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Seasonal variation effect on different *Physalis peruviana* L. (Solanaceae) waste extracts and investigation of their efficacy against *Culex pipiens* and *Musca domestica*

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Disease-carrying insects transmit many of the most serious human diseases. After decades of repeated use of insecticides, all of these vector species have demonstrated the ability to develop resistance to insecticides. This has necessitated the development of more efficient and environmentally safe alternatives in the form of biopesticides. Plants contain a wide range of potential phytochemicals that target a specific target, are rapidly biodegradable, environmentally friendly, and have a variety of therapeutic effects, making them a treasure trove of biological materials. Moreover, this has led to the creation of highly effective new drugs. The present study aims to demonstrate the specific active components in *Physalis peruviana* calyces that were collected in two consecutive fruiting seasons through UPLC/MS and multivariate data analyses. The extracts were prepared using 70% methanol/water and petroleum ether for each season, then evaluated against disease-carrying vectors, *Culex pipiens* and *Musca domestica*. The UPLC/MS analysis resulted in the tentative identification of fifty-four secondary metabolites belonging mainly to flavonoids, phenolic acids, withanolides, triterpenoids, phenyl propanoids, and many others. After various intervals of exposure, plant extracts in this study showed high insecticidal activity against mosquito and housefly larvae, *Cx. pipiens*, and *M. domestica*. Data showed that *P. peruviana* methanol extract (POM) appeared to be most effective (MO%) against *Cx. pipiens* (LC_{50} = 8.18 mg/ml) and *M. domestica* larvae (LC_{50} = 9.87 mg/ml), 24 h post-treatment. The relative toxicity revealed that the old *P. peruviana* extract (POM) was the most effective in killing larvae, followed by the POP extract, while the modern extracts (PNM and PNP) were less successful on mosquito and housefly larvae. Thus, *Physalis peruviana* calyx extracts can act as a potential biocontrol agent against certain medical insects.

Keywords *Physalis*, *Peruviana*, Solanaceae, Bioinsecticide, Waste, *Culex*, *Musca*

Among the horticulture crops, fruits and vegetables are the most widely consumed food items. Depending on their nature and method of preparation, these foods are eaten raw, partially cooked, or completely cooked.

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Throughout the supply chain, wastes from fruits and vegetables might vary greatly based on how they are processed. In both solid and liquid form, the fruit and vegetable processing industries produced 10–60% waste or by-products. The best advantages should be derived from waste material in a way that minimizes financial loss and poses no environmental risks. Due to their perishable nature, fruits and vegetables soon decay. Waste disposal is therefore a major issue as it attracts rats and insects. *Physalis peruviana* also known as golden berry or goose berry carries edible berry-like fruits which upon consumption results in its calyx being left over as waste[1–3].

Genus *Physalis* (Solanoideae, Solanaceae) includes approximately 120 species distributed mainly in South and North America. Five species of *Physalis* are found in China. *Physalis peruviana* (Chinese name: deng-long-guo) is cultivated in China for its edible fruits. *Physalis peruviana* (goose berry or golden berry) is widely grown in Egypt and is one of the folk edible fruits of Egypt[4,5]. It is also used in traditional medicine to treat cancer, asthma, malaria, dermatitis, hepatitis, and rheumatism. In addition to its antispasmodic, diuretic, antiseptic, antibacterial, analgesic, sedative, antioxidant, helping to build the optic nerve, throat pain relief, elimination of intestinal parasites, and amoeba. *Physalis peruviana* has health benefits such as purifying the blood, lowering albumin in the kidneys, rebuilding and fortifying the optic nerves, eradicating intestinal parasites, soothing throat infections, immune system support, diabetes control, digestive health, and digestive health[6]. *Physalis angulata,* a folk medicine called as 'Ku-Zhi' in Chinese, was used to cure impaludism, tracheitis, dermatitis, rheumatism, and hepatitis, and was also used in Mexico, Indonesia, Peru, and Brazil. In traditional Chinese medical theory, the calyxes and fruits of *P. alkekengi* var. *franchetii,* known as 'Jin-Deng-Long' in China, were often accepted for the treatment of excessive phlegm, cough, pharyngitis, pemphigus, dysuria, etc. The fruits, calyxes, and leaves of *P. philadelphica* are used in South American traditional medicine to treat a variety of human diseases. The fruits were used to treat fever, coughing, and amygdalitis; the calyxes were used to treat diabetes; and the leaves were potentially used to treat gastrointestinal issues. *Physalis*, as a whole plant, has many medicinal properties, such as antipyretic, purifying, diuretic, diaphoretic, and vermifuge effects. Arab physicians of the past highly valued this plant for its potential in treating kidney and urinary tract diseases, due to its alleged ability to dissolve kidney stones. Recently, a number of studies have talked about how *Physalis* can be used as a medicine and how it can fight parasites, viruses, tumors, free radicals, and leukemia[7].

Withanolides are the primary chemical ingredients of the genus *Physalis* and the primary biologically active compounds that underpin the traditional usage of these plants, according to phytochemical and pharmacological studies[8].

Physalis peruviana is an annual fruit in temperate countries and a perennial plant in the tropics; it can withstand temperatures of up to 30 °C. The plant's annual average temperature is between 13 and 18 °C. The plant requires between 800 and 4300 mm of growing rainfall in well-drained soil. It also enjoys either full or partial shade, and sandy loam soil is ideal for plant growth. *Physalis peruviana* possesses different phytochemical compounds: phenolics, tannins, terpenes, saponins, flavonoids, carotenoids, withanolides, and alkaloids. Each of the aforementioned phytochemical components can be found in the root, bark, stem, flowers, leaves, fruits, and seeds, among other plant parts. It has been suggested that *P. peruviana* is useful in treating various bacterial diseases because research reveals that it contains a variety of phytochemical compounds with therapeutic qualities[9].

