, 72 h post-treatment), and the LC₅₀ values were 142.8, 189.5, 95.4, and 71.1 for *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis*, respectively. Plant extracts tested in this study showed high insecticidal and antimicrobial potential. *L. camara* acetone extract inhibited all the tested microbial isolates. *D. regia* showed higher antioxidant activity than other plant extracts in scavenging free radicals. GC-MS results showed the acetone extract had a higher number of chemical classes, with more terpene compounds. HPLC analysis showed the presence of flavonoids and phenolic compounds in *L. camara* and *P. orientalis* acetone extracts, and Syllimarine and Rutin were the most abundant. ADME analysis of elemene, caryophyllene oxide, caryophyllene, and copaene showed drug-likeness and revealed better gastrointestinal absorption and permeation of the blood-brain barrier by all the tested phytochemicals. Our results confirm that the diversity of phytochemicals found in ornamental plants has promising larvicidal activity and is a safe and effective alternative to chemical insecticides and biotechnology.

Keywords: *Culex pipiens*; Larvicidal; Antimicrobial; GC-MS, HPLC; ADMET analysis; Phytochemicals; Ornamental plants.

Introduction

Mosquitoes are vectors of many of the world's serious human and veterinary diseases [1], including malaria, dengue, yellow fever, filariasis, Japanese encephalitis, chikungunya, and streptoderma of cattle [2]. Mosquito-borne diseases have an economic impact including loss of commercial production and employment, the burden of disease and death, poverty, and social debilitation worldwide, especially in countries with tropical and subtropical climates; however, no part of the world is free of vector-borne diseases [3,4].

Several methods are widely used to control the threats posed by mosquitoes. Over the years, synthetic insecticides were introduced, but although these are effective, the insect is forced to adapt to those pesticides and, over time, develop resistance to the pesticide products, along with environmental risks from frequent pesticide use [5,6]. The most important alternatives to synthetic insecticides are phytochemicals (plant extracts or essential oils). Phytochemicals have a wide range of effects on mosquitoes, including being ovicidal, larvicidal, adulticidal, oviposition deterrent, developmental toxic, antifeedants, repellents, hatching blockers, and emergence blockers [7,8]. Many medicinal plant products have yet to be tested for their potency against mosquitoes.

Recently, great attention has been given to insecticides of plant origin and those that do not harm the ecosystem. Therefore, natural products are the most suitable alternative to use because they cause less harm to the environment and to non-target species and are eco-friendly, bio-sourced, and safe to use. Besides, many extracts and compounds from several plant groups have been investigated as potential new larvicides [9]. Studies have shown that saponin [10], steroids [11], isoflavonoids [12], essential oils [13], alkaloids [14], and tannins [15] can all be used to kill mosquito larvae. Many alternative sources of insect repellents come from plant chemicals and their essential oils. However, the botanicals have not been fully approved due to challenges in formulation and marketing, which are attributed to a lack of chemical data and positive controls. Besides, many publications have included botanical pesticides with a skewed interest in insect pest management [16]. Also, many research papers did not adequately address the effect of biopesticides derived from ornamental plants on insects.

Plants that are known to contain a treasure trove of sustainable and renewable bioactive compounds are many. One of these plants, *Lantana camara* Linn (Verbenaceae), is known as wide sage or lantana weed. It is a hefty, thorny, extensive evergreen shrub that can reach a height of 1-2 m, grows erect, and has a strong scent. The branches and stems have angular shapes and are organized along the margins with curving spines. Leaves are simple, opposite, oval, regularly dentate, and have an acute apex [17]. *L. camara* has also been used in traditional herbal medicines for the treatment of ailments, including cancer; skin itching, leprosy, rabies, chickenpox, measles, asthma, and ulcers [18,19].

All parts of this plant have been used conventionally for numerous diseases all over the world. The leaves of this plant were used as an antibacterial and antihypertensive agent, and the roots were used for the treatment of malaria, rheumatism, and skin rashes [20]. Extract from the leaves of *L. camara* possessed larvicidal activity, while extract from the flowers of the plant showed repellent activity against adult mosquitoes [21,22].

Thuja, or *Platyclusus orientalis*, is a bushy, thick, and conical to columnar evergreen and coniferous tree that belongs to the Cupressaceae family and is characterized by its resin and oil content in various forms. The leaves have triangular scales with a blunt point and are arranged in decussate pairs. Leaves emit an odor when bruised. In the winter, the foliage may turn bronze. cones, oval, up to 3/4" long, rusty brown bark [23]. Around the world, this tree flourishes in both warm and temperate climates. According to folk medicine, *P. orientalis* is used as an herbal remedy for uterine cancer, psoriasis, amenorrhea, enuresis, rheumatism, cystitis, bronchial catarrh, and antimicrobial activity [24,25].

Bougainvillea glabra wild, also known as paper flower or Bougainvillea, is a woody vine belonging to the Nyctaginaceae family. It is native to South America, but it is spread all over the world for its wonderful ornamental properties. In fact, this plant blooms regularly, and the bracts are a stunning shade of purple, red, or white [26]. Plants have simple, alternate, ovate-acuminate leaves that are alternate and 4–13 cm long, and their flowers are pink, magenta, purple, red, orange, white, or yellow [27]. There are 18 species in the genus Bougainvillea, some of which are used in traditional

medicine to cure conditions like diarrhea, stomach acidity, sore throat, cough, leucorrhoea, and hepatitis. They also possess anti-inflammatory, antiviral, and antibacterial properties [27,28] and are larvicidal [29].

Delonix regia Rafin is a nearly evergreen, shallow-rooted legume tree found in temperate and tropical zones and widely grown throughout Africa and Southeast Asia. It is often referred to as the "flamboyant flame tree." It is a medium-sized decorative tree, and it is cultivated in gardens and avenues. The blooms with panicles, which are abundant in large, upright clusters along the branches and range in color from deep crimson to flaming orange to delicate salmon, make for a stunning appearance [30]. *D. regia* extract was reported to have a wide range of bioactivities. The plant has shown promising antioxidant [31], antimicrobial [32], antidiabetic [33], anti-inflammatory [34], and larvicidal [35,36] activities.

The screening of compounds with potential bioactivity is both expensive and time-consuming. However, computer-aided drug design (CADD) could save time and money on molecule synthesis, thereby lowering the cost of research. Such studies would culminate in the development of novel drug molecules at a faster pace against infectious pathogens. Additionally, the physicochemical properties of the molecule would provide vital information on the initial phase of drug development [37]. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis is a critical component of computational drug design, and ADMET research evaluates a drug's pharmacokinetics. Prediction of a drug's fate and effects inside the body, such as how much medication is absorbed if delivered orally and how much is absorbed in the gastrointestinal tract, is an essential element of drug discovery. Similarly, if absorption is poor, distribution and metabolism are impaired, which can result in neurotoxicity and nephrotoxicity [38].

In this work, we hypothesize that ornamental plant extracts *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis* have many biologically active phytochemical compounds with lethal effects against *Cx. pipiens* mosquito larvae and different multidrug resistant (MDR) pathogenic microbes (*Candida albicans*, *Listeria monocytogenes*, *Salmonella sp.*, and *Pseudomonas aeruginosa*). Therefore, we aim to identify the phytochemical profile of these plants in acetone and aqueous extracts through GC-MS and HPLC analysis, then evaluate the larvicidal, antimicrobial, and antioxidant activities of these extracts, and assess the bioactivity and pharmacokinetic indices related to the ADMET aspects in light of the possible therapeutic powers of the selected plant metabolites. The flow chart of the article summarizes the steps for our study as shown in Fig. 1.

Materials and Methods

Plant materials and analysis

Plant collection

Leaves of ornamental plants, *B. glabra* Choisy, *D. regia* Bojer, *L. camara* L., and *P. orientalis* Franco, were collected from different areas in farmlands at the Faculty of Agriculture, Qalyubiya Governorate, Egypt, during September–November 2021 (Table 1, Fig. 2). Plants were identified by Dr. Ahmed Moubarak at the Flora and Phytotaxonomy Section, Botany Department, Faculty of Science, Benha University, Egypt, according to reference books of Egyptian flora (Boulos, 1999, 2009). This comment has been mentioned in other articles because we rely on Dr. Ahmed Moubarak in the classification. The study plant specimens were deposited in an herbarium of botany department, Faculty of science with respective voucher numbers for *Bougainvillea glabra* C. (B87), *Delonix regia* B. (B65), *Lantana camara* L. (B112), and *Platyclusus orientalis* F. (B365). The plant materials were shade-air-dried at room temperature until the plant became well-dried 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 [39].

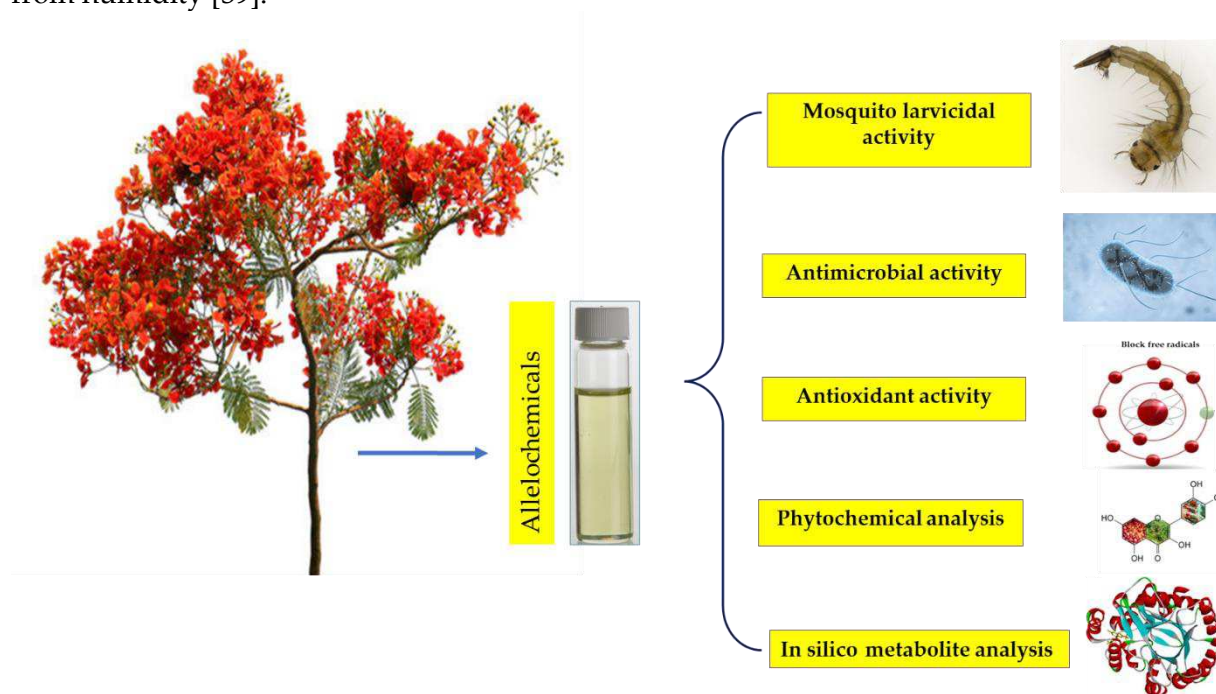


Fig. 1. Flowchart of research structure

Table 1 List of plant species and plant parts tested against *Culex pipiens* larvae and selected microbial species.

No.	Common Name	Botanical Name	Family	Part used
1	Paperflower	<i>Bougainvillea glabra</i>	Nyctaginaceae	Leaf
2	Royal poinciana	<i>Delonix regia</i>	Fabaceae	Leaf
3	Largeleaf lantana	<i>Lantana camara</i>	Verbenaceae	Leaf
4	Thuja	<i>Platycladus orientalis</i>	Cupressaceae	Leaf

Plant extraction

The acetone extract was prepared by soaking 20 g of dry plant materials in 100 to 150 mL of acetone at a conical flask (cap. 250 mL) at room temperature 27 ± 2 °C. Another 20 g of plant materials were extracted using warm water (60 °C) at room temperature for preparing the aqueous extract. The extraction was done after 48 h then the solution was filtered using Whatman No. 1 filter paper through a Buchner funnel. The extracts were concentrated using a rotary evaporator, then stored [8].

Mosquito larvicidal assay

Rearing of *Culex pipiens*

Cx. pipiens larvae were reared in the insectary, and they were maintained at 27 ± 2 °C and $75 \pm 5\%$ relative humidity for a photoperiod of 12:12 h (light/dark). The larvae were fed fish food (Tetramin) and ground bread at a 3:1 ratio. The pupae were then moved from the enamel pans to a cup filled with dechlorinated water and put in screened cages (35x35x40 cm in size), where the adults eventually emerged. Female mosquitoes got periodic blood feeds from a hamster rat while the adults' colony was given a 10% sugar solution. Larvae and pupae, which are two stages of development, were always available for the tests and kept in the same laboratory [40].

