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Repellent and insecticidal activities of *Pinus halepensis* and *Cupressus sempervirens* extracts against the West Nile virus vector *Culex pipiens*

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Mosquitoes are major disease vectors that pose serious public health threats. Extensive use of synthetic insecticides against mosquitoes has caused resistance, environmental pollution, and health risks, making it imperative to use natural alternatives. This study evaluated the larvicidal and repellent activities of *Pinus halepensis* and *Cupressus sempervirens* leaf extracts and essential oils against *Culex pipiens*, the vector of West Nile virus, and profiled their phytochemical composition. Methanol, acetone, ethyl acetate, and *n*-hexane extracts, along with EOs, were tested for larvicidal toxicity and analyzed by UPLC/MS and GC/MS. EOs exhibited the highest larvicidal potency, with LC_{50} values of 71.96 ppm for *P. halepensis* and 96.38 ppm for *C. sempervirens*, followed by solvent extracts in the order acetone > *n*-hexane > methanol > ethyl acetate. Both oils provided complete repellency (100%) for 150 min at 1% concentration. UPLC/MS identified 58 secondary metabolites, mainly flavonoids and diterpenoids, while GC/MS revealed 20 components from monoterpenes, sesqui-, di-, and triterpenes side by side with fatty acids. Biochemical assays showed that *P. halepensis* extract caused greater inhibition of acetylcholinesterase, esterases, and antioxidant enzymes (SOD, GST, CAT, and GSH), with elevated oxidative stress markers (LPO and TPC) compared to *C. sempervirens*. These findings demonstrate the potential of *P. halepensis* and *C. sempervirens* extracts, particularly EOs, as eco-friendly botanical larvicides and repellents against *Cx. pipiens*.

Keywords *Pinus halepensis*, *Culex pipiens*, *Cupressus sempervirens*, Essential oil, Larvicidal activity, Phytochemistry profile

Mosquitoes are among the most significant public health pests worldwide, responsible for transmitting numerous pathogens that cause severe diseases such as malaria, dengue, Zika virus, chikungunya, yellow fever, lymphatic filariasis, and West Nile virus[1]. Collectively, mosquito-borne diseases affect well over 700 million people annually, leading to over one million deaths, particularly in tropical and subtropical regions. Beyond their impact on human health, these diseases impose substantial economic burdens due to healthcare costs, loss of productivity, and expenditures on control programs[2,3]. Chemical insecticides have long been the primary tool for mosquito control, including organophosphates (e.g., temephos, malathion), pyrethroids (e.g., permethrin, deltamethrin), carbamates (e.g., propoxur, bendiocarb), and organochlorines (e.g., DDT)[4–7]. These compounds

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act mainly by disrupting the insect nervous system, leading to paralysis and death. Despite their initial success in reducing mosquito-borne disease transmission, the continuous and indiscriminate use of these chemicals has resulted in several drawbacks. These include the rapid development of insecticide resistance in mosquito populations, bioaccumulation of toxic residues in the environment, adverse effects on beneficial and non-target organisms, and potential risks to human health through water and food contamination[8]. Given these limitations, plant-derived compounds have gained increasing attention as promising alternatives for mosquito control. Consequently, there is an urgent demand for eco-friendly alternatives based on plant-derived bioactive compounds that are biodegradable, less toxic to humans, and effective against resistant mosquito strains[9,10].

In this context, plants represent a rich source of bioactive compounds that can serve as potential natural insecticides. Many plant species produce secondary metabolites, such as alkaloids, terpenoids, flavonoids, and phenolics, that exhibit insecticidal, repellent, and growth-regulating properties. These natural compounds are biodegradable, less toxic to humans, and often effective against resistant mosquito strains[11,12].

The active natural compounds in plants can kill eggs[4], eliminate larvae, and control insect growth[13], as well as kill adult insects and keep them away[14] as well as their safety to non-target organisms and biodegradability[15,16]. Also, essential oils (EOs), which are extracted from leaves and seeds through steam distillation, contain bioactive compounds with a wide variety of medicinal and aromatic plants.

Among such promising botanical sources are coniferous plants belonging to the families Pinaceae and Cupressaceae, which are known for their high content of terpenes, diterpenoids, and phenolic compounds with antimicrobial, antioxidant, and insecticidal activities. Conifers belong to the Pinophyta (Coniferophyta) division of the Plantae kingdom. They are cone-bearing gymnosperm plants, growing as magnificent trees or small shrubs. Various conifers are natural sources of highly efficient bioactive molecules. The essential oils from various conifers have been used since ancient times and nowadays, due to their efficient antioxidant, antimicrobial, cytotoxic, and other bioactive properties. Cedar oil is obtained from conifers belonging to the Cupressaceae (*Juniperus*, *Cryptomeria*, *Cupressus*, *Calocedrus*, *Chamaecyparis*, *Austrocedrus*, and *Thuja* species) and Pinaceae (*Cedrus* and *Pinus species*) families[17].

The Pinaceae family is the most extensive conifer family, second only to the Gymnospermae. With only one equatorial crossing species, this family consists of monoecious, resinous trees that are extensively dispersed throughout the Northern Hemisphere[18–22].

The genus *Pinus* comprises about 110 known species and is considered the oldest and the widest extant conifer genus. In terms of the economy, pines, especially their seeds, are an essential source of food, paper, timber, charcoal, resins, and decorations. In European folk medicine, pine bark has long been utilized as a food supplement. *Pinus oleoresins* have a significant role in medicinal and cosmetic applications. *Pinus* is found natively in Sumatra, south of the equator, and in the Northern Hemisphere. However, some other species are cultivated worldwide. In certain nations, the inner portion of the bark, known as cambium, is edible.

P. Brutia Ten, *P. halepensis* Miller (Aleppo pine), *P. roxburghii* Sarg., *P. canariensis* C. Sm., and *P. pinea* L. (stone pine, umbrella pine) are among the five species of the genus *Pinus* that are grown in Egypt. *Pinus* species are important economically in the pharmaceutical and cosmetic industries. Pine is utilized as an herbal antirheumatic, analgesic, and anticancer agent in Chinese medicine. It is generally recognized that *Pinus turpentine* can be used as a topical counterirritant to treat rheumatic illnesses and muscle pain, as well as in anti-aging treatments. Terpenes, flavonoids, lignans, phenols, and coumarins with a variety of bioactivities were found in *Pinus* species, according to chemical analyses. Several phenolic components, including phenolic acids, epicatechin, catechin, and taxifolin, are present in the bark extracts. Additionally, some pine species, such as *P. sibirica*, have significant levels of carotenes and vitamin C[23].

Plants from the genus *Cupressus* are members of the Cupressaceae family, which comprises coniferous plants. The genus *Cupressus* (cypress) is the second largest genus belonging to the family Cupressaceae. It comprises about 25 species of monoecious trees or large shrubs. It is mostly distributed in the northern hemisphere, including Asia, the Mediterranean region, Northwest Africa, southern China, and North and Central America. Adult trees have scale-like leaves, whereas young seedlings (up to two years old) have opposing decussate leaves that resemble needles. The woody, terminal seed cones take 1–2 years to mature after pollination. Cones of pollen are often golden and terminal. The seeds are tiny, winged, flat or angled, and numerous per scale. Because of their pyramidal or columnar crown shape, *Cupressus* species are decorative and have historically been used to cure respiratory issues, pertussis, and rheumatoid arthritis. It is well recognized that *Cupressus* members produce both volatile (EOs) and nonvolatile compounds, primarily diterpenes, flavonoids, and biflavonoids[24].

Traditional medicine employed various *Cupressus* species to cure a range of conditions, such as infection and inflammation. *Cupressus macrocarpa* Hartw. ex Gord is a well-known ornamental tree that is grown for its aesthetic qualities. Few studies have been published on the non-volatile components of C. macrocarpa, despite several contributions on the composition of the plant's EOs. Lignans, biflavonoids, and diterpenes are the main chemotaxonomic secondary metabolites of *Cupressus* plants. A panel of cancer cell lines was subjected to cytotoxic and apoptotic effects by extracts of various *Cupressus* plants, including *C. lusitanica*. Several labdane diterpenes showed notable cytotoxic effects[25].

Cupressus L., also known as Cypress, is a genus of evergreen trees that are part of the gymnosperms' Cupressaceae family. From a morphological perspective, when the trees are mature, they have little, scale-like leaves, while when the trees are young (less than two years old), they are needle-like. In both situations, they are grouped in opposite decussate pairs. After pollination, the tall, globose, or ovoid cones mature in 18 to 24 months. Each side of the tiny seeds has a single, slender wing. Despite being extensively grown, *Cupressus* species are indigenous to a few temperate regions of the Northern Hemisphere, such as western North America, Central America, northwest Africa, the Middle East, the Himalayas, southern China, and northern Vietnam[26]. However, comparative studies evaluating the larvicidal and biochemical activities of different solvent extracts and EOs of *Cupressus sempervirens* and *Pinus halepensis* against *Culex pipiens* are still lacking.

This study presents an integrated evaluation of the larvicidal, repellent, and biochemical effects of different solvent extracts (methanol, acetone, ethyl acetate, *n*-hexane) and EOs of *C. sempervirens* and *P. halepensis* against *Cx. pipiens* larvae. Unlike previous studies that have primarily examined a single extract type or EOs, this work combines chemical profiling with bioassays and enzymatic analyses to elucidate the underlying mechanisms of toxicity. The extracts were characterized using UPLC/MS and GC/MS to identify major phytoconstituents responsible for larvicidal activity. In addition to determining lethal concentrations (LC_{50}, LC_{90}) for each extract, the study assessed alterations in key metabolic and antioxidant enzymes to clarify how these coniferous plant derivatives disrupt mosquito physiology. This comprehensive approach offers fresh viewpoints about the potential of *P. halepensis* and *C. sempervirens* as eco-friendly, plant-based larvicides for sustainable vector control.

Materials and methods

Plant materials and analysis

Plant collection

Needles from two coniferous species, *Pinus halepensis Mill*. (Aleppo pine) and *Cupressus sempervirens* L. (Mediterranean cypress), were gathered in August 2024 from agricultural fields belonging to the Faculty of Agriculture in Qalyubiya Governorate, Egypt (30° 21'16" N; 31° 13'21" E). The collection of plants was conducted in accordance with all relevant institutional, national, and international guidelines and legislation. Approval for the collection of plant material was obtained from the relevant authorities at the Faculty of Agriculture, Qalyubia Governorate, Egypt. The botanical identity of the collected specimens was verified by Dr. Trease Labib, a specialist affiliated with the Egyptian Ministry of Agriculture. A voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, under the reference number (*Cupressus*: PHG-P-CS-553; *Pinus*: PHG-P-PH-554). No formal ethical approval was required in accordance with local regulations.

Plant extraction

The collected leaves were air-dried in the shade at ambient temperature (27 ± 2 °C) for 8–10 days, then finely ground using a stainless-steel electric grinder. The resulting powder was stored in airtight containers to prevent moisture absorption. Extraction was carried out using a Soxhlet apparatus at approximately 27 ± 2 °C, employing various solvents (methanol, acetone, ethyl acetate, and *n*-hexane). For each solvent, 30 g of the ground leaf material was extracted separately using 200 milliliters of solvent. After a 48-hour extraction period, the extracts were concentrated using a rotary evaporator and stored in dark glass bottles to protect them from light exposure[27]. Plant extract EOs was purchased from Dow Egyptian companies.

Phytochemical identification and analysis

UPLC/MS analysis

The UPLC/ESI/MS analysis was performed for different *Pinus* and *Cupressus*extracts using the method of El-Tabakh et al.[28], Elhawary et al.[29], Shehata et al.[30] and Sobeh et al.[31]. UPLC/ESI/MS in both positive and negative ion acquisition modes was carried out on a XEVO TQD triple quadrupole instrument, Waters Corporation, Milford, MA 01757, U.S.A., mass spectrometer. Chromatographic separation of the sample was done by injecting 10 µl into a UPLC instrument equipped with a reverse-phase C-18 column (ACQUITY UPLC—BEH, 2.1 × 50 mm column; 1.7 µm particle size). The sample (100 µg/mL) solution was prepared using HPLC-grade methanol, filtered using a membrane disc filter (0.2 µm), degassed by sonication before injection, and then subjected to LC/ESI/MS analysis. The gradient mobile phase comprises two eluents: eluent A is H_2O acidified with 0.1% formic acid, and eluent B is MeOH acidified with 0.1% formic acid. Elution was made at a flow rate of 0.2 mL/min as follows: (10% B) from 0 to 5 min.; (30% B) from 5 to 15 min.; (70% B) from 15 to 22 min.; (90% B) from 22 to 25 min.; and (100% B) from 25 to 29 min. The analysis was accomplished using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were recorded in electrospray ionization (ESI) (negative and positive ion modes) (m/z 100–1000). UPLC/MS data were processed using Masslynx 4.1 software, and tentative identification was done by comparing their retention times (Rt), mass spectra, and fragmentation patterns with reported data[32].

GC/MS analysis

Pinus and *Cupressus* EOs and *n*-hexane extracts were injected into a gas chromatography coupled to mass spectrometry (Shimadzu GCMS-QP 2010, Kyoto, Japan) operating in EI mode at 70 eV, and mass spectrum acquisition was performed in the mass range of 35–500 amu. The instrument was equipped with an Rtx-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness: Restek, USA). One microliter sample was injected in a split injection mode with a split ratio of 10:1. Separation was achieved using an initial oven temperature at 45 °C for 2 min (isothermal), then gradually increased to 300 °C at a rate of 5 °C/min (ramp) and kept constant at 300 °C for another 5 min (isothermal). Helium was used as a carrier gas with a flow rate set at 1.4 mL/min. Injector temperature was maintained at 250 °C. The mass unit interface temperature was set at 280 °C, and the ion source temperature was adjusted to 200 °C. Retention indices (RI) were calculated relative to a homologous series of n-alkanes (C8-C30) injected under the same GC conditions. Identification of the compounds was performed by comparing their mass spectra and retention indices with the data reported in the NIST-17 and Wiley library databases[33].

Multivariate data analysis using PCA and clustered heat map

The unsupervised principal component analysis (PCA) was performed using Unscrambler X 10.3 (CAMO SA, Oslo, Norway). A clustered heat map was built using NCSS, 12 software with Euclidean distances, and the unweighted pair group method[34].

Mosquito larvicidal assay

Rearing of Culex pipiens

Culex pipiens larvae were reared under controlled insectary conditions, maintained at a temperature of 27 ± 2 °C with relative humidity of 75 ± 5%, and a consistent 12:12 h light-to-dark cycle. The larvae were nourished with a mixture of Tetramin fish food (47% crude protein, 16% crude fat and fiber, 9% ash, 6% phosphorus, and essential vitamins and minerals) and finely ground bread in a 3:1 proportion. Once they reached the pupal stage, they were transferred from enamel trays into containers with dechlorinated water and placed inside mesh cages measuring 35 × 35 × 40 cm, where adult mosquitoes subsequently emerged. Female mosquitoes were periodically offered blood meals from a hamster, while the adult population was continuously supplied with a 10% sugar solution. All bioassays were performed in the same laboratory, involving both larval and adult stages of the mosquito[34,35].

Larvicidal activity

Larvicidal activity of different solvent extracts and EOs of *P. halepensis* and *C. sempervirens* was evaluated against *Cx. pipiens* 3rd instar larvae according to WHO[36]. Larvae were exposed to a range of extract concentrations: 25, 50, 125, 250, 500, and 1000 ppm (equivalent to 1 g per 1000 mL of distilled water). Essential oils were emulsified with 0.2 mL ethanol and 0.05% tween-80 in distilled water to ensure proper dispersion. For each concentration, twenty-five larvae were placed in glass beakers containing 250 mL of distilled water, with four replicates prepared per treatment level (n = 100 larvae per concentration). Mortality rates were assessed at 24 and 48 h following exposure and after post-treatment intervals[36,37]. Percentage mortality was calculated and corrected using Abbott's formula[38] when necessary. Larvae were exposed to water containing 0.2 mL of alcohol in distilled water as a negative control, and Temephos (97.5% purity) was used as the positive control.

Biochemical and enzymatic and oxidative stress assays

Preparation of tissue homogenates

For biochemical analysis, 3rd instar *Cx. pipiens* larvae were collected 24 h post-treatment from each test concentration, corresponding approximately to the LC_{50} value of each extract or EOs. Only live larvae that survived exposure were selected to evaluate sublethal biochemical changes. For each replicate, 50–70 larvae (≈0.5 g fresh weight) were pooled and homogenized in 5 mL of ice-cold phosphate buffer (0.1 M, pH 7.4) using a glass homogenizer. Homogenization was performed in Eppendorf tubes using a manual homogenizer at approximately 4 °C. The homogenates were centrifuged at 10,000 × g for 10 min at 4 °C to remove debris, and the resulting clear supernatants were used for subsequent enzyme assay.

Activity of acetylcholinesterase (AChE) enzyme

The activity of AChE was assessed using a modified Ellman's method[39]. In brief, the AChE activity was determined by adding 0.3 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich) and 0.3 mM acetylthiocholine iodide (ATChI, Sigma-Aldrich) at 30 °C and measuring absorbance at 412 nm over a 5-minute period using a spectrophotometer.