Physalis peruviana L. is a perennial herb, 45–90 cm tall. Stems erect, sparingly branched, densely pubescent. petiole 2–5 cm, leaf blade broadly ovate to cordate, 6–15 _ 4–10 cm, thickly pubescent, base cordate, margin entire or with a few scattered teeth, apex short acuminate. Pedicel ca. 1.5 cm. Calyx broadly campanulate, 7–9 mm. Corolla yellow, spotted in throat, 1.2–1.5 _ 1.2–2 cm. Blue-purple filaments and 1–4 mm-long anthers. Fruiting calyx: green, oval, 2.5–4 cm, pubescent, with 5–10 narrow angles. 1–2 cm in diameter, golden berry. Yellow seeds with a diameter of roughly 2 mm summer flowering and fall fruits. Usually considered to be a hazardous weed in Africa[4,10–12].

Withanolides, also known as withasteroids, derived from plants are a kind of bioactive steroidal lactones that are arranged on a polyoxygenated C-28 ergostane skeleton. To present, there are known to be more than 300 withanolide compounds and their derivative forms, and new compounds are always being found[13]. All molecules have oxygenated carbon atoms at positions 1, 22, and 26. The side chain of ergostane-type steroids has a lactone/lactol ring attached to the backbone, which is a distinguishing feature of these compounds[14]. Since withanolides appear to occur almost exclusively in the nightshade family (Solanaceae), knowledge of their diversity and overall distribution within the plant kingdom is still fragmentary, a general classification of them remains challenging due to their functional and structural complexity, ongoing discoveries of novel withanolide compounds, and inconsistent nomenclature. Within the solanaceous clade, members of the genera *Acnistus*, *Datura, Dunalia, Deprea*, *Iochroma, Exodeconus, Lycium, Mandragora, Jaborosa, Physalis*, *Nicandra, Trechonaetes*, *Salpichroa*, *Withania,* Vassobia, and *Witheringia* have been found to produce withanolides[13]. Interestingly, it has also been found that the sea coral species *Paraminabea acronocephala* synthesises several withanolides. Both seasonal variations and geographic considerations appear to affect the quantity and variety of withanolide variants[15]. Withaferin A, the principal constituent of this class, was the first withanolide to be isolated. For ages, Withaferin A has been utilized in traditional Indian folk medicine[16].

Chemical ecology is the study of intricate interactions between and among plants, animals, and microbes that are mediated by chemicals. It incorporates studies on the molecular underpinnings of communication, defense, and signaling and covers both direct and indirect interactions. Chemical ecologists have a keen interest in identifying and characterizing particular molecules that are essential to these processes. Since chemical signals are widely used, fundamental research methods in this area have opened the door to new medicine discoveries, crop production advancements, and applied agricultural pest management. The study of plant–herbivore interactions is a fundamental area of chemical ecology that sheds light on the dynamics of speciation and evolutionary co-adaptation[16].

Plants produce waste as by-products after they are used as food or after industrial processes, and people often neglect their functions. Due to the increasing environmental burdens, including the increasingly severe effects of global warming and the excessive use of pesticides that have led to many insect species becoming resistant to these insecticides, significant efforts have been made to address this problem. These efforts include reducing and recycling garbage or agricultural waste. Secondary metabolites, which can be found in plants that produce waste, such as cabbage and taro leaves, orange and pomegranate peels, and plant cobs, like gooseberries, kill insects, taking the place of synthetic insecticides in pest contro[17].

Worldwide, vector-borne illnesses continue to be a major public health concern, particularly in tropical and subtropical regions. Over three billion people live in unhealthy environments, further endangering public health. Arthropod vectors have the ability to transmit a wide range of harmful pathogens, which can result in the spread of infectious diseases that can harm both humans and animals[18,19].

Under certain conditions, many diseases can spread directly from person to person. These conditions include interactions between viruses, hosts, vectors, susceptible populations, and the presence of disease reservoirs[20].

Mosquito-transmitted diseases such as filaria, dengue, and malaria have long documented numerous fatalities from mosquito-transmitted illnesses. According to a recent study, 88 (2.5%) of the 3578 species of mosquitoes are carriers for 78 different human diseases[21]. Furthermore, researchers believe that 243 mosquito species, accounting for 6.8%, could potentially transmit human diseases. Nonetheless, the chance of mosquito-borne viruses (MBVs) spreading to nations that may not have a history of MBVs is growing due to the sharp increase in worldwide travel. Female mosquitoes feed on vertebrate blood to get the necessary nutrients for laying eggs, which spreads hundreds of virus particles that may be present in their saliva[22].

The *Culex* mosquito species is the most prevalent globally[23,24] and serves as a significant vector for West Nile Virus, Usutu virus, St. Louis Encephalitis Virus, Rift Valley fever virus, Western and Eastern equine encephalitis viruses and Japanese Encephalitis Virus, as well as filarial parasites[25,26]. *Culex* mosquitoes are a recognized significant global source of annoyance for biting humans[27]. *Cx* mosquitoes, notably *Cx quinquefasciatus* and *Cx. pipiens*, are especially abundant in tropical and subtropical metropolitan areas where environmental factors and urban expansion promote their growth[28,29].

The house fly is wide distribution and can mechanically carry a variety of diseases to humans[30]. This is because adult houseflies have a keen sense of smell and feed on things like human food, animal excrement, perspiration, trash, and moist or decaying material from pet waste[30]. Also, its vomit or excrement can carry viruses, helminthic protozoa, and bacteria including *E. coli*, *Shigella* species, and *Salmonella*, among almost a hundred diseases that can infect humans and animals.

Various classes of synthetic insecticides have been widely used to control disease vectors, including pyrethroid insecticides and organophosphate insecticides, to control mosquitoes and houseflies[31]. However, despite these insecticides' ability to reduce the invasiveness of disease vectors, particularly during disease outbreaks or increased pest density, they also pollute the environment, endanger human health, harm non-target animals, and, most importantly, increase the resistance of these insects to the insecticides used[32].