Larvicidal activity

The plant leaf acetone and aqueous extracts of *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis* were evaluated against 4th larval instar of *Cx. pipiens* under laboratory conditions. The 4th larval instar was treated with the following concentrations: 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, 48 h and 72 h after the initial exposure [41] and post-treatments (PT).

Antimicrobial assay

Collection of microbial pathogens

Multidrug-resistant bacteria such as *Listeria monocytogenes* (ATCC 19155), *Pseudomonas aeruginosa* (ATCC 9027), and *Salmonella sp.* (ATCC 14028), in addition to the pathogenic fungus *Candida albicans* (ATCC 10231) were utilized in the current study.

Antimicrobial activity

Antimicrobial activity of the acetone and aqueous extracts of *L. camara*, *D. regia*, *B. glabra*, and *P. orientalis* was tested against unicellular fungi (*C. albicans*), Gram-positive bacteria (*L. monocytogenes*), and Gram-negative bacteria (*Salmonella sp.* and *P. aeruginosa*) via the agar diffusion approach according to Aletayeb et al. [42]. The bacteria were grown in a nutrient-liquid medium on a shaker bed at 200 rpm for 24 h at 37 °C. The bacteria (1.5×10^8 CFU) were swabbed on nutrient agar plates; subsequently, 200 μ L of plant extract were deposited in wells (diameter of 7 mm) cut into agar plates. For 24 h, agar plates were incubated at 37 °C. All the studies were carried out in triplicate for all tested strains, and the inhibition zones surrounding the discs were quantified in millimeters.

Antioxidant DPPH Assay

1,1-diphenyl-2-picryl hydrazyl (DPPH) was used to measure how well different extracts of plant leaves got rid of free radicals. In brief, a 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml of different extracts in ethanol at different concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 μ g/ml). Here, only those extracts are used that are solubilized in ethanol, and their various concentrations were prepared by the dilution method. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, absorbance was measured at 517 nm by using a spectrophotometer (UV-VIS Milton Roy). The reference standard compound being used was ascorbic acid, and the experiment was done in triplicate. The IC₅₀ value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using the log dose inhibition curve. DPPH scavenging effect (%) is calculated according to the following equation: DPPH scavenging effect (%) or Percent inhibition = $(A_0 - A_1) / A_0 \times 100$; Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of a test or standard sample [43].

Phytochemical identification and in silico analysis

GC/MS analysis

For the biochemical analyses of the plant leaf acetone and aqueous extracts, Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary columns, 0.1 mm, 0.251 mm, and 30 m thick, were used. It was done using an electronic ionizer with 70 eV ionization energy. As a carrier gas, helium gas was used (flow rate: 1 ml/min). The MS transmission line and injector were both set to 280

207 °C. The oven was preheated to 35 °C, then increased to 150 °C at a rate of 7 °C per min, 270 °C at a rate
 208 of 5 °C per minute (pause for two minutes), and lastly 310 °C at a rate of 3.5 °C per minute (continued
 209 for 10 min). A relative peak area was employed to explore the quantification of all components
 210 discovered. The chemicals were at least partially identified by comparing the retention times and mass
 211 spectra of the chemicals to those of NIST and Willy Library data from the GC/MS instrument.
 212 Identification was done using the aggregate spectrum of user-generated reference libraries. To evaluate
 213 peak homogeneity, single-ion chromatographic reconstructions were performed. To verify GC
 214 retention times, co-chromatographic analysis of reference substances was used whenever possible [44].
 215



216
 217 **Fig. 2.** Ornamental plants, *Bougainvillea glabra* (a), *Delonix regia* (b), *Lantana camara* (c), and *Platycladus orientalis*
 218 (d).
 219

220 **HPLC measurement of the concentration of polyphenol contents**

221 Using an Agilent 1260 series, high-performance liquid chromatography was carried out for polyphenol
 222 detection analysis in the *L. camara* and *P. orientalis* acetone extracts (the most efficient plant leaf extracts
 223 in sections “2.3 & 2.4”). An Eclipse C18 column (4.6 mm x 250 mm i.d., 5 m) was used for the separation
 224 procedure. A mixture of water (solution A) and 0.05% trifluoroacetic acid in acetonitrile (solution B),
 225 injected at a flow rate of 0.9 mL/min, made up the mobile phase. The linear gradient of the following
 226 time intervals was programmed for the mobile phase in order: 0 min (82% A), 0-5 min (80% A), 5-8 min
 227 (60% A), 8-12 min (60% A), 12-15 min (82% A), 15-16 min (82% A), and 16-20 min (82% A). A 280 nm
 228 wavelength was selected for the multi-wavelength detector. Five liters of each sample solution were
 229 injected. To keep the column temperature at 40 °C, an adjustment was made.
 230

231 **In silico absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis**

232 The identified phytochemicals in *L. camara* acetone extract (the most efficient plant in
 233 antimicrobial analysis) were analyzed for their ADMET analysis using the Swiss ADME server
 234 (<http://www.swissadme.ch/index.php>). This server evaluates the compounds for their physicochemical
 235 properties, lipophilicity, water solubility, pharmacokinetics, drug likeness, and medicinal chemistry.

Further, the toxicity properties of the identified phytochemicals were analyzed using online servers, Protox-II ([https:// tox-new. chari te. de/ protox_ II/](https://tox-new.charite.de/protox_II/)) [45] and StopTox ([https:// stopt ox. mml. unc. edu/](https://stoptox.mml.unc.edu/)) [46]. Protox-II predicted the LD50 (mg/kg) of the identified ligands and toxicity class, whereas StopTox identified cardiotoxicity (hERG liability prediction).

Statistical analysis

The data were analyzed by the software, SPSS V23 (IBM, USA), for doing the Probit analyses to calculate the lethal concentration (LC) values and 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 this study, plant extracts from *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis* were evaluated on 4th instar larvae of *Cx. pipiens*. All the tested plant extracts in this study showed high insecticidal activity against mosquito larvae, *Culex pipiens*, after different intervals of exposure. The results showed that acetone extracts of plant materials in this study had more toxic effects against mosquito larvae, *Cx. pipiens* than aqueous extracts.

The mortality percent (MO%) at 24 h post-treatment (PT) of *Cx. pipiens* with 1500 ppm acetone extracts of *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis* was 99, 94, 100, and 100% (MO%) (Table 2) with LC₅₀ (50%, median lethal concentration) = 270.1, 357.0, 183.2, and 148.1 ppm, respectively (Table 3); whereas those of aqueous extracts were 93, 90, 96, and 87 (MO%) with LC₅₀ values = 382.0, 444.3, 335.7, and 502.8 ppm.

The highest larval mortality was recorded after 48 h of PT, where the mortality reached 100% in acetone extracts for *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis* and 100, 99, 100, and 93% in aqueous extracts, respectively, at 1500 ppm. In terms of lethal concentrations, LC₅₀ (50%, median lethal concentration) for *Platycladus orientalis* acetone extracts appeared to be most effective against *Cx. pipiens* larvae (LC₅₀ = 98.5 ppm), followed by *L. camara* (LC₅₀ = 127.4 ppm) and *B. glabra* (LC₅₀ = 194.2 ppm) (Table 3, Fig. 3a), while *D. regia* was the least effective on *Cx. pipiens* (LC₅₀ = 254.1 ppm). Among those of aqueous extracts, *L. camara* appeared to be most effective against *Cx. pipiens* (LC₅₀ = 268.0 ppm), followed by *B. glabra* (LC₅₀ = 292.7 ppm) and *D. regia* (LC₅₀ = 319.6 ppm), while *Platycladus orientalis* was the least effective on *Cx. pipiens* larvae (LC₅₀ = 376.1 ppm) (Table 4, Fig. 3b).

It is obvious from Tables 3,4 and Fig. 3 that the LC₅₀ values of acetone of *Bougainvillea glabra* (142.8 ppm), *D. regia* (189.5 ppm), *L. camara* (95.4 ppm), and *Platycladus orientalis* (71.1 ppm) were more effective than aqueous extracts (LC₅₀ = 177.7, 228.5, 164.8, and 263.5 ppm, respectively, against the 4th larval instar of *Cx. pipiens*, 72 h PT).

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Table 2 Efficacy of *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis* extracts on *Culex pipiens* larval mortality, 24, 48, and 72 h post-treatment (mean±SE).

Plant species	Conc. (ppm)	Acetone			Aqueous		
		24	48	72	24	48	72
<i>Bougainvillea glabra</i>	0	0±0 ^{gA}	0±0 ^{gA}	0±0 ^{fA}	0±0 ^{gA}	0±0 ^{gA}	0±0 ^{fA}
	62.5	11±1.87 ^{fC}	17±1.22 ^{fB}	22±1.22 ^{eA}	8±2.55 ^{fC}	11±1.87 ^{fB}	19±1.87 ^{eA}
	125	24±1.87 ^{eC}	33±2.55 ^{eB}	42±3.39 ^{dA}	16±2.92 ^{eC}	25±2.24 ^{eB}	34±2.92 ^{dA}
	250	42±4.36 ^{dC}	57±2.55 ^{dB}	66±3.67 ^{cA}	32±2.55 ^{dC}	41±1.87 ^{dB}	59±4.30 ^{cA}
	500	66±1.87 ^{cC}	80±3.54 ^{cB}	94±3.67 ^{bA}	57±4.64 ^{cC}	66±3.32 ^{cB}	82±2.55 ^{bA}
	1000	92±1.22 ^{bC}	98±1.22 ^{bB}	100±0.00 ^{aA}	78±4.36 ^{bC}	88±3.39 ^{bB}	98±1.22 ^{aA}
	1500	99±1.00 ^{aA}	100±0.00 ^{aA}	100±0.00 ^{aA}	93±3.00 ^{aC}	100±0.00 ^{aA}	100±0.00 ^{aA}
<i>Delonix regia</i>	0	0±0 ^{gA}	0±0 ^{gA}	0±0 ^{fA}	0±0 ^{gA}	0±0 ^{gA}	0±0 ^{gA}
	62.5	11±1.87 ^{fC}	18±1.22 ^{fB}	21±1.00 ^{eA}	7±1.22 ^{fC}	8±1.22 ^{fB}	14±1.00 ^{fA}
	125	22±2.55 ^{eC}	29±1.87 ^{eB}	34±2.92 ^{dA}	14±1.87 ^{eC}	21±2.45 ^{eB}	26±1.87 ^{eA}
	250	36±4.85 ^{dC}	41±3.67 ^{dB}	54±4.85 ^{cA}	28±1.22 ^{dC}	34±1.87 ^{dB}	49±1.87 ^{dA}
	500	55±5.00 ^{cC}	66±3.67 ^{cB}	82±2.55 ^{bA}	50±2.24 ^{cC}	64±4.30 ^{cB}	78±2.00 ^{cA}
	1000	75±4.74 ^{bC}	90±3.54 ^{bB}	99±1.00 ^{aA}	74±3.67 ^{bC}	84±2.45 ^{bB}	90±2.24 ^{bA}
	1500	94±2.45 ^{aB}	100±0.00 ^{aA}	100±0.00 ^{aA}	90±1.58 ^{aB}	99±1.00 ^{aA}	100±0.00 ^{aA}
<i>Lantana camara</i>	0	0±0 ^{gA}	0±0 ^{fA}	0±0 ^{eA}	0±0 ^{gA}	0±0 ^{gA}	0±0 ^{fA}
	62.5	16±1.87 ^{fC}	23±1.22 ^{eB}	30±2.24 ^{dA}	9±1.87 ^{fB}	14±1.87 ^{fB}	20±1.58 ^{eA}
	125	34±2.92 ^{eC}	48±2.55 ^{dB}	61±2.92 ^{cA}	18±1.22 ^{eB}	26±2.92 ^{eB}	37±3.39 ^{dA}
	250	59±4.85 ^{dC}	75±2.24 ^{cB}	91±4.00 ^{bA}	37±2.00 ^{dB}	44±4.30 ^{dB}	62±7.18 ^{cA}
	500	82±2.55 ^{cC}	95±2.74 ^{bB}	100±0.00 ^{aA}	60±2.24 ^{cB}	69±6.60 ^{cB}	84±4.85 ^{bA}
	1000	98±2.00 ^{bB}	100±0.00 ^{aA}	100±0.00 ^{aA}	82±3.39 ^{bB}	91±3.32 ^{bB}	100±0.00 ^{aA}
	1500	100±0.00 ^{aA}	100±0.00 ^{aA}	100±0.00 ^{aA}	96±1.87 ^{aB}	100±0.00 ^{aA}	100±0.00 ^{aA}
<i>Platycladus orientalis</i>	0	0±0 ^{fA}	0±0 ^{eA}	0±0 ^{dA}	0±0 ^{gA}	0±0 ^{gA}	0±0 ^{gA}
	62.5	18±1.22 ^{eC}	27±3.74 ^{dB}	43±3.39 ^{cA}	7±2.00 ^{fC}	13±1.22 ^{fB}	16±1.00 ^{fA}
	125	39±2.92 ^{dC}	61±3.32 ^{cB}	80±2.74 ^{bA}	15±4.18 ^{eC}	21±2.92 ^{eB}	27±3.00 ^{eA}
	250	71±3.67 ^{cC}	91±2.92 ^{bB}	100±0.00 ^{aA}	28±4.36 ^{dC}	35±4.18 ^{dB}	43±6.82 ^{dA}
	500	93±3.39 ^{bB}	100±0.00 ^{aA}	100±0.00 ^{aA}	46±6.40 ^{cC}	52±5.39 ^{cB}	64±2.92 ^{cA}
	1000	100±0.00 ^{aA}	100±0.00 ^{aA}	100±0.00 ^{aA}	65±5.70 ^{bC}	72±2.55 ^{bB}	84±4.30 ^{bA}
	1500	100±0.00 ^{aA}	100±0.00 ^{aA}	100±0.00 ^{aA}	87±5.83 ^{aC}	93±2.55 ^{aB}	100±0.00 ^{aA}

