Parasitology International 111 (2026) 103178

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Efficiency of *Datura stramonium* metabolites as a promising insecticide against the vector-borne diseases *Culex pipiens* and *Aedes aegypti*

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ARTICLE INFO

Keywords:
Culex pipiens
Datura
Larvicidal
Antimicrobial
UPLC/MS
Alkaloids
Withanolides
insecticide

ABSTRACT

Mosquitoes, one of the deadliest animals on the planet, cause millions of fatalities each year by transmitting several human illnesses. Therefore, it was necessary to use many synthetic pesticides to prevent the spread of diseases, which led to an increase in the effective use of synthetic pesticides to protect humans. The extensive use of pesticides caused serious health problems for humans, environmental damage, and mosquito resistance to synthetic insecticides. This study aimed to evaluate the larvicidal efficacy of *Datura stramonium* extracts (methanol, ethyl acetate, *n*-hexane, and aqueous) against larvae and adults of *Culex pipiens* and *Aedes aegypti* with phytochemical profiles. Bioassays were conducted under laboratory conditions, and lethal concentrations (LC_{50} and LC_{90}) were calculated after 24 and 48 h. Methanol and ethyl acetate extracts showed the strongest ability to kill larvae, with LC_{50} values of 40.19 ppm for *Cx. pipiens* and 71.46 ppm for *Ae. aegypti*. In adulticidal assays, aqueous and ethyl acetate extracts induced the highest mortality rates (up to 88 %) at 100 ppm. The methanol extract exhibited strong antimicrobial activity, recording the largest inhibition zone (27 mm) against *Klebsiella pneumoniae*, followed by *Staphylococcus aureus* (25 mm) and *Candida albicans* (22 mm). highlighting its potential as a dual-function natural agent with both insecticidal and antimicrobial properties. UPLC/MS analysis identified several bioactive phytochemicals, including alkaloids withanolides, alkaloids, phenolic acids, cinnamic acid derivatives and flavonoids, that may contribute to the observed effects. The n-hexane extract revealed twelve volatile components from the mono-, di-, and sesquiterpenoid classes, along with volatile alkaloids such as nicotine and scopolamine. Antimicrobial tests also revealed moderate to high inhibition against selected bacterial and fungal pathogens. Quantitative real-time PCR revealed that the genes Apismin, Defincin 1, SFCYP1, SFCYP4, SFCYP5, and SFRYR were significantly increased, while SFCYP3 was consistently decreased in both *Datura* and pesticide. However, SFCYP2 is believed to increase in pesticide and decreased in *Datura*. These findings highlight *Datura stramonium* as an available, eco-friendly, and promising natural alternative to synthetic insecticides for vector control.

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https://doi.org/10.1016/j.parint.2025.103178
Received 4 July 2025; Received in revised form 24 September 2025; Accepted 29 September 2025
Available online 3 October 2025
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1. Introduction

Mosquito-borne diseases remain a critical global health challenge, especially in tropical and subtropical regions. The two major vectors, *Culex pipiens* and *Aedes aegypti*, are responsible for transmitting diseases such as West Nile virus, dengue, and chikungunya [1,2]. Growing concerns over insecticide resistance and environmental safety have driven research toward plant-derived bioinsecticides as alternative solutions. Therefore, many strategies focus on suppressing mosquito populations, eliminating individuals at different life stages such as larvae and adults, and limiting their ability to spread. Such measures are essential in breaking the transmission cycle and ultimately preventing the spread of these diseases [3].

Medicinal plants have long been known to contain many beneficial substances that can help treat illnesses and also serve as natural pesticides, which can help deal with pesticide resistance and pollution while being safe for other living organisms. One of these medicinal flowering plants is *Datura*, which belongs to the Solanaceae family and is widely grown in regions such as Europe, Asia, the Americas, and both North and South Africa in tropical and subtropical zones with warm, humid conditions [4–7].

The family Solanaceae (Nightshade) is well-known for producing the toxins named tropane alkaloids, and the most commonly investigated are (−)-hyoscyamine and (−)-scopolamine. Atropine is the racemic mixture of (−)-hyoscyamine and (+)-hyoscyamine, of which only the (−)-hyoscyamine enantiomer is found to have anticholinergic activity [8]. The annual or perennial herbaceous plant belonging to the genus *Datura* (Solanaceae) is widely distributed throughout the world, including India. This genus of plants is well-known for its many valuable chemical ingredients that have both therapeutic and commercially significant uses.

Although the plant carries many interesting phytochemical classes including alkaloids, tannins, flavonoids, steroids, and phenolic chemicals. Alkaloids which are has a powerful effect on the nervous system, these same substances can also provide important health benefits when used carefully [9,10]. The health benefits of *Datura stramonium* may involve nerve signaling, reduce inflammation, combat germs, and provide various therapeutic effects such as alleviating asthma symptoms, protecting against damage, relieving pain, killing insects, harming cancer cells, healing wounds, and safeguarding the nervous system; thus, it is useful for treating a range of human health issues [11,12].

boiling neutralizes the harmful compounds. In Ayurvedic practices, *Datura stramonium* is used to address ailments such as wounds, ulcers, rheumatism, inflammation, asthma, fever, and toothaches [13,14]. Internally, leaf extracts are used to manage respiratory problems like asthma and sinus infections, while bark preparations applied to the skin help treat burns and swelling. Still, the toxic potential of *D. stramonium* often outweighs its therapeutic uses in modern clinical settings. High doses can severely disrupt the central nervous system, leading to confusion, hallucinations, memory loss, and abnormal behavior [15]. Acute poisoning may also result in dry mouth, dilated pupils, blurred vision, difficulty urinating, dry skin, and a rapid heart rate [16].

The LC-MS/MS technology was used to assess the amounts of atropine and scopolamine in the plant. Atropine and scopolamine in *Datura* species were even analyzed using sophisticated Liquid Chromatography–Mass Spectrometric technique. UPLC-MS/MS was used to characterize withanolides from six distinct plant sections. Following the solid phase extraction methodology, an analytical method for estimating atropine and scopolamine was also published. In a different study, the amount of atropine and scopolamine (hyoscine) in the plant was examined. The results indicated that the concentration increased with the plant's growth stage and peaked toward the conclusion of its reproductive period. Many major and minor tropane alkaloids found in the plant are responsible for its various pharmacological properties. The roots contained alkaloids such as scopolamine and hyoscine, which demonstrated antibacterial, phytochemical, and antioxidant properties [11].

Numerous plants with therapeutic qualities have also been shown to have larvicidal potential. The larvicidal qualities of several commonly used plants, including *Azadirachta indica*, *Momordica charantia*, and *Nicotiana tabacum*, have been well studied [17–19]. Acaricidal activity of *D. innoxia* ethanol extract was reported against *Rhipicephalus microplus* [20]. Effective acaricides include biologically active limonoids, polyphenols, terpenoids, quinines, nicotine, amygdalin, sapotin, and saponin. Several components of the intricate variety of secondary metabolites found in nature exhibited larvicidal and acaricidal action. It is impossible to overlook the vital role those secondary metabolites of the plant kingdom play in a variety of biological processes. Consequently, it was essential to identify these possible phytoconstituents in order to clarify their bioactivities [21].

Medicinal plants of the genus *Datura* have been widely used in folk medicine to relieve pain and treat skin conditions, mental disorders, and organophosphorus poisoning. *Datura* leaves were smoked like cigarettes to alleviate asthma. Historically, the plant has been used medicinally worldwide; the Chinese used it for colds and nervous conditions, while the Zuni Indians and Mexicans applied it as an ointment for wounds and to relieve pain during bone setting. These plants produce compounds that can cause genetic alterations, potentially leading to diseases or teratogenic and carcinogenic effects. Although some substances have been studied, toxicological and genotoxicological research is still insufficient, necessitating further investigation into the genotoxic and cytological effects of medicinal plants [22–24].

Besides its famous medical uses, *Datura* may also work well to fight different insect pests, like mosquitoes, which can spread serious diseases such as malaria, dengue fever, yellow fever, lymphatic filariasis, Japanese encephalitis, and chikungunya, affecting both humans and animals. This was corroborated by several researchers, including Mohamed, Kehail, Hilmi, Homida and Abdelrahim [18]. It is concluded that the ether extract of *D. stramonium* is more effective against *Anopheles*, *Culex*, or *Aedes* larvae. Mosquito-borne diseases cause major economic and social problems around the world, resulting in lost work, higher death and illness rates, and worsening poverty, particularly in tropical and subtropical areas, although these diseases can happen anywhere [25].

Datura is also recognized for its insecticidal properties against pests such as the cattle tick (*Hyalomma dromedarii*) and the red flour beetle (*Tribolium castaneum*) and as a mosquito repellent [26]. People have historically used parts of the *Datura* plant to relieve pain from venomous bites, including snakebites. Beyond its medicinal uses, *Datura* is known for its role in traditional spiritual and religious rituals, with seeds commonly inhaled for their hallucinogenic effects. However, there have been numerous reports worldwide of both accidental and intentional poisoning from *D. stramonium*, often due to recreational use or its combination with herbal drugs to induce euphoria [27]. Therefore, we must handle the plant with caution, despite its promising medicinal value. Thorough research into its toxicological profile is necessary, and any use should be undertaken with awareness of its potential risks. Recently, the use of plant-based, environmentally friendly pesticides has received a lot of attention. These natural alternatives are considered safer and less damaging to ecosystems and non-target organisms. Many plants are being studied for their ability to kill mosquito larvae, with substances like saponins, alkaloids, flavonoids, essential oils, tannins, and steroids proving effective against them [3,28]. Even though many plant-based repellents have been found, getting them approved for use is still difficult because of issues with how they are made and a lack of reliable chemical information and testing methods.

Gene expression analysis is essential for better understanding the potential molecular effects and resistance mechanisms induced by *D. stramonium* extracts. Quantitative real-time PCR (qRT-PCR) is a useful molecular technique for measuring and monitoring gene expression due to its high sensitivity, specificity, reproducibility, reliability, and ability to perform high-throughput analysis [29]. Cytochrome P450 enzymes are important for the growth and survival of insects, and many of them

likely play a role in breaking down insecticides and helping insects resist them [30]. Cytochrome P450 enzymes, also known as CYPs, are heme-containing monooxygenases found in all kingdoms of life [31].

Despite various studies on the therapeutic and chemical properties of *D. stramonium*, research on its extracts as eco-friendly insecticides against disease vectors like *Cx. pipiens* and *Ae. aegypti* remains limited. Furthermore, there is a lack of research on the genetic and protein-level effects on mosquitoes, as well as the antimicrobial activity of these extracts. This study aims to address these gaps by evaluating the efficacy of multi-solvent extracts on entomological and molecular targets, alongside chemical and antimicrobial analyses.

This study aimed to evaluate how well *Datura stramonium* extract, using different solvents, can kill the larvae and adults of *Culex pipiens* and *Aedes aegypti*, which are important carriers of diseases. Using UPLC/MS, the chemical makeup of the plant extracts was examined, and methods were used to explore how the active compounds interact with a fatty acid binding protein (FABP) in insect muscle (PDB ID: 2FLJ). The study also determined the antimicrobial activity of *Datura stramonium*.

2. Materials and methods

2.1. Plant materials and analysis

2.1.1. Plant collection

Mature leaves of *D. stramonium* were gathered from various locations within the agricultural fields of the Faculty of Agriculture, Qalyubiya Governorate, Egypt, during the period of September to October 2024 (Fig. 1). The plant has large, trumpet-shaped blossoms that are white and purple in color, emit a strong fragrance, and can reach lengths of around six inches (approximately 15 cm). Its dark green leaves are broad and lush, and its fruit is a prickly capsule ranging from 3 to 10 cm in size [32]. The plant that was collected was identified and confirmed by Dr. Trease Labib, a plant expert at the Egyptian Ministry of Agriculture, and a sample was stored at the herbarium of the Pharmacognosy Department at Ain Shams University, with the code (PHG-P-DS-538). The harvested plant material was air-dried in the shade at ambient room temperature until fully dehydrated and reduced in weight. The dried leaves were then ground using a stainless-steel electric mixer and stored in sealed containers to protect them from humidity.

2.1.2. Plant extraction

The plant leaves were first washed with tap water and left to dry naturally in shaded conditions. Once dried, the leaves are ground into a fine powder to ensure extraction. Different solvents, including methanol (99.9 %), acetone (50 %), ethyl acetate (96.5 %), hexane (93 %), and distilled water, were used for extraction with the aid of a Soxhlet apparatus. For each extraction cycle, 40 g of the powdered leaf material were processed with 200 mL of the respective solvent separately. The extraction procedure lasted for 7 to 12 h, or until the solvent became colorless. After extraction, the extracts were concentrated using a rotary evaporator and then stored. The prepared extracts were stored in airtight containers at 4 °C and kept for further use [33].

Fig. 1. A photograph showing *Datura stramonium*.

2.2. Mosquito larvicidal assay

2.2.1. Rearing of Culex pipiens and Aedes aegypti mosquito

Culex pipiens and *Aedes aegypti* larvae were reared in an insectary under controlled environmental conditions of 27 ± 2 °C temperature and 75 ± 5 % relative humidity, with a 12-h light and 12-h dark cycle. They were nourished with a mixture of Tetramin fish food and finely ground bread in a 3:1 ratio. Once pupation occurred, individuals were transferred from enamel trays into cups containing dechlorinated water and placed in mesh cages measuring 35 × 35 × 40 cm, allowing for adult emergence. Female mosquitoes were routinely provided blood meals from a hamster, while all adults had continuous access to a 10 % sugar solution. Both larval and pupal stages were consistently maintained in the same laboratory setting to ensure a steady supply for experimental purposes [34].

2.2.2. Larvicidal activity

Under controlled laboratory settings, methanol, acetone, ethyl acetate, hexane, and distilled water of *D. stramonium* were tested for their effectiveness against 4th instar larvae of *Culex pipiens* and *Aedes aegypti*. A stock solution was made by dissolving 1 g of extract in 1000 mL of distilled water to obtain a 1000 ppm solution, and serial dilutions were performed from the stock solution to achieve the lower concentrations. The larvae were exposed to six different concentrations: 25, 50, 125, 250, 500, and 1000 ppm. For each concentration, twenty larvae were placed in a 250 mL glass beaker filled with distilled water. Untreated or negative control groups using water only. All tests were conducted in triplicate. Larval mortality was monitored at 24 and 48 h following initial exposure and during the post-treatment period [35].

2.3. Antimicrobial assay

To evaluate the antimicrobial activity of plant extracts, the technique of agar well diffusion is frequently utilized. In this method, a microbial suspension is evenly spread across the surface of an agar plate to inoculate it with bacteria or fungi, following a process like the disk diffusion technique. Next, punch a 6 mm diameter well into the agar using a sterile cork borer or pipette tip. Each well is subsequently filled with 100 μL of the plant extract or antimicrobial agent prepared at the required concentration. The plates are incubated under conditions suitable for the specific microorganisms being tested. During incubation, the antimicrobial compound diffuses through the agar and inhibits microbial growth [36]. After an incubation period of 16–24 h for bacteria and up to 48 h for fungi, the inhibition zones—clear areas around the wells where microbial growth has been halted—are measured in millimeters. Gentamicin (1.0 mg/mL) served as the positive control for bacterial strains, while fluconazole (1.0 mg/mL) was used for fungal strains. The plant extracts were prepared by dissolving 50 mg of extract in 1.0 mL of distilled water. All microbial cultures used in the experiment were obtained from Thermo Fisher Specialty Diagnostics Ltd., located in Hampshire, UK.