As a result, it was necessary to find a suitable alternative agent to synthetic pesticides, which represent biopesticides. Biopesticides have enormous potential and provide a more cost-effective, accessible, and environmentally safe alternative to conventional insecticides; botanicals have attracted much attention in many medical and industrial fields[33]. These alternative techniques can be a powerful tool in integrated pest management plans, delaying the development of resistance to conventional insecticides[34]. Several studies have documented the insecticidal properties of plant extracts and essential oils against houseflies[35]. Limonene, myrcene, terpineol, linalool, and pulegone are some of the monoterpenoids that can kill houseflies. Therefore, plant extracts or essential oils can replace synthetic insecticides to eliminate houseflies and other harmful insects[36].

Recent studies with extracts of *Physalis* leaves have detailed its role as molluscicide and insect repellent, due to bioactive compounds as whitanolides, phenols and ethanolics[37]. Moreover, the larvicidal activity of the ethanol extracts of leaves and fruits of *P. angulata* L. from Nigeria on the larvae of *Anopheles* mosquitoes were investigated[38]. In addition, *Physalis mollis* (smooth ground cherry) act as a fly poison due to its alkaloidal content[39].

In this study, extracts of different polarities of the Egyptian plant, *Physalis peruviana* calyx, through two seasons were phytochemically evaluated using UPLC/MS analysis followed by multivariate data analysis through PCA and clustered heatmap to explore the phytochemical values. Besides, the efficacy of *P. peruviana* extracts on two vector-borne diseases, *Culex pipiens* and *Musca domestica*, was evaluated as green insecticides extracted from agricultural waste.

Materials and methods

Plant collection

Physalis peruviana whole fruit (with calyx) was collected during two consecutive fruiting seasons, February–April and August–October of 2022–2023 and 2023–2024, from El-Laboudy farm, Abou Rawash, Imbaba, Giza Governorate (30.057891122623356, 31.093088056635292). The collected plant was then identified and authenticated by Dr. Trease Labib, a plant taxonomy consultant at the Egyptian Ministry of Agriculture and a voucher specimen was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University under the code: (PHG-P-PP-500). The climatic conditions of the plant collection area, including average temperature (°C), relative humidity (RH), and rainfall (mm), were monitored according to data from the Egyptian Meteorological Authority.

Extract preparation

The calyces were removed from the collected fruits then they were dried in shade. About (1.5 kg) from the dried calyces was soaked separately in 70% methanol and in 100% petroleum ether then filtered and the excess solvent

was evaporated using Rotavap. The extraction was repeated using the same solvents for the two seasons and the extraction process was repeated three times. The resulting extracts were weighed and recorded as: old for season 2022–2023 and new for season 2023–2024 where their weights were as followed; old methanol extract (POM, 18 g), new methanol extract (PNM, 15 g), old petroleum ether extract (POP, 22 g) and new petroleum ether extract (PNP, 10 g). The prepared extracts were dried till no residual solvent and kept in freezer for further use.

UPLC/MS analysis

The UPLC/ESI/MS analysis was performed for the four *Physalis peruviana* calyx extracts using the method of[40–44]. 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 H_2O 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.

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[41,42,45].

Larvicidal assay

Mosquito colony

Culex pipiens mosquito larvae were obtained from the Medical Entomology Division, Entomology Department, Faculty of Science, Benha University, Egypt, under laboratory conditions (27 ± 2 °C, 75–80% RH, and light period 12:12 h (L/day). Larvae were reared in enamel dishes measuring 25 × 20 × 10 cm with 2 L of dechlorinated water and fed with fish food (Tetramin) and ground dog biscuits every other day, with observation of water content and growth of larvae and avoiding the formation of a gelatinous layer on the surface that hinders larval respiration and growth. Adults were provided with 8–10% sucrose solution as a food source. For current and future scientific experiments, adults and larvae were kept under the same laboratory conditions[46].

Housefly colony

Adult houseflies were collected from the vegetable market in Benha, Qalyubiya, Egypt. They were then placed in 40 × 30 × 30 cm^3 wooden cages with wire tops and kept at room temperature (28–30 °C) in the insect rearing laboratory, Medical Entomology Division, Department of Entomology, Faculty of Science, Benha University. Cotton wool absorbed a mixture of 10% syrup and 10% milk, which formed their diet. Besides, 300 g of mackerels were carefully cooked in a tray measuring 18 × 25 × 9 cm^3 with a mixture of dry bread and mashed potatoes, which created an ideal environment for houseflies to feed and lay their eggs[47].

Larvicidal bioassays

Culex pipiens

Plant extracts (1 g/1000 ml distilled water) were tested according to WHO[48] to see if they might effectively control *Cx. pipiens* third larval instar. A glass beaker containing 250 ml of different concentrations (125, 250, 500, 1000, and 2000 ppm) with twenty-five mosquito larvae were added (dipping technique). The control groups were treated with water alone, without the addition of any plant extracts. The experiments were conducted three times. The mortality rates of *Cx. pipiens* larvae were recorded after 24 and 48 h after treatment (PT) at 27 ± 2 °C and 70–80% RH. The group without treatment received only distilled water as a control.

Musca domestica

Bioassays were performed to determine the effects of plant extracts on fly larvae using the contact method, placing the larvae in a treated culture medium[34]. We placed 25 early third-instar larvae in small paper cups (5 cm in diameter and 7 cm high) filled with 5 g of rearing medium. The cups were then treated with 2.5, 5, 15, 30, and 60 mg/mL plant extracts. Untreated groups were treated with water only. The treated and untreated cups were covered with a cotton cloth tied with a rubber band to prevent larvae from escaping. The experiment was repeated three times. Dead larvae were counted after 24, and 48 h, and then 3 g of sawdust was added to each Petri dish for pupation.

Statistical analysis

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

formula: RE for LC = LC_{50} (LC_{90} or LC_{99}) for reference oil/LC_{50}(LC_{90} or LC_{99}) for reference plant/LC_{50}(LC_{90} or LC_{99}) for plant extracts. A clustered heat map was constructed utilizing NCSS 12 software.