Activity of esterase enzyme

The clear supernatants were used for α- and β-esterase activity assays using the method described by Penilla et al.[40] with minor modifications in a 96-well microplate. For the α-esterase assay, 200 μL of α-naphthyl acetate (α-NA) solution (100 µL of 30 mM α-NA in acetone in 10 mL of 0.02 M sodium phosphate buffer, pH 7.2) was added to 10 μL of homogenate in a well. Similarly, for the β-esterase assay, 200 µL of β-NA solution (100 μL of 30 mM β-NA in acetone in 10 mL of 0.02 M sodium phosphate buffer, pH 7.2) was added to 10 μL of homogenate in another well simultaneously. The reactions were incubated for 15 min at room temperature and then stopped by adding 50 μL of *O*-dianisidine stain (a mixture of 22.5 mg *O*-dianisidine in 2.25 mL distilled water and 5.25 mL of 5% sodium lauryl sulfate in 0.1 M sodium phosphate buffer, pH 7.0). Control wells contained 10 μL of distilled water in place of homogenate, 200 μL of α-NA or β-NA solution, and 50 µL of *O*-dianisidine stain. Endpoint enzyme activity was measured at 570 nm in an ELISA reader. The total protein was determined according to the method suggested by Bradford[41] using bovine serum albumin (BSA) as a protein standard. The activities of α- and β-esterase in the individual samples were expressed as mmoles of product formed/min/mg protein based on the α- and β-naphthol standard curves, respectively.

Activity of gamma amino butyric acid transaminase (GABA-T) enzyme

The activity of GABA-T was assessed using the method outlined by Boer and Bruinvels[42] with modifications proposed by Pandey and Singh[43]. The reaction buffer consisted of 50 mM Tris-HCl (pH 8.5) and included 100 μL of 2 mM α-ketoglutarate (pH 7), 100 µL of 20 mM 2-mercaptoethanol, and 20 μL of 1.1 mM β-NAD. The reaction was started by adding 200 µL of 3 mM *gamma*-aminobutyric acid (GABA). Following a 30-minute incubation at 25 °C, the absorbance was measured at 340 nm.

Activity of amylase enzyme

The amylase enzyme activity was assessed using the method outlined by[44]. The assay mixture comprised 1 mL of 1% soluble starch substrate, 1 mL of 0.1 M phosphate buffer at the appropriate pH, and 0.5 mL of supernatant.

The mixture was then incubated at 40 °C for 10 min. To stop the reactions, 2 mL of 3,5-dinitrosalicylic acid (DNSA) reagent and 2 mL of distilled water were added. The test tubes were heated in a boiling water bath for 55 min, cooled, and the product's color intensity was measured at 540 nm.

Activity of lipase enzyme

The lipase enzyme activity was assessed using the method demonstrated by Itaya[45]. The assay mixture contained 0.25 mL of substrate (a combination of gum acacia and olive oil) in an appropriate buffer, 0.25 mL of supernatant, and 1 mL of Tris-HCl buffer (pH 7.5). The mixture was incubated at 40 °C for 10 min. To stop the reactions, 2 mL of ATC reagent and 10 mL of chloroform were added. The reaction mixture was shaken and left to stand for 10 min. Subsequently, 2 mL of the chloroform layer was transferred to a well-stoppered tube, and 1.5 mL of lipase coloring reagent was added to detect liberated free fatty acids, producing a color change. The color intensity was measured colorimetrically at 550 nm.

Markers of the oxidative stress

Superoxide dismutase (SOD) activity It was assayed in the tissue homogenates by the method suggested by Nishikimi et al.[46]. Results were defined as units per milligram of protein (U/mg protein/min). The homogenates were prepared in cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 × g for 15 min at 4 °C. The reaction mixture contained 50 mM sodium carbonate solution, 0.1 mM EDTA, 13 mM methionine, 75 μM nitroblue tetrazolium (NBT), and 2 μM riboflavin. The samples were illuminated for 10 min, and the absorbance was measured at 560 nm. The SOD activity unit was defined as the amount of enzyme that inhibits NBT reduction by 50%.

Glutathione S-transferases (G-S-T) activity The activity of GST was determined according to the method suggested by Habig et al.[47] by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. which measures the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH). The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the G-S-T activity in the sample. The result was expressed as units per gram of tissue. The reaction mixture consisted of 1.0 mM GSH, 1.0 mM CDNB, and 100 mM phosphate buffer (pH 6.5). The enzymatic reaction was initiated by adding tissue supernatant and monitored at 340 nm for 3 min. GST activity was calculated using the extinction coefficient $\varepsilon = 9.6\ mM^{-1}\ cm^{-1}$.

Catalase (CAT) activity It was assayed in the tissue homogenates by the method suggested by Abedi and Pakniyat[48]. Results were defined as units per milligram of protein (U/mg protein/min). CAT activity was quantified by monitoring the decomposition of hydrogen peroxide (H_2O_2). The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and freshly prepared 30 mM H_2O_2. The decrease in absorbance at 240 nm was recorded for 1–3 min. Activity was calculated using the molar extinction coefficient for H_2O_2 (43.6 M cm^{-1}).

Reduced glutathione (GSH) It was quantified in the tissue homogenates by the method suggested by Beutler et al.[49]. Results were expressed as µg/mg protein. The assay was based on the reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) to form TNB, measured at 412 nm. Tissue homogenates were deproteinized using 5% sulfosalicylic acid and centrifuged at 10,000 × g for 10 min before analysis. GSH concentration was calculated from a standard curve (0–100 µg/mL).

Lipid peroxidation product (LPO) The LPO was expressed as malondialdehyde (MDA), which is the end product of the lipid peroxidation reaction. It was determined as a thiobarbituric acid reactive substance (TBARs) according to the method of Ohkawa et al.[50]. Tissue supernatant was mixed with 1% phosphoric acid and 0.6% thiobarbituric acid (TBA), then heated at 95 °C for 45 min. After cooling, samples were extracted with *n*-butanol and centrifuged. The absorbance of the organic phase was measured at 532 nm, using an MDA extinction coefficient of 1.56×10^5 M cm^{-1}. The results were expressed as nmol/mg protein.

Total protein carbonyl content (TPC) The TPC content was assessed using the protocol described by Levine et al.[51]. The results were expressed as nmol of reactive carbonyl compounds per mg of tissue protein. Protein carbonyls were derivatized using 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl and incubated for 1 h in the dark. After precipitation with 20% TCA and washing with ethanol–ethyl acetate (1:1), pellets were dissolved in 6 M guanidine hydrochloride. Absorbance was measured at 370 nm, using $\varepsilon = 22{,}000\ M\ cm^{-1}$ for calculation.

Statistical analysis

Data were expressed as mean ± standard error (SE). Statistical differences among treatments were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test to separate means when significant differences were detected ($p < 0.05$). LC_{50} and LC_{90} values with 95% confidence limits were calculated by probit analysis using SPSS software version V23 (IBM, USA).

Results

Effects of plant extract on *Culex pipiens*

The toxicological effects of *Cupressus sempervirens* and *Pinus halepensis* extracts against *Cx. pipiens* larvae at different concentrations were presented in Tables 1, 2, 3, and 4. The tested botanical insecticides appear to be especially promising as larvicidal agents against *Cx. pipiens* based on the type of extract and the concentration. The most effective plant extract was *P. halepensis*, followed by *C. sempervirens*. On the other hand, the essential oil of each tested larvicide exhibited higher efficiency than the other leaf extract solvents. The results showed that

Plant type	Solvent	Concentration (ppm)						
		0	25	50	125	250	500	1000
Cupressus sempervirens	Methanol	0 ± 0^{aG}	12 ± 1.63^{dF}	30 ± 1.15^{dE}	45 ± 1.00^{dD}	64 ± 1.63^{dC}	80 ± 2.83^{cB}	100 ± 0.00^{aA}
	Acetone	0 ± 0^{aG}	15 ± 2.52^{cF}	35 ± 1.91^{cE}	54 ± 3.83^{cD}	75 ± 3.42^{cC}	94 ± 2.58^{bB}	100 ± 0.00^{aA}
	Ethyl acetate	0 ± 0^{aG}	9 ± 1.00^{eF}	26 ± 2.00^{eE}	40 ± 1.63^{eD}	60 ± 2.31^{eC}	75 ± 4.43^{dB}	99 ± 1.00^{aA}
	n-Hexane	0 ± 0^{aF}	19 ± 1.91^{bE}	48 ± 2.83^{bD}	70 ± 2.58^{bC}	90 ± 2.00^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Essential oil	0 ± 0^{aE}	22 ± 2.58^{aD}	47 ± 3.00^{aC}	80 ± 1.63^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Pinus halepensis	Methanol	0 ± 0^{aG}	11 ± 1.91^{cF}	27 ± 1.00^{cE}	50 ± 2.58^{cD}	80 ± 2.83^{cC}	95 ± 2.52^{bB}	100 ± 0.00^{aA}
	Acetone	0 ± 0^{aF}	16 ± 1.63^{bE}	33 ± 2.52^{bD}	55 ± 3.00^{bC}	85 ± 3.00^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Ethyl acetate	0 ± 0^{aG}	9 ± 1.00^{dF}	21 ± 1.91^{dE}	33 ± 3.42^{eD}	60 ± 1.63^{eC}	72 ± 3.27^{dB}	98 ± 2.00^{bA}
	n-Hexane	0 ± 0^{aG}	10 ± 1.15^{cdF}	27 ± 1.00^{cE}	48 ± 1.63^{dD}	80 ± 2.31^{dC}	90 ± 4.16^{cB}	100 ± 0.00^{aA}
	Essential oil	0 ± 0^{aE}	31 ± 2.52^{aD}	68 ± 2.83^{aC}	94 ± 2.58^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Temephos (1 mg/L)		85%						

Table 1. Efficacy of *Cupressus sempervirens* and *Pinus halepensis* extracts on *Culex pipiens* larval mortality, 24 h post-treatment. a, b & c: There is no significant difference ($P > 0.05$) between any two means for each plant extract, within the same column have the same superscript letter. A, B & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter. No mortality was observed in negative controls and Temephos was used as positive control, inducing 85% mortality within 24 h post-treatment.

Plant type	Solvent	Concentration (ppm)						
		0	25	50	125	250	500	1000
Cupressus sempervirens	Methanol	0 ± 0^{aG}	14 ± 2.58^{dF}	32 ± 0.00^{dE}	50 ± 2.58^{dD}	77 ± 4.12^{dC}	97 ± 1.91^{bB}	100 ± 0.00^{aA}
	Acetone	0 ± 0^{aG}	17 ± 1.00^{cF}	36 ± 2.83^{cE}	55 ± 1.00^{cD}	80 ± 3.27^{cC}	97 ± 3.00^{bB}	100 ± 0.00^{aA}
	Ethyl acetate	0 ± 0^{aG}	11 ± 1.91^{eF}	28 ± 2.31^{eE}	42 ± 2.58^{eD}	75 ± 3.42^{eC}	90 ± 4.16^{cB}	100 ± 0.00^{aA}
	n-Hexane	0 ± 0^{aF}	22 ± 2.58^{bE}	51 ± 5.00^{bD}	75 ± 3.42^{bC}	96 ± 2.83^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Essential oil	0 ± 0^{aE}	30 ± 1.15^{aD}	65 ± 2.52^{aC}	90 ± 2.00^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Pinus halepensis	Methanol	0 ± 0^{aF}	19 ± 1.91^{cE}	37 ± 3.00^{cD}	62 ± 2.58^{cC}	90 ± 2.58^{cB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Acetone	0 ± 0^{aF}	21 ± 2.52^{bE}	40 ± 3.65^{bD}	70 ± 3.83^{bC}	95 ± 3.79^{bB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
	Ethyl acetate	0 ± 0^{aG}	11 ± 1.91^{eF}	30 ± 2.00^{eE}	50 ± 2.58^{eD}	75 ± 4.43^{dC}	95 ± 5.00^{cB}	100 ± 0.00^{aA}
	n-Hexane	0 ± 0^{aF}	13 ± 2.52^{dE}	35 ± 1.91^{dD}	59 ± 2.52^{dC}	89 ± 6.19^{cB}	99 ± 1.00^{bA}	100 ± 0.00^{aA}
	Essential oil	0 ± 0^{aE}	37 ± 3.00^{aD}	78 ± 2.58^{aC}	99 ± 1.00^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Temephos (1 mg/L)		100%						

Table 2. Efficacy of *Cupressus sempervirens* and *Pinus halepensis* extracts on *Culex pipiens* larval mortality, 48 h post-treatment. a, b & c: There is no significant difference ($P > 0.05$) between any two means for each plant extract, within the same column have the same superscript letter. A, B & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter. No mortality was observed in negative controls and Temephos was used as positive control, inducing 100% mortality within 48 h post-treatment.

Time (hr)	Treatment	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	X^2 (sign.)
24	Methanol	249.08 (149.40–389.39.40.39)	1364.05 (1001.49–3319.41.49.41)	2208.89 (1648.40–6348.93.40.93)	1.735 ± 0.124	16.112 (0.002)
	Acetone	181.77 (155.68–210.12.68.12)	817.77 (668.66–1046.42.66.42)	1251.77 (984.37–1693.70.37.70)	1.962 ± 0.135	8.813 (0.065)
	Ethyl acetate	296.94 (176.37–481.84.37.84)	1598.32 (1195.10–4234.99.10.99)	2575.62 (1977.19–8153.71.19.71)	1.753 ± 0.125	17.908 (0.001)
	n-Hexane	120.81 (103.69–138.95.69.95)	447.67 (373.59–559.38.59.38)	648.95 (523.22–852.47.22.47)	2.253 ± 0.164	5.979 (0.200)
	Essential oil	96.38 (82.33–112.18.33.18)	298.01 (246.63–353.43.63.43)	401.00 (325.61–510.08.61.08)	2.744 ± 0.213	5.983 (0.200)
48	Methanol	188.72 (164.66–220.47.66.47)	858.30 (671.78–1070.58.78.58)	1245.09 (975.99–1712.83.99.83)	2.018 ± 0.142	9.443 (0.050)
	Acetone	156.83 (133.32–181.94.32.94)	710.08 (601.12–884.32.12.32)	1072.97 (869.10–1400.28.10.28)	2.018 ± 0130	7.208 (0.125)
	Ethyl acetate	237.21 (203.23–275.09.23.09)	1154.30 (924.59–1522.95.59.95)	1807.67 (1385.24–2536.89.24.89)	1.865 ± 0.131	5.345 (0.253)
	n-Hexane	106.55 (91.37–122.26.37.26)	381.95 (326.64–459.45.64.45)	548.49 (456.26–686.93.26.93)	2.311 ± 0.154	2.726 (0.681)
	Essential oil	75.12 (64.34–86.93)	221.01 (186.52–275.97.52.97)	298.99 (243.75–394.45.75.45)	2.768 ± 0.255	2.446 (0.654)

Table 3. Lethal concentrations (ppm) *Cupressus sempervirens* extracts on *Culex pipiens* mortality, 24 and 48 h post-treatment.

Time (hr)	Treatment	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	X^2 (sign.)
24	Methanol	210.41 (152.72–291.50)	876.02 (651.45-152.89.45.89)	1307.28 (952.73–2508.07.73.07)	2.089 ± 0.145	9.590 (0.047)
	Acetone	141.33 (101.56–202.28.56.28)	553.90 (416.83–960.43.83.43)	815.32 (600.51–1580.36.51.36)	2.286 ± 0.154	12.473 (0.014)
	Ethyl acetate	336.34 (202.23–552.26.23.26)	1756.98 (1327.30–4749.16.30.16)	2807.36 (2180.31–9069.92.31.92)	1.785 ± 0.127	18.364 (0.001)
	n-Hexane	190.46 (162.19–303.72.19.72)	914.98 (686.43–1518.15.43.15)	1361.01 (1002.67–2468.74.67.74)	2.106 ± 0.139	9.683 (0.046)
	Essential oil	71.96 (60.92–82.89)	200.72 (168.56–230.11.56.11)	258.47 (220.01–330.00)	2.876 ± 0.236	0.896 (0.925)
48	Methanol	140.68 (121.77–160.67.77.67)	507.20 (424.76–629.98.76.98)	779.56 (591.36–949.37.36.37)	2.301 ± 0.161	9.017 (0.060)
	Acetone	102.70 (91.37–128.08.37.08)	390.77 (327.88–480.30)	563.99 (455.58–741.25.58.25)	2.427 ± 0.176	6.220 (0.183)
	Ethyl acetate	202.77 (175.94–232.09.94.09)	720.71 (644.58–990.63.58.63)	1151.84 (926.68–1525.58.68.58)	2.148 ± 0.142	8.902 (0.063)
	n-Hexane	128.00 (137.90–179.73.90.73)	537.40 (452.74–661.85.74.85)	760.33 (621.40–977.41.40.41)	2.412 ± 0.163	6.763 (0.148)
	Essential oil	56.55 (49.74–68.90)	115.54 (108.47–146.56.47.56)	166.14 (132.46–210.26.46.26)	3.703 ± 0.434	0.147 (0.797)

Table 4. Lethal concentrations (ppm) *Pinus halepensis* extracts on *Culex pipiens* mortality, 24 and 48 h post-treatment.

the mortality rate of the 3rd larval instars after 24 post-treatment (PT) of *C. sempervirens* extracts (methanol, acetone, ethyl acetate, *n*-hexane, and EOs) was expressed as 100% mortality at 1000 ppm except for ethyl acetate (99% mortality), while they reached 80, 94, 75, 100, and 100% at 500 ppm, respectively (Table 1). The corresponding results for *P. halepensis* extracts (methanol, acetone, ethyl acetate, *n*-hexane, and essential oil) are expressed as 95, 100, 72, 90, & 100% and 100, 100, 98, 100, & 100% for 500 and 1000 ppm, respectively (Table 1).