2.4. Phytochemical identification

2.4.1. UPLC/MS analysis

The UPLC/ESI/MS analysis was performed for the four *D. stramonium* extracts using the method of [37–39]. UPLC/ESI/MS in both positive and negative ion acquisition modes were carried out on a XEVO TQD triple quadrupole instrument, Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer. Chromatographic separation of the sample was done by injecting 10 μL into UPLC instrument equipped with 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) disc and degassed by sonication before injection then subjected to LC/ESI/MS analysis. The gradient mobile phase comprising two eluents: eluent A is H2O acidified with 0.1 % formic acid and eluent B is MeOH acidified with 0.1 % formic acid. Elution was made at flow rate 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) 25–29 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.

2.4.2. GC/MS analysis

Datura stramonium n-hexane extract were injected to a gas chromatography coupled to mass spectrometry (Shimadzu GCMS-QP 2010, Kyoto, Japan) operating in EI mode at 70 eV and mass spectrum acquisition 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. 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 NIST-17 and Wiley library databases [40].

2.5. 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 distance and the unweighted pair group method [41,42].

2.6. UV–Vis spectroscopic measurements

Uv-Vis absorption spectra of extracts from *D. stramonium* were acquired using double-beam UV–visible spectrometer (Jasco spectrophotometer (Model V-670, Japan) using a 1 cm path length quartz cuvette. A matching quartz cuvette filled with (methanol), the solvent used for *Datura* extract, served as blank reference. Spectra were recorded in the range of 200–800 nm at room temperature. The absorption coefficient was then calculated directly from the measured absorption (A) using rearranged form of the Beer-Lambert Law using the following equation: The absorption data was used to calculate the optical properties which include transmittance, absorbance (A) and energy bandgap over the whole tested wavelength range. The optical absorption coefficient (α) as a function of wavelength can determine from the optical spectra using the following formula [43] as follows.

$$\alpha = \left(\frac{2.303 \times A}{d}\right) \tag{1}$$

d is the cuvette thickness and A is a function of the intensity of the transmitted (I) and incident beams (I_o) [$A = \log\left(\frac{I}{Io}\right)$].

The optical bandgap energy was measured in order to ascertain the amount of energy absorbed or accomplished in the system during sample exposure time. The optical bandgap energy determines how much energy is needed or generated for the photon to excite or release. The relationship between the incident photon energy and the absorption coefficient in various electronic transitions can be used to calculate the optical bandgap [44].

$$\alpha h\nu = \beta\left(h\nu - E_g\right)^e \tag{2}$$

"E_g" is the optical bandgap energy, "β" is a constant and "e" determines the type of electronic transition which equals to 1/2 for the direct transition or 2 for indirect transition band gap, "h" is the Planck constant, and ν is the photon's frequency.

2.7. Molecular evaluations

To assess the impact of insecticide and datura on the examined *Culex pipiens* mosquito after – days of treatment, a set of genes (Apismin, Defincin 1, SFCYP1, SFCYP2, SFCYP3, SFCYP4, SFCYP5, and SFRYR) were evaluated. The primers of the eight examined genes are listed in Table 1. After the complete treatment period, RNA extraction was performed using an RNA extraction kit (QIAGene, USA). RT-qPCR was carried out to measure the expression levels of these genes in the treated larvae relative to the non-treated (control), and the β-actin gene was used as a reference gene [45].

2.7.1. RNA extraction and cDNA synthesis

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The obtained RNA concentration and purity were measured using a NanoDrop apparatus (NanoDrop 2000 spectrophotometer, Thermo Scientific, USA). For cDNA synthesis, a total volume of 20 μL was carried out using a SureCycler 8800 thermocycler (Agilent Technologies, Origin, USA) in the presence of oligo (dT) primer (10 pmol/μL). The reaction component was 3 μL RNA (500 ng), 2.0 μL dNTPs (10 mM), 2.0 μL buffer, 5 μL primer (10 pmol/μL), 0.3 μL reverse transcriptase enzyme (Biolabs, New

Table 1
The oligonucleotide sequences of the primers used in real-time qPCR reactions to determine the expression level study in *Cx. pipiens*.

Gene	Primer sequences
Apismin	F: 5′-TGAGCA AAATCGTTG CTGTC-3′ R: 5′-AAC GACATC CAC GTTCGATT-3′
Defencin-1	F: 5′-TGC GCTGCTAAC TGTCTCAG-3′ R- 5′-AATGGCACT TAACCGAAACG-3′
SFCYP1 Cytochrome 1	F- 5` GAGCTTACTTCGGCACGTTG 3` R- 5` CAACACTTTCGAGCGGTCG 3`
SFCYP2 Cytochrome 2	F- 5` GAAGCGTGGCGTAAAGTTCT 3` R- 5` AAGAGCGCAGGTGTTAGGAC 3`
SFCYP3 Cytochrome 3	F- 5` GAGAAAGTATCCGCCGGGTT 3` R- 5` ACAAGCTCTCCATTCCGATCC 3`
SFCYP4 Cytochrome 4	F- 5` AAGAACCCTTGGAAACCG 3` R- 5` ATGGGAACACTAAGTCGGGG 3`
SFCYP5 Cytochrome 5	F- 5` TGTTATCCAAGCAAATTGATCGAG 3` R- 5` GTCAAATGCGGCTGATGACG 3`
SFRYR ryanodine receptor	F- 5` CACAGGTGGATCTCTCCCAG 3` R- 5` GCGTCCAACGTAGACACCTT 3`
ß actin Housekeeping gene	F- 5` GTGGGCCGCTCTAGGCACCAA 3` R- 5` CTCTTTGATGTCACGCACGATTTC 3`

England, Ipswich, MA, USA) (5 U/μL), and the volume was completed with ddH_2O. The cDNA synthesis reaction conditions were a first cycle of enzyme polymerization at 42 °C for 1 h and a final cycle of enzyme inactivation at 95 °C for 5 min.

2.7.2. qRT-PCR analysis

RT-qPCR was performed using the SYBR Green PCR Master Mix (Fermentas, USA). Each reaction consisted of a 25 μL mixture, which contained 1.5 μL of 10 pmol L − 1 forward primer, 1.5 μL of 10 pmol L − 1 reverse primer, 1 μL of template cDNA (50 ng), 12.5 μL of 2 × SYBR Green, and 3.5 μL of nuclease-free water. The used primer sequences were listed in Table 1. For each reaction, three biological replicates and three technical replicates were established. The reaction was done via a Rotor-Gene 6000 (QIAGEN, ABI System, USA), and the amplification program included an initial denaturation step at 95 °C for 10 min., followed by 40 cycles of denaturation at 95 °C for 15 s, then annealing at 60 °C for 30 s., and a final extension at 72 °C for 30 s. After the end of 40 cycles, the melting curves were obtained to eliminate the inclusion of non-specific products. Data are expressed as the expression relative to β-actin as a housekeeping gene using the 2ˆ-ΔΔCT method [46].

2.8. Statistical analysis

Statistical analysis was conducted using SPSS version 23 (IBM, USA), applying Probit analysis to determine lethal concentration (LC) values. A one-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test) was also performed. A significance threshold of $P < 0.05$ was used for all tests. Principal component analysis (PCA) was conducted using Unscrambler X 10.3 (CAMO SA, Oslo, Norway). A clustered heat map was generated with NCSS 12 software using Euclidean distance and the unweighted pair group method.

3. Results

3.1. Mosquito larvicidal activity

In this study, the larvicidal effect of *D. stramonium* extracts was evaluated on *Culex pipiens* and *Aedes aegypti* with varying concentrations, and larval mortality was recorded 24 and 48 h post-treatment. The plant extract tested in this study was very effective at killing mosquito larvae, *Culex pipiens,* and *Aedes aegypti*, after being exposed for different amounts of time, as shown in Tables 2-5. The results showed that the methanol extract and ethyl acetate of the tested larvicides exhibited higher efficiencies than the other leaf extract solvents for *Cx. pipiens* and *Ae. aegypti*, respectively. On the other hand, the most effective concentrations were 125, 250, 500, and 1000 ppm for botanical insecticides. The results indicated that the mortality rate of *Cx. pipiens* after 24 h post-treatment (PT) with *D. stramonium* extracts (methanol, acetone, ethyl acetate, *n*-hexane, and aqueous) was expressed as 100, 92, 100, 88, & 74.67 %; and 100, 100, 100, 100, & 88 % for 500 and 1000 ppm, respectively. The results for the mortality rate after 48 h post-treatment was 100, 100, 100, 100, and 88 % for 500 ppm and 100 % for 1000 ppm, respectively (Table 2).

The mortality rate for *Aedes aegypti* at 24 h PT of *D. stramonium* extracts (methanol, acetone, ethyl acetate, hexane, and aqueous) was 88, 92, 85, & 64 % and 100, 100, 100, 100, & 84 % for 500 and 1000 ppm, respectively. The larvicidal activity obtained after exposing for 48 h was 93.33, 100, 100, 88, & 72 %; and 100, 100, 100, 100, & 95 % for 500 and 1000 ppm, respectively (Table 3).

The LC_{50} values for *D. stramonium* extracts (methanol, acetone, ethyl acetate, *n*-hexane, and water) on *Cx. pipiens* were 67.19, 104.09, 81.59, 134.93, & 219.92 ppm, and 40.19, 61.75, 45.34, 72.89, & 123.58 ppm after 24 and 48 h, respectively (Table 4). For *Ae. aegypti*, the values were 120.33, 131.85, 98.17, 164.85, & 289.79 ppm and 93.20, 90.99, 71.46, 110.87, & 195.13 ppm after 24 and 48 h, respectively (Table 5). The results indicated that methanol extract was more toxic to *Cx. pipiens* larvae (LC_{50} = 67.19; 40.19 ppm) at 24 and 48 h PT, respectively. However, ethyl acetate extract exhibits more toxic effects on *Ae. aegypti* than other solvents (LC_{50} = 98.17; 71.46 ppm) at 24 and 48 h PT, respectively (Fig. 2)

3.2. Mosquito adulticidal activity

The study evaluated the adulticidal effect of *D. stramonium* extracts against *Cx. pipiens* and *Ae. aegypti*, and the highest mortality percentage (MO%) was obtained with ethyl acetate extracts at 76.0 and 88.0 % (MO %), respectively, at a concentration of 500 ppm, followed by methanol and acetone at (64.0, 80.0 %) and (64.0, 80.0 %), respectively, at the same concentration. The aqueous extract yielded the lowest mortality rate (44.0, 54.67 %). However, the hexane extract showed mild effect for both mosquito species with 60.0 and 72.0 % (MO%), respectively (Table 6).

3.3. Antimicrobial activity of D. stramonium leaves extract

The result evaluated the antibacterial activity of *D. stramonium* methanol extract against *Bacillus subtilis*, *Staphylococcus aureus, Klebsiella pneumoniae,* and *Salmonella typhi* and antifungal activity against *Candida albicans*, and *Penicillium glabrum*, after an incubation period of 16–24 h for bacteria and up to 48 h for fungi. The bacteria *Klebsiella pneumoniae* and *Staphylococcus aureus* were highly sensitive to the methanol extract with maximum zone of inhibition (27, 25 mm), respectively. However, the lowest zone of inhibition was observed against *Bacillus subtilis* (23 mm). There is antifungal activity was observed against *Candida albicans*, and *Penicillium glabrum* with inhibition zone (22, 15 mm), respectively (Table 7, Fig. 3).

The antimicrobial activities of the crude extracts of the plant were tested by paper disc diffusion method. The crude extracts of

Table 2
Efficacy of *Datura stramonium* extracts on *Culex pipiens* larval mortality, 24, and 48 h post-treatment.

Time (hr)	Solvent	Concentration (ppm)						
		0	25	50	125	250	500	1000
24	Methanol	0 ± 0^{aF}	16.00 ± 2.31^{aE}	40.00 ± 4.62^{aD}	68.00 ± 2.31^{aC}	92.00 ± 2.31^{aB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Acetone	0 ± 0^{aG}	13.33 ± 1.33^{bF}	25.33 ± 3.53^{cE}	52.00 ± 4.00^{cD}	76.00 ± 4.62^{cC}	92.00 ± 2.31^{bB}	100.0 ± 0.00^{aA}
	Ethyl acetate	0 ± 0^{aF}	12.00 ± 2.31^{bE}	32.00 ± 2.31^{bD}	60.00 ± 2.31^{bC}	88.00 ± 2.31^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Hexane	0 ± 0^{aG}	9.33 ± 1.33^{cF}	21.33 ± 1.33^{dE}	42.67 ± 2.67^{dD}	64.00 ± 2.31^{dC}	88.00 ± 2.31^{cB}	100.0 ± 0.00^{aA}
	Aqueous	0 ± 0^{aG}	8.00 ± 0.00^{cF}	14.67 ± 1.33^{eE}	28.00 ± 2.31^{eD}	52.00 ± 2.31^{eC}	74.67 ± 1.33^{dB}	88.00 ± 0.00^{bA}
48	Methanol	0 ± 0^{aF}	28.00 ± 2.31^{aE}	60.00 ± 2.31^{aD}	92.00 ± 4.00^{aC}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Acetone	0 ± 0^{aF}	20.00 ± 2.31^{cE}	44.00 ± 2.31^{cD}	76.00 ± 2.31^{cC}	96.00 ± 2.31^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Ethyl acetate	0 ± 0^{aF}	24.00 ± 2.31^{bE}	56.00 ± 2.31^{bD}	88.00 ± 2.31^{bC}	96.00 ± 2.31^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Hexane	0 ± 0^{aF}	18.67 ± 1.33^{dE}	40.00 ± 2.31^{dD}	60.00 ± 2.31^{dC}	84.00 ± 4.00^{cB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Aqueous	0 ± 0^{aG}	12.00 ± 2.31^{eF}	22.67 ± 1.33^{eE}	49.33 ± 3.53^{eD}	64.00 ± 2.31^{dC}	88.00 ± 0.00^{bB}	100.0 ± 0.00^{aA}

a, b & c: There is no significant difference ($P > 0.05$) between any two means, 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.

Table 3
Efficacy of *Datura stramonium* extracts on *Aedes aegypti* larval mortality, 24, and 48 h post-treatment.