Results

UPLC/MS analysis of *Physalis peruviana* calyx extracts using solvents of different polarities

The UPLC/MS analysis was performed for the four calyx extracts of *Physalis peruviana* namely, *Physalis* old season 70% methanol extract (POM), *Physalis* new season 70% methanol (PNM), *Physalis* old season petroleum ether (POP), *Physalis* new season petroleum ether (PNP) as presented in (Table 1). Fifty-four secondary metabolites were tentatively identified from the four extracts and their retention times, molecular ion peaks, chemical class and molecular formula were recorded in Table 1. The main identified classes were flavonoids, phenolic acids, withanolides, triterpenoids, phenyl propanoids and many others (Fig. 1). The UPLC/MS chromatograms for the four tested samples were shown in Figs. 2 and 3. It is worthy noted here that this is the first study comparing the seasonal variation in phytochemical content of *Physalis peruviana* calyx extract using solvents of different polarities. The new season petroleum ether extract showed higher number and percentage of identified phytochemical components compared to the old season extracts while the number and percentage of identified components were higher for the old season methanol extract compared to the new season extract.

The tentatively identified components were grouped into the following classes

Flavonoids

Eighteen flavonoids and their derivatives were detected (Table 1), they were mainly localized in the methanol samples (POM and PNM). A deprotonated peak was traced at *m/z* 377 (−ve mode) and *m/z* 381 (+ve mode) with molecular formula $C_{19}H_{18}O_{10}$ where it was defined as tetrahydroxy trimethoxy dihydroxyflavone[41]. Another peak was detected at *m/z* 609 (611), which was found as major components in the methanol samples only, was assigned to the famous flavonoid, rutin (previously reported from genus *Physalis*)[49]. From the same genus, another flavonoid was identified as nicotiflorin (also known as kaempferol-*O*-rutinoside) with parent peak at *m/z* 593[49,50]. Certain aglycones were detected many times in many compounds such as quercetin, luteolin and apigenin where the aglycone itself or its derivatives were previously reported from genus *Physalis*. Compounds 8, 15 and 50 shared quercetin as a common aglycone. They presented a deprotonated molecular ion peaks at *m/z* 549, 303(+ve) and 465(583) which were identified as quercetin malonyl-hexoside (1.56%, PNM only)[51], quercetin aglycone (6.28%, PNP only)[52] and quercetin-*O*-hexoside (hyperoside) (10.37(2.41)% POP & 3.61% PNP)[53], respectively.

In addition to that, compounds 12, 20, 29 and 34 shared apigenin in common. Their deprotonated molecular ion peaks were detected at *m/z* 563(704), 693(695), 609 (+ve) and 607 (+ve), respectively thus they were tentatively assigned to apigenin-*C*-hexoside-*C*-pentoside (12.62% POM only)[54,55], apigenin-*C*-pentoside-*C*-pentose-*O*-pentoside (7.81% POM only)[56,57], apigenin-*C*-(*O*-feruloyl)- hexoside (3.51% POM only)[58] and apigenin-*C*-pentoside-*C*-hexoside X″-*O*-acetyl (3.34% POM & 3.11% POP)[56], respectively (Table 1).

In a similar fashion, luteolin was a common aglycone for four of the identified components namely; compounds 16, 30, 45 and 47 which had their deprotonated peaks at m/z 449(+ve), 463, 463 and 477(491), respectively thus they were tentatively defined as luteolin-7-O-hexoside (previously reported from genus *Physalis*)[52], luteolin hexuronide[59–61], hydroxy-luteolin-O-hexoside[40–42,44,59] and hydroxy-luteolin-O-hexuronide[62], respectively. Other examples of flavonoids and their derivatives were listed in Table 1.

Phenolic acids

Six compounds were detected from the phenolic acids class (Table 1) with three of them previously reported from genus *Physalis*. The previously reported components were denoted as compound 3 with *m/z* 353 (chlorogenic acid, only from PNM & PNP)[40,44], compound 9 with *m/z* 327 (coumaroyl hexoside, only from PNM & POM)[52] and compound 22 with *m/z* 311 (caffeoyl-pentose, only from PNM & PNP)[57,63]. Other examples of phenolic acids and their derivatives were listed in Table 1.

Withanolides

Withanolides are usually listed under the class of triterpenoids with some structural differences which gave them special phytochemical and biological effects. In Table 1, many examples of withanolides were detected from the four *Physalis* samples which can be detailed as followed. It is also worthy noted that all of them were previously isolated from genus *Physalis*. A peak was traced at *m/z* 501 (3.58% POM only) and was assigned to the steroidal lactone withanolide, 4*β*-hydroxy withanolide E[64]. Other derivatives of the aforementioned withanolide were also tentatively identified as withalongolide F[65], withanolide F[66] and withanolide D[49] with *m/z* values at 425 (+ve), 469 and 339(413), respectively. An andosterone-type withanolide was detected at *m/z* 330 (3.74% PNP only) which was defined as irinan A[66]. Similarly, visconolide presented a deprotonated peak at *m/z* 485 in positive mode[64,67]. Meanwhile, compounds 26, 32 and 35 showed peaks at *m/z* 529, 513 and 541, respectively which gave rise to their identification as physalin O[52], physalin M[52] and 4-hydroxyneophysalin A isomer[52], respectively (Table 1).

Triterpenoids

Four triterpenoids were tentatively detected (Table 1) from which two were previously reported from genus *Physalis* and were named as (3*β*,24R)-ergost-5-en-3-ol, acetate (*m/z* 441)[68] and squalene (*m/z* 410)[68].