All leaf extract solvents and EOs from *C. sempervirens* and *P. halepensis* killed all the test larvae (100% MO) at a concentration of 1000 ppm, 48 h post-treatment. At a concentration of 500 ppm, the mortality rates for *C. sempervirens* extracts were 97%, 97%, 90%, and 100% for methanol, acetone, ethyl acetate, *n*-hexane, and EOs, while the rates for P. halepensis extracts were 100%, 100%, 95%, 99%, and 100% (Table 2). The positive control (temephos) caused 85% and 100% larval mortality within 24 and 48 h ($p < 0.05$), respectively, while no mortality was recorded in the negative control (dechlorinated water).

The LC_{50} values for *C. sempervirens* extracts (methanol, acetone, ethyl acetate, *n*-hexane, and EOs) were 249.08, 181.77, 296.94, 120.81, and 72.96 ppm after 24 h post-treatments, and 192.73, 166.83, 237.21, 106.55, and 60.55 ppm after 48 h post-treatments (Table 3; Fig. 1a). The LC_{50} values for *P. halepensis* extracts (methanol, acetone, ethyl acetate, *n*-hexane, and EOs) were 213.41, 161.33, 336.34, 225.46, and 100.38 ppm after 24 h post-treatments, and 140.68, 122.70, 202.77, 158, and 76.12 ppm after 48 h post-treatments (Table 4; Fig. 1b). The data showed that the EOs from both larvicides tested was more harmful to *Cx. pipiens* larvae than other leaf extract solvents. *C. sempervirens* EOs worked better ($LC_{50} = 72.96$) than *P. halepensis* EOs oil ($LC_{50} = 100.38$ ppm) (Tables 3 and 4).

Efficiency of essential oils on *Culex pipiens* larvae

At 60 min of exposure, the effectiveness of the tested plant EOs against the *Cx. pipiens* adult was tested. The knockdown rate (K) and 50% knockdown time (LT_{50} min) values were determined. Mosquito adult were more susceptible to *C. sempervirens* ($LT_{50} = 18.54$ at 4% conc. with knockdown = 100). The knockdown % for *Cx. pipiens* adult exposed to *C. sempervirens* EOs was 26.67, 33.33, 53.33, 100 and 100 with LT_{50} (min) rate were 176.56, 142.04, 59.51, 24.69, and 18.54 for 0.1, 0.5, 1.0, 2.0, and 4.0%, respectively. The corresponding results for *Cx. pipiens* adult exposed to *P. halepensis* EOs was 17.78, 26.67, 42.22, 42.22 and 91.11 knockdown % and 287.15, 196.06, 92.06, 100.26 and 29.72 for knockdown time at 0.1, 0.5, 1.0, 2.0, and 4.0%, respectively (Table 5).

Repellence activity

The repellency activity test showed the repellency percentage values exhibited for the tested plant EOs against *Cx. pipiens* adult at four concentrations (0.05, 0.25, 0.5 and 1%). The repellency activity of the EOs was time and concentration dependent. Complete protection was obtained at all test concentrations for 60 min, except for the lowest concentration (0.05 and 0.25%) of *C. sempervirens* and *P. halepensis* EOs. The highest concentration (1%) provided 100% protection for 150 min for two tests botanical EOs (Table 6).

Biochemical and oxidative stress of treated mosquito larvae

The data presented in Table 7 showed that the levels of acetylcholinesterase (AChE), α-esterase (α-EST), and β-esterase (β-EST) in treatment larvae with *P. halepensis* oil led to a significant ($p \leq 0.05$) reduction in the activities of AChE, α-EST, and β-EST (5.01 ± 0.03 nmol AChE hydrolyzed/min/mg protein, 1.13 ± 0.04 mmol/min/mg protein, and 1.59 ± 0.02 mmol/min/mg protein, respectively). Similarly, with exposure to *C. sempervirens* oil, there was a significant ($p \leq 0.05$) decrease in the activities of AChE, α-EST, and β-EST (7.81 ± 0.05 nmol AChE hydrolyzed/min/mg protein, 1.77 ± 0.07 mmol/min/mg protein, and 2.47 ± 0.03 mmol/min/mg protein, respectively) compared to the control larvae, 9.77 ± 0.06 nmol AChE hydrolyzed/min/mg protein, 2.21 ± 0.08 mmol/min/mg protein, and 3.09 ± 0.04 mmol/min/mg protein, respectively. Upon. Therefore, *P. halepensis* oil demonstrated higher biological efficiency against the activities of AChE, α-EST, and β-EST enzymes.

Data presented in Table 8 showed that the levels of gamma-aminobutyric acid transaminase (GABA-T), amylase, and lipase enzymes in treated larvae with *P. halepensis* oil also caused a significant ($p \leq 0.05$) decrease in enzyme activities (2.34 ± 0.02 mg/L, 10.21 ± 0.02 units/g tissue, and 1.03 ± 0.02 units/g tissue, respectively) compared to the control larvae (4.56 ± 0.04 mg/L, 19.90 ± 0.03 units/g tissue, and 2.01 ± 0.03 units/g tissue,

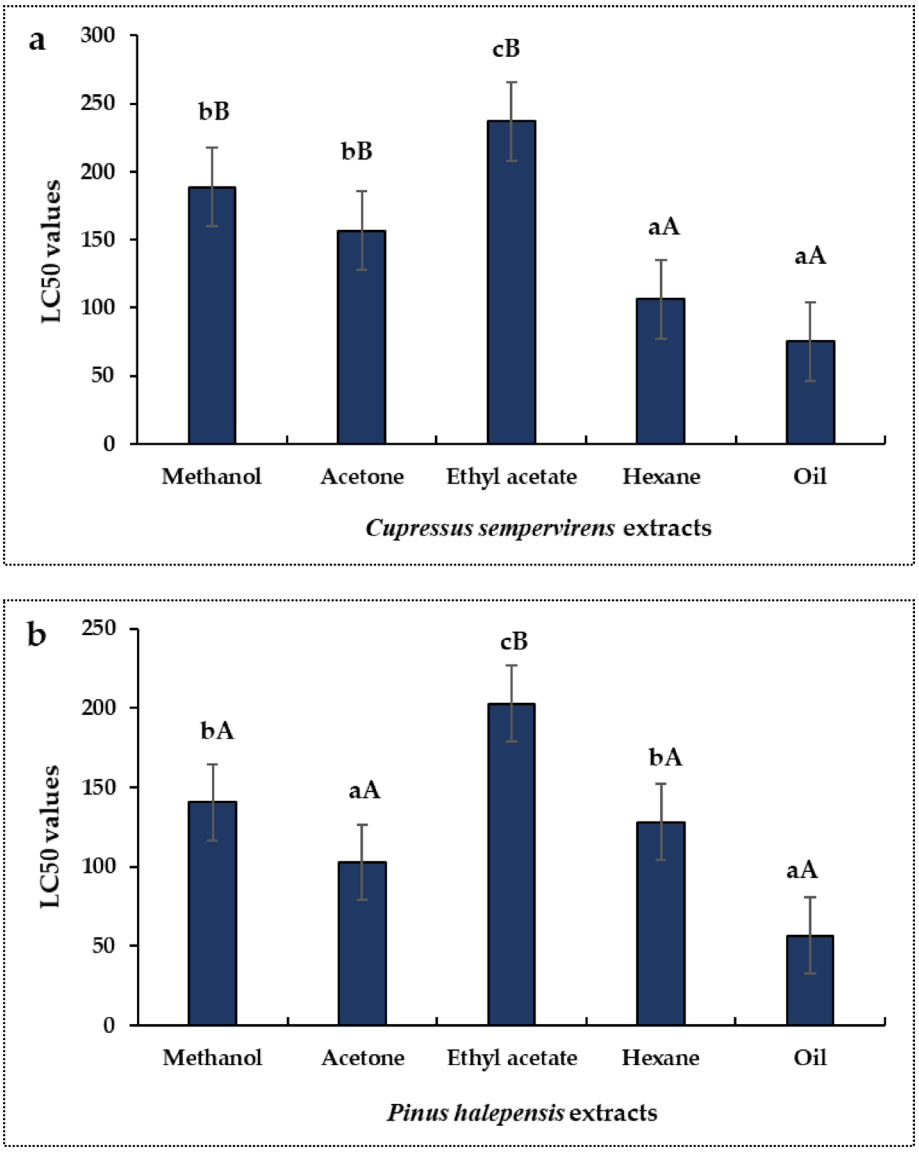

Fig. 1. Larvicidal activity of different solvent extracts and essential oils of *Pinus halepensis* and *Cupressus sempervirens* against 3rd instar larvae of *Culex pipiens* (mean ± SE) 48 h post-exposure. Different lowercase letters above bars indicate statistically significant differences among treatments according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Statistical analyses were performed using SPSS version V23 (IBM, USA).

Plant tested	Conc.%	Knockdown %[a]	LT_{50} (Low. - Up.)	LT_{95} (Low. - Up.)	Slope ± SE	Chi (Sig.)	mortality %[b]
Cupressus sempervirens	0.1	26.67 ± 3.85^{dB}	176.56 (102.94–512.36.94.36)	2925.08 (848.86–37238.23.86.23)	1.349 ± 0.2405	0.282 (0.963)	37.78 ± 4.45^{cA}
	0.5	33.33 ± 0.00^{cB}	142.04 (87.17–355.13.17.13)	2806.23 (848.95–29567.00)	1.269 ± 0.214	1.325 (0.723)	53.33 ± 3.85^{bA}
	1	53.33 ± 3.85^{bB}	59.51 (46.13–86.65)	699.83 (349.35–2181.00)	1.536 ± 0.193	1.784 (0.618)	97.78 ± 2.22^{aA}
	2	100 ± 0.00^{aA}	24.69 (20.11–27.15)	108.56 (103.33–119.34.33.34)	2.557 ± 0.210	50.27 (0.000)	100 ± 0.00^{aA}
	4	100 ± 0.00^{aA}	18.54 (13.44–22.14)	96.79 (90.12–122.00)	2.291 ± 0.191	31.679 (0.000)	100 ± 0.00^{aA}
Pinus halepensis	0.1	17.78 ± 2.22^{dB}	287.15 (277.45–295.11.45.11)	4425.57 (979.30–17555.67.30.67)	1.384 ± 0.298	0.313 (0.957)	28.89 ± 2.22^{dA}
	0.5	26.67 ± 0.00^{cB}	196.06 (101.16–778.70)	5937 (1255.19–177403.92.19.92)	1.110 ± 0.213	1.456 (0.723)	42.22 ± 2.22^{cA}
	1	42.22 ± 4.45^{bB}	92.06 (64.72–165.34.72.34)	1324.78 (536.04–6616.05.04.05)	1.420 ± 0.206	0.990 (0.503)	64.45 ± 2.22^{bA}
	2	42.22 ± 2.22^{bB}	100.26 (66.81–204.01.81.01)	1083.59 (720.78–16074.24.78.24)	1.239 ± 0.194	1.767 (0.622)	100 ± 0.00^{aA}
	4	91.11 ± 2.22^{aB}	29.72 (24.25–33.75)	139.62 (130.55–145.12.55.12)	2.244 ± 0.213	29.585 (0.000)	100 ± 0.00^{aA}

Table 5. Knockdown time and mortality % of *Culex pipiens* adult mosquitoes exposed to different concentrations of essential oils for 60 min. (mean ± SE). a: knockdown % for 60 min; b: mortality % for 24 h exposure.

Plant tested	Conc.	Repellency % (mean ± S.E)						
		5*	15	30	60	90	120	150
Cupressus sempervirens	0.05	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.55 ± 2.22^{bB}	64.44 ± 4.44^{cC}	46.67 ± 0.00^{dD}	31.11 ± 2.22^{dE}
	0.25	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	88.89 ± 2.22^{bB}	60.00 ± 3.85^{cC}	48.89 ± 2.22^{cD}
	0.5	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	91.11 ± 2.22^{bB}	77.78 ± 2.22^{bC}
	1.0	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}
Pinus halepensis	0.05	100 ± 0.00^{aA}	100 ± 0.00^{aA}	95.56 ± 4.44^{bB}	75.55 ± 2.22^{dC}	53.33 ± 3.85^{dD}	33.33 ± 3.85^{dE}	20.00 ± 0.00^{dF}
	0.25	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	88.89 ± 2.22^{cB}	77.78 ± 2.22^{cC}	53.33 ± 0.00^{cD}	35.55 ± 2.22^{cE}
	0.5	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	97.78 ± 2.22^{bB}	93.33 ± 3.85^{bC}	82.22 ± 2.22^{bD}	62.22 ± 2.22^{bE}
	1.0	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}	100 ± 0.00^{aA}

Table 6. Repellent activity of *Cupressus sempervirens* and *Pinus halepensis* essential oils against adult mosquitoes *Culex pipiens* exposed to 150 min. (mean ± SE). a, b & c: There is no significant difference ($P > 0.05$) between any two means for each plant tested, within the same column have the same superscript letter. A, B & C: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter. *: time after application (minutes).

Plant extracts		Acetylcholinesterase (AChE) (nmol ACh hydrolyzed/min/mg protein)	α-esterase (α-EST) (mmol/min/mg protein)	β-esterase (β-EST) (mmol/min/mg protein)
Control		9.77 ± 0.06^{a}	2.21 ± 0.08^{a}	3.09 ± 0.04^{a}
Treated	*C. sempervirens*	7.81 ± 0.05^{ab}	1.77 ± 0.07^{ab}	2.47 ± 0.03^{ab}
	P. halepensis	5.01 ± 0.03^{b}	1.13 ± 0.04^{b}	1.59 ± 0.02^{b}

Table 7. The effect of *Cupressus sempervirens* and *Pinus halepensis* essential oils against the activities of different esterase enzymes in mosquitoes. Values were calculated from three replicates and expressed as mean ± SE, , a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter

Plant extracts		Gamma amino butyric acid transaminase (GABA-T) (mg/L)	Amylase (units/g tissue)	Lipase (units/g tissue)
Control		4.56 ± 0.04^{a}	19.90 ± 0.03^{a}	2.01 ± 0.03^{a}
Treated	*C. sempervirens*	3.65 ± 0.03^{ab}	15.92 ± 0.03^{ab}	1.61 ± 0.02^{ab}
	P. halepensis	2.34 ± 0.02^{b}	10.21 ± 0.02^{b}	1.03 ± 0.02^{b}

Table 8. The effect of *Cupressus sempervirens* and *Pinus halepensis* essential oils against the activities of infection response enzymes in mosquitoes. Values were calculated from three replicates and expressed as mean ± SE, a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter

respectively), with levels still significantly lower than those in larvae treated with *C. sempervirens* oil. Similarly, *C. sempervirens* extract resulted in a significant ($p \leq 0.05$) reduction in the activities of these enzymes (3.65 ± 0.03 mg/L, 15.92 ± 0.03 units/g tissue, and 1.61 ± 0.02 units/g tissue, respectively) compared to untreated larvae. Therefore, *P. halepensis* oil exhibited higher biological efficiency against the activities of these enzymes.

The antioxidant enzyme activities (SOD, GST, and CAT) and GSH levels in mosquito larvae treated with *C. sempervirens* (1.96 ± 0.03 units/g tissue, 45.03 ± 0.43 units/g tissue, 2.73 ± 0.03 U/mg protein/min, and 1.09 ± 0.01 µg/mg protein, respectively) and in *P. halepensis* (1.26 ± 0.02 units/g tissue, 28.87 ± 0.27 units/g tissue, 1.75 ± 0.02 U/mg protein/min, and 0.70 ± 0.01 µg/mg protein, respectively) extracts were significantly reduced ($p \leq 0.05$) compared to untreated controls (2.45 ± 0.04 units/g tissue, 56.29 ± 0.53 units/g tissue, 3.41 ± 0.04 U/mg protein/min, and 1.36 ± 0.02 µg/mg protein, respectively) (Table 9). Lipid peroxidation product (LPO) and total protein carbonyl content (TPC), the products of peroxidation reactions, show an inverse relationship with the activities of enzymatic and non-enzymatic antioxidants, respectively. Data showed that *C. sempervirens* and *P. halepensis* essential oils significantly increased lipid peroxidation (LPO) (4.34 ± 0.06 and 6.74 ± 0.10 nmol/mg protein, respectively) and protein carbonyl (TPC) (7.01 ± 0.05 and 10.88 ± 0.08 nmol/mg protein, respectively) levels in mosquito larvae, indicating enhanced oxidative damage (Table 9).