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)

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291 **Table 3** Lethal concentrations (ppm) of *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis* acetone extracts against *Culex*
292 *pipiens*, 24, 48 and 72 h post-treatment

Time (h)	Plant species	LC ₅₀ (Low. - Up.)	LC ₉₀ (Low. - Up.)	LC ₉₅ (Low. - Up.)	Equation**	X ² (Sig.)
24	<i>B. glabra</i>	270.1 (237.9-305.2)	1022.6 (860.9-1258.6)	1491.4 (1216.0-1917.3)	2.216±0.138	6.106 (0.191)
	<i>D. regia</i>	357.0 (308.0-413.8)	1841.6 (1436.1-2533.4)	2932.1 (2173.6-4328.3)	1.798±0.132	7.794 (0.099)
	<i>L. camara</i>	183.2 (160.6-207.3)	623.2 (526.0-767.7)	881.8 (720.8-1136.5)	2.410±0.168	4.429 (0.351)
	<i>P. orientalis</i>	148.1 (130.8-166.3)	429.2 (367.0-521.5)	580.2 (481.8-735.5)	2.774±0.206	2.448 (0.654)
48	<i>B. glabra</i>	194.2 (168.3-221.8)	767.5 (636.0-969.1)	1133.2 (904.5-1508.6)	2.147±0.152	4.268 (0.370)
	<i>D. regia</i>	254.1 (217.5-295.0)	1285.8 (1012.8-1747.8)	2036.0 (1524.7-2973.2)	1.820±0.139	8.114 (0.087)
	<i>L. camara</i>	127.4 (111.1-144.0)	397.9 (344.1-473.7)	549.5 (462.6-680.2)	2.591±0.179	1.939 (0.918)
	<i>P. orientalis</i>	98.5 (87.1-109.9)	237.0 (205.1-286.5)	303.9 (255.6-384.4)	3.363±0.308	1.285 (0.863)
72	<i>B. glabra</i>	142.8 (125.1-161.3)	444.8 (378.0-544.6)	613.7 (505.6-786.3)	2.598±0.194	6.497 (0.165)
	<i>D. regia</i>	189.5 (128.3-263.1)	848.4 (630.7-1637.9)	1297.6 (948.9-2870.8)	1.968±0.154	10.375 (0.034)
	<i>L. camara</i>	95.4 (83.6-107.0)	240.6 (207.4-292.4)	312.8 (261.6-398.7)	3.190±0.295	3.065 (0.546)
	<i>P. orientalis</i>	71.1 (62.0-79.4)	149.2 (130.7-179.5)	184.0 (156.7-233.1)	3.987±0.464	2.491 (0.646)

293
294 **Table 4** Lethal concentrations (ppm) of *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis* aqueous extracts against *Culex*
295 *pipiens*, 24, 48 and 72 h post-treatment

Time (h)	Plant species	LC ₅₀ (Low. - Up.)	LC ₉₀ (Low. - Up.)	LC ₉₅ (Low. - Up.)	Equation**	X ² (Sig.)
24	<i>B. glabra</i>	382.0 (333.8-437.3)	1635.8 (1315.8-2151.5)	2470.6 (1905.6-3442.6)	2.028±0.141	4.147 (0.386)
	<i>D. regia</i>	444.3 (387.4-511.3)	1981.6 (1567.9-2670.4)	3027.6 (2289.9-4341.7)	1.973±0.141	4.383 (0.356)
	<i>L. camara</i>	335.7 (293.9-382.9)	1376.5 (1122.1-1776.0)	2053.5 (1609.6-2796.3)	2.091±0.143	5.274 (0.260)
	<i>P. orientalis</i>	502.8 (433.3-588.8)	2657.8 (2010.0-3828.8)	4260.6 (2050.4-6626.6)	1.772±0.136	5.581 (0.232)
48	<i>B. glabra</i>	292.7 (252.3-338.9)	1380.5 (1087.2-1878.2)	2142.6 (1607.2-3123.4)	1.903±0.145	1.884 (0.756)
	<i>D. regia</i>	319.6 (280.8-363.0)	1218.3 (1004.7-1547.2)	1780.3 (1415.9-2376.6)	2.205±0.149	7.815 (0.098)
	<i>L. camara</i>	268.0 (229.6-311.3)	1333.5 (1044.8-1828.5)	2101.5 (1564.9-3099.0)	1.839±0.143	5.140 (0.273)
	<i>P. orientalis</i>	376.1 (261.3-546.0)	2150.9 (1542.6-4769.9)	3526.2 (2453.8-9169.7)	1.692±0.129	10.195 (0.037)
72	<i>B. glabra</i>	177.7 (155.0-201.8)	631.9 (531.0-782.9)	905.4 (735.7-1176.4)	2.326±0.164	6.027 (0.197)
	<i>D. regia</i>	228.5 (199.9-259.5)	858.1 (715.4-1073.3)	2523.0 (1889.5-3653.2)	2.230±0.151	6.512 (0.163)
	<i>L. camara</i>	164.8 (143.9-186.9)	563.7 (475.5-695.5)	798.8 (652.1-1032.7)	2.400±0.171	7.667 (0.104)
	<i>P. orientalis</i>	263.5 (174.2-380.9)	1225.0 (915.0-2496.5)	1893.7 (1411.7-4411.7)	1.920±0.137	13.403 (0.009)

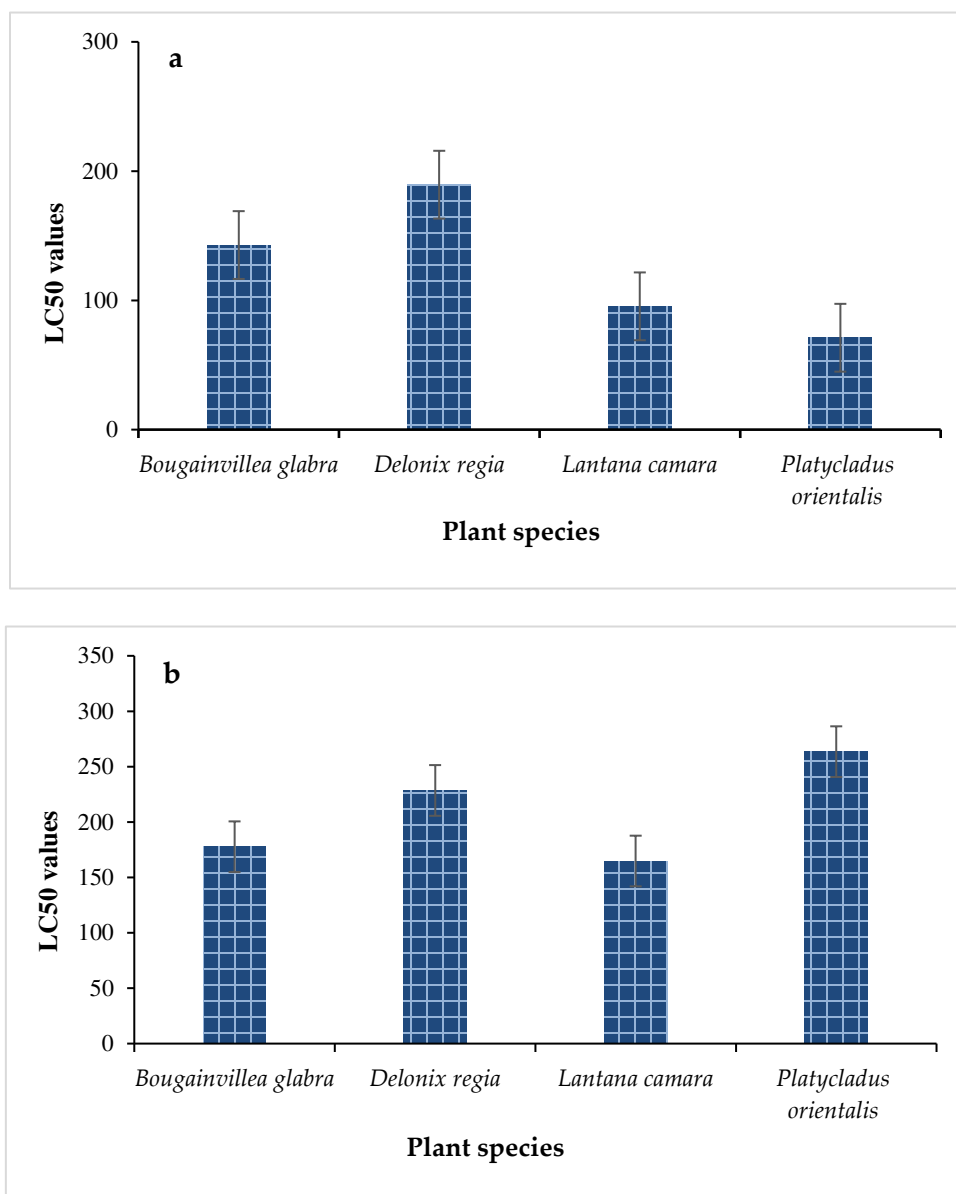


Fig. 3. The mean number of larval mortalities induced by the effects of *Lantana camara*, *Delonix regia*, *Bougainvillea glabra*, and *Platycladus orientalis* acetone (a) and aqueous (b) extracts against 4th larval instars of *Culex pipiens*, 72 h post-exposure.

Biological characteristics of the plant extracts

Antimicrobial activity

To investigate the antimicrobial activity of the aqueous and acetone extracts of the four plants, four different pathogenic microbes were used in the antimicrobial activity analysis. The diameters of the inhibition zones served to express the results of antimicrobial activity. Data in **Table 5** demonstrated that *L. camara* acetone extract inhibited all of the tested microbial isolates. Divergence in the extent of the inhibition area among the diverse groups of bacteria can be relatively disclosed. The highest inhibition zone was observed for *L. monocytogenes* at 20 mm. On the other hand, the minimum inhibition zone was detected for *C. albicans*, which was 13 mm. Acetone extracts of *D. regia* and *P. orientalis* inhibited the growth of *L. monocytogenes* and *P.*

aeruginosa, giving inhibition zones of 18 and 14 mm, respectively. No antibacterial activity was recorded for *B. glabra* (Fig. 4). The results also showed that the antimicrobial activity was not effective in the aqueous extracts.

Table 5 Antimicrobial activities of acetone extracts of *L. camara*, *D. regia*, *B. glabra*, and *P. orientalis* against several pathogenic microorganisms.