Time (hr)	Solvent	Concentration (ppm)						
		0	25	50	125	250	500	1000
	Methanol	0 ± 0^{aG}	13.33 ± 1.33^{aF}	29.33 ± 1.33^{bE}	44.00 ± 2.31^{bD}	64.00 ± 2.31^{bC}	88.00 ± 4.62^{bB}	100.0 ± 0.00^{aA}
	Acetone	0 ± 0^{aG}	9.33 ± 1.33^{bF}	22.67 ± 1.33^{cE}	40.00 ± 4.62^{cD}	64.00 ± 4.62^{bC}	92.00 ± 2.31^{aB}	100.0 ± 0.00^{aA}
24	Ethyl acetate	0 ± 0^{aG}	13.33 ± 1.33^{aF}	32.00 ± 2.31^{aE}	52.00 ± 4.00^{aD}	76.00 ± 2.31^{aC}	92.00 ± 2.31^{aB}	100.0 ± 0.00^{aA}
	Hexane	0 ± 0^{aG}	8.00 ± 0.00^{bF}	18.67 ± 1.33^{dE}	36.00 ± 2.31^{dD}	60.00 ± 2.31^{cC}	85.33 ± 1.33^{cB}	100.0 ± 0.00^{aA}
	Aqueous	0 ± 0^{aG}	5.33 ± 1.33^{cF}	12.00 ± 2.31^{eE}	24.00 ± 2.31^{eD}	44.00 ± 2.31^{dC}	64.00 ± 2.31^{dB}	84.00 ± 2.31^{bA}
	Methanol	0 ± 0^{aG}	14.67 ± 2.67^{aF}	32.00 ± 2.31^{bE}	52.00 ± 4.62^{cD}	80.00 ± 2.31^{bC}	93.33 ± 6.67^{bB}	100.0 ± 0.00^{aA}
	Acetone	0 ± 0^{aF}	12.00 ± 2.31^{bE}	30.67 ± 3.53^{bD}	56.00 ± 2.31^{bC}	80.00 ± 4.62^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
48	Ethyl acetate	0 ± 0^{aF}	16.00 ± 2.31^{aE}	36.00 ± 2.31^{aD}	68.00 ± 4.62^{aC}	88.00 ± 4.00^{aB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}
	Hexane	0 ± 0^{aG}	10.67 ± 1.33^{bF}	28.00 ± 2.31^{cE}	52.00 ± 2.31^{cD}	72.00 ± 4.62^{cC}	88.00 ± 2.31^{cB}	100.0 ± 0.00^{aA}
	Aqueous	0 ± 0^{aG}	8.00 ± 2.31^{cF}	16.00 ± 2.31^{dE}	33.33 ± 1.33^{dD}	54.67 ± 1.33^{dC}	72.00 ± 4.62^{dB}	95.00 ± 6.67^{bA}

a, b & c: There is no significant difference (P > 0.05) between any two means, 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.

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

Time (h)	Treatment	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Slope ± SE	X^2 (sign.)
	Methanol	67.19 (58.44–76.62)	226.47(189.85–281.62)	319.60 (259.39–416.33)	2.428 ± 0.175	4.098 (1.024)
	Acetone	104.09 (89.83–119.84)	442.02 (361.81–565.37)	686.00 (524.90–897.91)	2.040 ± 0.138	4.202 (0.379)
24	Ethyl acetate	81.59 (71.44–92.66)	268.69 (226.00–332.09)	376.69 (307.32–486.08)	2.476 ± 0.171	5.947 (0.203)
	Hexane	134.93 (116.88–155.21)	580.08 (471.99–747.80)	877.09 (686.71–1192.22)	2.023 ± 0.135	8.187 (0.085)
	Aqueous	219.92 (187.45–259.24)	1280.63 (968.56–1832.36)	2110.22 (1511.44–3255.99)	1.674 ± 0.122	3.233 (0.519)
	Methanol	40.19 (35.01–45.47)	108.00 (91.62–134.07)	142.93 (117.38–186.76)	2.985 ± 0.273	1.009 (0.908)
	Acetone	61.75 (52.35–72.11)	251.28 (198.41–344.78)	374.05 (281.15–553.12)	2.102 ± 0.189	9.274 (0.054)
48	Ethyl acetate	45.34 (39.00–51.88)	143.40 (120.37–179.29)	198.75 (161.18–261.93)	2.562 ± 0.215	1.133 (0.283)
	Hexane	72.89 (50.31–99.43)	295.21 (220.23–507.48)	438.87 (322.99–834.75)	2.109 ± 0.151	10.067 (0.039)
	Aqueous	123.58 (106.34–142.90)	574.10 (463.07–749.02)	887.31 (686.70–1225.85)	1.921 ± 0.131	8.531 (0.073)

Table 5
Lethal concentrations (ppm) *Datura stramonium* extracts on *Aedes aegypti* 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.)
	Methanol	120.33 (83.39–167.38)	625.75 (454.32–1129.77)	998.55 (708.17–2013.74)	1.789 ± 0.120	9.740 (0.044)
	Acetone	131.85 (90.97–186.59)	542.48 (403.12–989.99)	810.08 (597.41–1635.01)	2.086 ± 0.138	12.180 (0.016)
24	Ethyl acetate	98.17 (84.22–113.53)	442.58 (359.93–571.22)	678.25 (529.89–925.90)	1959 ± 0.135	5.075 (0.279)
	Hexane	164.85 (141.08–192.67)	827.15 (643.48–1139.83)	1306.63 (967.91–1928.52)	1.829 ± 0.135	3.334 (0.503)
	Aqueous	289.79 (245.61–346.12)	1772.87 (1297.93–2664.53)	2962.48 (2043.48–4838.64)	1.629 ± 0.124	1.975 (0.740)
	Methanol	93.20 (80.05–107.61)	406.45 (332.06–52.40)	617.04 (484.86–835.96)	2.003 ± 0.138	5.312 (0.256)
	Acetone	90.99 (79.34–103.51)	341.00 (289.12–414.53)	495.91 (408.49–627.28)	2.233 ± 0.136	5.552 (0.235)
48	Ethyl acetate	71.46 (62.06–81.61)	249.60 (208.66–311.23)	355.80 (287.82–465.02)	2.359 ± 0.168	3.787 (0.435)
	Hexane	110.87 (95.33–128.14)	504.65 (409.11–653.93)	775.45 (603.73–1062.94)	1.947 ± 0.133	4.726 (0.316)
	Aqueous	195.13 (167.19–228.18)	1048.42 (811.29–1450.86)	1688.60 (1243.34–2502.64)	1.755 ± 0.124	6.144 (0.188)

D. stramonium leaves have potent antibacterial activity against *S. aureus, B. subtilis, S. typhi,* and *E. coli* and antifungal activity against *F. oxysporum, A. niger, and F. solani* at concentrations of 20 and 40 mg/mL. The antibacterial activity of different solvent leaves extracts of *D. stramonium* were summarized against four bacteria in Table 7. Petroleum ether extract was produced maximum zone of inhibition (19.30 ± 0.18 mm) against *S. aureus* while minimum zone of inhibition (12.30 ± 0.16 mm) against *S. typhi*. Hexane extract leaves of the plant revealed that maximum zone of inhibition (18.00 ± 0.27 mm) against *S. aureus* while minimum zone of inhibition (11.05 ± 0.62 mm) against *E. coli.* Chloroform extract of the plant showed maximum zone of inhibition (18.43 ± 0.57 mm) against *B. subtilis* while minimum zone of inhibition (11.51 ± 0.54 mm) against *S. typhi*. Acetone extract of the plant exhibited maximum zone of inhibition (15.60 ± 0.21 mm) against *S. aureus* while minimum zone of inhibition (9.52 ± 0.22 mm) against *S. typhi*. Ethanol extract of the plant also exhibited maximum zone of inhibition (15.50 ± 0.55 mm) against *S. aureus* while minimum zone of inhibition (8.74 ± 0.22 mm) against *E. coli.*

3.4. *Tentative metabolites identification from different extracts of Datura stramonium using UPLC/MS analysis*

Through UPLC/MS analysis, four extracts of different polarities of *D. stramonium* were analyzed, from which one hundred and thirteen metabolites were tentatively identified and quantified (listed in Table 8). The classes of the identified components were mainly flavonoids, cinnamic acid derivatives, phenolic acids, and tannins in the ESI negative ion mode, while the positive ion mode presented mainly alkaloids (tropane type and others), anthocyanins, steroids, fatty acids, and others (Fig. 4). The % identification ranged from 21.03 % to 97.42 % for the ESI negative mode and from 28.44 % to 61.56 % for the ESI positive ion mode. It is worthy of note that the highest number of identified compounds were detected in the aqueous extract, followed by the ethyl acetate and methanol extracts, while the acetone extract showed a scarce number of components. Solanaceae, being a famous and well-studied plant family, is well-known for the alkaloids and triterpenoids that are scattered through a lot of genera belonging to this family. Tropane alkaloids and the withanolides (a class of triterpenoids) are the most famous members of plant metabolites from the Solanaceae plants. Table 8 represented the main identified components from the four

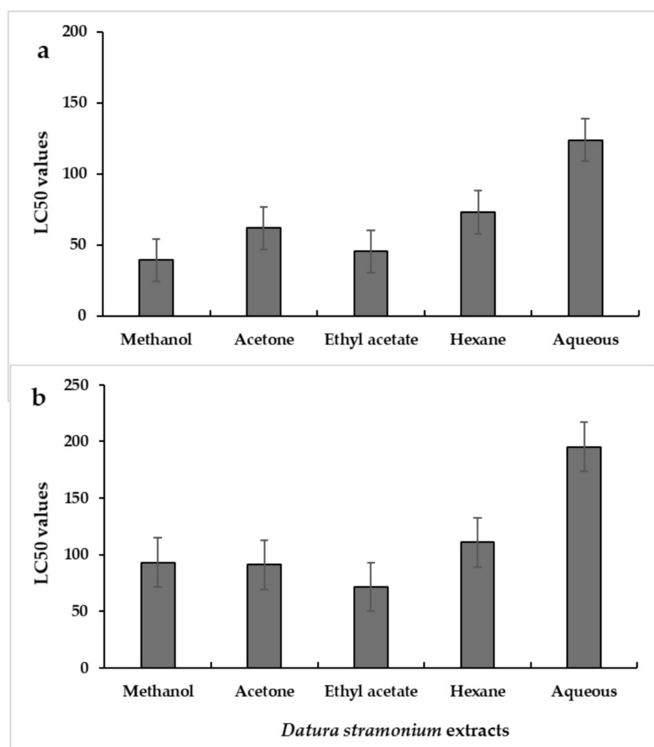

Fig. 2. The mean number of larval mortalities induced by the effects of *Datura stramonium* extracts against 4th larval instars of *Culex pipiens*, (a) and *Aedes aegypti* (b), 48 h post-exposure.

Table 6
Mean % adult mortality of *Datura stramonium* solvent extracts at different concentrations against *Culex pipiens* and *Aedes aegypti*.

Species	Solvents	% mean mortality ± S.E				
		Conc. (ppm)				
		25	50	100	250	500
Culex pipiens	Methanol	0 ± 0^{aG}	13.33 ± 1.33^{aF}	29.33 ± 1.33^{bE}	44.00 ± 2.31^{bD}	64.00 ± 2.31^{bC}
	Acetone	0 ± 0^{aG}	9.33 ± 1.33^{bF}	22.67 ± 1.33^{cE}	40.00 ± 4.62^{cD}	64.00 ± 4.62^{bC}
	Ethyl acetate	0 ± 0^{aG}	13.33 ± 1.33^{aF}	32.00 ± 2.31^{aE}	52.00 ± 4.00^{aD}	76.00 ± 2.31^{aC}
	Hexane	0 ± 0^{aG}	8.00 ± 0.00^{bF}	18.67 ± 1.33^{dE}	36.00 ± 2.31^{dD}	60.00 ± 2.31^{cC}
	Aqueous	0 ± 0^{aG}	5.33 ± 1.33^{cF}	12.00 ± 2.31^{eE}	24.00 ± 2.31^{eD}	44.00 ± 2.31^{dC}
Aedes aegypti	Methanol	0 ± 0^{aG}	14.67 ± 2.67^{aF}	32.00 ± 2.31^{bE}	52.00 ± 4.62^{cD}	80.00 ± 2.31^{bC}
	Acetone	0 ± 0^{aF}	12.00 ± 2.31^{bE}	30.67 ± 3.53^{bD}	56.00 ± 2.31^{bC}	80.00 ± 4.62^{bB}
	Ethyl acetate	0 ± 0^{aF}	16.00 ± 2.31^{aE}	36.00 ± 2.31^{aD}	68.00 ± 4.62^{aC}	88.00 ± 4.00^{aB}
	Hexane	0 ± 0^{aG}	10.67 ± 1.33^{bF}	28.00 ± 2.31^{cE}	52.00 ± 2.31^{cD}	72.00 ± 4.62^{cC}
	Aqueous	0 ± 0^{aG}	8.00 ± 2.31^{cF}	16.00 ± 2.31^{dE}	33.33 ± 1.33^{dD}	54.67 ± 1.33^{dC}

a, b & c: There is no significant difference ($P > 0.05$) between any two means, 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.

extracts of *D. stramonium* with their retention times, area %, molecular formulas, and molecular ion peaks. The identified components can be detailed as follows.

Flavonoids represented the main identified class of secondary metabolites in the negative ion mode. Certain flavonoid aglycones were

Table 7
Antimicrobial activities of *Datura stramonium* methanol extract against several pathogenic microorganisms.

Microorganism	Inhibition zone (mm)	
	Treated	Control
Bacillus subtilis (ATCC 6633)	23 ± 0.6	21 ± 0.2
Staphylococcus aureus (ATCC 6538)	25 ± 0.2	22 ± 10
Klebsiella pneumoniae (ATCC 13883)	27 ± 0.08	25 ± 0.6
Salmonella typhi (ATCC 6539)	24 ± 10	24 ± 0.8
Candida albicans (ATCC 10221)	22 ± 0.7	21 ± 10
Penicillium glabrum (Op 694,171)	15 ± 0.1	37 ± 0.8

detected along with their glycosides or other derivatives, *viz.*, quercetin (m/z 301), naringenin (m/z 271) [47], kaempferol (m/z 285), limocitrol (m/z 375), and myricetin (m/z 317). Three quercetin derivatives, previously reported from the family Solanaceae, were tentatively detected at m/z 433, 463, 447, and 317, which were assigned to quercetin-oxyloside (5.17 % ethyl acetate) [47], quercetin-hexoside [47], quercitrin (2.13 % methanol extract), and quercetagetin (13.68 % ethyl acetate extract) [48], respectively. Two luteolin derivatives appeared at m/z 581 only in the ethyl acetate extract and were tentatively defined as *di*-hydro-luteolin-*C*-hexosyl-*C*-pentoside [49], while another luteolin derivative was shown at m/z 519 with an area % of 3.55 % only in the aqueous extract [50]. Similarly, kaempferol derivatives appeared at different m/z values where a deprotonated peak was detected at m/z 287 with a molecular formula of $C_{18}H_{16}O_8$, which was tentatively defined as dihydrokaempferol [51]. Likewise, a deprotonated peak was found at m/z 431 and was identified as kaempferol-pentoside [47]. In addition, another kaempferol derivative appeared at m/z 609.6 (ESI + ve mode), which was defined as kaempferol-hexoside-coumaroyl ester, also known as tiliroside (previously documented in genus *Datura*) [52]. In a similar way, limocitrol-hexoside [53] and limocitrol-hexoside-rutinoside [54] appeared at m/z 537 and m/z 681 (734.6), respectively. On the other hand, two acacetin derivatives, previously identified from the family Solanaceae, showed their peaks at m/z 591 and m/z 531, which were assigned to acacetin-rutinoside and acacetin-malonoyl-hexoside [55], respectively.