UPLC/MS tentative metabolite profiling for *Cupressus* and *Pinus* extracts

Different solvent extracts (methanol, ethyl acetate, acetone) from *Pinus halepensis* and *Cupressus sempervirens* were metabolically profiled and compared using UPLC/MS analysis in ESI positive (Figs. 2 and 3) and negative ion modes (Figs. 4 and 5). Fifty-eight metabolites were tentatively assigned to various phytochemical classes,

Plant extracts		SOD (units/g tissue)	G-S-T (units/g tissue)	CAT (U/mg protein/min)	GSH (µg/mg protein)	LPO (nmol/mg protein)	TPC (nmol/mg protein)
Control		2.45 ± 0.04^{a}	56.29 ± 0.53a	3.41 ± 0.04^{a}	1.36 ± 0.02^{a}	3.42 ± 0.05^{b}	5.52 ± 0.04^{b}
Treated	*C. sempervirens*	1.96 ± 0.03^{ab}	45.03 ± 0.43^{ab}	2.73 ± 0.03^{ab}	1.09 ± 0.01^{ab}	4.34 ± 0.06^{ab}	7.01 ± 0.05^{ab}
	P. halepensis	1.26 ± 0.02^{b}	28.87 ± 0.27^{b}	1.75 ± 0.02^{b}	0.70 ± 0.01^{b}	6.74 ± 0.10^{a}	10.88 ± 0.08^{a}

Table 9. The effect of *Cupressus sempervirens* and *Pinus halepensis* essential oils against markers of the antioxidant defense in mosquitoes. Values were calculated from three replicates and expressed as mean ± SE, a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter

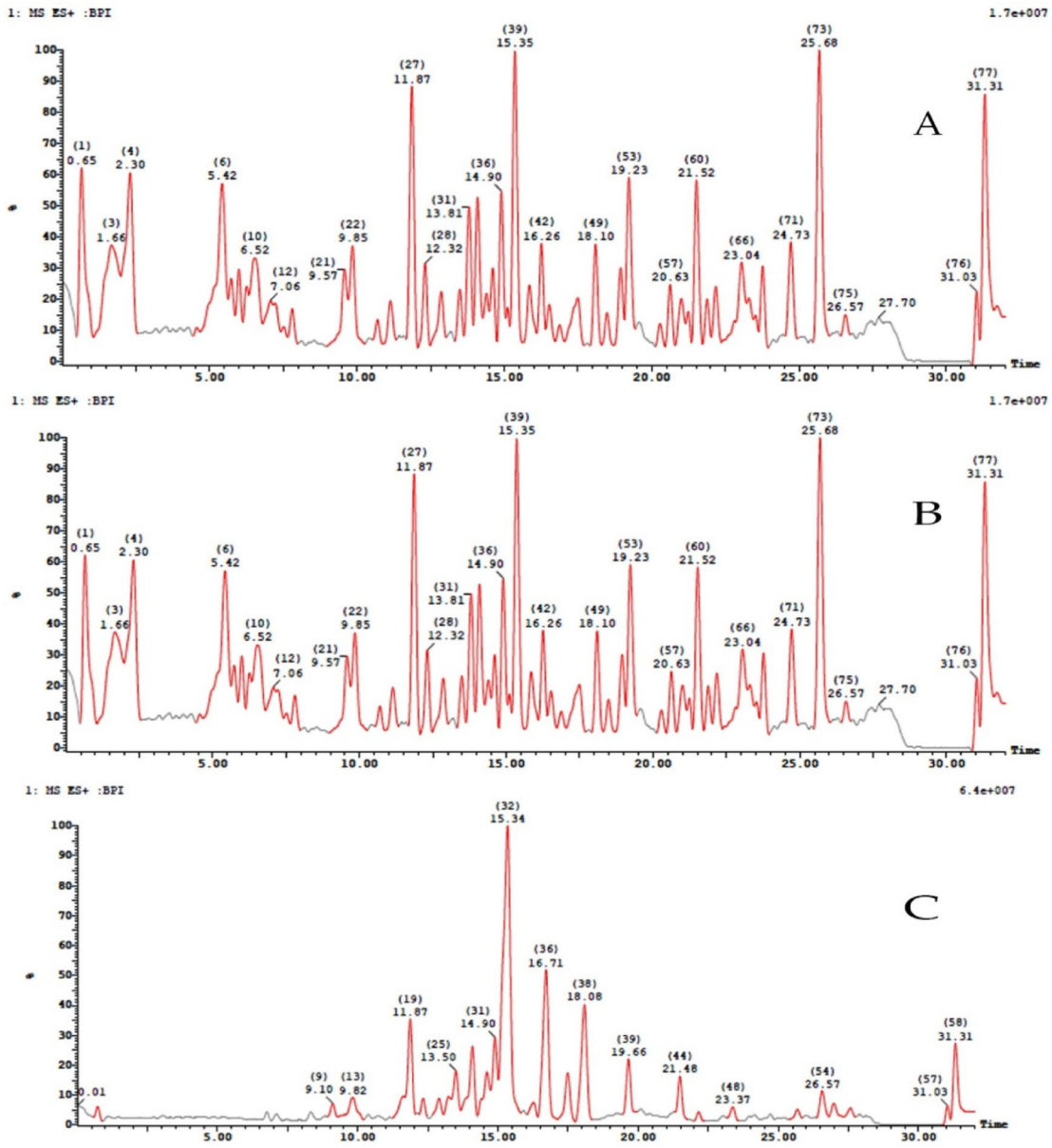

Fig. 2. BPI chromatogram of *Pinus halepensis* in *ESI positive* ion mode for (A) methanol, (B) ethyl acetate and (C) acetone extracts.

viz., flavonoids (the predominant class), diterpenoids, cinnamic acid derivatives, prenol lipids, lignans, tannins, alkaloids, stilbenes, chalcones, and others (Fig. 6).

A Venn diagram was constructed illustrating the distribution of identified metabolites between *Cupressus* (CU) and *Pinus* (GA) extracts. A total of fifty-eight metabolites were detected across both sources, from which

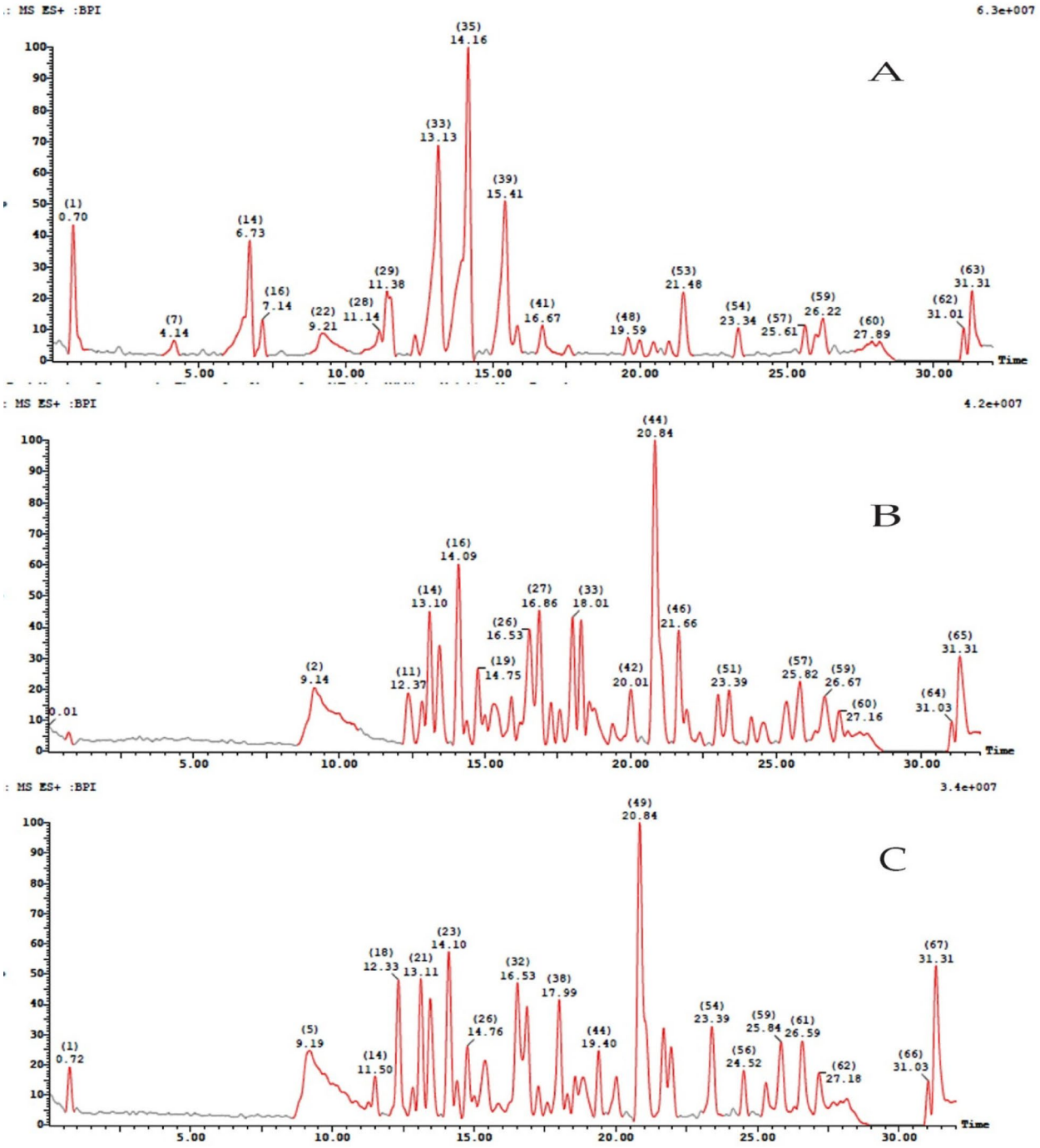

Fig. 3. BPI chromatogram of *Cupressus sempervirens* in *ESI positive* ion mode for (A) methanol, (B) ethyl acetate and (C) acetone extracts.

twenty-one metabolites were only detected from CU, while seventeen metabolites showed unique distribution to the GA extracts. Moreover, twenty other metabolites were found in common between the two groups of extracts and were assigned to the intersected area between the two groups (Fig. 7).

The CU-specific metabolites were mainly presented as catechin gallate, catechin-caffeic acid adduct, chrysocauloflavone I, cupresin A, and cyanidin-pentoside. Thus, CU extracts were mainly showing a distinctive profile of flavonoids and phenolic derivatives. On the other hand, the GA-specific metabolites were centered in 18-nor-8(17),13E-labdadiene-4α,15-diol, caffeoyl hexose, chrysin, cubebin, and phloretic acid hexoside; thus, the GA phytochemical profile mainly involved diterpenoids, phenolics, and glycosides (Fig. 7). The twenty common metabolites were mainly presented by abietic acid, amentoflavone, apigenin hexoside, caffeoyl-shikimic acid, and caftaric acid. These shared compounds suggest biochemical similarities, while the unique metabolites underline distinctive phytochemical signatures (Fig. 7).

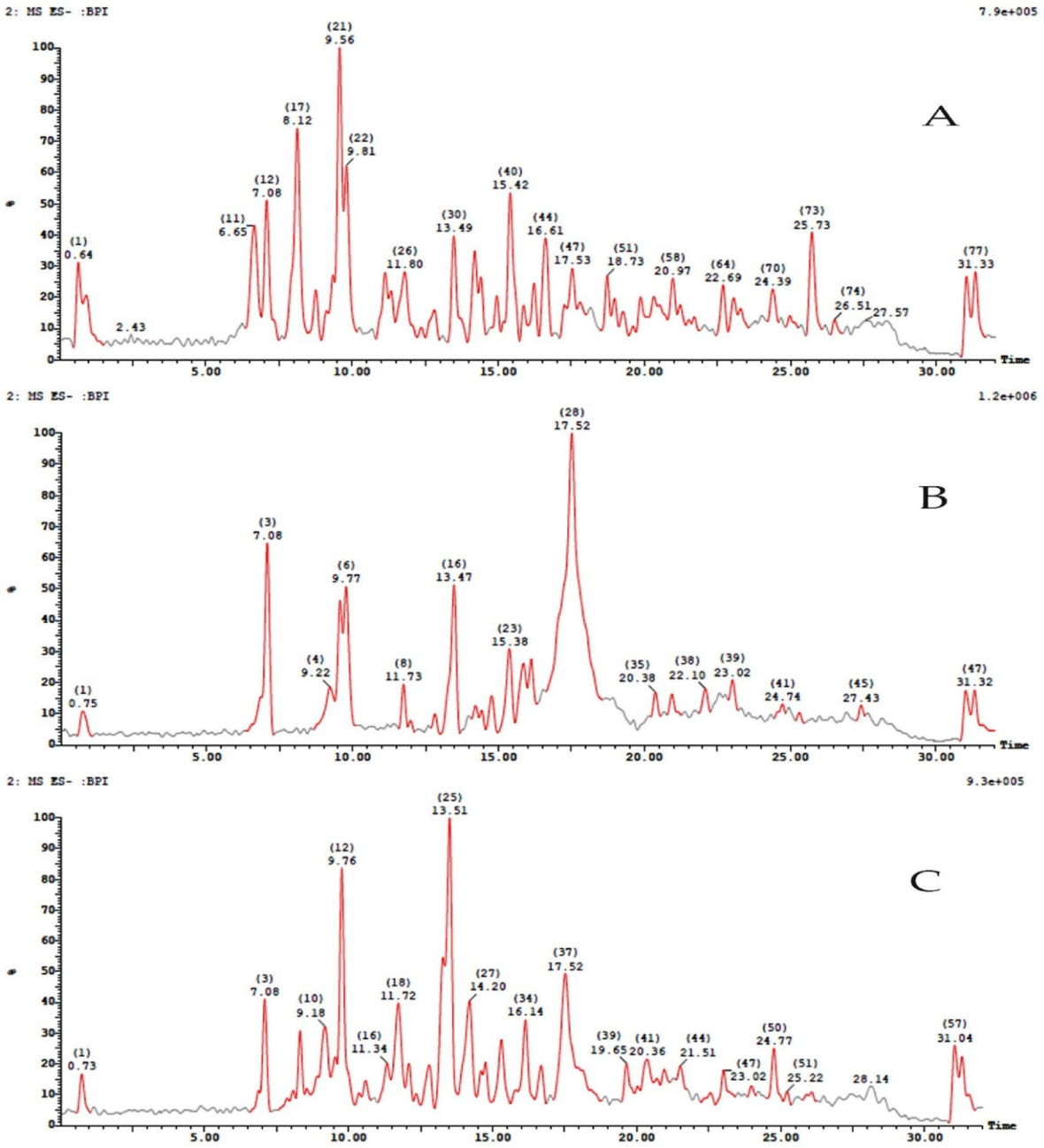

Fig. 4. BPI chromatogram of *Pinus halepensis* in *ESI negative* ion mode for (A) methanol, (B) ethyl acetate and (C) acetone extracts.

The interpretation of UPLC/MS *m/z* values provides essential insights into the structural characterization of the detected metabolites. Flavonoids represented the main identified class of secondary metabolites herein, with flavonoid glycosides, acylated derivatives, and bioflavonoids as the main subclasses. Flavonoid glycosides such as apigenin hexoside (*m/z* 431)[23], quercetin-hexoside (*m/z* 463)[23,52,53], isorhamnetin-pentoside (*m/z* 461), and laricitrin-hexoside and its sugar isomer (*m/z* 493)[54] were tentatively identified. Moreover, acetylated flavonoids, including quercetin acetyl-hexoside, presented a deprotonated peak at $[M\text{-}H]^-$ *m/z* 505[55]. While bioflavonoids were mainly detected as amentoflavone *m/z* 537(539) in both *Pinus* and *Cupressus* genera[56] and *iso*-ginkgetin *m/z* 565(567) only from *Cupressus* extracts[24].