Microorganism	Inhibition zone (mm)			
	<i>D. regia</i>	<i>B. glabra</i>	<i>L. camara</i>	<i>P. orientalis</i>
<i>L. monocytogenes</i> ATCC 19155	18±0.0	0±0.0	20±0.28	0±0.0
<i>P. aeruginosa</i> ATCC 9027	0±0.0	0±0.0	15±0.30	14±0.52
<i>Salmonella</i> sp. ATCC 14028	0±0.0	0±0.0	14±0.27	0±0.0
<i>C. albicans</i> ATCC 10231	0±0.0	0±0.0	13±0.39	0±0.0

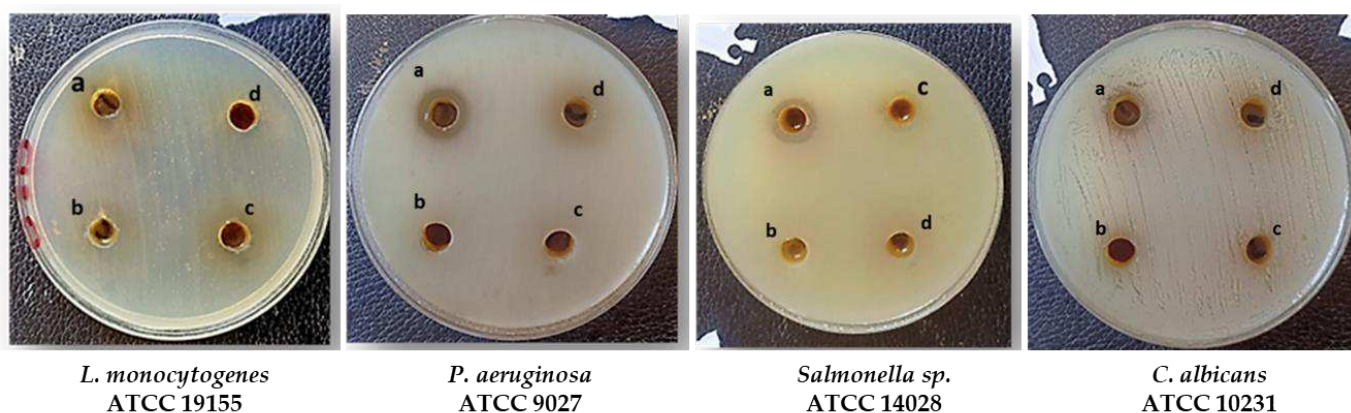


Fig. 4. Antimicrobial activities of acetone extract of *Lantana camara* (a), *Delonix regia* (b), *Bougainvillea glabra* (c), and *Platycladus orientalis* (d), against *L. monocytogenes* ATCC 19155, *P. aeruginosa* ATCC 9027, *Salmonella* sp. ATCC 14028, and *C. albicans* ATCC 10231 microorganisms

Antioxidant Activity—DPPH Assay

To investigate the antioxidant capacity of acetone extracts as most efficient than aqueous extracts in the antimicrobial activity, DPPH scavenging was used. The DPPH radical scavenging activity values of the acetone extracts of *D. regia*, *B. glabra*, *L. camara*, and *P. orientalis*, along with standard Ascorbic acid, are shown in Table 6 and Fig. 5. The highest DPPH free radical scavenging activity was obtained from the extract of *D. regia*, followed by *P. orientalis* and *B. glabra*, while the lowest DPPH free radical scavenging activity was recorded from *L. camara*. As shown in Fig. 5, the DPPH scavenging assay of the extract increases in a concentration-dependent manner, as exhibited by the standard, ascorbic acid. The IC₅₀ values of *D. regia*, *B. glabra*, *L. camara*, and *P. orientalis* extract were found to be 5.95, 22.65, 29.80, and 15.25 µg/ml, respectively, whereas the IC₅₀ value of Ascorbic acid was 2.52 µg/ml. The free radical activity of DPPH ranged from 5.95 µg/ml to 29.80 µg/ml. The *D. regia* extract (IC₅₀ = 5.95 µg/ml) was found to be more effective than the other plant extracts. The extracts of *D. regia* and *P. orientalis* were moderately good compared to ascorbic acid.

Table 6 The DPPH scavenging for *D. regia*, *B. glabra*, *L. camara* and *P. orientalis* acetone extracts.

Concentrate (ug/ml)	DPPH scavenging %				
	Ascorbic acid St.	<i>D. regia</i>	<i>B. glabra</i>	<i>L. camara</i>	<i>P. orientalis</i>
1000	99.3	97.1	92.6	90.5	96.3
500	96.3	94.4	85.4	82.5	91.3
250	94.8	91.6	77.6	74.3	83.0
125	91.9	83.5	69.5	66.6	74.8
62.5	84.2	75.4	61.6	58.9	67.2
31.25	76.1	67.7	53.4	50.9	58.7
15.625	67.6	60.1	45.8	42.6	50.7
7.8125	60.4	52.4	38.0	34.4	42.2
3.9	52.1	45.0	30.0	26.3	33.8
1.95	43.7	36.8	22.2	18.8	25.5
0.0	0.0	0.0	0.0	0.0	0.0
IC	2.52	5.95	22.65	29.80	15.25

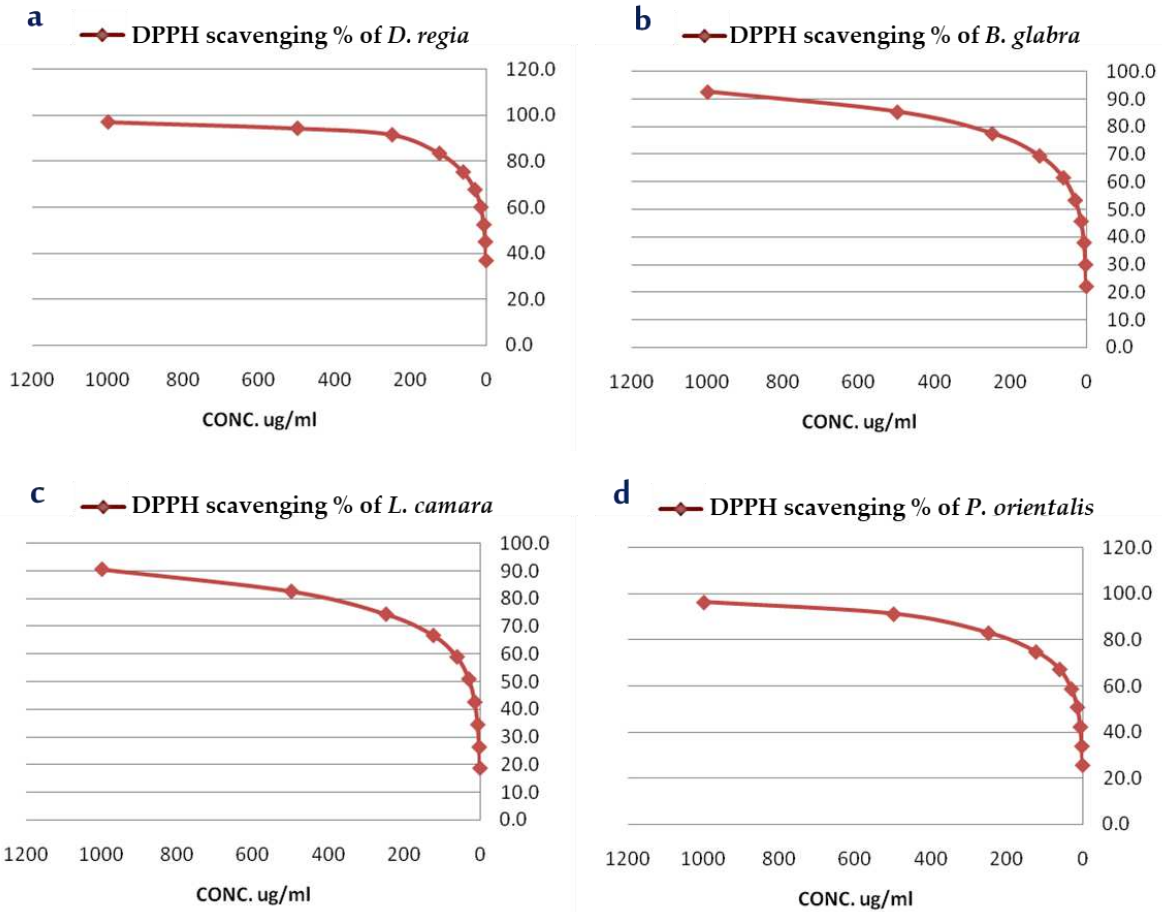


Fig. 5. Antioxidant activities of acetone extract of *D. regia* (a), *B. glabra* (b), *L. camara* (c), and *P. orientalis* (d), using DPPH Assay

Metabolomic analysis of four plant extracts.

GC-MS data analysis

Metabolomic analysis of the eight extracts and comparison between aqueous and acetone extracts were done by the help of GC-MS analysis. Results of GC-MS analysis in our study led to the identification of various compounds such as terpenes, fatty acids, esters, ketone, alkane, steroids, aliphatic amine, and phenols in the leaves of *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis* by using two different solvents (acetone and aqueous) (**Table 7–14 and Fig. 6**).

B. glabra acetone leaf extract contained 19 compounds (**Table 7**), while the aqueous leaf extract contained 12 compounds (**Table 8**), in which *B. glabra* showed abundance of Pimaric acid (24.65%), 2-Pentanone, 4-hydroxy-4-methyl (16.49%), and Caryophyllene (14.61%) in the acetone extract, while in the aqueous extract it was Cis-13-Eicosenoic acid (36.78%), 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (15.74%), and Oleyl oleate (12.61%).

D. regia acetone leaf extract had 22 compounds, as shown in **Table (9)**, while aqueous leaf extract had 12 compounds (**Table 10**). The highest concentrations of the detected compounds in acetone were DL- α -tocopherol (28.39%) and squalene (20.83%), while in aqueous extract they were Erucic acid (30.92%) and (E)-13-Docosenoic acid (20.26%).

L. camara acetone leaf extract had 17 compounds, as shown in **Table (11)**, while aqueous leaf extract had 13 compounds (**Table 12**). The highest concentrations of the detected compounds in acetone were: 1-Dodecanamine, N,N-dimethyl- (29.24%), and Caryophyllene (26.71%), while in aqueous extract were 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (20.53%), and 2-Hydroxy-3-[(9e)-9-octadecenoyloxy]propyl (9e)-9-octadecenoate (16.28%).

P. orientalis acetone leaf extract had 14 compounds, as shown in **Table (13)**, while aqueous leaf extract had 11 compounds (**Table 14**). The highest concentrations of the detected compounds in acetone were: Cedrol (41.58%), 1,4,8-cycloundecatriene, 2,6,6,9-tetramethyl-, (e,e,e)- (31.88%), while in aqueous extract, they were: Epicedrol- (38.25%) and Caryophyllene (24.62%). There is a variety in percentage and number of secondary metabolites in the acetone and water extracts of four plants, where the highest yield (52.6%), (40.70%), (38.70%), and (35.60%) with *P. orientalis*, *L. camara*, *D. regia*, and *B. glabra*, respectively.

The Venn diagram in **Fig. 6** summarizes the conservation and occurrence of chemical compounds extracted from different plants in acetone and aqueous extracts. It was observed that there was an increase in the number of compounds extracted from each plant using acetone compared to water extraction (**Fig. 6a,b**). In acetone and aqueous extracts, there was a different signature in each plant while there were some conserved compounds in two or three plants in the case of acetone and aqueous extracts, respectively. Four common fatty acids and esters (erucic acid, linoleic acid methyl ester, 9-octadecenoic acid methyl ester, and 9-hexadecenoic acid, 9-octadecenyl ester) have been identified in aqueous extracts of *B. glabra*, *D. regia*, and *L. camara*, whereas there was no shared compound in these three plants in the case of acetone extract. Two terpenes (1-heptatriacotanol and caryophyllene) were identified in *B. glabra* and *D. regia* acetone extracts (**Fig. 6a**), while three different compounds (cis-11-enicosenoic acid, cis-13-eicosenoic acid, and E,E,Z-1,3,12-nonadecatriene-5,14-diol) are shared in the aqueous extracts of *B. glabra* and *D. regia* (**Fig. 6b**). Interestingly, data showed that the chemical parts of the *P. orientalis* plant do not overlap with those of *B. glabra*, *D. regia*, or *L. camara*, either in acetone or water extracts (**Fig. 6a,b**). In addition, the Venn diagram showed that *B. glabra* was overlapped with *L. camara* in Eucalyptol, and *D. regia* was overlapped with *L. camara* in two organic constituents (Squalene and Ethyl iso-allocholate). The differential and conserved occurrence of chemical constituents in acetone and aqueous extracts of four plants is detailed in supplementary **Table (S1 & S2)**.

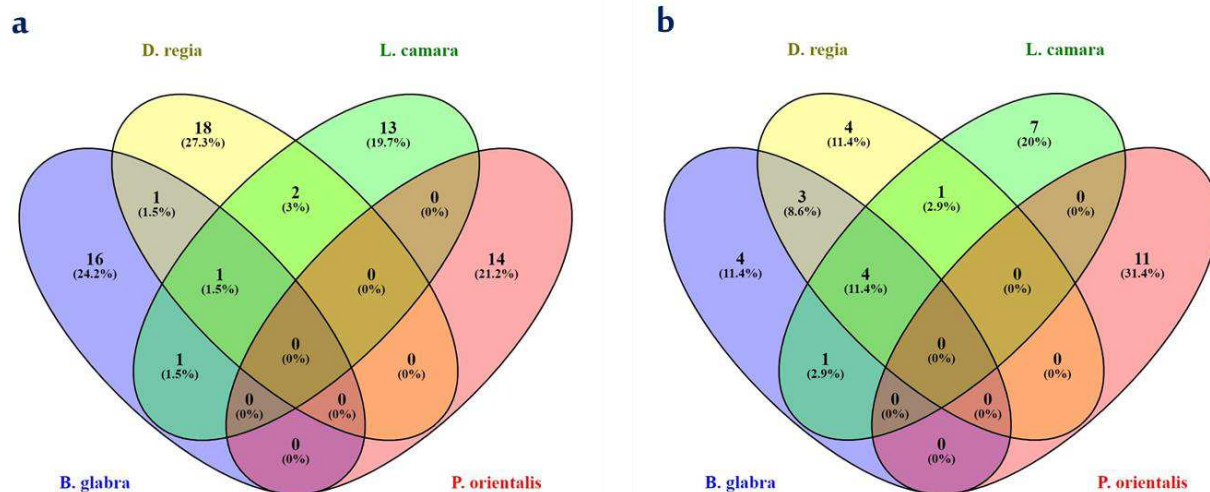


Fig. 6. A Venn diagram showed the relationships among chemical constituents in acetone (a), and aqueous (b) extracts of *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis*.