Different peaks belonging to isoflavones and isoflavonoids were also present. One peak was detected at m/z 407 with molecular formula $C_{23}H_{20}O_7$, which led to its identification as the isoflavone, didehydro-amorphigenin [56]. In addition to that, an isoflavonoid appeared at m/z 297 only in the aqueous extract with an area of 8.05 %, which was defined as methylretusin [57]. Besides, two other deprotonated ion peaks were traced at m/z 417 (ESI + ve) and m/z 443 (483.5) and were tentatively defined as the isoflavonoids daidzin [58] and formononetin-hexouronide [59], respectively, and it is worthy to note that both of them were previously identified from the family Solanaceae.

Triterpenoids represent a predominant part of family Solanaceae and genus *Datura* literature. Various triterpenoids and withanolides were among the main identified components from the four *D. stramonium* extracts discussed herein, with eight triterpenoids and ten withanolides tentatively recognized as listed in Table 7. Among the triterpenoids, stigmasterol (m/z 414, +ve mode) [60], sitosterol (m/z 414, +ve mode) [61], and daturadiol (m/z 442, +ve mode) were previously identified from the genus *Datura*, while lucyin A (m/z 485) and carboxy urosolic acid [m/z 499(501), 27.55 % only in the acetone extract with reported anti-feedant activity] [62], ursolic acid (m/z 455, 6.77 % only in the acetone extract) [63], ecdysone (m/z 509 + ve mode) [64], and carnosol (phenolic triterpenoid, m/z 329) [65,66] were listed as part of family Solanaceae literature.

Withanolides represent a special class of triterpenoids that are uniquely present in certain plant families, including the family Solanaceae, and they were also reported in the phytochemical literature of the genus *Datura*. Herein, ten withanolides were tentatively detected from the four extracts of *D. stramonium*. Withanolides showed better

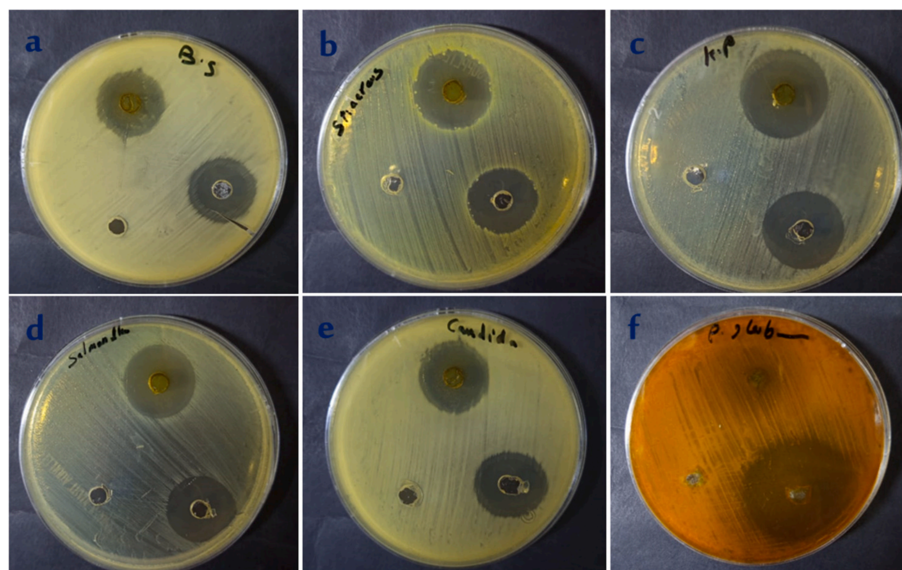

Fig. 3. Antimicrobial activities of petroleum ether extract of *Datura stramonium* against (a) *Bacillus subtilis* (ATCC 6633), (b) *Staphylococcus aureus* (ATCC 6538), (c) *Klebsiella pneumoniae* (ATCC 13883), (d) *Salmonella typhi* (ATCC 6539), (e) *Candida albicans* (ATCC 10221), and (d) *Penicillium glabrum* (Op 694,171) microorganisms.

ionization in the ESI positive mode owing to their chemical nature. Certain withanolide members were previously detected from the genus *Datura* and were also identified herein, like withaferin A [*m/z* 503 (505)]. [67], hyoscyamilactol (*m/z* 471), penta-hydroxy-(20*S*,22*R*)-with-α-5,24-dienolide-hexoside $[M\text{-}H]^-$ *m/z* 635 and $[M + H + Na]^+$ *m/z* 671, baimantuoluoside J *m/z* 547 [67], baimantuoluoline D *m/z* 504 (+ve mode) [68], daturameteloside J $[M + COOH]^+$ *m/z* 651.9 (10.58 %, ethyl acetate extract) [67], and withanolide *m/z* 472 (ESI + ve) (24.03 % ethyl acetate and 1.30 % methanol) [69]. On the other hand, certain other withanolides were reported before from the family Solanaceae, *viz.*, withanolide A *m/z* 493 (0.94 % Eth. Ac. and 8.92 % methanol), withasomniferolide B *m/z* 437, and daturametelin D *m/z* 437 [70]. It is worthy of note here that the majority of the withanolides reported here may show low abundance; however, their biological effects are pronounced due to their high toxicity profile.

Tropane alkaloids, together with certain other classes of alkaloids, are well-known metabolites from the family Solanaceae and genus *Datura*. Herein, five tropane alkaloids were tentatively identified, namely meteloidine, hyoscine/scopolamine, atropine, apoatropine, and datumetine. All of the aforementioned alkaloids were traced before from the genus *Datura*. In addition to that, other alkaloids, *viz.*, N-(2,14-eicosadienoyl)-piperidine, sinomenine (morphinan alkaloid), and tomatidine, were also detected, and they were documented before from the family Solanaceae. Moreover, other varying classes of secondary metabolites were tentatively detected and detailed in (Table 8), including tannins, *viz.* catechin-caffeic adduct, fragment of (*epi*)gallocatechin, hydroxy-gallic acid derivative, fragment of procyanidin trimer, epigallocatechin, *di*-galloyl hexoside, and procyanidin B7. Fatty acids in the form of arachidonic acid, oxiranedioctanoic acid, N-(2-hydroxyethyl)-palmitamide, hydroxy-octadecatrienoic acid, linolenic acid, hydroxy-octadecadienoic acid, and oleic acid.

Two phenolic acids, namely, quinic acid and caftaric acid. In addition to several cinnamic acid derivatives, including caffeoyl-hexoside derivative, caffeoyl–feruloyl-quinic acid, *di*-caffeoyl-quinic acid, caffeoyl-N-tryptophanrhamnoside, caffeoyl hexoside, feruloyl-caffeoyl-quinic acid derivative, cinnamoyl hexose, *tri*-methoxy-cinnamoyl-quinic acid, caffeoyl hexose conjugate, chlorogenic acid, caffeoyl-2-hydroxyethane-1,1,2-tricarboxylic acid, caffeic acid-hexuronide, *p*-coumaroyl-hexoside, caffeic acid derivative, and caffeoyl-*di*-hydroxyphenyllactoyltartaric acid. Limonoids such as obacunone and hydroxy-deoxo-*di*-hydrodeoxygedunin, which were both reported in genus *Datura*.

3.5. GC/MS identified components from the n-hexane extract of Datura stramonium

The *n*-hexane extract of *D. stramonium* was subjected to GC/MS analysis, where twelve volatile metabolites were identified. The main identified classes were *mono*-, *sesqui*-, *di*-, and triterpenoids in addition to two volatile alkaloids. The % identification reached 97.59 % (Table 9). The most abundant volatile components were *n*-hexadecanoic acid (53.81 %), oleic acid (20.29 %), stearic acid (4.25 %), hexadecanoic acid methyl ester (4.11 %), and nicotine (4.00 %).

3.6. Multivariate data analysis using principal component analysis (PCA) and clustered heat map

Multivariate data analysis was performed for the UPLC/MS tentatively identified metabolites. The identified metabolites were split into two groups based on how they were detected in the ESI ionization mode. Metabolites like flavonoids, phenolic acids, and cinnamic acid derivatives were mainly found in the ESI negative mode, while withanolides, alkaloids, triterpenoids, and anthocyanins were more clearly seen in the ESI positive mode. Principal component analysis (PCA) as an unsupervised data analysis technique was selected for the multivariate data analysis. PCA focuses on identifying patterns and reducing the dimensionality of data by finding the principal components that capture the most variance. It's often used for dimensionality reduction, exploratory data analysis, and data compression. While a clustered heat map was constructed for better differentiation between the two ESI ionization modes regarding their metabolite accumulation.

PCA analysis for the compounds detected in positive ion mode gave rise to three clusters (Fig. 5A & B) where the variance was 47 % for PC1 and 40 % for PC2. One cluster was located in the upper left quadrant for the ethyl acetate and aqueous extracts, while the other two were located in the lower right and left quadrants for the methanol and acetone extracts, respectively. Moreover, PCA analysis for the identified compounds in negative ion mode showed the presence of three distinct clusters (Fig. 7A & B) with different distribution compared to the positive ion mode components, where the variance was 67 % for PC1 and 18 % for PC2. One cluster was located in the upper left quadrant for the methanol extract, while the aqueous and acetone extracts formed

Table 8
UPLC/MS tentatively identified metabolites from four *Datura stramonium* leaf extracts.

No.	Compound name	Class	Molecular formula	R_t (min.)	$[M-H]^-$ m/z	$[M+H]^+$ m/z	% composition Eth. Ac.	Meth.	Acet.	Aq.	Ref.
1	Arbutin	Phenolic glycoside	$C_{12}H_{16}O_7$	0.62	**273**	**327**	–	–	–	2.46 **(1.70)**	[107]
2	Medioresinol	Lignan	$C_{21}H_{24}O_7$	0.70	**387**	**389**	2.77 **(1.87)**	–	–	tr. **(2.23)**	[108,109]
3	Caffeoyl-hexoside deriv.	Cinnamic acid deriv.	–	0.79	**377**	**381**	–	**9.02 (28.59)**	–	–	[110]
4	*Desmethyl*-Hydroxycolumbin	Furanoid diterpene	$C_{15}H_{14}O_5$	0.93	**358**	**434**	–	–	–	0.32 **(3.40)**	[111]
5	Quinic acid[f]	Phenolic acid	$C_7H_{12}O_6$	0.99	**191**	**–**	0.68	2.02	–	–	[48]
6	Withanolide A[f]	Triterpenoid (withanolide)	$C_{28}H_{38}O_6$	1.15	**–**	**493**	(0.94)	**(8.92)**	–	–	[70]
7	(*di*-Hydroxy-phenyl) valerolactone	Resorcinol	$C_{11}H_{12}O_4$	1.22	**–**	**209**	–	(0.64)	–	–	[112]
8	*didehydro*-Amorphigenin	Isoflavone	$C_{23}H_{20}O_7$	1.28	**407**	**–**	0.97	–	–	–	[56]
9	Catechin-caffeic adduct[f]	Tannin	–	1.51	**467**	**–**	1.28	–	–	0.49	[113]
10	Stigmasterol*	Triterpenoid	$C_{29}H_{48}O$	1.66	**–**	**414**	(0.44)	–	–	–	[60]
11	Secoisolariciresinol guaiacylglyceryl ether	Secolignan	$C_{30}H_{40}O_7$	1.77	**557**	**–**	0.78	–	–	–	[114]
12	Meteloidine*	Tropane alkaloids	$C_{13}H_{21}NO_4$	1.95	**–**	**256**	–	(1.25)	–	(1.69)	[21,115]
13	Resveratrol deriv.	Flavonoid	–	2.07	**571**	**–**	1.45	–	–	–	[116]
14	Quercetin-oxyloside[f]	Flavonoid	$C_{20}H_{18}O_{11}$	2.08	**433**	**–**	**5.17**	0.42	–	–	[47]
15	*di*-Coumaroyl spermidine	Polyamine	$C_{25}H_{31}N_3O_4$	2.33	**436**	**–**	0.38	–	–	–	[117]
16	Lucyin A[f]	Triterpenoid	$C_{30}H_{46}O_5$	2.50	**485**	**–**	–	–	–	0.63	[48]
17	Caffeoyl–feruloyl-quinic acid	Cinnamic acid deriv.	$C_{26}H_{26}O_{12}$	2.64	**529**	**–**	2.28	–	–	–	[56]
18	Hyoscine/scopolamine*	Tropane alkaloid	$C_{17}H_{21}NO_4$	2.77	**–**	**304**	–	–	–	**(6.39)**	[21]
19	Arachidonic acid*	Fatty acid	$C_{20}H_{32}O_2$	2.80	**–**	**305**	–	(2.85)	–	–	[118]
20	*di*-Hydro-luteolin-*C*-hexosyl-*C*-pentoside	Flavonoid	$C_{27}H_{30}O_{15}$	2.92	**581**	**–**	0.83	–	–	–	[49]
21	5,6,12,21,27-*penta*Hydroxy-(22*R*)-1-oxowitha-2,24-dienolide (withaferin A)*	Triterpenoid (withanolide)	$C_{28}H_{38}O_6$	3.52	**503**	**505**	0.65	(0.49)	–	0.71 (0.56)	[67]
22	Limocitrol-hexoside	Flavonoid	$C_{24}H_{26}O_{14}$	3.93	**537**	**–**	1.43	–	–	–	[53]
23	*O*-Methylaloesin-*penta* acetate	Chromone	$C_{33}H_{52}O_4$	4.13	**616**	**–**	0.30	–	–	–	[119]
24	Quercetin-hexoside[f]	Flavonoid	$C_{21}H_{20}O_{12}$	4.57	**463**	**–**	1.28	1.85	–	–	[47]
25	5,6,7-*tri*-Hydroxy-2,3-dihydrocyclopenta[*b*]chromene-1,9-dione-3-carboxylic acid hexoside	Chromene	$C_{26}H_{30}O_{13}$	5.06	**453**	**494**	1.80 (0.26)	–	–	–	[120]
26	Atropine*	Tropane alkaloid	$C_{17}H_{23}NO_3$	5.29	**–**	**291**	–	(1.41)	(2.80)	(2.80)	[121]
27	Gerberinol[f]	Hydroxycoumarin	$C_{21}H_{16}O_6$	5.66	**363**	**–**	–	1.33	–	–	[48]
28	Fragment of (epi)gallocatechin	Tannin	–	6.14	**261**	**–**	–	–	–	0.43	[122]
29	Delphinidin-hexoside (Myrtillin)	Anthocyanin	$C_{21}H_{21}ClO_{12}$	6.33	**–**	**465**	–	1.26	–	–	[123]
30	Fragment of fatty acid	Fatty acid	–	6.43	**201**	**259**	–	–	–	2.73	[117]
31	Sitosterol*	Triterpenoid	$C_{29}H_{50}O$	6.47	**–**	**414**	(0.26)	–	–	–	[61]
32	Hyoscyamilactol*	Triterpenoid (withanolide)	–	6.67	**471**	**–**	0.88	–	–	–	[67]
33	Quercitrin[f]	Flavonoid	$C_{21}H_{20}O_{11}$	6.68	**447**	**–**	–	2.13	–	–	[47]
34	*di*-Hydrokeampferol	Flavonoid	$C_{18}H_{16}O_8$	6.82	**287**	**–**	–	–	–	0.88	[51]
35	Rutin[f] $[M+H+Na]^+$	Flavonoid	$C_{27}H_{30}O_{16}$	6.88	**–**	**624**	–	–	–	(0.26)	[124]
36	*di*-Caffeoyl-quinic acid[f]	Cinnamic acid deriv.	$C_{25}H_{24}O_{12}$	6.94	**515**	**–**	–	2.03	–	–	[125]
37	Torachrysone[f]	Naphthalenol	$C_{14}H_{14}O_4$	7.04	**245**	**269 or 334**	–	–	–	**5.39** (0.15)	[126]
38	Acacetin-rutinoside[f]	Flavonoid	$C_{28}H_{32}O_{14}$	7.07	**591**	**–**	0.61	–	–	–	[47]
39	Kaempferol-pentoside[f]	Flavonoid	$C_{21}H_{20}O_{10}$	7.13	**431**	**–**	–	0.67	–	–	[47]
40	Tanacetolide A	Sesquiterpene lactone	$C_{15}H_{20}O_3$	7.14	**–**	**301**	–	–	–	(0.08)	[125]
41	Carboxy ursolic acid[f]	Triterpenoid	–	7.32	**499**	**501**	–	–	**(27.55)**	–	[50,62]
42	Hydroxy-gallic acid deriv.	Tannin	–	7.33	**313**	**–**	1.98	–	–	2.36	[125]
43	Hydroxy-pinoresinol-hexoside	Lignan	$C_{26}H_{32}O_{12}$	7.47	**535.5**	**584**	–	–	–	1.84 (0.26)	[127]
44	Maltitol[f]	Sugar	$C_{12}H_{24}O_{11}$	7.64	**343**	**–**	–	–	–	1.38	[47]
45	*penta*-Hydroxy-(20*S*,22*R*)-With-α-5,24-dienolide-hexoside*	Triterpenoid (withanolide)	–	7.58	**635**	**671**	–	2.52 (0.77)	–	–	[67]
46	Limocitrol-hexoside-rutinoside	Flavonoid	$C_{29}H_{34}O_{17}$	8.24	**681**	**734.6**	–	**3.45** (0.80)	–	–	[54]
47	Acacetin-malonyl-hexoside	Flavonoid	$C_{34}H_{48}O_{21}$	8.45	**531**	**–**	–	0.42	–	1.52	[55]
48	Asterosterol[f]	Hydroxysteroids	$C_{26}H_{42}O$	8.49	**369**	**–**	0.86	–	–	–	[48]
49	N-(2,14-Eicosadienoyl)-piperidine[f]	Alkaloid	$C_{25}H_{45}NO$	8.65	**–**	**376**	–	–	–	(0.13)	[48]
50	Oxiranedioctanoic acid[f]	Fatty acid	$C_{18}H_{32}O_5$	8.76	**327**	**336**	0.99	**8.77**	–	**6.10**	[128]
51	Luteolin derivative	Flavonoid	–	9.24	**519**	**–**	–	–	–	**3.55**	[50]
52	*tri*-Hydroxy dimethoxy flavone	Flavonoid	$C_{17}H_{14}O_7$	9.43	**329**	**353**	–	1.70 (2.40)	–	2.65 (0.26)	[125]
53	Sinomenine[f]	Morphinan alkaloid	$C_{19}H_{23}NO_4$	9.50	**329.6**	**–**	0.74	–	–	–	[129]