Diterpenoids represented the second most abundant class, with bicyclic diterpenoids such as methyl-*iso*-*cupressate*, which appeared at *m/z* 333 and was mainly related to the genus *Pinus* as a major metabolite[23]; phenolic diterpenoids like carnosol, which was phytochemically related to both *Cupressus* and *Pinus* genera and had its deprotonated peak at *m/z* 329[23,24]; diterpene ester in the form of methyl-sciadopate (*m/z* 349)[23]; and other diterpenoids, including *iso*-cupressic acid (*m/z* 319(321))[25], hydroxy-abietatrienoic acid (*m/z* 315)[24],

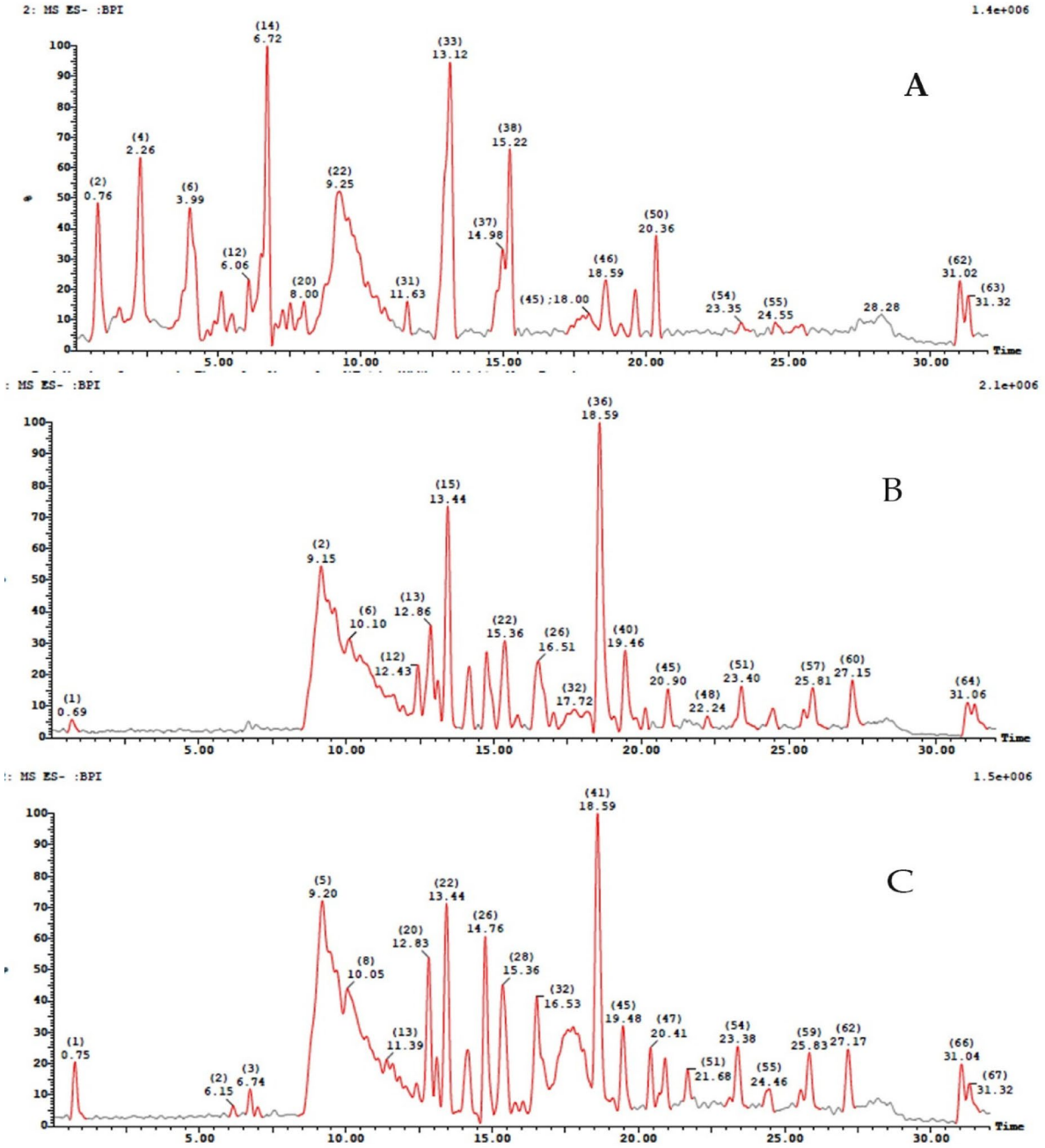

Fig. 5. BPI chromatogram of *Cupressus sempervirens* in *ESI negative* ion mode for (A) methanol, (B) ethyl acetate and (C) acetone extracts.

18-nor-8(17),13E-labdadiene-4*α*,*15-diol* (*m/z* 291)[54], and abietic acid (*m/z* 301(303))[57], were annotated (Table 10).

Proanthocyanidins and tannins, such as gallocatechin (*m/z* 305)[53], catechin gallate (*m/z* 441)[58], and procyanidin B-type dimers *m/z* 577 (579)[52], were also recognized (Table 10). Cinnamic acid derivatives, including caffeoyl-hexose (*m/z* 341)[24], caffeoyl-shikimic acid *m/z* 335 (337)[24], and *p*-coumaroyl-quinic acid derivative (*m/z* 511), were assigned tentatively. In addition, phenolic acids such as caftaric acid were tentatively presented at *m/z* 311 in negative ion mode[53]. Anthocyanidins, exemplified by cyanidin-pentoside, appeared at *m/z* 483[54]. Lignans, as a rare class of secondary metabolites, were also detected in the form of hydroxylariciresinol I (*m/z* 375)[18] and secoisolariciresinol guaiacylglyceryl ether (*m/z* 557)[18]. Prenol lipids and fatty acids such as pinifolic acid (*m/z* 335(337)) and its derivative dehydro-pinifolic acid (*m/z* 333(353)), hydroxy-palmitic acid (*m/z* 271)[23], methyl-octadecanoic acid (*m/z* 297, only from GA-Me)[54], and *di*-oxo-octadecanoic acid (*m/z* 311)[24]were recognized in negative mode as carboxylate anions. Stilbenes such as piceatannol-hexoside, also known as astringin, appeared at *m/z* 405[52], and chalcones such as phloretic acid hexoside (*m/z* 327)[24] were annotated. In

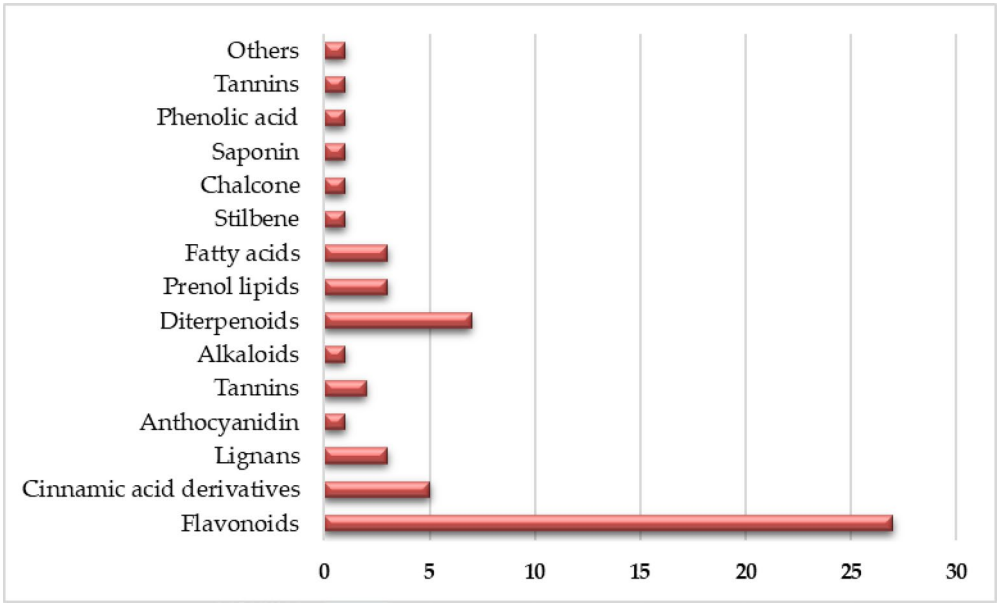

Fig. 6. Bar chart showing the phytochemical classes tentatively detected from *Pinus* and *Cupressus* extracts.

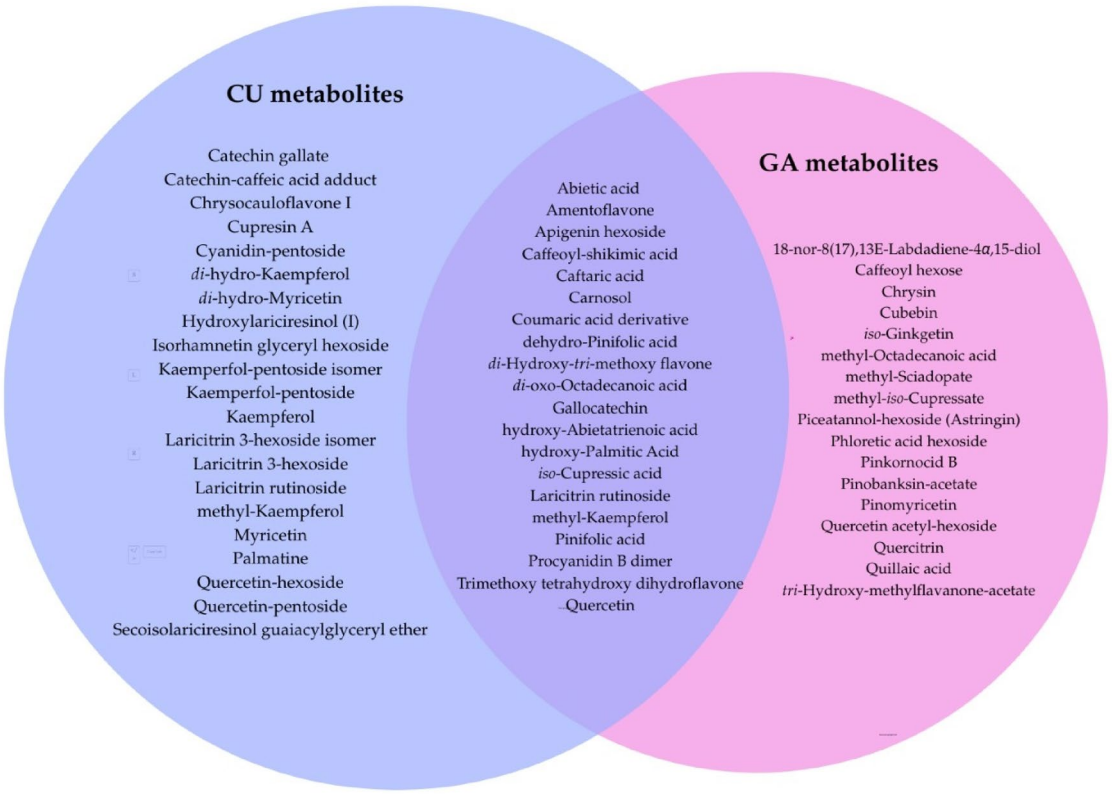

Fig. 7. Venn diagram illustrating the overlap and unique distribution of metabolites between *Cupressus* and *Pinus* extracts.

No.	Compound name	Class	Molecular formula	[M-H]$^-$ *m/z*	[M+H]$^+$ *m/z*	R_t (min.)	% Composition						Ref.
							CU-Me	CU-Eth.	CU-Ac.	GA-Me	GA-Eth.	GA-Ac.	
1	Apigenin hexoside[g]	Flavonoid	$C_{21}H_{20}O_{10}$	**431**	**453**	0.70	–	**0.41** (0.23)	–	**4.37** (1.21)	**12.12** (1.97)	**5.57**	23
2	Trimethoxy tetrahydroxy dihydroflavone	Flavonoid	$C_{18}H_{18}O_9$	**377**	**419**	0.76	**4.51 (5.76)**	–	–	2.41	–	–	91
3	Isorhamnetin-pentoside[c]	Flavonoid	$C_{22}H_{22}O_{11}$	**461**	-	1.53	0.28	–	–	**3.51**	–	–	24
4	Quercetin-pentoside[g]	Flavonoid	$C_{20}H_{18}O_{11}$	**433**	-	5.10	1.38	–	–	–	–	–	52
5	Hydroxylariciresinol (I)[g]	Lignan	$C_{20}H_{24}O_7$	**375**	-	5.47	0.61	–	–	–	–	–	18
6	*di*-Hydroxy-*tri*-methoxy flavone[g, c]	Flavonoid aglycone	$C_{18}H_{16}O_7$	**345**	**357**	6.04	1.44	–	–	0.88 **(6.52)**	–	–	23
7	Quercetin-hexoside[g, c]	Flavonoid	$C_{21}H_{20}O_{12}$	**463**	-	6.14	–	–	0.17	–	–	–	24,52,53
8	Kaemperfol-pentoside[g]	Flavonoid	$C_{21}H_{20}O_{10}$	**417**	**419**	6.51	2.85 **(3.12)**	–	–	(2.95)	–	–	52
9	Quercitrin[c]	Flavonoid	$C_{21}H_{20}O_{11}$	**447**	-	6.65	–	–	–	**4.77**	–	–	92
10	Kaemperfol-pentoside isomer[g, p]	Flavonoid	$C_{21}H_{20}O_{10}$	**417**	**419**	6.72	**8.16 (5.31)**	–	–	–	–	–	52
11	Isorhamnetin glyceryl hexoside[c]	Flavonoid	$C_{22}H_{22}O_{12}$	**551**	-	6.74	–	–	0.42	–	–	–	24
12	Catechin-caffeic acid adduct	Cinnamic acid deriv.	$C_{24}H_{22}O_{10}$	**467**	-	7.00	1.63	–	–	–	–	–	93
13	Caffeoyl hexose[c]	Cinnamic acid deriv.	$C_{15}H_{18}O_9$	**341**	-	7.29	–	–	–	0.37	–	–	24
14	Laricitrin-hexoside[g, p]	Flavonoid	$C_{22}H_{22}O_{13}$	**493**	-	7.83	0.51	–	–	–	–	–	54
15	Laricitrin-hexoside isomer[g, p]	Flavonoid	$C_{22}H_{22}O_{13}$	**493**	-	8.00	1.14	–	–	–	–	–	54
16	*tri*-Hydroxy-methylflavanone-acetate[g]	*O*-methylated flavonoid	$C_{16}H_{14}O_5$	**327**	**329**	8.77	2.30	1.02 **(5.89)**	**4.73 (5.35)**	1.61	–	–	54
17	Amentoflavone[c]	Biflavonoid	$C_{30}H_{18}O_{10}$	**537**	**539**	9.25	**3.71** (2.70)	**6.57** (1.06)	**13.25 (10.14)**	0.74	–	–	56
18	Procyanidin B dimer[g, c]	Tannin	$C_{30}H_{26}O_{12}$	**577**	**579**	9.34	–	–	–	**15.95 (6.19)**	**12.72** (2.68)	1.76	24,52
20	*di*-hydro-Kaempferol[c, g]	Flavonoid	$C_{15}H_{12}O_6$	**287**	-	9.70	–	–	**4.30**	–	–	–	88
21	Chrysocauloflavone I[c]	Flavonoid	-----	**539**	-	10.10	–	**8.90**	–	–	–	–	24
22	Caffeoyl-shikimic acid[g, c]	Hydroxycinnamic acid deriv.	$C_{16}H_{16}O_8$	**335**	**337**	10.74	**4.74**	2.24 **(3.56)**	–	–	–	0.42	23,24
23	Palmatine[c]	Berberine alkaloid	$C_{21}H_{22}NO_4$+	**351**	-	10.85	2.78	–	**3.38**	–	–	1.40	54
24	Chrysin[g]	Flavonoid	$C_{15}H_{10}O_4$	**253**	**255**	11.15	–	–	–	2.51 (0.77)	–	–	57,88
25	Cupresin A[c]	Miscellaneous	$C_{20}H_{32}O_3$	-	**343 + Na**	11.27	–	–	(0.39)	–	–	–	25
26	methyl-*iso*-Cupressate[g]	Bicyclic diterpnoid	$C_{21}H_{34}O_3$	**333**	-	11.34	–	–	–	**5.26**	**8.90**	**20.95**	23
27	Pinkornocid B[g]	Prenol lipid	$C_{20}H_{30}O_6$	**365**	-	11.39	–	–	1.53	–	**5.70**	**17.27**	54
28	*di*-hydro-Myricetin[g]	Flavonoid	$C_{15}H_{12}O_8$	**319**	-	11.63	0.72	1.21	0.45	–	–	–	54
29	hydroxy-Palmitic Acid[g]	Fatty acid	$C_{16}H_{32}O_3$	**271**	**299**	11.86	0.53	0.36	**4.16**	2.98 **(4.69)**	(2.97)	1.43	23
30	methyl-Sciadopate[g]	Diterpene ester	$C_{12}H_{14}O_5$	**349**	-	11.98	–	–	–	–	0.33	**3.29**	23
31	*iso*-Ginkgetin[c]	Biflavonoid	$C_{32}H_{22}O_{10}$	**565**	**567**	12.32	–	–	–	0.32 (1.46)	–	–	24
32	Carnosol[g, c]	Phenolic diterpenoid	$C_{20}H_{26}O_4$	**329**	-	12.43	–	**4.17**	–	**9.30**	–	–	23,24
33	*dehydro*-Pinifolic acid[g]	Prenol lipid	$C_{20}H_{30}O_4$	**333**	**353**	12.84	–	1.31 (1.30)	**3.83** (0.70)	**4.03** (0.88)	2.14	**7.22**	54