Figure 7 shows the heatmap obtained for the analysis of organic compounds in the acetone and aqueous extracts of *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis*. The scale shows the percentage of chemical classes resembled in each extract; a low percentage is indicated by a pale red color, while a darker red indicates a higher percentage. In general, the acetone extract showed a higher number of chemical classes, with a higher chemical abundance of the terpene compounds, while fatty acid compounds were most common in the aqueous extract, followed by terpenes (**Fig. 7**).

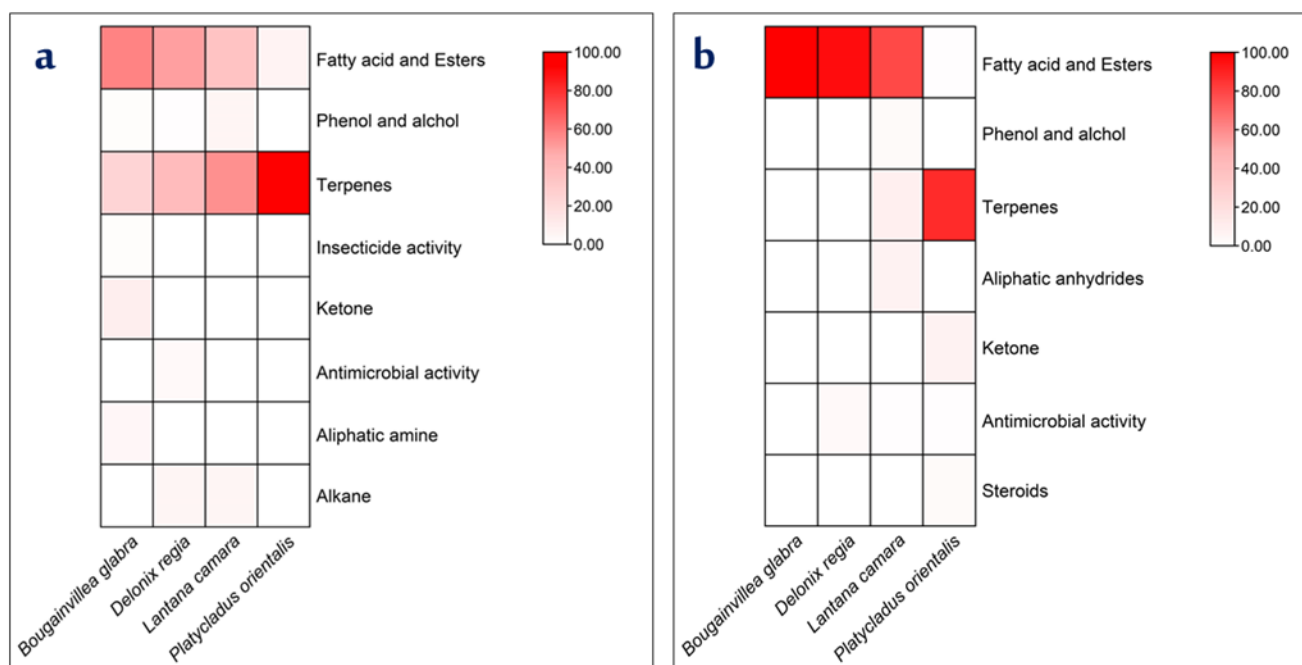


Fig. 7. Heatmap showing the percentage of chemical constituents of acetone (a) and aqueous (b) leaves extracts of *Bougainvillea glabra*, *Delonix regia*, *Lantana camara*, and *Platycladus orientalis*. In the heat map, red and pale pink colors indicate higher and lower chemical abundance, respectively.

426 **Table 7** The major chemical constituents of *B. glabra* leave acetone extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Antimalarial activity					
1	4.03	1.11	Halofantrine	C ₂₆ H ₃₀ C ₁₂ F ₃ NO	499
Aliphatic amine					
2	4.55	0.57	N,1-Dimethylhexylamine	C ₈ H ₁₉ N	129
3	4.62	0.65	2-Methyl-2-propanamine	C ₄ H ₁₁ N	73
4	5.26	0.26	Urea, N,N'-dimethyl-	C ₃ H ₈ N ₂ O	88
5	5.67	2.92	Benzeneethanamine, à,à-dimethyl-	C ₁₀ H ₁₅ N	149
Phenol					
6	6.74	1.68	à-Pinene, 10-(dimethylaminomethyl)-	C ₁₃ H ₂₃ N	193
7	8.64	0.38	Benzene, 1,2-dimethyl-	C ₈ H ₁₀	106
Terpene (Monoterpene and Sesquiterpene)					
8	9.34	2.0	à-Pinene	C ₁₀ H ₁₆	136
9	13.84	0.86	Eucalyptol	C ₁₀ H ₁₈ O	154
10	20.52	0.95	Nerolidol-epoxyacetate	C ₁₇ H ₂₈ O ₄	296
11	27.49	14.61	Caryophyllene	C ₁₅ H ₂₄	204
12	35.61	6.08	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536
Fatty acid and Esters					
13	8.97	16.49	2-Pentanone, 4-hydroxy-4-methyl-	C ₆ H ₁₂ O ₂	116
14	13.22	0.36	Cyclohexene, 4-isopropenyl-1-methoxymethoxymethyl-	C ₁₂ H ₂₀ O ₂	196
15	23.34	2.33	Methyl 10,12-pentacosadiynoate	C ₂₆ H ₄₄ O ₂	388
16	24.73	6.51	10,12-Tricosadiynoic acid, methyl ester	C ₂₄ H ₄₀ O ₂	360
17	27.18	7.74	10-12-Pentacosadiynoic acid	C ₂₅ H ₄₂ O ₂	374
18	37.73	24.65	Pimaric acid	C ₂₀ H ₃₀ O ₂	302
Ketone					
19	36.15	9.85	2-Butyne-1,4-dione, 1-(2,3-dihydro-3,3-dimethyl-1h-inden-5-yl)-4-phenyl-	C ₂₁ H ₁₈ O ₂	302

427
428 **Table 8** The major chemical constituents of *B. glabra* leave aqueous extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Terpene (Monoterpene and Sesquiterpene)					
1	27.15	1.14	Caryophyllene	C ₁₅ H ₂₄	204
Fatty acid and Esters					
2	28.71	1.45	Linoleic Acid methyl ester	C ₁₉ H ₃₄ O ₂	294
3	28.89	2.1	9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296
4	38.46	1.83	Erucic acid	C ₂₂ H ₄₂ O ₂	338
5	40.37	36.78	Cis-13-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310
6	40.97	15.74	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	C ₃₄ H ₆₄ O ₂	504
7	41.19	11.25	(Z)-(Z)-Docos-13-en-1-yl icos-11-enoate	C ₄₂ H ₈₀ O ₂	616
8	43.47	12.61	Oleyl oleate	C ₃₆ H ₆₈ O ₂	532
9	43.33	0.78	9-Octadecenoic acid (z)-, 9-octadecenyl ester, (z)-	C ₃₆ H ₆₈ O ₂	532
10	44.89	4.43	(Z)-(Z)-icos-11-en-1-yl icos-11-enoate	C ₄₀ H ₇₆ O ₂	588
11	45.05	7.08	(Z)-Icos-11-en-1-yl oleate	C ₃₈ H ₇₂ O ₂	560
12	39.93	4.81	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C ₁₉ H ₃₄ O ₂	294

429 **Table 9** The major chemical constituents of *D. regia* leave acetone extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Terpene (Monoterpene and Sesquiterpene)					
1	38.94	1.88	Isochiapin b	C ₁₉ H ₂₂ O ₆	346
2	39.93	1.38	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536
3	14.97	2.16	Caryophyllene	C ₁₅ H ₂₄	204
4	24.42	2.00	Neophytadiene	C ₂₀ H ₃₈	278
5	24.92	1.14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296
6	27.69	0.70	Ethylene brassylate	C ₁₅ H ₂₆ O ₄	270
7	29.66	8.13	Phytol	C ₂₀ H ₄₀ O	296
8	30.66	1.95	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284
9	35.06	2.55	Ethanol, 2-(9-octadecenyl)-, (Z)-	C ₂₀ H ₄₀ O ₂	312
10	39.82	20.83	squalene	C ₃₀ H ₅₀	410
Phenol					
11	11.69	0.27	Cyclohexanol, 2-(1,1-dimethylethyl)-	C ₁₀ H ₂₀ O	156
12	11.86	0.68	Phenol, 2-methyl-5-(1-methylethyl)-	C ₁₀ H ₁₄ O	150
Fatty acid and Esters					
13	13.10	0.64	Triacetin	C ₉ H ₁₄ O ₆	218
14	26.20	1.57	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270
15	26.97	3.76	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256
16	29.33	10.14	Methyl 10-trans,12-cis-octadecadienoate	C ₁₉ H ₃₄ O ₂	294
17	30.19	3.13	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	278
18	40.76	2.67	ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436
19	42.34	0.42	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	C ₃₄ H ₆₄ O ₂	504
20	43.04	28.39	DL-α-tocopherol	C ₂₉ H ₅₀ O ₂	430
Alkane					
21	40.61	3.16	Tetratetracontane	C ₄₄ H ₉₀	838
22	42.81	2.45	17-pentatriacontene	C ₃₅ H ₇₀	490

430

431 **Table 10** The major chemical constituents of *D. regia* leave aqueous extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Fatty acid and Esters					
1	25.68	1.05	Palmitic Acid methyl ester	C ₁₇ H ₃₄ O ₂	270
2	28.69	9.44	Linoleic Acid methyl ester	C ₁₉ H ₃₄ O ₂	294
3	28.87	14.02	9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296
4	29.43	1.71	Stearic Acid Methyl Ester	C ₁₉ H ₃₈ O ₂	298
5	40.27	3.21	Cis-13-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310
6	42.74	2.69	Ethanol, 2-(9-octadecenyl)-, (Z)-	C ₂₀ H ₄₀ O ₂	312
7	44.07	2.18	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	C ₅₇ H ₁₀₄ O ₆	884
8	44.25	1.68	Cis-11-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310
9	44.42	9.38	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	C ₃₄ H ₆₄ O ₂	504
10	44.56	30.92	Erucic acid	C ₂₂ H ₄₂ O ₂	338
11	45.08	20.26	(E)-13-Docosenoic acid	C ₂₂ H ₄₂ O ₂	338
12	44.88	3.46	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C ₁₉ H ₃₄ O ₂	294

432 **Table 11** The major chemical constituents of *L. camara* leave acetone extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Terpene (Monoterpene and Sesquiterpene)					
1	3.15	7.59	Eucalyptol	C ₁₀ H ₁₈ O	154
2	10.24	26.71	Caryophyllene	C ₁₅ H ₂₄	204
3	10.92	7.48	Humulene	C ₁₅ H ₂₄	204
4	12.94	1.24	ξ-Elemene	C ₁₅ H ₂₄	204
5	13.95	8.22	Caryophyllene oxide	C ₁₅ H ₂₄ O	220
6	14.55	1.56	á-copaene	C ₁₅ H ₂₄	204
7	31.98	1.88	Squalene	C ₃₀ H ₅₀	410
Fatty acid and Esters					
8	11.90	29.24	1-Dodecanamine, N,N-dimethyl-	C ₁₄ H ₃₁ N	331
9	18.06	1.32	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280
10	22.31	0.54	9-Octadecenoic acid (Z)-, methylester	C ₁₉ H ₃₆ O ₂	296
11	22.98	1.18	Oleic Acid	C ₁₈ H ₃₄ O ₂	282
12	33.92	1.57	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436
13	35.95	0.75	Oleic acid, 3-(octadecyloxy)propylester	C ₃₉ H ₇₆ O ₃	592
Phenol					
14	3.96	0.81	Benzene, (chloromethyl)-	C ₇ H ₇ Cl	126
15	22.48	4.44	2-Methyleneborexane	C ₁₀ H ₁₄	134
Alkane					
16	14.34	2.79	Cyclooctenone, dimer	C ₁₆ H ₂₄ O ₂	248
17	32.79	2.68	Dotriacontane	C ₃₂ H ₆₆	450