(*continued on next page*)

Table 8 (*continued*)

No.	Compound name	Class	Molecular formula	R_t (min.)	$[M-H]^-$ *m/z*	$[M+H]^+$ *m/z*	% composition				Ref.
							Eth. Ac.	Meth.	Acet.	Aq.	
54	Baimantuoluoside J*	Triterpenoid (withanolide)	$C_{30}H_{44}O_9$	9.62	**547**	–	–	–	–	1.20	[67]
55	Ursolic acid[f]	Triterpenoid	$C_{30}H_{48}O_3$	9.86	**455**	–	–	–	**6.77**	–	[63]
56	*di*-ketospirilloxanthin/ 2,2′-*di*-ketospirilloxanthin[f]	Carotenoid	$C_{42}H_{56}O_4$	9.90	**623.8**	**625.5**	0.41 (1.01)	0.58 (2.54)	–	–	[48]
57	Gibberellin A4[f]	Plant hormone	$C_{19}H_{24}O_5$	9.93	**331**	–	–	–	–	1.87	[47]
58	Caffeoyl-N-tryptophan-pentoside[f]	Cinnamic acid deriv.	$C_{26}H_{30}N_2O_{10}$	10.33	**511**	–	0.96	–	–	–	[109]
59	Daidzin[f]	Isoflavonoid	$C_{21}H_{20}O_9$	10.77	–	**417**	–	–	–	(0.62)	[58]
60	*di*-Hydrocapsiate[f]	Capsinoids	$C_{18}H_{28}O_4$	10.81	**307**	–	0.32	0.61	–	–	[117]
61	Baimantuoluoline D*	Triterpenoid (withanolide)	$C_{28}H_{42}O_8$	11.19	–	**504**	–	–	–	(0.23)	[68]
62	Fragment of procyanidin trimer	Tannin	–	11.21	**697**	–	0.47	–	–	–	[130]
63	Apoatropine*(mass 271)	Tropane alkaloid	$C_{17}H_{21}NO_2$	11.33	–	**274**	–	**(3.44)**	–	(1.79)	[118]
64	Caffeoyl hexoside[f]	Cinnamic acid deriv.	$C_{15}H_{18}O_9$	11.42	**341**	–	0.39	0.33	–	–	[125]
65	2-(3-(1-Benzyl-5-(2-ethoxyphenyl)-1*H*-pyrazol-3-yl)-4-(3-chlorobenzyloxy)phenyl) acetic acid	Miscellaneous	$C_{33}H_{26}O_4N_2Cl$	11.45	–	**553**	–	(1.82)	–	(1.22)	[131]
66	Epigallocatechin[f]	Tannin	$C_{15}H_{14}O_7$	11.70	**305**	–	2.43	2.93	–	0.75	[59]
67	Datumetine*	Tropane alkaloid	$C_{16}H_{21}NO_3$	11.86	–	**276**	–	–	(2.21)	(0.16)	[115]
68	Withasomniferolide B[f]	Triterpenoid (withanolide)	$C_{28}H_{36}O_4$	11.88	–	**437**	–	–	–	(0.21)	[70]
69	N-(2-Hydroxyethyl)-palmitamide[f]	Fatty acid	$C_{18}H_{37}NO_2$	12.42	–	**299**	–	–	–	(0.15)	[132]
70	Ecdysone	Triterpenoid	$C_{27}H_{44}O_6$	12.52	–	**509**	(0.18)	–	–	–	[64]
71	Feruloyl-caffeoyl-quinic acid deriv.[f]	Cinnamic acid deriv.	–	12.57	**265**	–	**4.38**	1.29	**3.08**	0.34	[40]
72	Daturametelin D[f]	Triterpenoid (withanolide)	$C_{26}H_{38}O_4$	12.92	–	**437**	–	–	–	(0.10)	[70]
73	*di*-Hydrochalcone phloretin	Flavonoid	$C_{30}H_{28}O_6$	13.69	–	**437**	–	–	–	(0.17)	[109]
74	Hydroxy-octadecatrienoic acid[f]	Fatty acid	$C_{18}H_{30}O_3$	14.42	**293.5**	–	**26.57**	**3.90**	–	0.27	[133]
75	Cinnamoyl hexose[f]	Cinnamic acid deriv.	$C_{15}H_{18}O_7$	14.96	**309**	–	2.63	2.99	–	0.45	[59]
76	*tri*-Methoxy-cinnamoyl-quinic acid	Cinnamic acid deriv.	$C_{18}H_{22}O_9$	15.00	**411**	–	–	–	2.56	–	[134]
77	Linolenic acid[f]	Fatty acid	$C_{18}H_{30}O_2$	15.08	–	**279**	–	–	–	(0.15)	[70]
78	Naringenin[f]	Flavonoid	$C_{15}H_{12}O_5$	15.23	**271**	–	–	–	–	1.85	[47]
79	Carnosol[f]	Phenolic triterpenoid	$C_{20}H_{26}O_4$	15.29	**329**	–	0.74	–	–	–	[65,66]
80	Hydroxy-octadecadienoic acid[f]	Fatty acid	$C_{18}H_{32}O_3$	15.61	**295**	–	**10.59**	0.34	–	1.00	[133]
81	Apigenin-pentoside	Flavonoid	$C_{20}H_{20}O_{10}$	15.93	**401**	–	–	–	**7.63**	0.39	[65,135]
82	Methylretusin	Isoflavonoid	$C_{17}H_{14}O_5$	16.05	**297**	–	–	–	–	**8.05**	[57]
83	Oleic acid* (mass 282)	Fatty acid	$C_{18}H_{34}O_2$	16.50	–	**284**	–	–	–	(0.38)	[118]
84	2,2-*di*-bromo Cholestanone*	Steroid	$C_{27}H_{44}OBr_2$	17.03	**541**	–	–	0.44	–	–	[136]
85	Quercetin*	Flavonoid	$C_{15}H_{10}O_7$	17.13	**301**	–	–	–	–	0.68	[137]
86	Caftaric acid[f]	Phenolic acid	$C_{13}H_{12}O_9$	17.58	**311**	–	–	2.21	–	1.61	[40]
87	Enterolactone[f]	Lignin	$C_{18}H_{18}O_4$	17.72	**299**	–	–	–	–	1.18	[59]
88	Miraxanthin-I[f]	Tetracarboxylic acid	$C_{14}H_{18}N_2O_7$ S	17.89	–	**359**	(0.33)	–	–	–	[48]
89	Tomatidine[f]	Alkaloid	$C_{27}H_{45}NO_2$	19.62	–	**398**	–	–	–	(2.32)	[138]
90	Caffeoyl hexose conjugate[f]	Cinnamic acid deriv.	–	19.73	**377**	**399**	0.60 (1.28)	1.06 **(3.05)**	–	0.91 (0.10)	[139]
91	Chlorogenic acid[f]	Cinnamic acid deriv.	$C_{16}H_{18}O_9$	19.77	**353**	–	–	2.31	–	–	[48]
92	Caffeoyl-2-hydroxyethane-1,1,2-tricarboxylic acid[f]	Cinnamic acid deriv.	$C_{14}H_{12}O_{10}$	20.66	**339**	–	1.83	–	0.99	–	[40]
93	Tiliroside*	Flavonoid	$C_{30}H_{26}O_{13}$	21.86	–	**609.6**	(1.14)	–	–	–	[52]
94	Caffeic acid-hexuronide[f]	Cinnamic acid deriv.	$C_{15}H_{16}O_{10}$	23.47	**355**	–	1.26	–	–	0.33	[59]
95	Daturadiol*	Triterpenoid	$C_{30}H_{50}O_2$	25.01	–	**442**	(0.75)	–	–	–	[61]
96	Lactinolide-hexoside	Monoterpene glycoside	$C_{24}H_{28}O_{10}$	25.15	**361**	–	–	0.19	tr.	–	[140]
97	*p*-Coumaroyl-hexoside[f]	Cinnamic acid deriv.	$C_{15}H_{18}O_8$	25.48	**325.5**	–	0.56	0.95	tr.	–	[40,125]
98	Obacunone*	Limonoids	$C_{26}H_{30}O_7$	26.26	–	**455**	**(4.48)**	–	(0.40)	(0.18)	[52]
99	Esculin[f]	Coumarin glucoside	$C_{15}H_{16}O_9$	26.37	**339.7**	–	2.88	–	–	–	[47]
100	28-isoFucosterol*	Sterol	$C_{29}H_{48}O$	26.57	–	**414**	(1.43)	–	–	–	[118]
101	Formononetin-hexouronide[f]	Isoflavonoid	$C_{22}H_{20}O_{10}$	26.76	**443**	**483.5**	1.03 **(3.45)**	–	–	–	[59]
102	*di*-Galloyl hexoside	Tannin	$C_{20}H_{20}O_{14}$	27.47	**465**	–	–	–	–	1.31	[141]
103	Daturameteloside J	Triterpenoid (withanolide)	$C_{34}H_{52}O_{12}$	27.63	–	**651.9**	**(10.58)**	–	–	(0.75)	[67]
104	2′-deamino-2′-Hydroxyparomamine*	Miscellaneous	$C_{12}H_{24}N_2O_8$	27.72	**325**	–	–	1.87	–	1.06	[52]
105	Withanolide*	Triterpenoid (withanolide)	$C_{28}H_{38}O_6$	28.15	–	**472**	**(24.03)**	(1.30)	–	–	[69]
106	Caffeic acid deriv.*	Cinnamic acid deriv.	–	28.32	**326**	–	–	–	–	**3.75**	[142]
107	Quercetagetin[f]	Hexahydroxyflavone	$C_{15}H_{10}O_8$	28.45	**317**	–	**13.68**	–	tr.	–	[48]
108	Procyanidin B7[f]	Tannin	$C_{30}H_{26}O_{12}$	28.71	**577**	–	–	**4.51**	–	–	[40,143]
109	Myricetin[f]	Flavonoid	$C_{15}H_{10}O_8$	28.76	**317**	–	2.75	–	–	–	[47]
110	Hydroxy-deoxo-*di*-hydrodeoxygedunin*	Limonoid	$C_{28}H_{38}O_6$	29.22	–	**471**	(0.35)	(1.29)	–	–	[99]

(*continued on next page*)

Table 8 (*continued*)

No.	Compound name	Class	Molecular formula	R_t (min.)	[M-H]$^-$ *m/z*	[M + H]$^+$ *m/z*	% composition				Ref.
							Eth. Ac.	Meth.	Acet.	Aq.	
111	Caffeoyl-*di*-hydroxyphenyllactoyltartaric acid*	Cinnamic acid deriv.	$C_{13}H_{12}O_9$	29.24	**491**	–	–	–	–	**4.63**	[144]
112	*dimethyl*-Oleuropein[f]	Terpene glycoside	$C_{24}H_{30}O_{13}$	30.55	**525**	–	–	0.77	–	–	[59]
113	Ligustroside	Secoiridoid	$C_{25}H_{31}O_{12}$	30.79	**523**	–	–	–	–	2.55	[145]
No. of identified compounds							**51**	**42**	**12**	**56**	
% Identification											
ESI –ve mode							**97.42 %**	**70.04 %**	**21.03 %**	**67.62 %**	
ESI + ve mode							**52.78 %**	**61.56 %**	**32.96 %**	**28.44 %**	

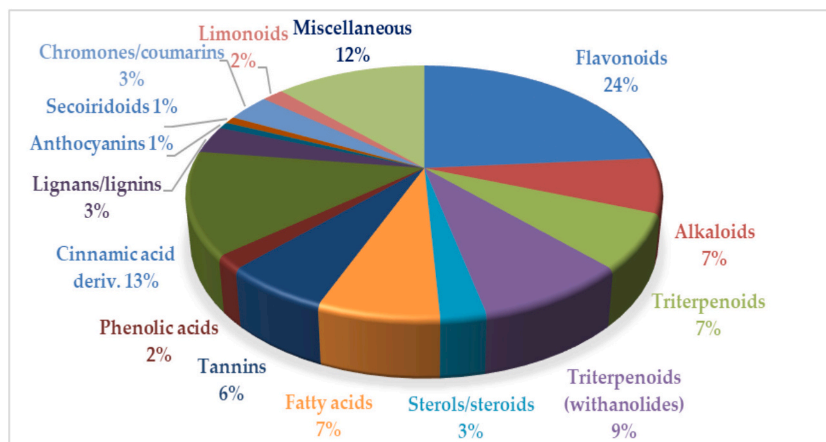

Fig. 4. Classification of the different phytochemical classes detected by UPLC-MS in positive and negative Ionization modes.