Continued

No.	Compound name	Class	Molecular formula	[M-H]$^-$ *m/z*	[M+H]$^+$ *m/z*	R_t (min.)	% Composition						Ref.
							CU-Me	CU-Eth.	CU-Ac.	GA-Me	GA-Eth.	GA-Ac.	
34	*iso*-Cupressic acid[g, c]	Diterpenoid	$C_{20}H_{32}O_3$	**319**	**321**	13.17	**14.15 (16.01)**	**6.94 (4.16)**	1.19 **(3.79)**	–	–	–	25
35	Pinifolic acid[g]	Prenol lipid	$C_{20}H_{32}O_4$	**335**	**337**	13.44	–	–	**4.83 (3.75)**	–	–	–	54
36	Kaempferol[g]	Flavonoid	$C_{15}H_{10}O_6$	**285**	**287**	14.16	**(15.86)**	**3.51**	2.62	–	–	–	53
37	Hydroxy-Abietatrienoic acid[g, c]	Diterpene acid	-----	**315**	-	14.20	–	–	–	2.93	–	**8.26**	23,24
38	Pinobanksin-acetate[g]	Flavonoid	$C_{17}H_{14}O_6$	**313**	-	14.61	–	–	–	–	–	1.01	18
39	18-nor-8(17),13*E*-Labdadiene-4*α*,15-diol[g]	Diterpenoid	$C_{19}H_{32}O_2$	**291**	-	14.77	–	–	–	–	1.65	0.65	54
40	methyl-Kaempferol[g]	Flavonoid	$C_{16}H_{12}O_6$	**299**	**317**	14.78	0.93 **(10.04)**	–	–	0.31	–	–	54
41	Pinomyricetin[g, c]	Flavonoid	-----	**331**	**357**	15.34	–	–	–	–	–	**3.17 (28.63)**	24,54
42	Secoisolariciresinol guaiacylglyceryl ether[g]	Lignan	-----	**557**	-	16.06	–	–	0.40	–	–	–	18
43	Methyl-Octadecanoic acid[g]	Fatty acid	$C_{19}H_{38}O_2$	**297**	**309**	16.26	–	–	–	**8.23** (2.91)	–	–	54
44	Piceatannol-hexoside (Astringin)[g]	Stilbene	$C_{20}H_{22}O_9$	**405**	-	16.71	–	–	–	–	–	1.50	52
45	Quillaic acid[g]	Saponin	$C_{30}H_{46}O_5$	**485**	-	17.03	–	0.44	–	–	–	–	88
46	Quercetin acetyl-hexoside[g]	Flavonoid	$C_{23}H_{22}O_{13}$	**505**	-	17.79	–	–	–	0.56	–	–	55
47	Quercetin[c, g]	Flavonoid	$C_{15}H_{10}O_7$	**301**	-	18.18	0.97	1.59	–	0.19	–	–	94
48	Cyanidin-pentoside[g]	Anthocyanidin	$C_{21}H_{21}O_{10}$+	**483**	-	18.60	0.28	0.34	–	–	–	–	54
49	Abietic acid[c, g]	Diterpenoid	$C_{20}H_{30}O_2$	**301**	**303**	19.03	0.69 (0.78)	(0.89)	**7.89** (1.02)	–	–	**1.37 (3.05)**	57
50	Caftaric acid[g]	Phenolic acid	$C_{13}H_{12}O_9$	**311**	-	20.73	–	–	0.72	–	–	1.52	53
51	Catechin gallate[g, p]	Tannin	$C_{22}H_{18}O_{10}$	**441**	-	20.90	–	1.11	1.07	–	–	–	58
52	*di*-oxo-Octadecanoic acid[c]	Fatty acid	$C_{18}H_{32}O_6$	**311**	-	21.24	–	–	–	0.85	–	–	24
53	Phloretic acid hexoside[c]	Dihydrochalcone	$C_{15}H_{20}O_8$	**327**	-	22.69	–	–	–	1.46	–	–	24
54	Cubebin[g]	Lignan	$C_{20}H_{20}O_6$	**355**	-	23.04	–	–	–	1.09	–	–	54
55	*p*-Coumaroyl-quinic acid derivative[g]	Hydroxycinnamic acid	-----	**511**	-	24.46	–	0.71	0.60	–	–	–	23
56	Coumaric acid derivative[g]	Cinnamic acid deriv.	-----	**475**	-	25.48	0.51	–	–	–	–	0.28	52
57	Laricitrin rutinoside[g]	Methylated flavonoid gly.	$C_{28}H_{32}O_{17}$	**639**	-	25.50	–	0.49	0.33	–	–	–	23
58	Myricetin[g]	Flavonoid	$C_{15}H_{10}O_8$	**317**	-	31.02	1.94	–	1.03	0.77	–	–	58
Continued													

No.	Compound name	Class	Molecular formula	[M-H]$^-$ *m/z*	[M+H]$^+$ *m/z*	R_t (min.)	% Composition						Ref.
							CU-Me	CU-Eth.	CU-Ac.	GA-Me	GA-Eth.	GA-Ac.	
59	Gallocatechin[g, c]	Tannin	$C_{15}H_{14}O_7$	**305**	**307**	31.32	1.13 **(3.10)**	1.15 **(4.18)**	0.91	2.58	2.79	2.19	[24,53]
	No. of identified compounds						**25**	**19**	**22**	**26**	**9**	**18**	
	% Identification in ESI negative mode						**57.89%**	**42.47%**	**57.81%**	**77.98%**	**46.35%**	**79.26%**	
	% Identification in ESI positive mode						**62.68%**	**21.27%**	**25.14%**	**27.58%**	**7.62%**	**31.68%**	

Table 10. UPLC/MS tentatively identified metabolites from *Pinus* and *Cupressus* extracts. [c] for compounds reported from genus *Cupressus*, [g] for compounds reported from genus *Pinus*, [f] for compounds reported from family Cupressaceae and [P] for compounds reported from family Pinaceae. Ref: references, R_t: retention time. CU-Me; *Cupressus* methanol extract, CU-Eth.; *Cupressus* ethyl acetate extract, CU-AC.; *Cupressus* acetone extract, GA-Me; *Pinus* methanol extract, G A-Eth.; *Pinus* ethyl acetate extract and GA-AC.; *Pinus* acetone extract.

addition, one berberine alkaloid peak appeared at *m/z* 351 and was assigned to palmatine, which was previously identified from the genus *Cupressus*[54].

Multivariate data analysis using PCA and clustered heat map

Principal component analysis (PCA) was selected as unsupervised data discrimination for the differentiation between the metabolite distribution between the analyzed *Pinus* and *Cupressus* extracts, which provided insights into their spatial phytochemical profiles. PCA was performed twice for the metabolites detected in the ESI positive ion mode and for the metabolites identified from the ESI negative ion mode due to the different ionization preferences for each phytochemical class, which gave rise to different metabolites upon changing the ionization mode. As illustrated in Fig. 8A, the PCA plot in positive mode showed three distinctive clusters: one in the left upper quadrant for CU-Me alone, another in the right upper quadrant for GA-AC, while the rest of the extracts (CU-Eth, CU-AC, GA-Eth, and GA-Me) were detected in the left lower quadrant, overlapping in one cluster. This clustering pattern could be explained through the PCA loading plot (Fig. 8B), where kaempferol and iso-cupressic acid were abundant in CU-Me, while the flavonoid pinomyricetin was selectively present in GA-AC, thus explaining its sole cluster. The rest of the extracts showed a collection of common metabolites, explaining their overlapping and clustering.

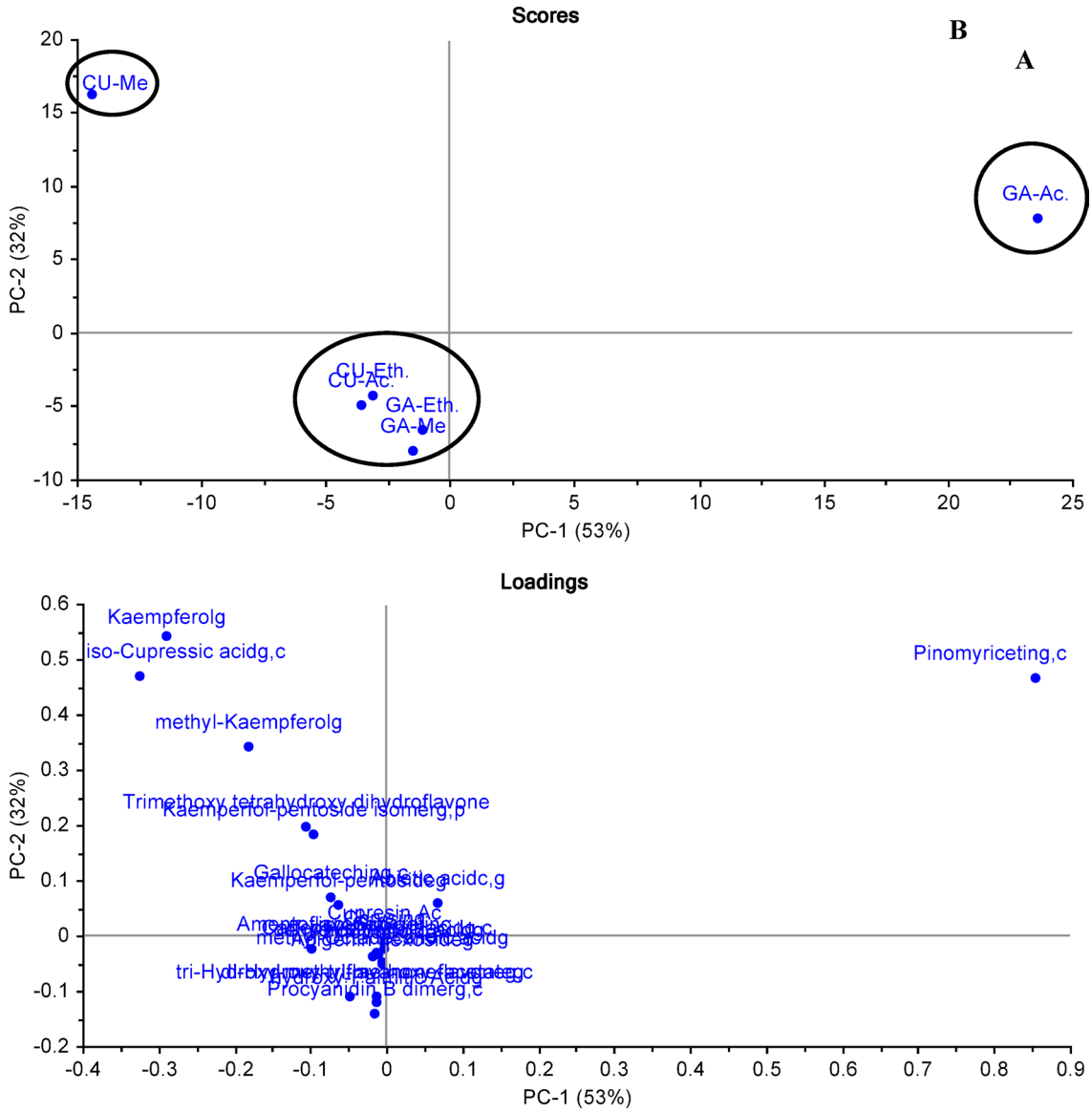

Fig. 8. (A) Score plot of PC1 *versus* PC2 of the identified secondary metabolites (positive ion mode) from *Pinus* and *Cupressus* extracts (area % as a variable). (B) Loading plot for PC1 and PC2 contributing metabolites and their assignments (area % as variable). CU-Me; *Cupressus* methanol extract, CU-Eth.; *Cupressus* ethyl acetate extract, CU-AC.; *Cupressus* acetone extract, GA-Me; *Pinus* methanol extract, GA-Eth.; *Pinus* ethyl acetate extract and GA-AC.; *Pinus* acetone extract.

The PCA score plot for the metabolites in the ESI negative ion mode (Fig. 9A) led to the detection of four main clusters appearing as two clusters in the right upper quadrant, one cluster in the intersection between the left upper and lower quadrants, and one last cluster in the right lower quadrant to the periphery. Upon examining the PCA loading plot (Fig. 9B), GA-Me formed one cluster due to the abundant presence of procyanidin B dimer, while GA-Eth formed a unique cluster due to its richness with apigenin hexoside. GA-Me and GA-Eth appeared in the same quadrant due to their near phytochemical composition. *iso*-Cupressic acid and amentoflavone were the main common clustering components, which led to the overlapping of CU-Eth., CU-Me, and CU-AC.

Applying further multivariate analysis with the help of a clustered heat map (double dendrogram), the color pattern ranged from blue for the lowest area percentage, and the color intensity increased gradually until red for the highest area percentage for the positive ion mode components. The color gradient for the negative ion mode components ranged from green for the lowest area percentage to red for the highest area percentage. The clustered heat map further confirmed the clustering results discussed above for PCA using area percentage as a variable (Figs. 10 and 11).

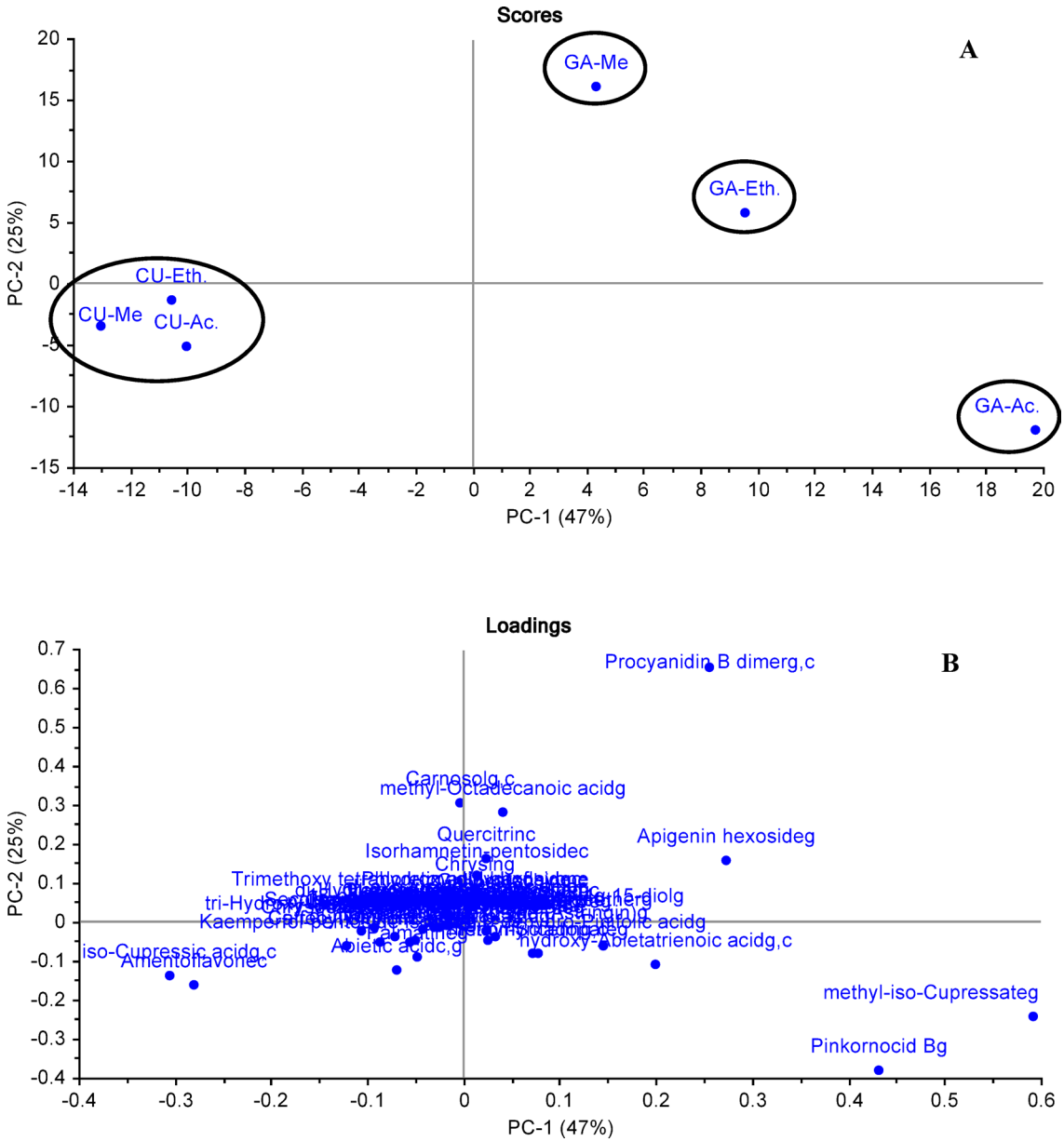

Fig. 9. (A) Score plot of PC1 *versus* PC2 of the identified secondary metabolites (negative ion mode) from *Pinus* and *Cupressus* extracts (area % as a variable). (B) Loading plot for PC1 and PC2 contributing metabolites and their assignments (area % as variable). CU-Me; *Cupressus* methanol extract, CU-Eth.; *Cupressus* ethyl acetate extract, CU-AC.; *Cupressus* acetone extract, GA-Me; *Pinus* methanol extract, GA-Eth.; *Pinus* ethyl acetate extract and GA-AC.; *Pinus* acetone extract.