433

434 **Table 12** The major chemical constituents of *L. camara* leave aqueous extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Fatty acid and Esters					
1	28.70	10.25	Linoleic acid methyl ester	C ₁₉ H ₃₄ O ₂	294
2	28.88	15.62	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296
3	40.67	20.53	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	C ₃₄ H ₆₄ O ₂	504
4	42.27	3.87	9-octadecenoic acid (z)-, 9-hexadecenyl ester, (z)-	C ₃₄ H ₆₄ O ₂	504
5	44.05	5.8	Erucic acid	C ₂₂ H ₄₂ O ₂	338
6	44.72	14.15	Oleyl oleate	C ₃₆ H ₆₈ O ₂	532
Phenol and alcohol					
7	43.45	2.33	Ethanol, 2-(9-octadecenyl)-, (Z)-	C ₂₀ H ₄₀ O ₂	312
8	41.68	1.05	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C ₁₆ H ₂₈ O ₃	268
Terpenes and carotenoids					
9	36.77	7.91	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	C ₃₀ H ₅₂ O ₂	444
10	38.55	0.28	1-Heptatriacotanol	C ₃₇ H ₇₆ O	536
11	38.48	0.95	7,8-Epoxy lanostan-11-ol, 3-acetoxy-	C ₃₂ H ₅₄ O ₄	502
12	38.69	0.98	Rhodopin	C ₄₀ H ₅₈ O	554
Glycerides					
13	41.05	16.28	2-Hydroxy-3-[(9e)-9-ctadecenoyloxy]propyl (9e)-9-octadecenoate	C ₃₉ H ₇₂ O ₅	620

435 **Table 13** The major chemical constituents of *P. orientalis* leave acetone extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Terpene (Monoterpene and Sesquiterpene)					
1	9.74	0.36	Cyclohexene, 1,5,5-trimethyl-6-methylene-	C ₁₀ H ₁₆	136
2	10.88	4.13	Cedrene	C ₁₅ H ₂₄	204
3	11.76	31.88	1,4,8-cycloundecatriene, 2,6,6,9-tetramethyl-, (e,e,e)-	C ₁₅ H ₂₄	204
4	12.99	7.86	ç-Murolene	C ₁₅ H ₂₄	204
5	13.19	2.65	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	C ₁₅ H ₂₄	204
6	13.79	3.51	Aromandendrene	C ₁₅ H ₂₄	204
7	14.83	41.58	Cedrol	C ₁₅ H ₂₆ O	222
Fatty acid and Esters					
8	8.34	0.44	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	C ₁₂ H ₂₀ O ₂	196
9	15.22	0.29	Doconexent	C ₂₂ H ₃₂ O ₂	328
10	24.92	0.80	Methyl 4,7,10,13,16,19-docosaheptaenoate	C ₂₃ H ₃₄ O ₂	342
11	25.40	2.67	Podocarp-7-en-3á-ol, 13á-methyl-13-vinyl-	C ₂₀ H ₃₂ O	288
12	26.33	0.73	5,8,11-Heptadecatriynoic acid, methyl ester	C ₁₈ H ₂₄ O ₂	272
13	28.23	1.85	Androstan-17-one, 3-ethyl-3-hydroxy-, (5à)-	C ₂₅ H ₄₀ O ₂ Si	400
Phenol					
14	32.07	1.25	Retinol	C ₂₀ H ₃₀ O	286

436

437 **Table 14** The major chemical constituents of *P. orientalis* leave aqueous extracts.

No	RT	Area %	Compound Name	M. F.	M.W
Terpene (Monoterpene and Sesquiterpene)					
1	11.07	24.62	Caryophyllene	C ₁₅ H ₂₄	204
2	11.76	12.35	Humulene	C ₁₅ H ₂₄	204
3	14.32	3.20	Caryophyllene oxide	C ₁₅ H ₂₄ O	220
4	14.82	38.25	Epicedrol	C ₁₅ H ₂₆ O	222
Fatty acid and Esters					
5	24.91	7.80	Doconexent	C ₂₂ H ₃₂ O ₂	328
Ketone					
6	12.42	1.01	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4ar-(4aà,7à,8aá)]-	C ₁₅ H ₂₄	204
7	15.76	4.58	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-à,à,4a,8-tetramethyl-, [2R-(2à,4aà,8aá)]-	C ₁₅ H ₂₆ O	222
8	28.25	0.86	Cholan-24-oic acid, 3,7,12-trihydroxy-, (3à,5á,7à,12à)-	C ₂₄ H ₄₀ O ₅	408
Steroids					
9	25.39	2.14	Androst-4-en-3-one, 17-hydroxy-, (17á)-	C ₁₉ H ₂₈ O ₂	288
Phenol					
10	26.22	1.83	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1à,4aà,10aá)]-	C ₂₀ H ₃₀ O	286
11	32.07	3.36	Retinol	C ₂₀ H ₃₀ O	286

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HPLC analysis

The phenolic acids and flavonoids in the acetone extracts of *L. camara* and *P. orientalis* were determined by HPLC analysis, and the results showed the presence of flavonoid compounds such as Syllimarine, Rutin, and Ellagic in both extracts. Six acids were identified in *L. camara*; Syllimarine acid (0.55141 mg/g), Rutin (0.12310 mg/g), and Thymol (0.10825 mg/g) were the major constituents found in the *L. camara* extract, while phenolic acids Ferulic (0.00035 mg/g) and Ellagic (0.00035 mg/g) were less abundant (Table 15). Similarly, Syllimarine (0.045114 mg/g), Rutin (0.18680 mg/g), and Myricetin (60.10311 mg/g) were the major constituents found in the *P. orientalis* extract, while Ellagic (0.00045 mg/g) and Amentoflavone (0.00024 mg/g) were the minor constituents (Table 16).

Table 15 HPLC analysis for Total polyphenolic compounds from *L. camara* acetone extract

No.	Reten. Time (min)	Amount (mg/ml)	Compound Name
1	1.793	0.00053	Gallic Acid
2	10.025	0.10825	Thymol
3	15.258	0.00035	Ferulic
4	23.917	0.55141	Syllimarine
5	26.188	0.00035	Ellagic
6	28.132	0.12310	Rutin

Table 16 HPLC analysis for Total polyphenolic compounds from *P. orientalis* acetone extract

No.	Reten. Time (min)	Amount (mg/ml)	Compound Name
1	4.812	0.00403	Para Amino Benzoic Acid
2	10.305	0.00457	Syringic Acid
3	15.968	0.18680	Rutin
4	20.387	0.01963	Apeinine
5	22.243	0.45114	Syllimarine
6	24.252	0.10311	Myricetin
7	26.515	0.00024	Amentoflavone
8	35.828	0.00045	Ellagic

ADMET analysis

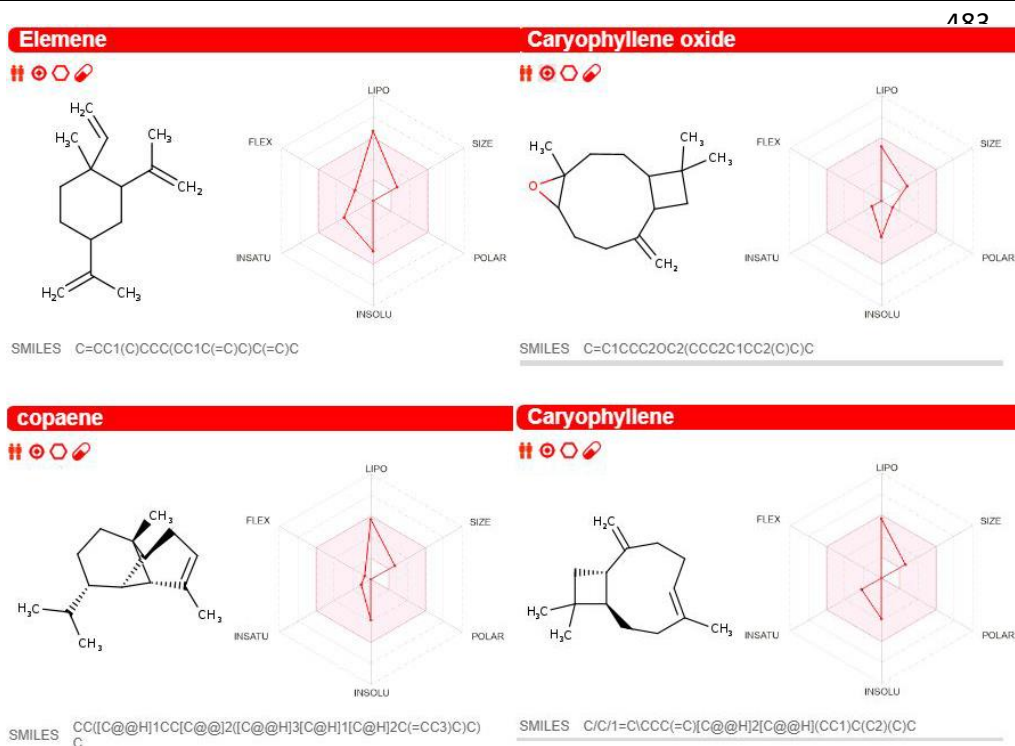
L. camara acetone extract showed the most effective antimicrobial activity against all the tested pathogenic organisms. So, four phytochemicals of identified metabolites in *L. camara* acetone extract (elemene, caryophyllene oxide, caryophyllene, and copaene) were evaluated for ADME aspects (physicochemical characteristics, water solubility, lipophilicity, pharmacokinetics, drug resemblance, and medicinal chemistry) using Swiss ADME software (Table 17). These phytochemicals have been selected based on their potential activity as antimicrobial or insecticidal compounds. All four phytochemicals studied exhibited drug-like properties without breaking Lipinski's rule of five. Furthermore, all of the phytochemical compounds had a bioavailability score of 0.55, indicating that they had drug-like characteristics. Furthermore, while all of the phytochemicals studied were found to be absorbed in the gastrointestinal system, caryophyllene oxide and copaene could only penetrate the blood-brain barrier. The topological polar surface area ranged from 0 to 12.532, while the consensus log Po/w (an indication of lipophilicity) ranged from 3.15 to 3.37. Furthermore, none of the substances examined showed

permeability to glycoprotein substrate (P-gp). Furthermore, all of the phytochemicals evaluated interacted with two isoenzymes of the cytochrome P family, CYP2C19 and CYP2C9, and only copaene interacted with CYP1A2, implying that they are efficacious while being low in toxicity (Table 18, Table S3). The bioavailability radar plots of the studied phytochemicals (Fig. 8) showed that the phytochemicals were mostly inside the pink area, indicating a better drug-likelihood profile. Furthermore, a red point on the boiled egg graph of elemene, caryophyllene oxide, caryophyllene, and copaene predicts their brain penetrability as a non-substrate of P-gp (Fig. S1).

Table 17 In silico ADME analysis of phytochemicals tested.

Descriptors	Elemene	Caryophyllene oxide	Copaene	Caryophyllene
SMILE	<chem>CC(=C)C1CCC(C(C1)C(=C)C)(C)C=C</chem>	<chem>CC12CCC3C(CC3(C)C)C(=C)CCC1O2</chem>	<chem>CC(C)[C@@H]1CC[C@@]3(C)[C@@H]2C(/C)=C\C[C@H]3[C@H]12</chem>	<chem>C1(=C)\CC/C=C(/CC[C@@H]2[C@@H]1CC2(C)C)C</chem>
Physicochemical properties				
Number of heavy atoms	15	16	15	15
Number of aromatic heavy atoms	0	0	0	0
Fraction Csp3	0.6	0.87	0.87	0.73
Number of rotatable bonds	3	0	1	0
Number of H-bond acceptors	0	1	0	0
Number of H-bond donors	0	0	0	0
Molar refractivity	70.42	68.27	67.14	68.78
Topological polar surface area (Å ²)	0	12.53	0	0
Lipophilicity				
Log Po/w (iLOGP)	3.37	3.15	3.4	3.25
Log Po/w (XLOGP3)	6.11	3.56	4.47	4.38
Log Po/w (WLOGP)	4.75	3.94	4.27	4.73
Log Po/w (MLOGP)	4.53	3.67	5.65	4.63
Log Po/w (SILICOS-IT)	4.5	4.07	3.73	4.19
Consensus log Po/w	4.65	3.68	4.3	4.24
Water solubility				
Log S (ESOL)	-4.76	-3.45	-3.86	-3.87
Solubility (mg/ml)	3.57E-03	7.84E-02	2.84E-02	2.78E-02
Class	Moderately soluble	Soluble	Soluble	Soluble
Log S (Ali)	-5.89	-3.51	-4.19	-4.1
Ali Solubility (mg/ml)	2.62E-04	6.83E-02	1.32E-02	1.64E-02
Class	Moderately soluble	Soluble	Moderately soluble	Moderately soluble
Log S (SILICOS-IT)	-3.58	-3.51	-3.07	-3.77
Solubility	5.36E-02	6.81E-02	1.74E-01	3.49E-02

Class	Soluble	Soluble	Soluble	Soluble
Pharmacokinetics				
GI absorption	Low	High	Low	Low
BBB permeant	No	Yes	Yes	No
P-gp substrate	No	No	No	No
CYP1A2 inhibitor	No	No	Yes	No
CYP2C19 inhibitor	Yes	Yes	Yes	Yes
CYP2C9 inhibitor	Yes	Yes	Yes	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
Log Kp (skin permeation) (cm/s)	-3.21	-5.12	-4.37	-4.44
Drug-likeness				
Lipinski	1	0	1	1
Ghose	0	0	0	0
Veber	0	0	0	0
Egan	0	0	0	0
Muegge	2	1	1	1
Bioavailability score	0.55	0.55	0.55	0.55
Medicinal chemistry				
PAINS	0	0	0	0
Brenk	1	2	1	1
Lead likeness	2	2	2	2
Synthetic accessibility	3.63	4.35	4.62	4.51



505 Fig. 8. Bioavailability radars of the phytochemicals tested (elemene, caryophyllene oxide, copaene, and caryophyllene).