Table 9
GC/MS identified metabolites from *Datura stramonium n*-hexane extract.

No.	Component	KI		Area %	Method of identification	Molecular formula	Nature
		Cal.	Rep.				
1	Nicotine	1351	1360	4.00	KI, MS	C10H14N2	Alkaloid
2	(1*S*,3*R*,5*S*,6*R*)-(−)-5-Caranol	1432	–	1.04	KI, MS	C10H18O	M.
3	Limonene glycol	1505	–	2.22	KI, MS	C10H18O2	M.
4	Tetradecanoic acid	1766	1765	1.95	KI, MS	C14H28O2	M.
5	Hexadecanoic acid, methyl ester	1927	1927	4.11	KI, MS	C17H34O2	Sesq.
6	*n*-Hexadecanoic acid	1968	1968	53.81	KI, MS	C16H32O2	Sesq.
7	Elaidic acid, methyl ester	2103	2109	3.26	KI, MS	C19H36O2	D.
8	Methyl stearate	2128	2128	1.50	KI, MS	C19H38O2	D.
9	Oleic Acid	2144	2146	20.29	KI, MS	C18H34O2	D.
10	Stearic acid	2166	2167	4.25	KI, MS	C18H36O2	D.
11	Scopolamine	2378	–	0.44	KI, MS	C17H21NO4	Alkaloid
12	β-Sitosterol acetate	3119	–	0.72	KI, MS	C31H52O2	T.
	% Identification			97.59 %			

The main identified components were in bold. KI: Kovats index, Cal.: calculated, Rep.: reported. M.: monoterpene, Sesq.: sesquiterpene, D.: diterpene, T.: triterpene.

another cluster in the lower left quadrant, and the third cluster was for the ethyl acetate extract. 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. 6 & 8).

3.7. UV–Vis absorption spectrum of Datura stramonium extracts

Datura extracts exhibit UV–Vis absorption spectra at six concentrations, spanning from 50 ppm to 2000 ppm (Fig. 9). The spectrum's most noticeable characteristic is a strong UV band that is centered at about 226 nm. For the 200 ppm concentration, this band shows a substantially greater absorption coefficient at higher concentrations. The higher the concentration, the higher the absorption intensity will be. There is a direct relationship between concentration and absorption intensity.

Data showed that around 280 nm, there is a second, smaller, yet still noticeable absorption band. This absorption band likewise exhibits concentration dependence, following the primary peak trend. There is a wide, relatively low absorption band in the visible light spectrum

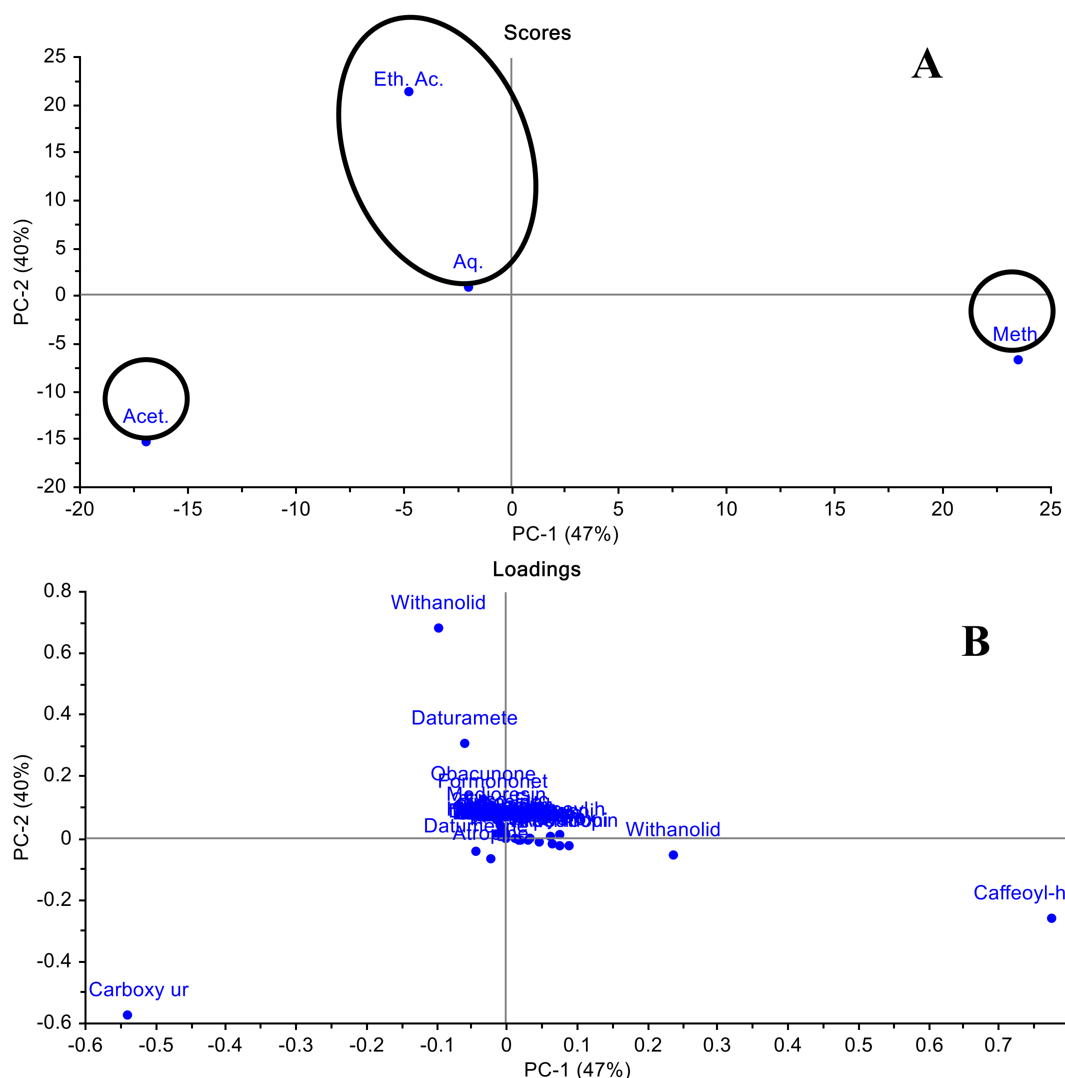

Fig. 5. (A) Score plot of PC1 versus PC2 of the identified secondary metabolites (positive ion mode) from *Datura stramonium* (area % as a variable). (B) Loading plot for PC1 and PC2 contributing metabolites and their assignments (area % as variable). Eth. Ac.; ethyl acetate extract, Aq.; aqueous extract, Meth.: methanol extract, Acet.: acetone extract.

approximately at 405 nm. As wavelength increased, the intensity of the band gradually decreased, particularly at higher concentration spectra (200 ppm, 1000 ppm, and 500 ppm). An additional peak can be detected at about 660 nm (Fig. 10).

Since we used the entire range of *Datura* leaf extract, the total absorbance at any wavelength is the combined absorbance of all the different parts in the mixture. Therefore, to follow the electronic transition that takes place in the extract due to photoactivity of light with the extract, the optical bandgap energies will contain whole and individual intrinsic transitions. A typical optical bandgap plot will give rise to various components: alkaloids (primarily absorbed in the UV range), flavonoids (near UV to visible light absorption range), chlorophylls (absorbed in the visible range), and phenolic components (far UV range). The following figure represents the optical bandgap plot of *Datura* extract with low (intrinsic) and high (whole) electronic transition (Fig. 11). The values of the optical bandgap energies are listed in Table 10.

Due to the nature of the *Datura* sample, the observed Eg shows varying data due to the fact that the extract includes all natural components of the chromophore, which demonstrates a whole optical bandgap of electronic transitions and an intrinsic electronic energy transition (HOMO-LUMO transitions). (E.g., data are shown in Table 10 and illustrated in Fig. 11) The optical bandgap energy did not change with changing concentration, showing structure stability of the extract at different concentrations. Due to the fact that *Datura* extract includes various components, their individual electronic transitions might overlap, leading to delocalized electrons across the aggregation region, which in turn can lower the energy shifting between HOMO and LUMO levels compared to the isolated molecules. This energy shifting results in optical bandgap red-shifting and consequently reducing the bandgap energy (Fig. 11).

3.8. Molecular qRT-PCR evaluations

The data in Fig. 12 showed that using pesticides and datura greatly increased the levels of Apismin, Defincin 1, SFCYP1, SFCYP4, SFCYP5, and SFRYR compared to the control, with SFCYP1 increasing by 612 times, SFCYP5 by 47.4 times, and SFRYR by 140 times. Pesticide use also raised SFCYP2 levels, which went up by 200 times steadily. However, SFCYP2 levels dropped significantly with datura, and SFCYP3 levels were consistently lower in both pesticide and datura treatments, decreasing to 0.05 times compared to the control. Pesticide use also raised the levels of SFCYP2, which increased by 200-fold in a consistent manner. In contrast, the SFCYP2 gene decreased significantly with

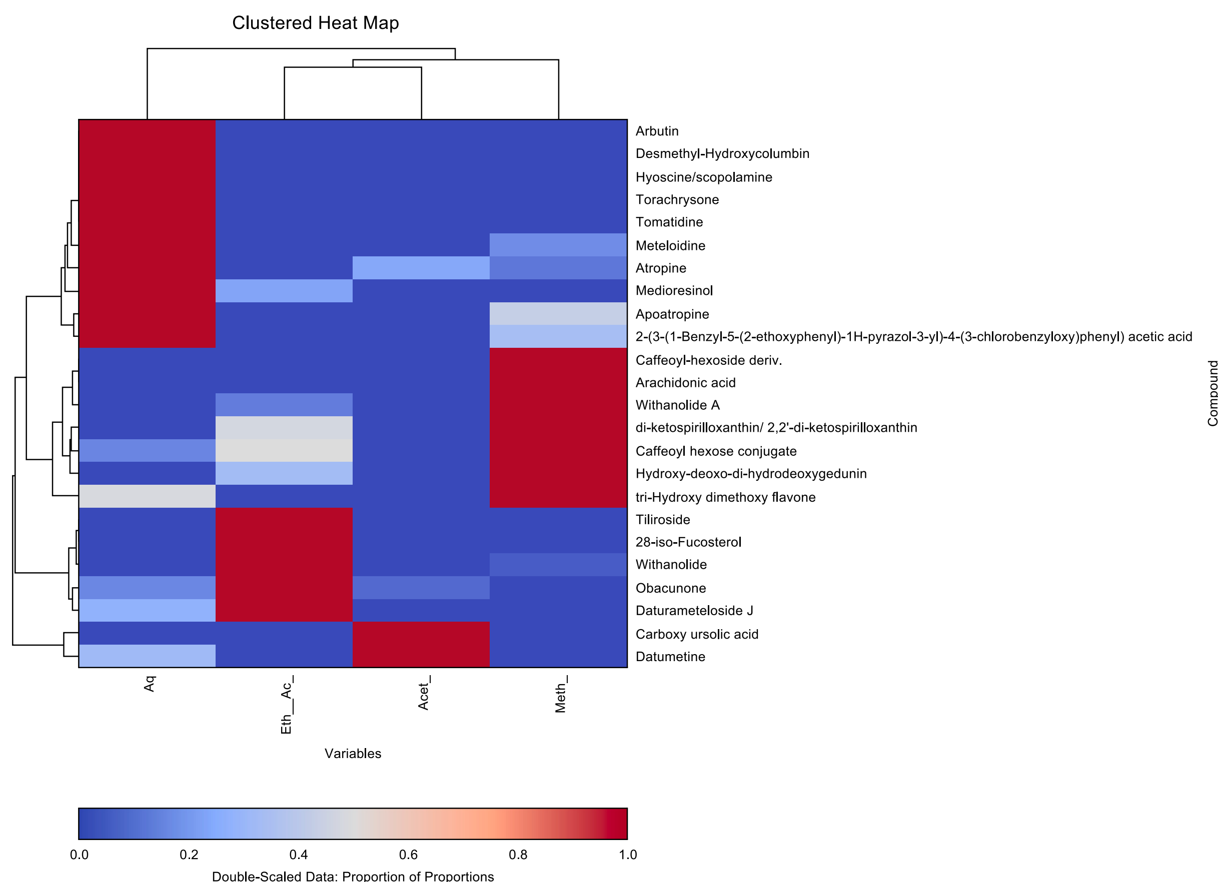

Fig. 6. Clustered heat map showing the identified components (positive mode) from *Datura stramonium*. A heat map was constructed using Euclidean distance and the unweighted group method. Eth. Ac.; ethyl acetate extract, Aq.; aqueous extract, Meth.: methanol extract, Acet.: acetone extract.

datura, and the SFCYP3 gene was consistently lower in both pesticide and datura treatments, showing a 0.05-fold decrease compared to the control.

4. Discussion

Plant extracts and essential oils are useful substances used in many areas, such as protecting plants and helping to keep humans and animals healthy from different pests [71]. These intricate combinations comprise volatile and nonvolatile chemical molecules generated as secondary metabolites in plants. Plant extracts or essential oils from plants may be effective in managing mosquito populations, as they include a plethora of bioactive components that can decompose into innocuous substances. They are ideally suited for implementation in integrated management methods for mosquito control [72,73]. Plant extracts have proven beneficial in managing insect pests, including mosquitoes, in comparison to synthetic insecticides [74,75].

For centuries, people have utilized plant extracts due to their rich content of natural compounds that help control both pests and plant-related diseases [76]. These plant-based substances are not only effective against insects but are also environmentally friendly and possess antimicrobial properties, making them valuable in medical applications as protective agents [77,78].

The current study indicated that extracts from *D. stramonium* using methanol and ethyl acetate were very effective at killing mosquito larvae. The evaluated methanol and ethyl acetate extracts from *D. stramonium* achieved 100 % mortality within 24 h at 500 ppm. It demonstrated significant larvicidal efficacy against *Culex pipiens*. The findings align with the study conducted by Yahaya, Bandiya, Ladan and Shindi [79] and Goyal and Shinde [80], which demonstrated the effectiveness of *Datura* leaves methanol extracts against mosquitoes.

The findings align with previous research demonstrating the larvicidal properties of extracts from *D. stramonium* leaves against *Culex* species [81]. Other studies have also shown that ethanolic extracts from *D. stramonium* leaves can kill larvae and repel *Aedes aegypti, Anopheles stephensi*, and *Culex quinquefasciatus* mosquitoes, and they can also kill their eggs. Additionally, Afolabi, Simon-Oke, Elufisan and Oniya [82] reported that essential oils derived from *D. stramonium* possess both adulticidal and repellent effects against *Anopheles gambiae* mosquitoes.