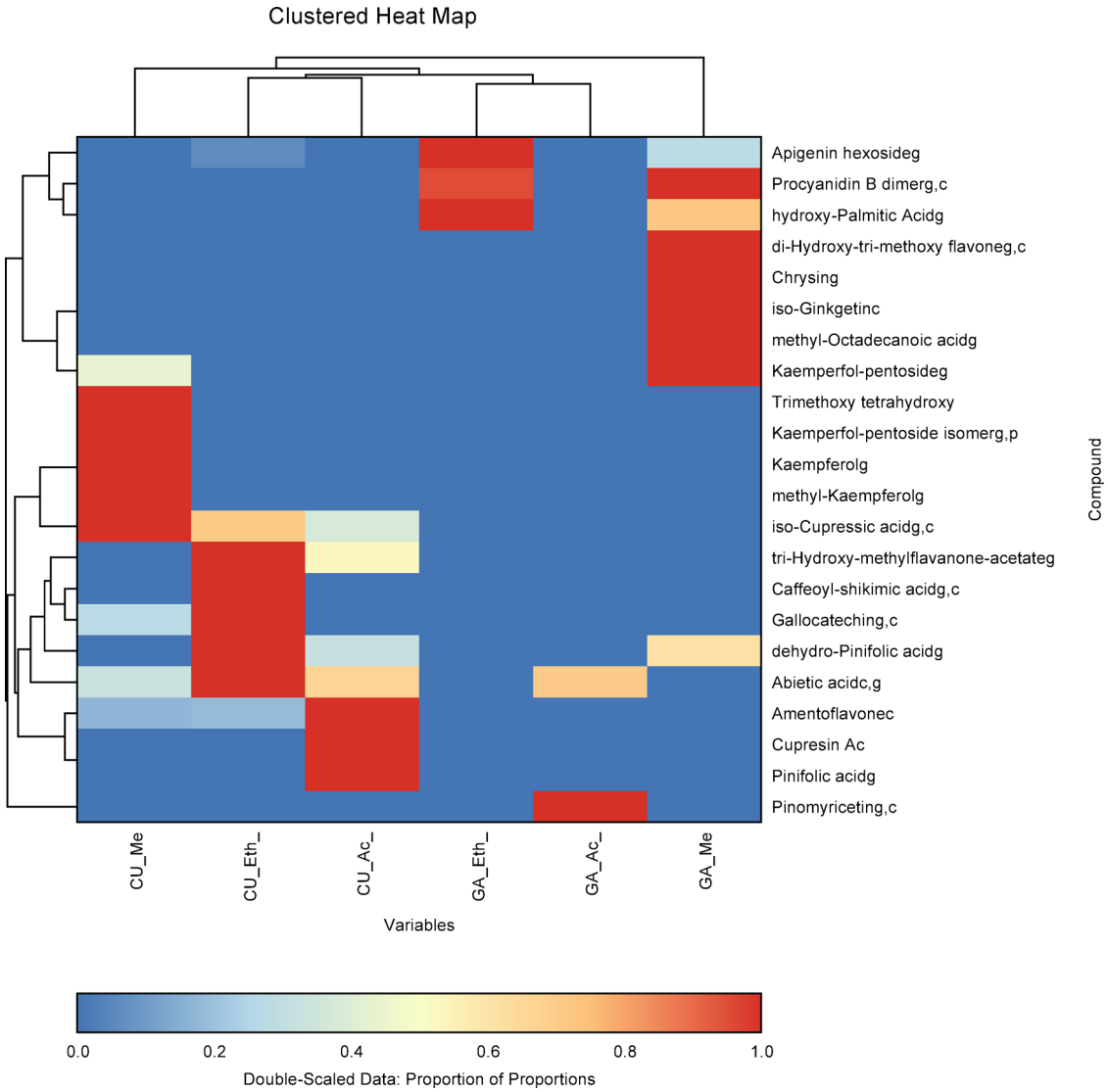

Fig. 10. Clustered heat map showing the identified components (positive mode) from *Pinus* and *Cupressus* extracts. A heat map was constructed using Euclidean distance and the unweighted group method. CU-Me; *Cupressus* methanol extract, CU-Eth.; *Cupressus* ethyl acetate extract, CU-AC.; *Cupressus* acetone extract, GA-Me; *Pinus* methanol extract, GA-Eth.; *Pinus* ethyl acetate extract and GA-AC.; *Pinus* acetone extract.

GC/MS identification of volatile metabolites from the oil and *n*-hexane extracts from *Pinus* and *Cupressus*

GC/MS analysis was performed to comparatively profile the volatile components from *Pinus* and *Cupressus n*-hexane extracts and oils. The percentage identification ranged from 79.98% to 90.64% (Table 11). 1,3-dimethyl-Adamantane, (-)-terpinen-4-ol, *cis*-totarol, ferruginol, hexatriacontane, tetracontane, and lupeol were among the main identified components. The *n*-hexane extracts from *Pinus* (GA-Hex) and *Cupressus* (CU-Hex) showed relatively similar phytochemical profiles with different compositions for the detected components. The same applies for their EOs. Certain components appeared solely in the CU-Hex, such as *O*-cymene, *γ*-terpinene, *cis*-totarol, and ferruginol, while ethyl-cyclohexane and *O*-xylene were selectively present in GA-Hex. The two EOs samples should have the same components in different % compositions.

Discussion

Over the past decade, there has been growing interest in using medicinal plants as alternatives to chemical insecticides. Environmental issues and insects' growing resistance to synthetic insecticides are the main drivers of this shift. Plant-based insecticides offer a natural source for pest control solutions[59]. This study seeks to control mosquito populations that have developed resistance to conventional insecticides and show adverse reactions to synthetic chemicals. Researchers have investigated bioactive compounds in select plant species to assess their potential for insecticidal, antimicrobial, and antioxidant properties. Many natural substances in plant extracts

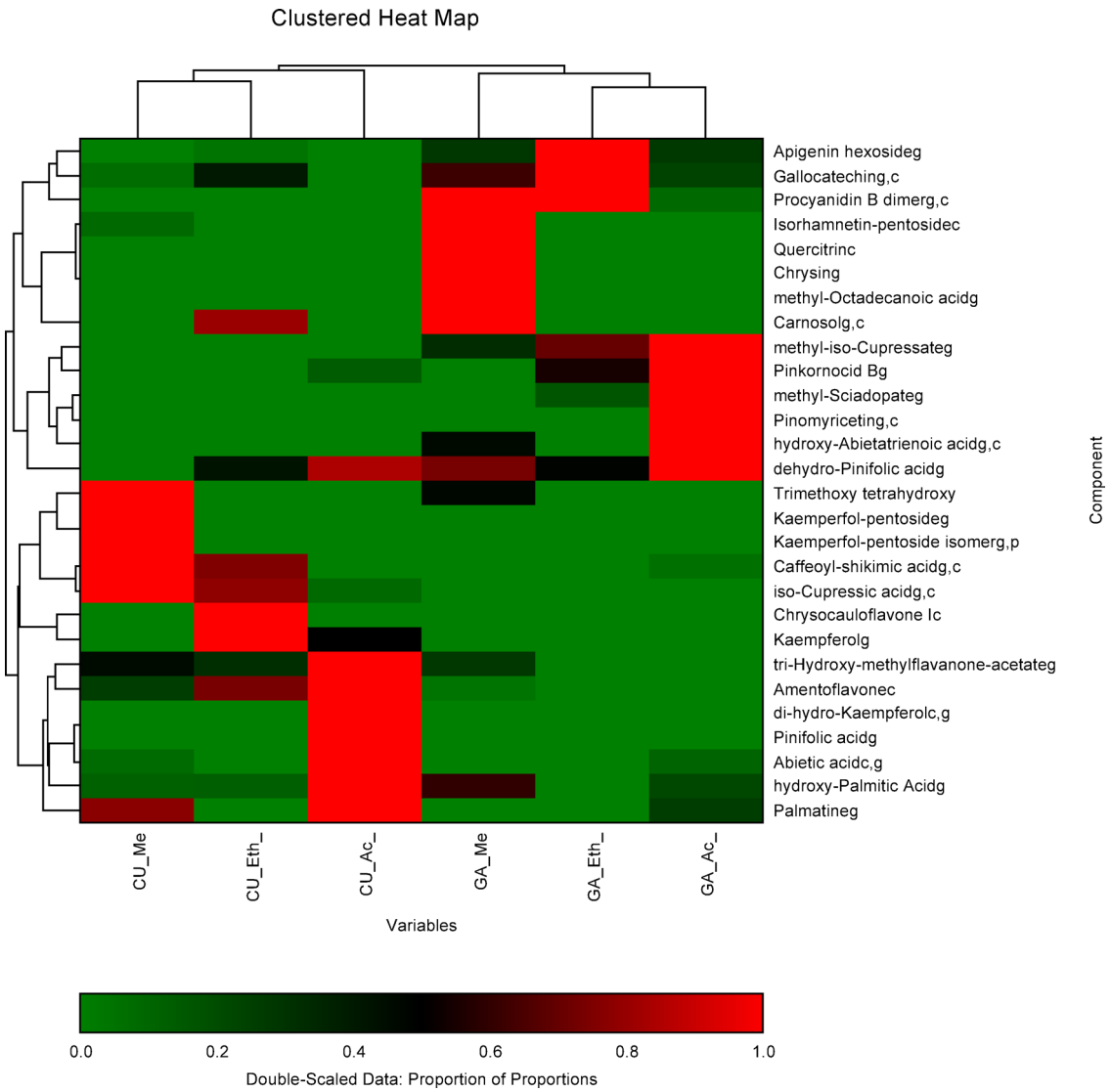

Fig. 11. Clustered heat map showing the identified components (negative mode) from *Pinus* and *Cupressus* extracts. A heat map was constructed using Euclidean distance and the unweighted group method. CU-Me; *Cupressus* methanol extract, CU-Eth.; *Cupressus* ethyl acetate extract, CU-AC.; *Cupressus* acetone extract, GA-Me; *Pinus* methanol extract, GA-Eth.; *Pinus* ethyl acetate extract and GA-AC.; *Pinus* acetone extract.

and EOs are considered safe for the environment and effective at controlling pests and diseases because they are strong and break down easily[60].

Coniferous plants, which belong to families that produce resinous compounds known as terpenoids, include the widely recognized Pinaceae and Araucariaceae families. There is limited evidence regarding the effectiveness of certain plant resins against *Cx. pipiens*, despite their demonstrated insecticidal properties. This study aims to evaluate how well some affordable plants that produce resin can kill mosquito larvae and to find out which chemicals in them are responsible for this effect.

To better understand the bioefficacy of these extracts, the larvicidal results were compared with those reported for other coniferous and resin-producing plants, and they revealed the chemical basis of their insecticidal potential. The present study demonstrated that both solvent extracts and essential oils of *P. halepensis* and *C. sempervirens* exhibited strong larvicidal and biochemical effects against *Cx. pipiens* larvae. Toxicity levels, measured by LC_{50} values, indicated *P. halepensis* and *C. sempervirens* oils were most effective and highly toxic overall on mosquito larvae, followed by leaf extract. Furthermore, the toxicity levels, shown by LC_{50} values, indicated that the leaf extracts from *P. halepensis* could be ranked from most to least effective as follows: the acetone extract was the most toxic, followed by the *n*-hexane extract, then methanol, and lastly ethyl acetate, while *C. sempervirens* extracts could be ranked as follows: the *n*-hexane extract was the most effective overall, followed by the acetone extract, then methanol, and lastly ethyl acetate. To better understand the bioefficacy of these extracts, the larvicidal results were compared with those reported for other coniferous and resin-producing

No.	Component	R_t (min.)	RI		Relative abundance %				Method of Identification	Molecular formula
			Cal.	Rep.	CU-Hex	CU-Oil	GA-Hex	GA-Oil		
1	Octane	4.103	802	–	15.76	–	22.53	–	RI, MS	C_8H_{18}
2	Ethyl-Cyclohexane	4.728	827	826	–	–	8.95	–	RI, MS	C_8H_{16}
3	*O*-Xylene	5.380	851	850	–	–	9.28	–	RI, MS	C_8H_{10}
4	1,1'-oxy-*bis*–2-Propanol	9.755	1000	–	–	7.77	–	10.70	RI, MS	$C_6H_{14}O_3$
5	*O*-Cymene	9.960	1004	1002	6.30	7.75	–	6.20	RI, MS	$C_{10}H_{14}$
6	2-(2-hydroxypropoxy)–1-Propanol	10.270	1010	1013	–	7.45	–	11.68	RI, MS	$C_6H_{14}O_3$
7	*γ*-Terpinene	11.151	1043	1042	6.28	7.48	–	5.52	RI, MS	$C_{10}H_{16}$
8	Linalool	12.450	1082	1082	–	7.38	–	4.94	RI, MS	$C_{10}H_{18}O$
9	1,3-dimethyl-Adamantane	12.938	1152	1151	15.52	7.08	23.08	–	RI, MS	$C_{12}H_{20}$
10	(-)-Terpinen-4-ol	14.721	1155	1160	–	10.62	–	12.01	RI, MS	$C_{10}H_{18}O$
11	L-*α*-Terpineol	15.080	1180	1172	–	–	–	4.94	RI, MS	$C_{10}H_{18}O$
12	Linalyl acetate	17.138	1236	1237	–	7.36	–	5.75	RI, MS	$C_{12}H_{20}O_2$
13	2-Methyl-*β*-ionone	27.758	1621	–	–	–	–	5.89	RI, MS	$C_{14}H_{22}O$
14	Tonalid	32.137	1818	–	–	–	–	6.21	RI, MS	$C_{18}H_{26}O$
15	Ethylene brassylate	34.935	1969	–	–	9.70	–	8.27	RI, MS	$C_{15}H_{26}O_4$
16	*cis*-Totarol	40.604	2266	2260	18.86	–	–	–	RI, MS	$C_{20}H_{30}O$
17	Ferruginol	40.872	2272	–	8.76	–	–	–	RI, MS	$C_{20}H_{30}O$
18	Hexatriacontane	56.034	3304	–	11.52	–	8.96	–	RI, MS	$C_{36}H_{74}$
19	Lupeol	56.835	3308	3270	–	7.39	–	4.81	RI, MS	$C_{30}H_{50}O$
20	Tetracontane	60.000	3594	–	7.64	–	12.28	–	RI, MS	$C_{40}H_{82}$
% Identification					**90.64**	**79.98**	**85.08**	**86.92**		

Table 11. GC/MS identified metabolites from the *n*-hexane and essential oil of *Pinus* and *Cupressus*. Cal.: calculated, Rep.: reported, RI: Retention index, R_t: retention time. CU-Hex.; *Cupressus n*-hexane extract, CU-Oil; *Cupressus* oil, GA-Hex.; *Pinus n*-hexane extract and GA-Oil; *Pinus* oil. Retention indices (RI) were calculated according to the Van den Dool and Kratz equation using a homologous series of n-alkanes (C8–C30) analyzed under the same temperature-programmed GC–MS conditions.

plants, and they revealed the chemical basis of their insecticidal potential. These findings are partially supported by Sukumar, et al.[61] and Maurya, et al.[62].

Similar with our work, El Omari, et al.[63] indicated that *P. halepensis* Mill. needle EOs showed larvicidal action on *Aedes albopictus* larvae survival and developmental time (DT) (LC_{50}= 70.21 mg/L). Also, Koutsaviti, et al.[64] showed that the needle EOs from *P. halepensis*, *P. brutia*, and *P. stankewiczii* against *Aedes albopictus*had larvicidal activity (LC_{50} ≈ 67–70 mg/L). Mitić, et al.[65] evaluated the *Pinus* species (Pinaceae) and showed that EOs extracted from aerial plant parts, including *P. halepensis*, showed high toxic activity against *Aedes* and *Culex* larvae, even at low concentrations of 0.2 µL/cm².

Also, the results of this study are consistent with several previous studies that demonstrated the potent toxic activity of the extracts and oils of *C. sempervirens* and *P. halepensis* against *Cx. pipiens*larvae, with the EOs of both species exhibiting higher toxicity compared to other extraction solvents. This work is consistent with the findings of Sukumar, et al.[61] and Hamrouni, et al.[66], who reported similar effects of medicinal plants as bio-vectors against mosquitoes. This study also supports the reports of Bousba and Yakoubi[67] on the effectiveness of *P. halepens* is oils in controlling the larvae of various mosquito species.

The higher toxicity observed for essential oils relative to solvent extracts indicates that the volatile fraction of these plants plays a crucial role in larval mortality. This finding aligns with previous reports on terpenoid-rich oil. These EOs showed higher larvicidal activity than the solvent extracts, which can be attributed to their richness in volatile monoterpenes such as α-pinene, β-pinene, limonene, and caryophyllene. compounds are known to penetrate the insect cuticle easily and interfere with neural transmission by inhibiting acetylcholinesterase, leading to paralysis and death. Similar findings were reported by Babu, et al.[68] and Onen, et al.[69], who observed high larvicidal activity of conifer-derived EOs against mosquito larvae.