Phenyl propanoids/phenyl ethanoids

Two phyl propanoids and one phenyl ethanoid were traced (Table 1). The two phenyl propanoids presented their peaks at *m/z* 265 and 501 (+ve) and were tentatively defined as feruloyl-caffeoyl-quinic acid derivative[69] and

No	Component	Chemical class	Molecular formula	R_t (min.)	$[M-H]^-$ *m/z*	$[M+H]^+$ *m/z*	Different extracts of *Physalis peruviana* (area between brackets for + ve mode)				Reference(s)
							Methanol		Petroleum ether		
							POM	PNM	POP	PNP	
1	Tetrahydroxy trimethoxy Dihydroxyflavone	Flavonoid	$C_{19}H_{18}O_{10}$	0.77	377	381	**4.65** (9.99)	**9.40** (22.87)	**10.85** (3.52)	**3.76** (4.64)	41
2	*di*-Hydroxy benzoic acid derivative	Miscellaneous	$C_9H_{10}O_5$	0.92	197	–	–	–	**10.22**	–	51
3	Chlorogenic acid*	Phenolic acid	$C_{16}H_{18}O_9$	1.44	353	–	–	**3.13**	–	**6.84**	40,44
4	Rutin*	Flavonoid	$C_{27}H_{30}O_{16}$	5.97	609	611	**5.86** (4.17)	**23.31** (11.22)	–	–	49
5	Nicotiflorin*	Flavonoid	$C_{27}H_{30}O_{15}$	6.28	593	–	–	0.70	–	–	49,50
6	Ursolic acid derivative	Triterpenoid	–	6.75	547	625	**7.73** (8.49)	**10.43** (6.89)	–	–	76
7	4*β*-Hydroxy withanolide E*	Steroidal lactones (Withanoloid)	$C_{28}H_{38}O_8$	8.58	501	–	**3.58**	–	–	–	64
8	Quercetin malonyl-hexoside	Flavonoid	$C_{24}H_{22}O_{15}$	8.59	549	–	–	1.56	–	–	51
9	Coumaroyl hexoside*	Phenolic acid	$C_{15}H_{18}O_8$	8.83	327	–	**4.98**	1.18	–	–	52
10	Icariside I	Flavonoid	$C_{27}H_{30}O_{11}$	8.98	531	750	**5.94** (5.76)	0.48	–	(4.11)	114
11	Buddlenol E*	Lignan	$C_{31}H_{36}O_{11}$	9.13	583	623	–	**2.84** (8.27)	–	–	71
12	Apigenin-*C*-hexoside-*C*-pentoside	Flavonoid	$C_{26}H_{28}O_{14}$	9.28	563	704	**12.62** (5.32)	–	–	–	54,55
13	Methoxy ursolic acid	Triterpenoid	–	10.69	487	511 (+ Na)	**6.30** (5.52)	–	–	–	76
14	Hepta decatrienyl resorcinol	Miscellaneous	–	11.54	–	343	–	–	(4.34)	–	115
15	Quercetin*	Flavonoid	$C_{15}H_{10}O_7$	13.55	–	303	–	–	–	(6.28)	52
16	Luteolin-7-*O*-hexoside*	Flavonoid	$C_{21}H_{20}O_{11}$	14.34	–	449	(5.74)	–	–	–	52
17	Feruloyl-caffeoyl-quinic acid derivative	Phenyl propanoid	–	14.39	265	–	–	**17.52**	**15.58**	**36.30**	69
18	Guaiacyl malvidin-*O*-hexoside	Anthocyanin	–	14.48	639	–	**4.41**	–	–	–	74
19	2'-Acetylpurpureaside A [1-*O*-[hexosyl-dihydroxy-cinnamoyl-*O*-acetyl-hexoside] derivative of hydroxytyrosol 1-hexosides]	Miscellaneous	$C_{31}H_{38}O_{17}$	14.63	682	720	**5.39** (3.83)	–	–	–	116
20	Apigenin-*C*-pentoside-*C*-pentose-*O*-pentoside	Flavonoid	–	15.10	693	695	**7.81** (5.02)	–	–	–	56,57
21	Tetramer of vanillic acid	Phenolic acid	–	15.58	673	–	–	–	–	**7.42**	117
22	Caffeoyl-pentose*	Phenolic acid	–	15.80	311	–	–	**8.64**	–	**9.45**	57,63
23	Irinan A*	Androstane-type Withanolide	–	15.90	–	330	–	–	–	(3.74)	66
24	Hispidulin	Flavone	$C_{16}H_{12}O_6$	16.62	–	301	–	–	(2.30)	–	59
25	(3*β*,24R)-Ergost-5-en-3-ol, acetate*	Triterpenoid	$C_{30}H_{50}O_2$	16.79	441	–	**3.54**	–	–	**3.38**	68
26	Physalin O*	Withanolides	$C_{28}H_{32}O_{10}$	17.17	529	–	–	1.56	–	–	52
27	Delphinidin hexosyl pentosyl malonate	Anthocyanin	$C_{29}H_{29}O_{19}$	20.84	681	701	–	–	–	**6.00** (7.65)	74,75
28	Lavandulifolioside	Phenyl ethanoid glycoside	$C_{34}H_{44}O_{19}$	20.99	–	757	(7.19)	–	–	–	57
29	Apigenin-*C*-(*O*-feruloyl)- hexoside	Flavonoid	$C_{31}H_{30}O_{14}$	21.29	–	609	(3.51)	–	–	–	58
30	Luteolin hexuronide	Flavonoid	$C_{21}H_{18}O_{12}$	22.38	–	463	–	–	(3.44)	–	59–61
31	Visconolide*	Withanolides	–	22.43	–	485	–	(8.26)	–	(4.40)	64,67
32	Physalin M*	Withanolides	$C_{28}H_{32}O_9$	23.65	–	513	–	(6.74)	–	(12.19)	52
33	*O*-Caffeoyl-pentosyl- hexoside	Phenolic acid	–	24.07	473	–	–	–	–	**3.24**	118
34	Apigenin-*C*-pentoside-*C*-hexoside X″-*O*-acetyl	Flavonoid	–	24.45	–	607	(3.34)	–	(3.11)	–	56
35	4-Hydroxyneophysalin A isomer*	Withanolides	$C_{28}H_{30}O_{11}$	24.72	–	541	(3.35)	–	–	(9.80)	52
36	*tri*-Hydroxy-*di*-hydrocyclo-*penta*[b]chromene-dione-carboxylic acid hexoside	Chromone	–	25.38	453	455	**3.91**	–	**10.37** (24.58)	–	73
37	Byzantionoside B*	Miscellaneous	$C_{19}H_{32}O_7$	25.40	372	391 (+ H_2O)	–	–	–	**3.41** (8.89)	50
38	Squalene*	Triterpenoid	$C_{30}H_{50}$	26.14	410	–	–	–	**10.42**	–	68
39	Withalongolide F*	Withanolides	–	26.77	–	425	–	–	(2.28)	–	65
Continued											