Earlier research by Maheshwari, Khan and Chopade [83] and Singh, Minj, Devhare, Uppalwar, Anand, Suman and Devhare [84] showed that *Datura* metabolites contain various bioactive compounds that generally help crude plant extracts work well against mosquitoes. Based on the findings, the chemical constituents identified in *D. stramonium* exhibit strong larvicidal properties, with their activity ranging from moderate to high depending on the solvent used, extract concentration, and exposure time. These findings are partially supported by Olofintoye, Simon-Oke and Omoregie [81] and Srivastava, Khandagle, Morey and Raut [85]. Many studies have indicated that certain phytochemicals such as alkaloids, tannins, flavonoids, terpenoids, and sterols can effectively disrupt insect development, including growth, physical formation, transformation, and reproduction [86]. These compounds, including saponins, are also found in *Datura stramonium* and showed insecticidal property against different stages of insects [87]. Previous studies evaluated the larvicidal effects of thymol-based compounds extracted from *Datura*, which caused mosquito deaths in a manner that increased with dosage, particularly *Culex* species [88].

Different studies indicated that mosquito species respond differently to various plant extracts [88,89]. In this study, extracts from *D. stramonium* were found to kill adult *Cx. pipiens* and *Ae. aegypti*

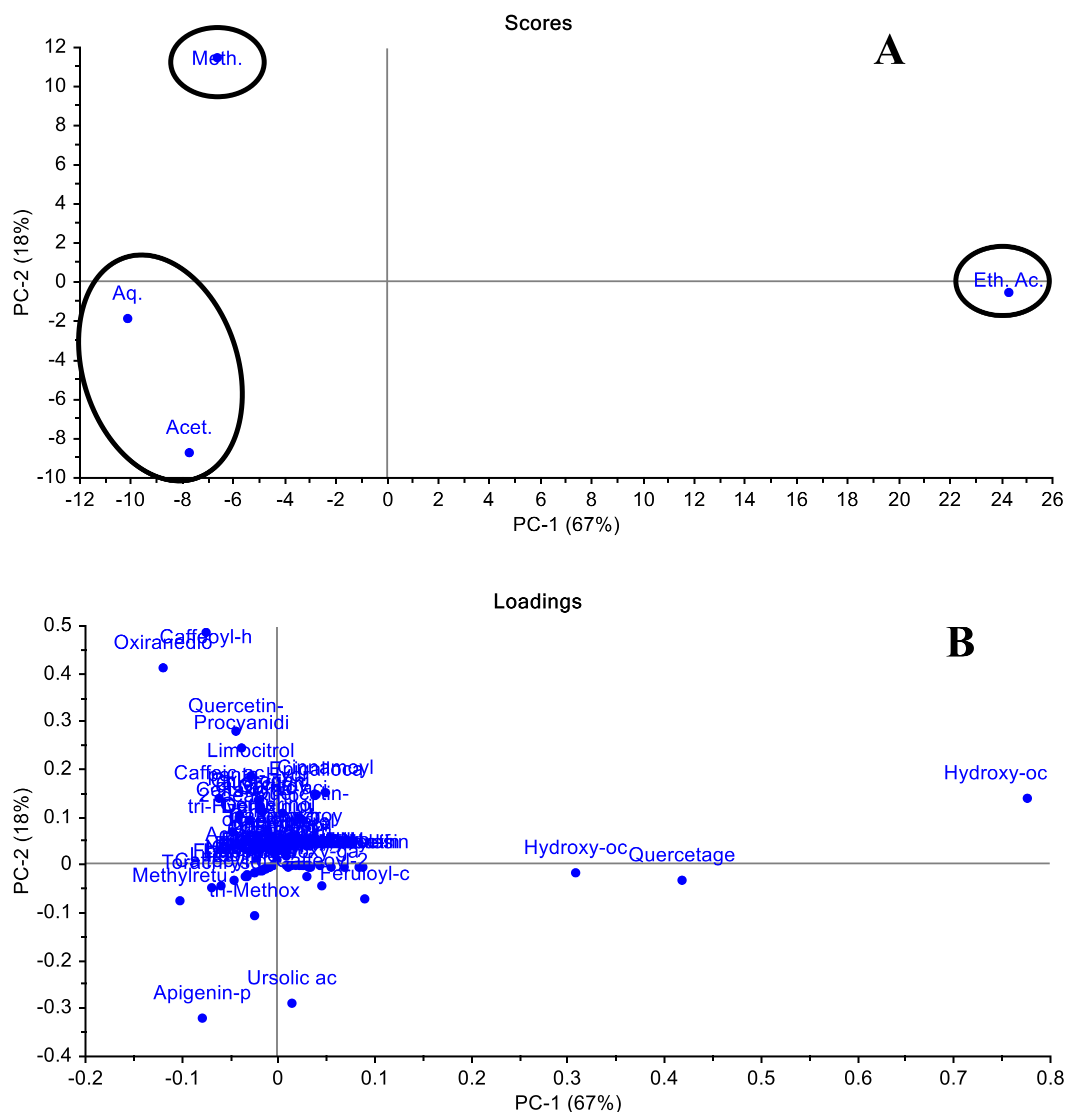

Fig. 7. (A) Score plot of PC1 versus PC2 of the identified secondary metabolites (negative ion mode) from *Datura stramonium* (area % as a variable). (B) Loading plot for PC1 and PC2 contributing metabolites and their assignments (area % as variable). Eth. Ac.; ethyl acetate extract, Aq.; aqueous extract, Meth.: methanol extract, Acet.: acetone extract.

mosquitoes, with death rates of 76 % and 88 %, respectively. Similar results indicated that essential oils from *D. stramonium* also kill and repel *An. gambiae* mosquitoes [82]. According to Mahanta and Khanikor [90], the same plant extract has proven to be effective in targeting multiple stages of mosquito development. It impacts not only larvae but also has ovicidal properties, deters egg-laying, and affects adult mosquitoes. Therefore, we can use this extract to control mosquito populations across all life stages. A study by Srivastava, Khandagle, Morey and Raut [85] showed that the essential oils from *Morus alba* and *Datura stramonium* can kill adult *Cx. quinquefasciatus* mosquitoes and repel An. gambiae mosquitoes.

The study found that the toxicity levels (LC_{50}) were different for *Cx. pipiens* and *Ae. aegypti* mosquito larvae when both were tested with the same plant extract from *D. stramonium* methanolic, with *Cx. pipiens* larvae being more affected than *Ae. aegypti* larvae. The difference could be due to several biological and physical factors that affect how the larvae respond to the active compounds, such as differences in detoxification enzyme activity, how easily substances can get through their outer layer, and genetic differences between the two species [91]. Liu [92] found that *Ae. aegypti* larvae possess higher activity of enzymes such as cytochrome P450 monooxygenases, glutathione-S-transferases (GSTs), and esterase's compared to *Cx. pipiens*. This gives them a greater ability to tolerate plant extracts or break them down more efficiently.

Our data showed that *D. stramonium* leaves can kill bacteria like *B. subtilis, S. aureus, K. pneumoniae,* and *S. typhi*, and can also fight fungi like *C. albicans* and *P. glabrum*. Previous research has shown that methanol extracts derived from *Datura stramonium* exhibit antimicrobial activity against a range of bacteria, including *Bacillus thuringiensis, Bacillus subtilis, Pseudomonas aeruginosa, Agrobacterium tumefaciens, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus,* and *Klebsiella pneumoniae* [93,94]. Furthermore, studies have indicated that extracts prepared with benzene, chloroform, and ethanol from the same plant are also effective in inhibiting the growth of *Enterobacter, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Micrococcus luteus* [95]. Additional findings suggest that extracts of *D. stramonium* made with ethanol, methanol, acetone, chloroform, and petroleum ether can kill bacteria like *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and similar strains found in hospitals. In another study, Khandare and Salve [96] demonstrated that leaf extracts from *D. stramonium* showed antifungal effects against *Fusarium oxysporum*, the causative agent of wilt disease in pigeon

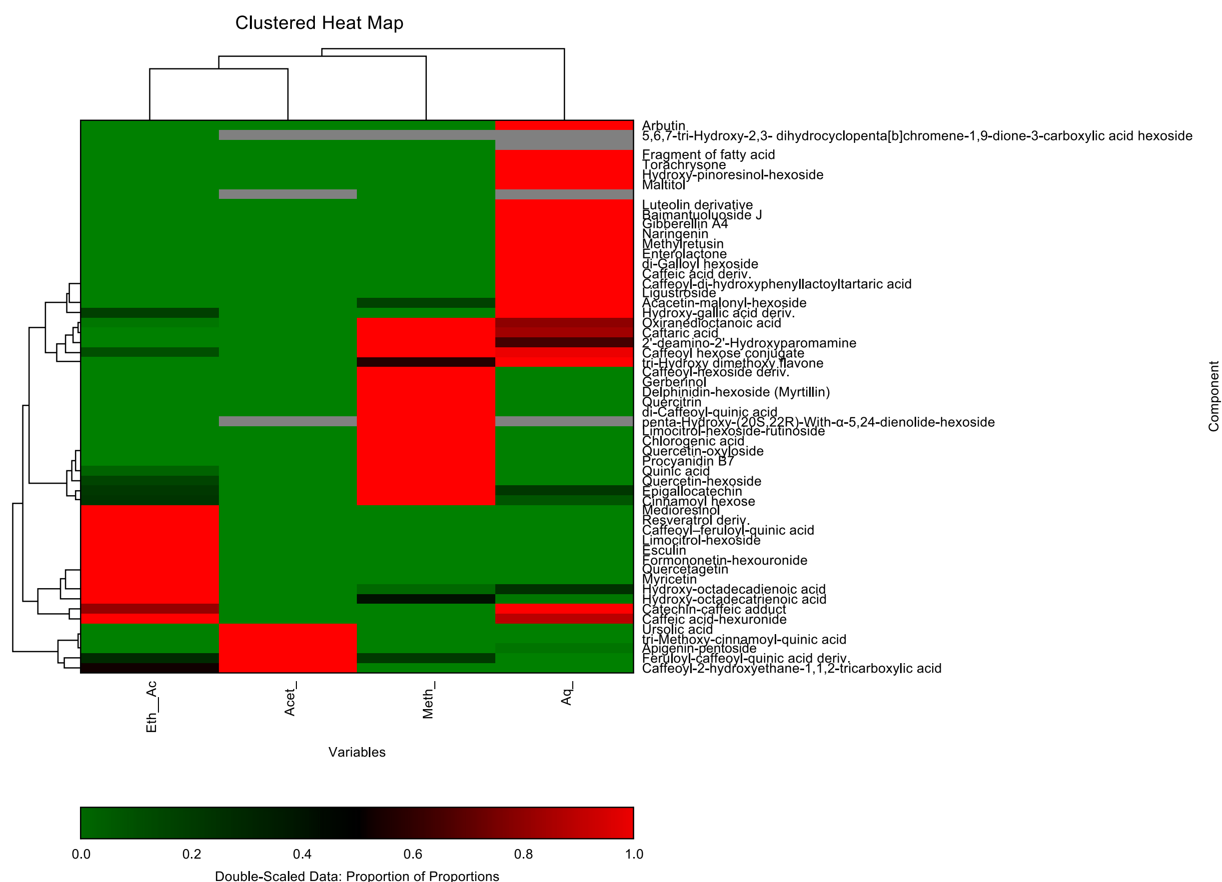

Fig. 8. Clustered heat map showing the identified components (negative mode) from *Datura stramonium*. A heat map was constructed using Euclidean distance and the unweighted group method. Eth. Ac.; ethyl acetate extract, Aq.; aqueous extract, Meth.: methanol extract, Acet.: acetone extract.

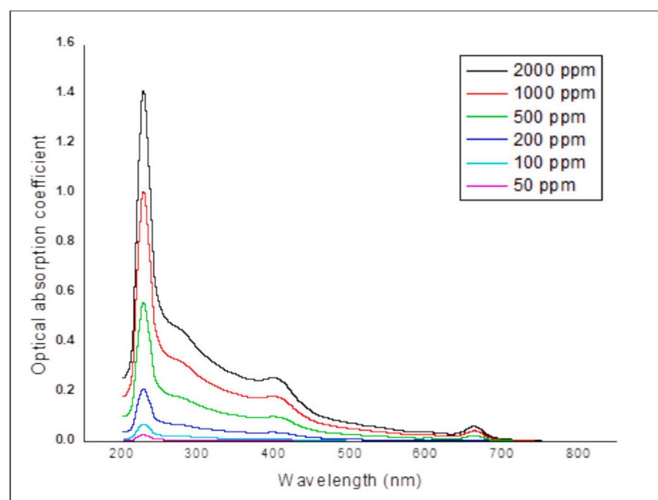

Fig. 9. Optical absorption coefficient of *Datura* extracts at different concentrations.

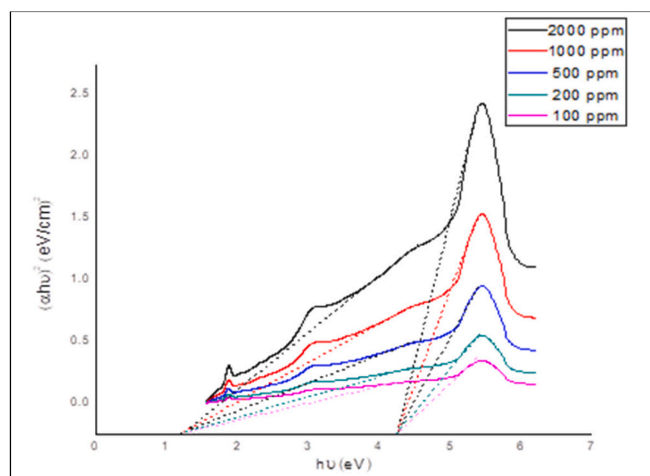

Fig. 10. Band gap energy of *Datura* extracts at provided concentrations. The direct optical bandgap (Eg) for each *Datura* extract concentration can be determined by extrapolating the linear portion of the absorption edge to the x-axis, when (ahu)2 axis = 0.

pea (*Cajanus cajan* L.).

Plants have always been an essential part of human existence and civilization, meeting our basic requirements for food, medicine, and shelter, but their significance extends beyond these uses. Because they provide vital elements including vitamins, minerals, and fiber that are necessary for healthy bodily function, plants are important for human health and wellbeing. We can enhance our general health and lower our chance of developing chronic illnesses like cancer, diabetes, and heart disease by include a range of plant-based foods in our diets. Plants can prevent and treat a number of ailments in addition to offering vital nutrients. For centuries, people have used plants to alleviate symptoms of illnesses and promote healing, and plant-based medicine is still a significant aspect of many traditional and modern medical practices [97].