Beyond reproducing known insecticidal trends, the present investigation expands the scope of prior research by offering a comparative and mechanistic interpretation of multiple extract types from both species. However, this study advances previous research by providing a comprehensive and systematic comparison of multiple extraction sources (methanol, acetone, ethyl acetate, *n*-hexane, and EOs) for both plants, with accurate LC_{50} estimation across multiple times (24 and 48 h), demonstrating the superiority of *P. halepensis* EOs in its low toxic potency (LC_{50} = 71.96 ppm). Additionally, the study included an in-depth analysis of the biochemical benefits, evaluating the effect of the extracts on important enzymes such as acetylcholinesterase, esterase, and oxidation enzymes, providing a deeper understanding of the biological mechanisms behind the toxic potency.

Our results match previous studies that looked at leaf extracts from the Egyptian *C. sempervirens* (Cupressaceae) plant, using solvents like ethanol, acetone, and petroleum ether, on 3rd instar *Cx. pipiens* larvae. The results indicated that the petroleum ether extract (LC_{50} = 37.8 ppm) was more potent than the acetone extract

(LC_{50} = 104.3 ppm) and the ethanol extract (LC_{50} = 263.6 ppm)[70]. The data indicated that EOs for both tested larvicides exhibited more toxicity to *Cx. pipiens* larvae compared to other leaf extract solvents. Furthermore, *C. sempervirens* essential oil was more effective (LC_{50} = 61.55 ppm) than *P. halepensis* EOs (LC_{50} = 76.12 ppm).

In agreement with our results, Mitić, et al.[65] showed that the oil of *P. halepensis* included the highest concentrations of sesquiterpenes and diterpenes, with (E)-caryophyllene and thunbergol being the most prevalent constituents (32.2% and 29.2%, respectively). More than half of the monoterpenes found in *P. heldreichii* oil were limonene (34.4%) and α-pinene (23.8%). Mitić et al. (2019). Furthermore *C. sempervirens* EO have different type of terpenes caused significant insecticidal bioactivity against *Cx. quinquefasciatus*[71]. *Cupressus sempervirens* EOs has shown insecticidal effects against pests like the maize weevil *Sitophilus zeamais*[72], rice weevil *Sitophilus oryzae*[73], and *Aedes albopictus*, the common mosquito vector of West Nile fever[74]. This EO, along with its oil-based nanoemulsion and main terpenes, especially α-cedrol, acted as a strong repellent against *Cx. quinquefasciatus*. Additionally, *C. sempervirens* EO exhibited repellent bioactivities against *Ae. albopictus*[74]. The development of eco-friendly, low-concentration, and non-toxic mosquito repellents is gaining interest as an alternative to harmful chemical pesticides[75].

The variations in larvicidal efficacy among different extracts can also be related to the polarity of the solvents used during extraction. Non-polar solvents tend to extract lipophilic terpenoids, which have stronger insecticidal potential, while polar solvents such as methanol and acetone extract phenolic and flavonoid compounds that may contribute to antioxidant and moderate larvicidal effects. The synergistic action of multiple phytoconstituents could further explain the observed differences between extracts[76].

Several other oils and plant extracts have shown similar larvicidal effects against *Cx. pipiens* larvae, including *Commiphora molmol*, *Araucaria heterophylla*, *Eucalyptus camaldulensis*, *Boswellia sacra*, and *Pistacia lentiscus* oil-resins. These demonstrated notable larvicidal activities with LC_{50} values were 300.63, 384.71, 628.65, 832.78 and 1185.69 ppm, 48 h post-treatment respectively[27]. Among these, *Artemisia* oils were particularly effective in controlling *Cx. pipiens* larvae, especially when formulated as a triple-blend EOs derived from different *Artemisia* species. The larvicidal potency of this blend was significantly enhanced by encapsulating it in β-cyclodextrin, which improved both its stability and insecticidal activity, resulting in a 48% increase in toxicity compared to the unencapsulated oil. This enhancement is attributed to its mechanism of action targeting the acetylcholinesterase enzyme[77].

The inclusion of both positive (Temephos) and negative (dechlorinated water) controls ensured the reliability of the bioassay results. The absence of mortality in the negative control confirmed that the observed effects were due to the plant extracts and EOs, while the complete mortality in the positive control validated the test procedure. These findings are consistent with standard WHO bioassay protocols and support the accuracy of the experimental design.

Certain plant compounds, such as elemene, caryophyllene, and copaene, can disrupt various physiological processes in insects, particularly by affecting their nervous systems and potentially causing nerve damage. This disruption can lead to dysfunction in nerve activity, impairing the insects' ability to move, feed, or reproduce. It can also affect their ability to avoid eating certain things, stop them from making chitin, which is important for their outer shell, and cause problems in their growth[78]. These compounds might block cholinesterase activity, harm cell membranes, and cause cells to leak and die; additionally, they could act as repellents by altering insect behavior and keeping them away from specific areas.

In the current study, the repellency activity of the EOs was time- and concentration-dependent. Complete protection was achieved at all test concentrations for 60 min, except for the lowest concentrations (0.05% and 0.25%) of *C. sempervirens* and *P. halepensis* EOs. The highest concentration (1%) provided 100% protection for 150 min for both tested botanical EOs.

These results agree with previous studies showing the repellent effects of various EOs on the movement behavior of insect pests in stored products. *P. halepensis* EO exhibited significant repellent activity against *T. castaneum* and *Rhyzopertha dominica*, with high repulsion rates of class IV at 63.60% against *T. castaneum* and 66.50% against *R. dominica*. The RI values, which were less than 1 for all concentrations applied over time (10 min–5 h), confirm these results and the EO's ability to repel both insects. Insects avoided the treated half-disks and moved more quickly toward the untreated half (control), demonstrating the repellent activity of this EO[79,80].

In addition to direct toxicity, the biochemical assays provided critical evidence of how these extracts interfere with the physiological and metabolic systems of mosquito larvae. The data showed that both *P. halepensis* and *C. sempervirens* and extracts significantly inhibited the activities of key neuroenzymes (AChE, α-EST, β-EST) and metabolic enzymes (GABA-T, amylase, and lipase) in mosquito larvae, with *P. halepensis* exhibiting a more pronounced inhibitory effect ($P \leq 0.05$). Additionally, both extracts significantly reduced antioxidant enzymes (SOD, GST, and CAT) and GSH levels, with *P. halepensis* again showing stronger activity. Concurrently, markers of oxidative stress—lipid peroxidation (LPO) and protein carbonyl content (TPC)—were significantly elevated, especially in larvae treated with *P. halepensis*. These results indicate that *P. halepensis* possesses superior larvicidal potential, likely through a multi-targeted mechanism involving enzymatic inhibition and oxidative stress induction. EOs can penetrate the cuticle of insects, enhancing their toxicity upon contact. This penetration facilitates absorption and amplifies the neurotoxic effects, potentially leading to paralysis or death[81,82]. Some substances in EOs, like eucalyptol, can block acetylcholinesterase, which messes up how nerves send signals and can kill the insect. Eucalyptol, for instance, interferes with the octopamine receptor, disrupting nerve signaling[83]. Fatty acids like palmitoleic, linolenic, palmitic, lauric, and myristic acids also possess insecticidal properties[84]. In this study, *Cx. pipiens* larvae showed a high sensitivity to EOs. According to Toews and Subramanyam[85], insects such as *Rhyzopertha dominica* may be more vulnerable due to faster cuticular penetration, heightened sensitivity at the target site, or reduced detoxification. The insect cuticle serves as a protective barrier, and EOs can compromise this barrier, allowing toxins to enter the body and ultimately leading to death[80].

To elucidate the relationship between chemical composition and larvicidal potency, the phytochemical profiling of both species was carried out using advanced chromatographic techniques. The current study investigated the phytochemical profiles of the methanol (Me), ethyl acetate (Eth.) and acetone (Ac) extracts of two conifer plants *P. halepensis* (GA) and *C. sempervirens* (CU) using UPLC/MS analysis which gave rise to fifty-eight metabolites as detailed in the results part. In addition, their *n*-hexane extracts and EOs were analyzed through GC/MS analysis leading to the identification of twenty components. The two plants showed an interesting phytochemical profile mainly centered around flavonoids as the main identified class with diterpenoids in the second place. *Cupressus* extracts were mainly rich with flavonoids and phenolic derivatives. On the other hand, *Pinus* extracts were abundantly presenting diterpenoids, phenolics and glycosides.

The number of identified components were varied between the extracts as well as the % composition for the metabolites between the extracts. For *Cupressus* (CU) extracts, the number of identified compounds was following CU-Me > CU-Ac > CU-Eth while for the *Pinus* (GA) extracts it showed the following order GA-Me > GA-Ac > GA-Eth. The % composition was the highest for CU-Me in ESI positive mode followed by CU-Me in –ve mode and CU-AC –ve mode while for GA extracts the GA-Ac showed the highest % (79.26%, ESI –ve mode) followed by GA-Me (-ve mode) and GA-Eth (-ve mode).

Multivariate data analysis remained a cornerstone for data analysis and discrimination especially for huge data sets and detailed data results. Herein, principal component analysis (PCA) and clustered heat map were utilized for data discrimination between the *Pinus* and *Cupressus* extracts metabolites in the positive and negative ion modes which gave rise to different clustering patterns that were changing upon changing the UPLC/MS ionization technique. Certain components were behind the unique clustering of each group of extracts.

Crude extracts prepared using different solvents such as methanol, acetone, and ethyl acetate hold substantial biological significance due to their rich content of diverse phenolic compounds, flavonoids, and alkaloids, many of which are absent or present in lower concentrations in volatile oils[86]. These bioactive constituents contribute critically to insecticidal and larvicidal properties by inhibiting essential enzymes and inducing oxidative stress within the target organism[87].

Confers are among the widely investigated plants and from their literature we can detail the following; profiling of five *Pinus* species identified 44 metabolites (flavonoids, phenolics, lignans, diterpenes, and fatty acids). Chemometric analyses revealed species-specific compounds and linked metabolite profiles to bioactivities. All extracts showed antiaging effects by elevating telomerase/TERT, while *P. pinea* and *P. canariensis* exhibited strong acetylcholinesterase and DPP-4 inhibition with antidiabetic activity comparable to sitagliptin, highlighting pine needles as promising sources for managing diabetes and Alzheimer's disease[23].

Comparative analysis of cones from 16 coniferous taxa showed that antioxidant levels (TPC, FRAP, DPPH) were highest in green cones, followed by mature and then opened cones. *Tsuga canadensis*, *Metasequoia glyptostroboides*, *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Thuja orientalis*, and *Picea abies* exhibited the greatest antioxidant potential in green and mature cones. HPLC-MS/MS profiling confirmed diverse polyphenols, providing a foundation for future bioactivity studies and valorization of conifer cone waste as a source of antioxidant compounds[52].

Ethanol extracts of *Pinus sylvestris* from different Turkish provinces showed antioxidant and enzyme inhibitory activities. Cones from Gumushane exhibited the strongest radical scavenging effects with IC_{50} values of 14.75 µg/mL (DPPH) and 12.56 µg/mL (ABTS). LC-HRMS profiling identified major metabolites including (+)-*trans* taxifolin, quercitrin, fumaric acid, (–)-epicatechin, nepetin-7-*O*-glucoside, and apigenin-7-*O*-glucoside, highlighting *P. sylvestris* cones as a source of bioactive compounds with potential health benefits[88].

UPLC-ESI-MS/MS analysis of *Pinus brutia* bark extract (ultrasonically extracted with 50% ethanol) identified 15 phenolic compounds, with catechin hydrate as the predominant metabolite (28.3 mg/100 g extract). Other compounds included gallic, protocatechuic, vanillic, caffeic, *p*-coumaric, and ferulic acids, as well as flavonoids such as myricetin, luteolin, naringenin, and kaempferol. Comparison with pycnogenol showed both extracts contained significant levels of phenolics, underscoring *P. brutia* bark as a rich source of bioactive compounds[58].

LC-DAD–ESI-MS/MS Profiling of branch wood extracts from 13 *Pinaceae* species revealed diverse phytochemicals, including lignans, stilbenes, flavonoids, diterpenes, and procyanidins. Species-specific differences were noted: *Picea abies* was richest in stilbenes, *Larix decidua* in flavonoids, and *Abies alba* in lignans. Ten lignans were isolated, confirming that conifer branch wood, an abundant wood industry byproduct, is a valuable and accessible source of bioactive compounds suitable for future isolation and applications[18].

Aqueous extracts from petal and core fractions of *Pinus halepensis*, *P. brutia*, and *P. pinea* were evaluated. *P. halepensis* petal water extract (APW) showed the highest polyphenol content (203.5 mg GAE/g DW) and strongest antioxidant activity (IC_{50} = 13.5 µg/mL). All extracts exhibited strong anticancer effects against HeLa and $HepG_2$ cells with minimal toxicity to normal HEK-293 cells. Only APW significantly inhibited α-glucosidase (77.2% at 50 µg/mL). HPLC-DAD identified 14 compounds, while GC-MS revealed 28 compounds, including 11 reported for the first time, highlighting pinecones as promising sources of therapeutic agents[89].

The phytochemical investigation of *Pinus cembra* heartwood using UHPLC-DAD-ESI-MS^n, HPLC-DAD, isolation, and NMR identified seven flavonoids, four stilbenes, two bibenzyls, three fatty acids, and one diterpenic acid. Key bioactive compounds such as dihydropinosylvin monomethyl ether and pinosylvin monomethyl ether were separated. confirmed and expanded upon earlier mid-20th-century studies, identifying substances 1–4 and 13 as newly reported in *P. cembra*, while disproving the earlier claim of pinobanksin presence[57].

LC-MS/MS profiling of *Cupressus arizonica* leaf extracts identified 67 metabolites, mainly phenolic acids, fatty acids, diterpene acids, proanthocyanidins, and flavonoid/biflavonoid glycosides. The aqueous extract showed strong antioxidant activity (FRAP, DPPH) and effectively inhibited skin-aging enzymes (collagenase, elastase, tyrosinase, hyaluronidase) with IC_{50} values comparable to standard drugs. Additionally, it suppressed the formation of Pseudomonas aeruginosa biofilms and reduced bacterial motility[24].

A phytochemical study of *Cupressus macrocarpa* leaves led to the isolation of ten secondary metabolites, including three new diterpenoids, four known diterpenoids, and three known lignans. Structural elucidation was achieved using 2D NMR and MS analyses. Cytotoxic assays showed that several compounds (1–3, 7–10) significantly inhibited HepG2, MDA-MB-231, and A549 cancer cell lines (IC_{50} = 0.004–19.9 μg/mL). Additionally, the lignan (–)-matairesinol exhibited strong anti-inflammatory effects by inhibiting superoxide anion generation (IC_{50} = 2.7 μM) and elastase release (IC_{50} = 6.6 μM)[25].

Needle extracts of *Cupressus torulosa* demonstrated strong antioxidant potential. The 25% aqueous methanol extract showed promising in vitro and in vivo activity, with reduced SGOT and SGPT levels comparable to glibenclamide. Among fractions, the ethyl acetate extract was most active, with a DPPH EC_{50} of 85.6 μg/mL, indicating effective free radical scavenging. UPLC-QTOF-MS analysis identified 34 metabolites, including 10 key antioxidant compounds[90].

Collectively, the integration of larvicidal, biochemical, and phytochemical findings highlight the multi-targeted action of these plant derivatives, which supports their practical relevance in vector management. Finally, the present findings highlight that *P. halepensis* and *C. sempervirens* extracts and EOs can be effectively integrated into vector management programs as eco-friendly alternatives to synthetic insecticides. Their strong larvicidal and repellent activities against *Cx. pipiens*, along with their safety, biodegradability, and availability from local plant sources, make them suitable for practical application in treating larval breeding sites and personal protection formulations. Therefore, these botanicals could contribute to sustainable and environmentally safe mosquito control strategies.

Conclusions

Cupressus sempervirens and *P. halepensis* extracts and EOs demonstrated strong larvicidal and repellent activities against *Cx. pipiens*, highlighting their potential as effective, eco-friendly alternatives to synthetic insecticides. Different classes of phytoconstituents were tentatively detected from the studied plants, viz., flavonoids as the main class, followed by diterpenoids, tannins, prenol lipids, fatty acids, alkaloids, and others. Furthermore, the *n*-hexane extract and EOs were abundant in various mono-, sesqui-, di-, and triterpenoids, along with fatty acids. The EOs from these plants demonstrated significant repellent and insecticidal properties. The effectiveness of these oils can be influenced by various factors, such as their chemical composition, the interactions between the compounds, the concentrations used, the duration of exposure, and the specific insect targeted. Higher concentrations were more effective in killing and repelling *Cx. pipiens*, especially with longer exposure. Even at moderate concentrations, the oils continued to offer strong repellency against adult mosquitoes over extended periods. These findings emphasize the practical value of utilizing locally available coniferous resources for the development of sustainable, biodegradable botanical larvicides that can be integrated into environmentally safe vector management programs and the non-target effects of these plant-based formulations to ensure their secure application in field conditions.

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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Declarations

Competing interests

The authors declare no competing interests.

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Additional information

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