No	Component	Chemical class	Molecular formula	R_t (min.)	$[M-H]^-$ *m/z*	$[M+H]^+$*m/z*	Different extracts of *Physalis peruviana* (area between brackets for + ve mode)				Reference(s)
							Methanol		Petroleum ether		
							POM	PNM	POP	PNP	
40	Glycitein-*O*-hexouronide	Flavonoid	$C_{22}H_{20}O_{11}$	26.95	459	–	–	–	–	**3.50**	60,119
41	Isopentyl dihexose	Miscellaneous	–	27.67	–	413	–	–	(2.86)	(7.00)	120
42	Withanolide F*	Withanolides	$C_{28}H_{38}O_6$	28.08	469	–	–	0.42	–	**4.09**	66
43	Isoscutellarein-*O*-(acetylallosyl) hexoside	Flavonoid	–	28.33	–	653	(4.17)	–	–	–	60
44	Caffeoyl-coumaroyl-quinic acid	Phenyl propanoid	$C_{25}H_{24}O_{11}$	28.38	–	501	–	(10.89)	–	–	70
45	Hydroxy-luteolin-*O*-hexoside	Flavonoid	$C_{21}H_{22}O_{13}$	28.50	463	–	**5.09**	–	**11.46**	–	40–42,44,59
46	Salvianolic acid A isomer	Benzofuran	–	28.82	493	–	–	0.43	–	–	61,76
47	Hydroxy-luteolin-*O*-hexuronide	Flavonoid	$C_{21}H_{22}O_{13}$	29.09	477	491	**9.37** (3.68)	–	–	–	62
48	(13*Z*)-*β*-carotene*	Carotenoids	$C_{40}H_{56}$	29.70	535	537	(3.49)	–	(2.82)	–	52
49	Tetrandrine	Miscellaneous	$C_{38}H_{42}N_2O_6$	30.06	–	623	(3.27)	–	–	–	121
50	Quercetin-*O*-hexoside (hyperoside)	Flavonoid	$C_{21}H_{20}O_{12}$	30.12	465	583	–	–	**10.37** (2.41)	**3.61**	53
51	*O*-Methyl-cyanidin-*O*-(galloyl)-pentoside	Tannin	–	30.43	613	–	–	–	**10.21**	–	72
52	Buddlenol C*	Lignan	–	30.52	–	615	(3.87)	–	–	–	71
53	Rosmarinic acid hexoside	Phenolic acid	$C_{24}H_{28}O_{14}$	30.93	521	–	–	**3.42**	–	–	61
54	Withanolide D*	Steroidal lactones (Withanolides)	$C_{24}H_{30}O_6$	31.11	339	413	–	–	(4.92)	(6.70)	49
	% Identification (No. of compounds)										
	Negative ion mode	**91.18** (15)	**85.02** (15)	**79.11** (8)	**91.00** (12)						
	Positive ion mode	**89.71** (18)	**75.14** (7)	**56.58** (11)	**75.40** (11)						

Table 1. The tentatively identified components from the calyx extracts of *Physalis peruviana* through UPLC/MS. *for the compounds previously identified from genus *Physalis* from literature, POM; *Physalis* old season 70% methanol extract, PNM; *Physalis* new season 70% methanol extract, POP; *Physalis* old season petroleum ether extract, PNP; *Physalis* new season petroleum ether extract.

caffeoyl-coumaroyl-quinic acid[70], respectively. Moreover, a peak was traced at *m/z* 757 in positive mode and was assigned to the phenyl ethanoid, lavandulifolioside[57].

Lignans
Two lignans, previously reported from genus *Physalis*, were tentatively identified as buddlenol E[71] and buddlenol C[71] (Table 1) where their peaks were detected at *m/z* 583(623) and 615 (+ve), respectively.

Tannins
Only one tannin peak was defined as *O*-methyl-cyanidin-*O*-(galloyl)-pentoside at *m/z* 613 (10.21% POP only) (Table 1)[72].

Chromones
Only one tannin peak (Table 1) was defined as *tri*-Hydroxy-*di*-hydrocyclo-*penta*[b]chromene-dione-carboxylic acid hexoside at *m/z* 453(455)[73].

Anthocyanins
Two anthocyanin peaks (Table 1) were shown at *m/z* 639 and 681(701) and were defined as guaiacyl malvidin-*O*-hexoside[74] and delphinidin hexosyl pentosyl malonate[74,75], respectively.

Carotenoids
A carotenoid, which was previously reported from genus *Physalis*, was traced at *m/z* 535(537) which was found to be (13*Z*)-*β*-carotene (Table 1)[52].

Benzofurans
A benzofuran peak was traced at *m/z* 493 and was defined as salvianolic acid A isomer (Table 1)[61,76].