Herein, four extracts of *Datura stramonium* namely; methanol, ethyl

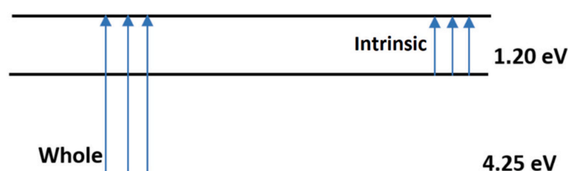

Fig. 11. Representation of optical bandgap energy when photon absorbs or generates energy.

acetate, acetone and aqueous were subjected to UPLC/MS analysis which resulted in the tentative identification of one hundred and thirteen metabolites. The compounds identification was varied according to the used solvent polarity and the ionization mode used for their detection. This was obvious through the accumulation of certain metabolites like flavonoids, phenolic acids and cinnamic acid derivatives in the methanol, ethyl acetate and aqueous extracts in the negative ionization mode while upon visualizing the UPLC/ESI/MS positive mode other classes were prominent including triterpenoids, withanolides and alkaloids. The number of identified components was the highest for the ethyl acetate extract compared to the other three. In addition to that, the n-hexane extract for *D. stramonium* was analyzed through GC/MS analysis where twelve volatile components were detected mainly from the mono-sesqui- and diterpenoids in addition to volatile alkaloids that are known to accumulate in genus *Datura* family Solanaceae.

The *Datura* plant has many active compounds, including alkaloids, saponins, flavonoids, tannins, steroids, and triterpenoids, with scopolamine being the main active ingredient in the alkaloid group. These constituents contribute to its therapeutic use in treating various conditions like asthma, bronchitis, diabetes, cardiovascular issues, mental disorders, epilepsy, skin diseases, fever, and diarrhea [9,10]. People have used *Datura* flowers, known for their withanolide content, to alleviate pain [98]. Despite its hallucinogenic nature, the seeds sometimes serve as a substitute for opium, and chewing the leaves is said to alleviate dental pain [10]. The literature reports on genus *Datura* and the

Table 10
Optical bandgap energies in electron volts (eV) for *Datura* whole extract.

Datura extract	Optical bandgap (eV)
Whole gab	4.25
Intrinsic gab	1.20

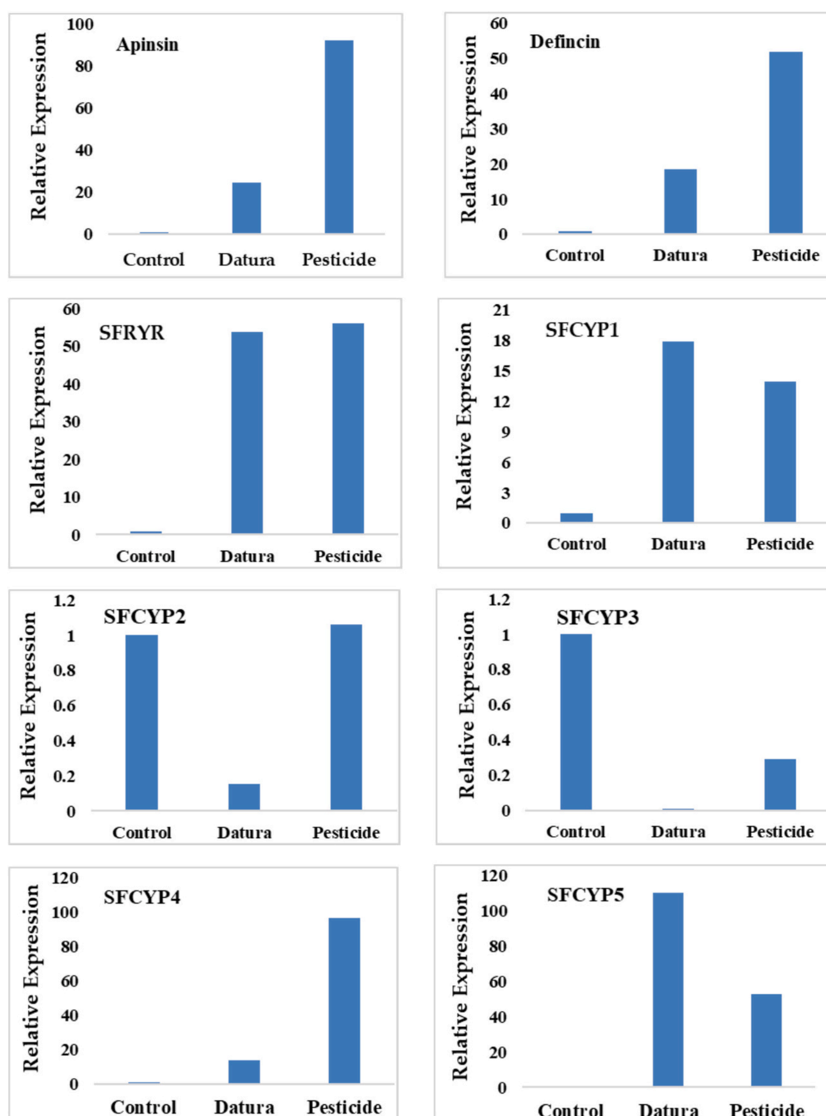

Fig. 12. The expression profiles of the 8 candidate genes under pesticide and datura application of *Cx. pipiens* larvae genes, Apismin, Defincin 1, SFCYP1, SFCYP2 SFCYP3 SFCYP4, SFCYP5 and SFRYR after treatment. Different letters on column indicate significant variations at 0.05.

nightshade family are rich with many reports on the phytochemical and biological activities that may be summarized as followed.

The immune modulatory effect of fractions of *D. stramonium* L. leaves on human peripheral blood mononuclear cells (PBMC) was assessed followed by assessment of cytotoxic abilities of immunomodulated PBMC toward cancer cells. Chromatographic fractionation guided by PBMC proliferation assay of *D. stramonium* extract resulted in bioactive fraction (fraction-10) exhibiting significant immunostimulatory activity [$EC_{50} = 19.1 \pm 1.5$(μg/ml)] on human blood lymphocytes. Fraction-10 pretreated PBMC displayed enhanced cytotoxicity toward A549 and MCF-7 (59 % ± 2.1 % and 62 % ± 2.3 % at 1:20 effector: target ratio respectively). Moreover, fraction-10 also enhanced the secretion of IL-2 (8-fold) and IFN-γ (10-fold) by human PBMC. LC-MS analysis depicted presence of four major phytoconstituents in fraction-10 as daturaolone, daturadiol, stigmasterol and sitosterol with corresponding mass spectrum (*m/z*) of 440, 442, 412 and 414 respectively [61]. In another study, HRLC-MS analysis of methanol leaf extract of *D. stramonium L. spectrum* profile gives presence of 100 compounds in the form of fatty acids, organic compounds, phenolics, alkaloids, phytoharmone, coenzyme, aminopyrimidines, dipeptide and tripeptides like important metabolites in these leaves [99].

Moreover, with an LC_{50} value of 46.1636 μg/ml, the cytotoxic activity test demonstrated the significant cytotoxic capability of *Datura metel* leaf extract. The outcome also demonstrated that the isolate was a steroid, specifically Baimantuoluoline D, which is a member of the Withanolide group. With daughter fragments creating 477.2583 *m/z* [M+ H- CH_3-OH] and 301.1780 [M+ H-$C_9H_{11}O_4$], the isolate's molecular weight was [M+] *m/z* 504.0591. The activities demonstrated the cytotoxic, anti-inflammatory, and anti-microbial properties of the withanolide that was extracted from it. Alkaloids, flavonoids, steroids, and saponins were found in the *Datura* leaves, according to the results of the phytochemical screening [68]. In addition to that, the ethanol extracts of *Datura alba* Ness exhibited significant efficacy in pain reduction by lowering C-reactive protein levels. This effect was especially enhanced when the 500 mg/kg dose of MEDAN was combined with the standard drug, Flexicam [60].

The phytochemical compounds mentioned show that the datura plant has several natural chemical compounds, like withanolides, scopolamine, and nicotine, which may help fight against mosquitoes and microbes. Extracts from this plant have also been shown to be capable of combating many dangerous pests. Being eco-benign natural products, *Datura* phytochemicals can serve as potential bio-acaricide, thereby, a smart delivery system could be developed comprising synergistic concoction of the alkaloid rich phytochemical fractions for efficient management of the notorious acarids [21]. Highest acaricidal activity was observed for root extract showing LC_{50} and LC_{90} of 112.5 and 317.3 μg/mL after 48 h of exposure, respectively [47].

UPLC-QTOF-ESI-MS analysis of the *Datura metel* extracts resulted tentative identification of eighteen tropane alkaloids such as tigloidin, hyoscyamine N-oxide, scopolamine N-oxide, hyocyamine, hydroxyapoatropine, tropinone, scopine, hyoscine, atropine, valtropine, ditigloyloxytropane, ditigloyloxytropanol, apohyoscine, tigloyloxytropane, norhyoscine, meteloidine, cuscohygrine and tropine in the aerial parts and roots. Further, nine phenolic acids such as gallic, protocatechuic, chlorogenic, vanillic, p-coumaric, ferulic, quinic, syringic, and sinapic acids along with fourteen flavonoids such as luteolin-7-glucoside, catechin-3-*O*-rhamnoside, kempferol- 3,7-*O*-diglucoside, naringenin-6-*β*-D-glucopyranoside, quercetin 3-*O*-glucosyl-xyloside, apigenin-7-*O*-glucoside, flavonol-3-glucoside, luteolin, epicatechin, chrysoeriol, diadzein, genistein, hesperidin and hydroxycoumarin were also identified.

Principal component analysis (PCA) is an advantageous statistical procedure that has found applications in various fields. It helps in reducing the dimensionality of the data set and identification of patterns in the data through graphical illustration. In nutshell, we can say that principal component analysis is a data reduction procedure [100,101]. In the present study, PCA was utilized as an unsupervised data analysis technique for data discrimination between the four studied *Datura* extracts (ethyl acetate, methanol, aqueous and acetone). Different clustering patterns were observed between the four extracts which could be linked to their varied phytochemical composition. PCA was constructed for the four extracts once for the metabolites identified in the ESI positive mode and another of the metabolites detected in the ESI negative mode. The observed variance for PC1 and PC2 were different for each mode together with the grouping of extracts in distinct clusters. For better data visualization and discrimination, another tool was utilized, the clustered heat map, which was also constructed twice for the tentatively identified components from the two ionization modes. The color gradients were differing upon changing the number, classes and area% between the two modes resulting in two distinct heat maps exploring the tentatively identified components from the four extracts.

The optical spectroscopic investigation of the extracts from *D. stramonium* revealed that these extracts cover a wide range of wavelengths because they contain all the natural components of *D. stramonium* [5]. Optical bandgap energies showed two values, one related to the whole electronic transitions and the other related to the tiny intrinsic electronic transition, which also shows the effect of having the extract as a whole in mosquito control. Furthermore, it shows that the optical bandgap energies are concentration independent, which proves that the extract possesses photostability and can be used in the field at the normal sunlight wavelengths, visible and UV ranges.

qRT-PCR, used to precisely monitor gene expression, has helped elucidate the role of cytochrome P450 enzymes, a group of proteins ubiquitous in living organisms, in insect development and resistance to pesticides. Insects possess a large number of P450 genes, particularly in mosquitoes, which contributes to their high ability to degrade and resist pesticides. Apismin and defensing 1 significantly contribute to insect resistance against pesticides [102]. Furthermore, Corbel, Nosten, Thanispong, Luxemburger, Kongmee and Chareonviriyaphap [103] proposed that identifying the molecular mechanisms causing resistance in mesquites is critical for tracking down resistance alleles and enhancing resistance management tactics. In the case of Apismin and Defincin 1, pesticides were unable to shut down both of them, but *Datura* succeeded to some extent, implying that the insects showed greater resistance to pesticides than *Datura* Muhammed, Dugassa, Belina, Zohdy, Irish and Gebresilassie [104] obtained the same observation when they employed various plant extracts as insecticides against Anopheles stephensi. Actually, it is widely understood that the mosquito's resistance mechanism is generated by the enhanced activity of detoxifying enzymes. These detoxifying enzymes include cytochrome P450 monooxygenases, which are referred to as P450s or CYPs when discussing their genes. *Datura* successfully shut down the most investigated cytochrome genes (detoxification genes), implying that *Datura* has more insecticidal effects than the insecticide utilized in this investigation. Our assumption is that the datura plant extract makes the insect more susceptible to its insecticidal properties. Şengül Demirak and Canpolat [105] and Ren, Li, Zhang, Zhu, Hou, Miao, Xu, Zhang and Sheng [106], revealed that insects can develop resistance to various insecticides if they carry cytochrome family genes and mutant alleles. Both the datura and the pesticide showed high levels of the pesticide-targeting gene (SFRYR), suggesting that the datura extract is important for targeting the receptors. It can be concluded that *Datura* extract could be an effective bioinsecticide alternative to chemical ones.

5. Conclusion

Mosquitoes are major carriers of life-threatening diseases that pose significant risks to human health, and managing these risks goes beyond simply eliminating the insects. However, the rise of insecticide-resistant mosquito populations, along with environmental and health concerns linked to chemical pesticides, has led to the search for safer and more sustainable alternatives. Among these, plant-based compounds are attracting interest due to their natural origin, lower toxicity,

affordability, and reduced risk of resistance development. In this context, researchers have investigated certain ornamental plants to identify potent bioactive substances with insecticidal and antimicrobial properties suitable for pest control in both human and animal health. The phytochemical analysis of the four *Datura* extracts (methanol, ethyl acetate, acetone and aqueous) revealed the tentative identification of 113 components from different classes *viz*. flavonoids, phenolic acids, withanolides, triterpenoids, alkaloids, phenolic acids and others together with twelve volatile metabolites solely detected from the n-hexane extract. The findings of the present study revealed that the leaf extracts of *D. stramonium* possess larvicidal and adulticidal activities against vector mosquitoes and medicinal values as it has antimicrobial activity. Furthermore, our findings showed that the extracts of *D. stramonium* have photostability at different concentrations, and two identical optical bandgaps cover a wide range of absorption frequencies. QRT-PCR analysis results indicate that *D. stramonium* and the pesticide clearly influence the regulation of resistance genes within insects, especially cytochrome P450 genes, suggesting that some of them may vary in diversity and resistance between organisms. Further studies should prioritize identifying plant-based substances that can be developed and promoted as dependable solutions for minimizing mosquito threats and preventing diseases they transmit.

Sample availability

Not applicable.

CRediT authorship contribution statement

Mohamed M. Baz: Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Randa I. Eltaly:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Ramy E. El-Ansary:** Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Data curation. **Moustafa Ibrahim:** Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Fatma H. Galal:** Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Hattan S. Gattan:** Writing – original draft, Validation, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mohammed H. Alruhaili:** Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Abdelfattah M. Selim:** Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Lamya Ahmed Alkeridis:** Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Yasser A. El-Sayed:** Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Esraa A. Elhawary:** Writing – original draft, Visualization, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Informed consent statement

Not applicable.

Consent for publication

Not applicable.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Benha University and was approved by the Ethics Committee of the Faculty of Science, Benha University (Code: BUFS-REC-2025-451 Ent). The research adhered to all institutional and ethical guidelines for invertebrate studies, ensuring humane treatment of treated insect with minimal suffering and environmental impact, in compliance with approved research ethics standards.

Funding

Not available.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgments

The authors wish to thank Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNU RSP2025 R82) Princess Nourah bint Abdulrahman university, Riyadh, Saudi Arabia.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Correspondence: mohamed.albaz@fsc.bu.edu.eg.

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