506 **Table 18** In silico toxicity analysis of phytochemicals tested.
507

no.	Phytochemicals	Oral toxicity of phytochemicals (PROTOX II)		Acute Inhalation Toxicity	Acute Oral Toxicity	Acute Dermal Toxicity
		Predicted LD50 (mg/kg)	Predicted toxicity class			
1	Elemene	5000	5	Non-cardiotoxic (66%)	Non-cardiotoxic (96%)	Non-cardiotoxic (74%)
2	Caryophyllene oxide	5000	5	Toxic (52%)	Non-toxic (93%)	Non-toxic (78%)
3	Copaene	3700	5	Non-toxic (56%)	Non-toxic (95%)	Non-toxic (75%)
4	Caryophyllene	5300	5	Non-toxic (61%)	Non-toxic (94%)	Non-toxic (79%)

508

509 Discussion

510 Mosquito larvicidal activity

511 Several important natural components are found in plant extracts and essential oils (EOs)
512 and can be safely used in pest and disease control due to their ability to achieve the purpose of
513 killing harmful pests and then their ability to degrade in nature in a smooth manner [47,48].
514 Despite their insecticide benefits, only 5% of the world's pesticides are biopesticides [49,50].
515 However, biopesticides are expanding rapidly and are expected to overtake chemical pesticides
516 in the near future at an average annual growth rate of 9–20% [49–52], due to the unique
517 characteristics of biopesticides that encourage their application, including their non-toxicity to
518 the environment.

519 In this study, all ornamental plant extracts tested showed strong insecticidal effectiveness
520 against mosquito larvae (moderate to high larvicidal activities). The plant acetone extracts of *B.*
521 *glabra*, *D. regia*, *L. camara*, and *P. orientalis* killed mosquito larvae with 94–100% mortality and 87-
522 96% mortality for aqueous extracts 24 h post-treatment.

523 Our data showed the most effective plant extracts against mosquito larvae were *P.*
524 *orientalis* and *L. camara*, followed by *B. glabra* and *D. regia*. Our data agreed with Bosly's findings
525 [53], who studied the effectiveness of *Thuja orientalis* leaf and fruit extracts against *Cx. pipiens*
526 larvae and found that all plant extracts killed all of the larvae (100% mortality), except for the
527 plant hexane extract, which killed only 98% of the larvae.

528 Similarly, 3rd larval instar of *Anopheles stephensi* and *Culex pipiens* died when the essential
529 oil from the leaves of *T. orientalis* was used to test it [54]. The author indicated the toxicity of the
530 Thuja plant because it contains several main components, mainly the compounds carene and
531 cedrol.

532 Evaluation of the larvicidal effects of *Chamaecyparis obtusa* and *Thuja orientalis* oils on *Aedes*
533 *aegypti* and *Culex pipiens* 4th instar larvae compared to stem, fruit, and seed oils found that, the
534 larvicidal effects of leaf oils from *C. obtusa* and *T. orientalis* were much higher. These findings
535 demonstrate that *C. obtusa* and *T. orientalis* have strong larvicidal action against *Ae. aegypti* and
536 *Cx. pipiens* in both the leaf part and age class II as natural larvicides [55].

537 In this study, one of the most effective plant extracts against mosquito larvae was *Lantana*
538 *camara*, either in acetone or aqueous extract. In a similar study, *L. camara* leaf extracts with
539 concentrations of 0.3, 0.6, 0.9, and 1.2 g/l were shown to kill *Aedes aegypti* larvae in the lab for 24
540 hours. The mortality reached 91.66 to 96.66% with ethanolic leaf extract, while less than 35% of
541 larvae died in aqueous extract. The outcome demonstrated that *A. aegypti* larvae were more fatally
542 affected by the ethanolic extract than the aqueous extract [56]. The previous findings may be

attributed to the fact that most of the active compounds are lipophilic in character and are therefore more readily extracted into an organic medium to be more efficient [57].

In a similar study, the effectiveness of *Lantana camara aculeata* leaf extracts was tested against 4th instar larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The mortality rate of different types of mosquitoes was evaluated in aqueous (LC₅₀ = 98.10), ethanol (LC₅₀ = 60.93), methanol (LC₅₀ = 39.54), acetone (LC₅₀ = 60.64), and chloroform (LC₅₀ = 86.91 ppm) extract 24 h post-treatment. The extracts of this plant showed strong efficacy in killing larvae, especially at methanol and acetone [3].

Another study conducted by Alghamdi and Beshr [58] on the effect of ethanol extracts from the leaves and flowers of *L. camara* and the ability of the extract to kill the larvae of *Anopheles arabiensis* and *Culex quinquefasciatus*. The results showed that the LC₅₀ values for *A. arabiensis* and *C. quinquefasciatus* were 15.84 ppm and 21.37 ppm in flower extracts and 9.54 ppm and 5.01 ppm in leaf extracts, which indicates the efficacy of plant leaves in killing mosquito larvae rather than flowers.

When we look at these ornamental plants, we find that they have been mentioned in the past in many treatments for human diseases, and due to the lack of technology, most treatments in the ancient system were derived from plants, and their effectiveness as medicines has also been proven. Which indicates its effectiveness in eliminating microbes and thus killing pests harmful to human health. Among these plants is the aromatic ornamental plant *Lantana camara*, which contains a wide variety of healing ingredients [59-61]. This herb has long been used to treat a variety of ailments, including malaria, fever, colds, and coughs [60-67].

Thuja or *P. orientalis* also referred to as the "tree of life" or "white cedar," is a well-known ornamental tree. In traditional medicine, it is frequently used to treat conditions like rheumatism, bacterial skin infections, cold sores, enuresis, amenorrhea, psoriasis, bullous bronchitis, and enuresis. It is also used as an insect repellent [68]. Among the aromatic ornamental plants, *Bougainvillea*, whose leaves, flowers, and stem bark have long been used as medicinal plants in the form of infusions, decoctions, and tinctures, Also used as a tea, *Bougainvillea* extracts are used to treat a variety of ailments, including cough, sore throat, fever, diarrhea, diabetes, hepatitis and liver problems, asthma, and bronchitis, as well as to regulate menstruation, stop leucorrhea, reduce stomach acidity, dissolve blood clots, and treat anemia linked to gastrointestinal bleeding and epigastric pain. Drinking a floral infusion can help decrease blood pressure [26,69].

D. regia is known as flame of the forest or royal Poinciana and has been widely used traditionally or in Ayurveda, an alternative medicine system, to treat various ailments, including jaundice, ulcers, wounds, arthritis, malaria, vomiting, infection, inflammation, pain, and diarrhea [30-32,34], due to the many bioactive compounds it contains, such as alkaloids, flavonoids, triterpenoids, sterols, tannins, saponins, steroids, and carotene hydrocarbons [70,71]. However, the effectiveness of this plant against insects, microbes, or biological aspects is scant, according to literary articles. This shows the ability of the current ornamental plant extracts to control mosquitoes, which is in line with the results of several studies on mosquitoes [72-75].

Antimicrobial activity

According to our results, *L. camara* possessed high antibacterial activity; this could be due to the presence of phenolics, anthocyanins, and proanthocyanins in *L. camara* leaves [61,76]. The active principle of the extracts disrupts the permeability barrier of cell membrane structures and thus inhibits bacterial growth [77]. Also, EOs may interact with and affect the plasma membrane, interfering with respiratory chain activity and energy production [78].

Sukul and Chaudhuri [79] tested petroleum ether, benzene, chloroform, and methanol from *L. camara* leaves on *Escherichia coli* (ATCC 10536), *Salmonella typhi* (ATCC 686), *S. aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 25619). Chloroform and methanol extracts showed activity against all the bacteria tested, while the petroleum ether fraction only showed activity against *P. aeruginosa* and the benzene fraction only against *S. typhi*. Also, Basu et al., [80] found that chloroform and methanolic extracts of *L. camara* were more specific toward the Gram-positive strains, although Gram-negative *P. aeruginosa* was also inhibited by the methanol extract, while the aqueous extract was found to be inactive. Furthermore, Amutha [81] investigated the antimicrobial activities of methanol, chloroform, acetone, petroleum ether, and hexane extracts of *L. camara* seed against *S. aureus*, *P. aeruginosa*, *P. vulgaris*, and *E. coli*. Methanolic extract showed maximum inhibition against *S. aureus*, *P. aeruginosa*, and *E. coli* and no inhibition against *P. vulgaris*.

According to reports by Weenen et al. [82], *L. camara* root extract possesses hepatoprotective and pancreatic properties and has antimalarial activity against *Plasmodium falciparum*. It is also a reliable source of oleanolic acid. The most important phytochemical groups are flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridescent glycosides, phenylethanoids, oligosaccharides, quinine, saponins, steroids, triterpenes, sesquiterpenoids, and tannin [83]. The compound Dotriacontane in the alkan group is associated with the antimicrobial effect, as reported by Gallo and Sarachine [84], who showed that dotriaconane is known to possess diverse biological activities such as antioxidant and antimicrobial.

Thuja orientalis has good antimicrobial activity against gram-positive bacteria, while all the tested gram-negative bacteria recorded weak or no susceptibility [85]. Due to the presence of *Thuja* essential oils, coumarins, flavonoids, tannins, and proanthocyanidins, the chemical components of *T. occidentalis* have attracted study attention for many years. Its pharmacology includes antibacterial, antifungal, anticancer, antiviral, antioxidant, gastrointestinal protective, anti-inflammatory, radioprotective, antipyretic, and lipid-metabolizing activities [53,68,86]. The discovery that *Bougainvillea* leaf extracts had antiviral activities resulted in the extraction and characterization of bouganin, a protein with traits common to type 1 ribosome-inactivating proteins (RIPs) [26]. Caryophyllene oxide compounds overlap with all study plants that possess anticarcinogenic, anti-inflammatory, and antibacterial properties [87]. Amalia et al. [88] showed that the bark of *D. regia* harbored antimalarial activity against *Plasmodium berghei* in mice. The antimicrobial activity of leaves extracts of *D. regia* against the tested microbes might be linked to the presence of phenolic acids such as gallic, protocatechuic, 3-hydroxybenzoic, chlorogenic acids and flavonoids [89].

Antioxidant activity

Reactive oxygen species (ROS) are involved in the pathogenesis of various diseases. Free radicals are the root cause of uncontrolled oxidation. Free radicals oxidize all major classes of biomolecules. The products of these oxidation reactions diffuse from the original site of attack and spread the damage all over the body, causing serious damage to almost all the cells. Some important biomolecules susceptible to free radical oxidation are Lipids, Proteins, Nucleic acids, and Carbohydrates. Thus, the need for antioxidant therapy arises.

Antioxidants are compounds that neutralize and interact with free radicals, thus preventing them from causing cell damage. The presence of these antioxidants can prevent the types of free radical damage that have been associated with cancer development and other diseases. The antioxidants work in different parts of cells and contribute to different chemical

reactions in the body. As a first line of defense, some antioxidants suppress the formation of free radicals, while others work to remove them before they do damage to cells or repair damage once it has been done. Numerous plants have phytochemical properties that act as antioxidants. In addition to some familiar nutrients that act as antioxidants, such as vitamins C and E and β -carotene, there are many others that are phytochemicals, such as quercetin and other flavonoids [90,91].

Soni and Sehrawat [92] found that the plant could be a source of natural antioxidants because it has a number of bioactive substances that neutralize free radicals, which can damage cell membranes and DNA and have other pharmacological effects. In the DPPH test, the ability of a compound to act as a donor for hydrogen atoms or electrons was measured spectrophotometrically. The results showed that the *D. regia* extract had a high level of antioxidant activity ($IC_{50} = 5.95 \mu\text{g/ml}$) and was more effective at getting rid of DPPH radicals than the other plant extracts, which were less effective. This could be credited to the presence of more phytochemicals (22 compounds) than the other three studied plant extracts (Fig. 6), including flavonoids, phenolics, fatty acids, terpenes, and alkanes. This is in agreement with the findings of Mariajancyran et al. [93], who revealed the presence of tannin, terpenoids, flavonoids, steroids, and fatty acids in the chloroform extract of *D. regia* leaves.

Moreover, the leaves of *D. regia* are valuable as drugs [94]. The leaves are accounted for their antimicrobial and antioxidant impacts [95]. It was reported that *D. regia* is utilized in numerous nations for the preparation of extracts having antifungal and antimicrobial activities [96].

Phytochemical analysis

Natural sources of bioinsecticides include pheromones, bacteria, fungi, viruses, and protozoa, as well as bioactive plant compounds, pheromones, and microbes. Phytochemicals, microbiological pesticides, plant-incorporated protectants, and pheromones are the four primary categories of bioinsecticides based on their source [97]. They have been employed to control pests with success [98]. They are better than synthetic molecules because they are biodegradable, less poisonous, target-specific, and very powerful in small quantities.