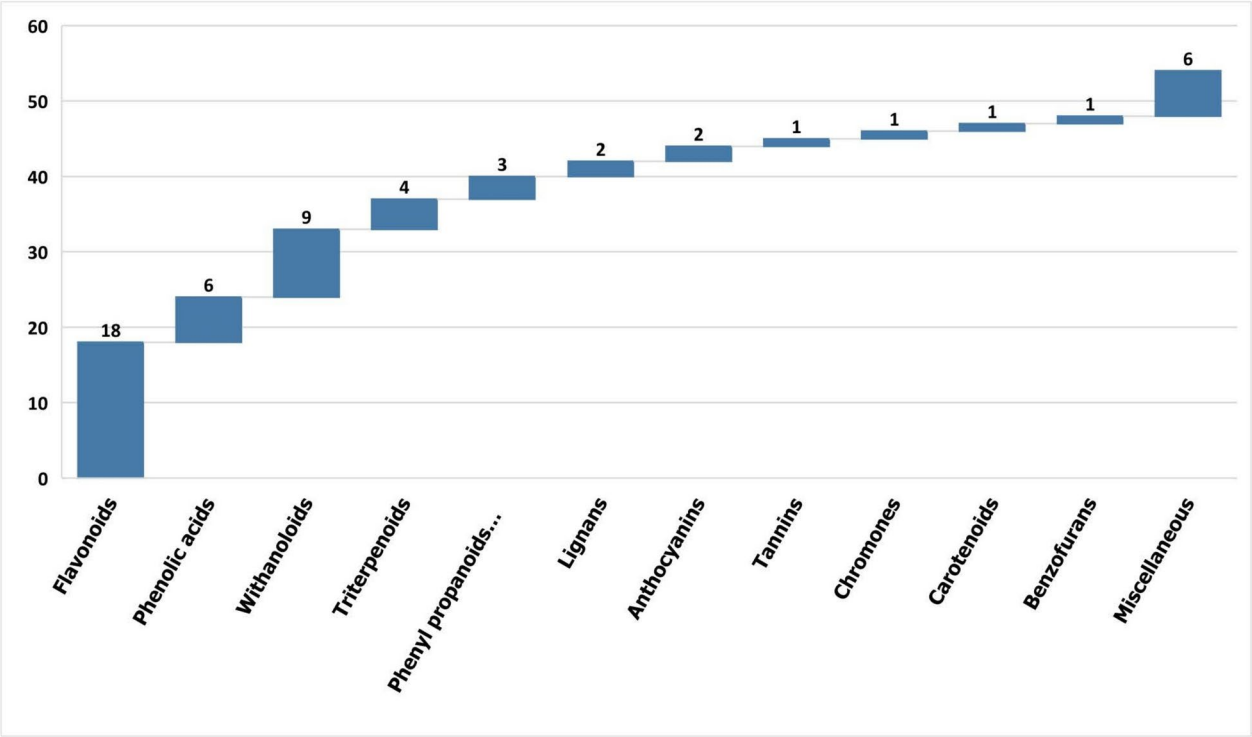

Fig. 1. Chart showing the number of compounds from each classes secondary metabolites identified from *Physalis peruviana* calyx extracts.

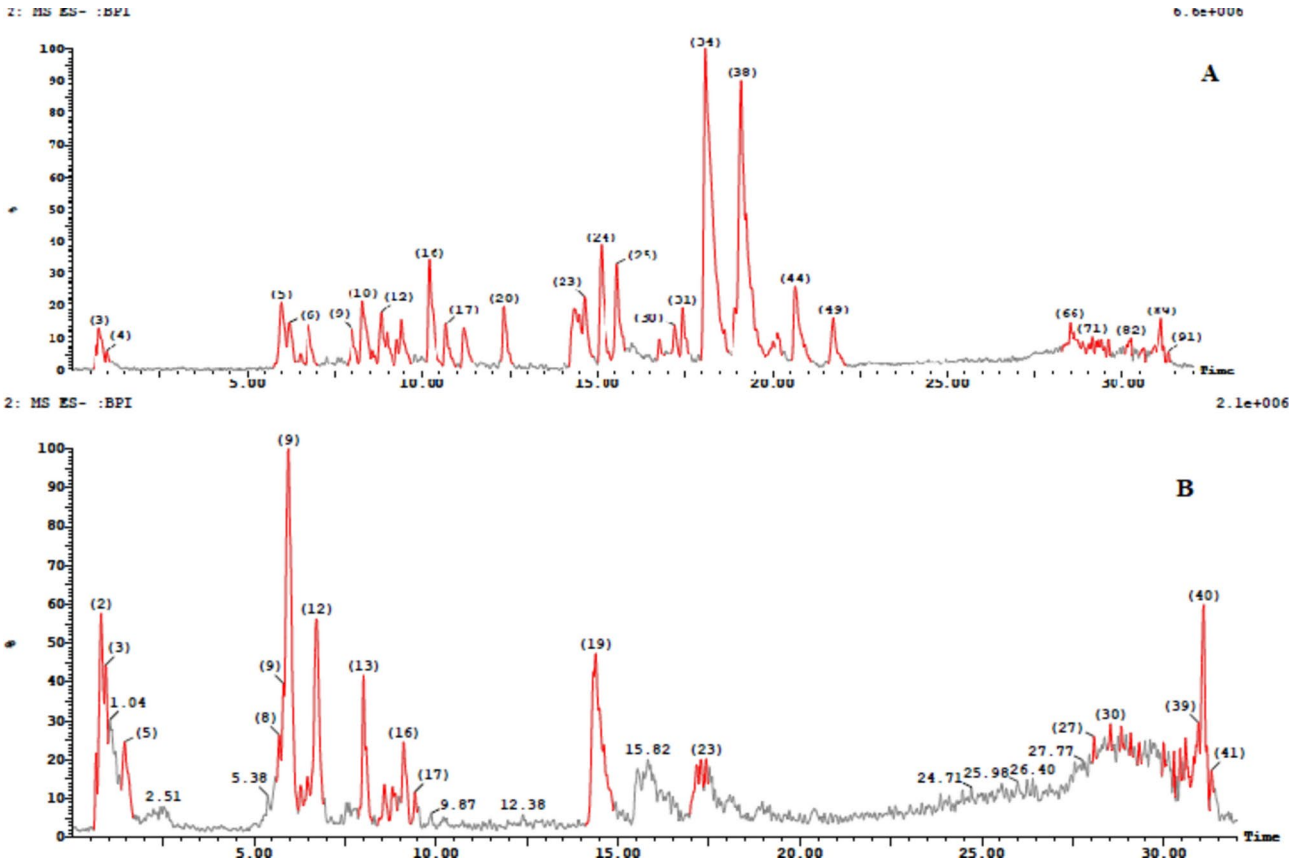

Fig. 2. UPLCMS chromatograms in −ve mode for (**A**) old methanol (POM) and (**B**) new methanol (PNM).