Plants are biological factories that produce various chemicals collectively known as secondary metabolites. Secondary metabolites like alkaloids, carbohydrates, flavonoids, saponins, tannins, and terpenoids are what give medicinal plants their pharmacological effects. Many diverse groups of bioactive substances extracted from or included in botanical insecticides can have the potential to cause adverse effects on pests or organisms that consume or are exposed to them.

In this work, the analysis of organic compounds in the acetone and aqueous extracts of *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis* revealed that the acetone extract showed a higher number of different organic compounds, with a higher chemical abundance of the terpene compounds, while fatty acid compounds were most common in the aqueous extract, followed by terpenes. The extraction yield, phytochemical content, and antioxidant properties were influenced by the polarity of the extracting solvents [99]. The different solvents resulted in various extraction yields. This is because differences in the polarity of the solvents could cause a wide variation in the level of bioactive compounds in the extract due to the plant materials containing high levels of polar compounds that are soluble in solvents with high polarity. The results revealed that alkanes, flavonoids, terpenes, ketone, and phenols were among the phytochemicals identified in acetone more than in aqueous extract [100].

Our results are in conformity with previous studies supporting the use of acetone as the best solvent to recover higher extractable compounds from various medicinal plants [101]. Similarly, previous studies have shown that the high polarity of acetone was found to exhibit better efficiency in extracting various polar phytochemicals, such as phenolics, from the leaves of *L. camara* [102]. In several studies, it was proven that the leaf extracts of *L. camara* have anti-inflammatory [103]; anticancer [104], antibacterial [105], antifungal, insecticidal [106], and nematocidal activities [107].

Also, the results showed that acetone extracts of plant materials were more toxic to mosquito larvae, *Cx. pipiens* than aqueous extracts. This is in line with the study of Bosly [53], who seemed to rank the leaf extracts in order of their toxicity to mosquito larvae as follows: acetone > methanol > aqueous > hexane. It was mentioned that the activities of the plant extracts against mosquito species depend on the solvent used in extracting the phytochemicals responsible for the responses [108,109]. Results of GC-MS analysis in our study led to the identification of various compounds such as terpenes, fatty acids, esters, ketone, alkane, steroids, aliphatic amines, and phenols in the leaves of *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis* by using two different solvents (acetone and aqueous). This agrees with Park et al. [110], who reported that the leaves of *B. glabra* contain a wide variety of secondary metabolites such as phenols, flavonoids, tannins, saponins, and proteins.

The qualitative analyses of the ethanolic extracts from *D. regia* bark and *Carica papaya* leaf showed the presence of phytochemical constituents, including flavonoids, alkaloids, triterpenoids, steroids, tannins, and glycosides [83]. The most significant phytochemical groups in *L. camara* are flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridescent phenylethanoids, oligosaccharides, quinine, saponins, steroids, triterpenes, sesquiterpenoids, and tannin. Pavela et al. [111] reported that *Thuja orientalis* contains bioflavonoids, terpenoids, fatty acids, and cytotoxic principles. In this study, all ornamental plant extracts tested showed strong insecticidal effectiveness against mosquito larvae (moderate to high larvicidal activities) and antibacterial activity, but the most effective plant extracts against mosquito larvae were *P. orientalis* and *L. camara*, followed by *B. glabra* and *D. regia*, and this may be due to the presence of insecticide and antimicrobial activity and other organic compounds such as fatty acids and esters, alkanes, flavonoids, ketone, phenols, monoterpenes, and sesquiterpenes. Also, data showed that the chemical parts of the *P. orientalis* plant do not overlap with those of *B. glabra*, *D. regia*, or *L. camara*, either in acetone or water extracts.

Thuja orientalis previously acquired cytotoxic principles and contained terpenoids, fatty acids, aliphatic compounds like alkanes, and bioflavonoids [112]; this is in line with our results. Fatty acids and esters are one of the secondary metabolites that gave our tested plants their insecticidal effect. The same result obtained by Schmidt et al. [113], in which Ethyl (E, Z, E)-2-decent (Zeder's) was an effective insecticide against the Cimex, was also suggested for fat methyl esters (derived from *Solanum chlamygyndense*) that have larvicidal properties for *Culex quinquefasciatus* [114]. In other studies, saturated and polyunsaturated acids (particularly C8, C9, and C10) showed toxic effects against houseflies, horn flies, and stable flies, respectively. A similar study proved that a fatty acid mixture (C8910) has been shown to be both toxic and refractive to an insecticide-resistant *Anopheles* mosquito strain. Our results also agree with Youssef et al. [115], who found that the larvicidal activity of linoleic acid was active against *S. littoralis* and that the larval weight was reduced.

The common monoterpenes and sesquiterpenes that were highly present in the tested four plant leaf extracts were cedrol, caryophyllene, caryophyllene oxide, phytol, squalene, and

Caryophyllene. The main components, such as Caryophyllene which is known as Isocaryophyllene; eucalyptol; and caryophyllene oxide, might be responsible for the observed insecticidal activity, and this agrees with Zoubiri and Baaliouamer [116], who reported that the main components, e.g., b-caryophyllene and caryophyllene oxide, had insecticidal activity. Also, Caryophyllene oxide, spathulenol, and germacrene-D are known to possess anticarcinogenic, anti-inflammatory, insecticide, pesticide, and antibacterial properties [87].

HPLC analysis showed the presence of flavonoids and phenolic compounds in *L. camara* and *P. orientalis* acetone extracts, whereas polyphenolic flavonoid (Sylimarine) and flavonoid glycoside (Rutin) were the most abundant constituents in *L. camara* and *P. orientalis*.

This result agrees with Ahmed et al. [117], who reported that both species of *Lantana* (*L. camara* and *L. montevidensis*) contained Flavonoid glycosides. In some cases, the antibacterial activities of flavonoid glycosides were equal to or higher than those of ciprofloxacin used as a reference antibiotic, suggesting that they might be effective antibiotics against these pathogenic bacteria [118]. Considering the medical importance of the test microbial species, the result can be considered promising for the development of new antimicrobial drugs.

Rutin (3, 3', 4', 5', 7-pentahydroxyflavone-3-rhamnoglycoside), also called rutoside, quercetin-3-rutinoside, or sophorin, is a flavonol category of flavonoid [119]. Chemically, rutin is a glycoside combining the flavonol quercetin with the disaccharide rutinose (rhamnose and glucose) [120]. Phytochemistry analyses of *P. orientalis* shed light on many chemical constituents, such as diterpenes and flavonoids [121]. Rutin has significant antioxidant capabilities due to its reducing characteristics on various oxidizing species such as superoxide, peroxy, and hydroxyl radicals [122]. Besides, it has pharmacological properties such as anticancer, antibacterial, and anti-inflammatory properties [123]. Due to its several pharmacological qualities, such as its anti-inflammatory, anticancer, antioxidant, analgesic, and antibacterial actions, thymol has been utilized in traditional medicine in many countries. Additionally, its safety as a natural plant chemical has also been shown [124].

Secondary metabolites are produced in amazing diversity in all plants. Phenolic compounds are one of the most important categories of these metabolites. Many of phenolic compounds have antioxidant properties [125,126]. There have been numerous instances of an increased buildup of phenolic compounds and peroxide activity in plants exposed to high metal concentrations. Phosphonic' antioxidant action is mostly owing to their redox characteristics, which allow them to operate as reducing agents, hydrogen donors, and singlet oxygen quenchers [127].

These phenolic chemicals, often known as polyphenols, include a wide variety of components, including simple flavonoids, phenolic acids, complex flavonoids, and colorful anthocyanins. These phenolic chemicals are usually associated with plant defense responses. However, phenolic metabolites play an essential role in other processes, such as incorporation of attractive chemicals to aid in pollination, coloration for masking and protection from herbivores, and antibacterial and antifungal activity [128-130].

Our data revealed that the ornamental plant extracts contain many biologically active compounds with antimicrobial and antiparasitic activity, as shown in the GC-MS results of *B. glabra* and *P. orientalis* plants, which contained antimalarial bioactive compounds such as halofantrine and retinol. They also contain caryophyllene (14.61%) and cedrol (41.58%), which have insecticidal effects. *D. regia* had E,E,Z-1,3,12-Nonadecatriene-5,14-diol (3.46%); Isochiapin (1.88%); and 1-Heptatriacotanol (1.38%); *L. camara* plant had 2-Hydroxy-3-[(9e)-9-ctadecenoyloxy]propyl (9e)-9-octadecenoate (16.28%); Tricyclo [20.8.0.0 (7,16)]

triacontane,1(22),7(16)-diepoxy- (7.91%), and Squalene (1.88%), where these compounds all have antimicrobial effects. BESIDE THAT the chemical analysis with HPLC of *L. camara* and *P. orientalis* plants' acetone extracts revealed the presence of phenolic and flavonoids. The presence of these metabolites in conjunction with fatty acids, tannins and terpenoids confers antibacterial and antioxidant activity to these extracts. Intriguingly, flavonoids can form compounds with biological components and microbial cell walls, potentially impairing their function and inhibiting microbial development.

ADME Analysis

The ADME profile of the monoterpenoids (elemene, caryophyllene oxide, caryophyllene, and copaene) that have been found in plants has rarely been studied alongside in vitro antimicrobial effectiveness studies against pathogenic microbes. The Swiss ADME server was utilized in this work to analyze various ADME characteristics such as physiochemical properties, pharmacokinetics, solubility, lipophilicity, drug-likeness based on Lipinski's rule of five, and medicinal chemistry [38]. The four phytochemical compounds in silico ADME prediction proved drug-likeness, as indicated by no violation of Lipinski's rule of five and the derived bioavailability score (0.55). Furthermore, the compound's red line in the bioavailability radar map must be inside the pink area to be considered drug-like. All compounds' radar plots were totally within the pink area. Furthermore, the ADME data, together with the boiled egg model, demonstrated that all the investigated phytochemicals had superior gastrointestinal absorption and permeability of the blood-brain barrier [38].

P-gp and cytochrome P450 (CYP) are known to aid in the biotransformation of xenobiotics to protect tissues [131]. The phytochemicals in this investigation did not have any P-gp substrates. The P-gp transporter is identified as the most important member of the ATP-binding cassette transporter family, which is critical for assessing the protective efflux of biological membranes (GI tract or brain) from xenobiotics [38,131]. Furthermore, the three phytochemicals evaluated interacted only with the CYP1A2 isoenzyme, delivering efficiency with minimal toxicity [132].

To study the in-silico toxicity parameters, the machine learning programs Protox-II [45,46] were utilized. The projected LD50 (mg/kg) for the three phytochemicals evaluated varied from 3700 to 5300, putting them in Protox-II toxicity class 5. Furthermore, StopTox software determined that all the investigated phytocompounds were non-cardiotoxic based on hERG liability prediction, with confidence levels ranging from 50 to 90%. However, before being assessed in appropriate laboratory animal models, the resulting toxicity findings must be correlated with the in vitro safety assessments.

Conclusion

Mosquitoes are an important vector of deadly diseases that threaten human health, and their dangers extend beyond eradication. The emergence or recurrence of mosquitoes in endemic, non-endemic, and new parts of the world has led to the widespread use of synthetic pesticides to reduce the transmission of mosquito-borne diseases. With the emergence of resistant mosquitoes and concerns about the toxicity of synthetic pesticides to both target and non-target organisms, safer, biodegradable alternatives, Eco-friendly [133] and more efficient methods of controlling mosquitoes and other medical and veterinary pests have been sought. As phytochemicals are gaining popularity in mosquito control because they are natural, environmentally safe, less toxic, less expensive, and, most importantly, less susceptible to insect resistance, selected ornamental plants were evaluated for the detection of effective chemicals used as insecticides and against

many microbes and pests. This was proven in the research, as the results of the research showed the efficiency of ornamental plants as insecticides, antimicrobials, and antioxidants, and the four phytochemical compounds in the ADME predicted silico test showed similarity and the possibility of the intervention of that plant in the manufacture of medicine. Our data revealed that ornamental plant extracts of *B. glabra*, *D. regia*, *L. camara*, and *P. orientalis* are rich with bioactive organic compounds in addition to phenolics and flavonoids. There is no doubt that more bioactive chemicals must be discovered, and future research should focus on the discovery of botanical products with the goal of disseminating them as a reliable treatment to reduce mosquito risks and prevent mosquito-borne diseases.

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Author details

¹ Entomology Department, Faculty of Science, Benha University, Benha 13518, Egypt; ² Botany and Microbiology Department, Faculty of Science, Benha University, Benha, Qalyubiya 13518, Egypt; ³ Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta, 34517, Egypt; ⁴ National Biotechnology Network of Expertise (NBNE), Academy of Scientific Research and Technology (ASRT), Cairo, Egypt; ⁵ Zoology Department, Faculty of Science, Suez University, Suez, 43518 Egypt

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