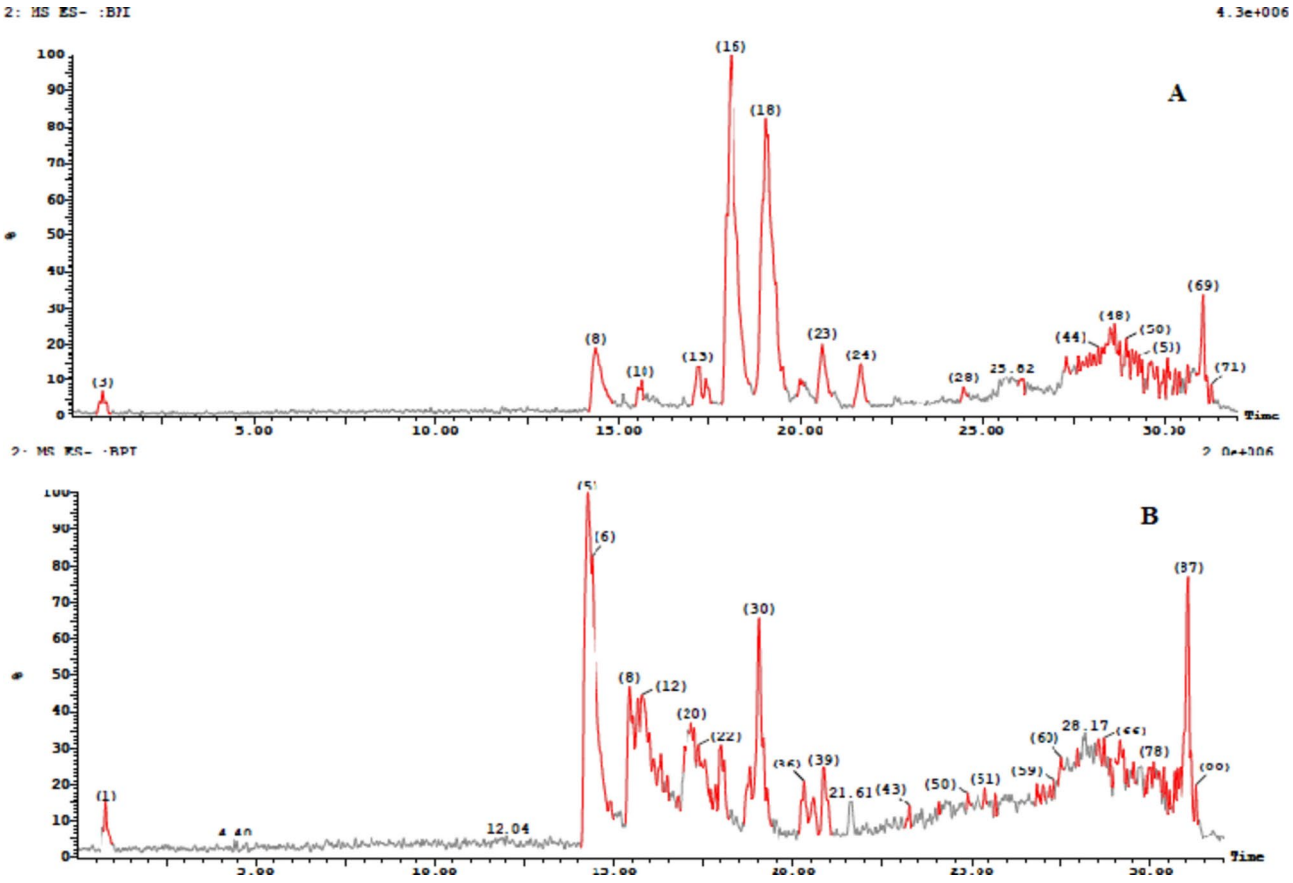

Fig. 3. UPLCMS chromatograms in −ve mode for (**A**) old petroleum ether (POP) and (**B**) new petroleum ether (PNP).

Multivariate data analysis using PCA and clustered heat map

PCA was utilized for data discrimination between the four *Physalis* samples namely; POM, PNM, POP, PNP. PCA was performed twice, one for the components identified in the negative ion mode and another for those identified in the positive ion mode in order to detect the best clustering and correlation behavior between the samples. PCA analysis for the compounds in negative ion mode gave rise to four different clusters (Fig. 4A,B) where the variance was 49% for PC1 and 33% for PC2. Three distinct clusters were located in the upper right and left quadrants for POM, PNM and PNP while POP formed a single cluster in the lower left quadrant which could be attributed to its unique chemical composition which discriminated it from the other clusters. In addition to that, PCA analysis for the compounds in positive ion mode gave rise to three distinct clusters (Fig. 5A,B) where the variance was 47% for PC1 and 31% for PC2. One cluster was located in the upper left quadrant for POM and PNM while POP and PNP formed two separate clusters in the upper right and the lower right quadrants, respectively.

Applying further multivariate analysis with the help of 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. The clustered heat map further confirmed the clustering results discussed above for PCA using area percentage as a variable (Figs. 6, 7).

Mean values of climatic conditions for the plant collection area, including temperature (°C), dew point (°C), relative humidity (RH), and rainfall (mm), were presented in Fig. 8. The climatic conditions in the plant collection area were monitored over two seasons, starting from February 10 to April 30 and August 7 to October 30 in the first and second seasons, 2022–2023 and 2023–2024. The average temperature values showed variation during the two seasons, as the average temperature during the first season was 27 °C and the second season was 25 °C, while the dew point was higher in the second season (54.6 °C) than the first (50.3 °C). The rainfall values recorded slight percentages during the two seasons but were much higher in the first season (7.2 mm) than in the second season (4.3 mm). The results showed that the relative humidity (RH%) was moderate during the first and second seasons (50.3 and 51.6 RH, respectively).

Mosquito larvicidal activity

This study evaluated *Physalis peruviana* extracts on 3rd instar larvae of *Cx. pipiens*. All the tested plant extracts in this study showed high insecticidal activity against mosquito larvae, *Cx. pipiens*, after different intervals of exposure. The mortality percent (MO%) at 24 h post-treatment (PT) of *Cx. pipiens* with 50 mg/ml methanol

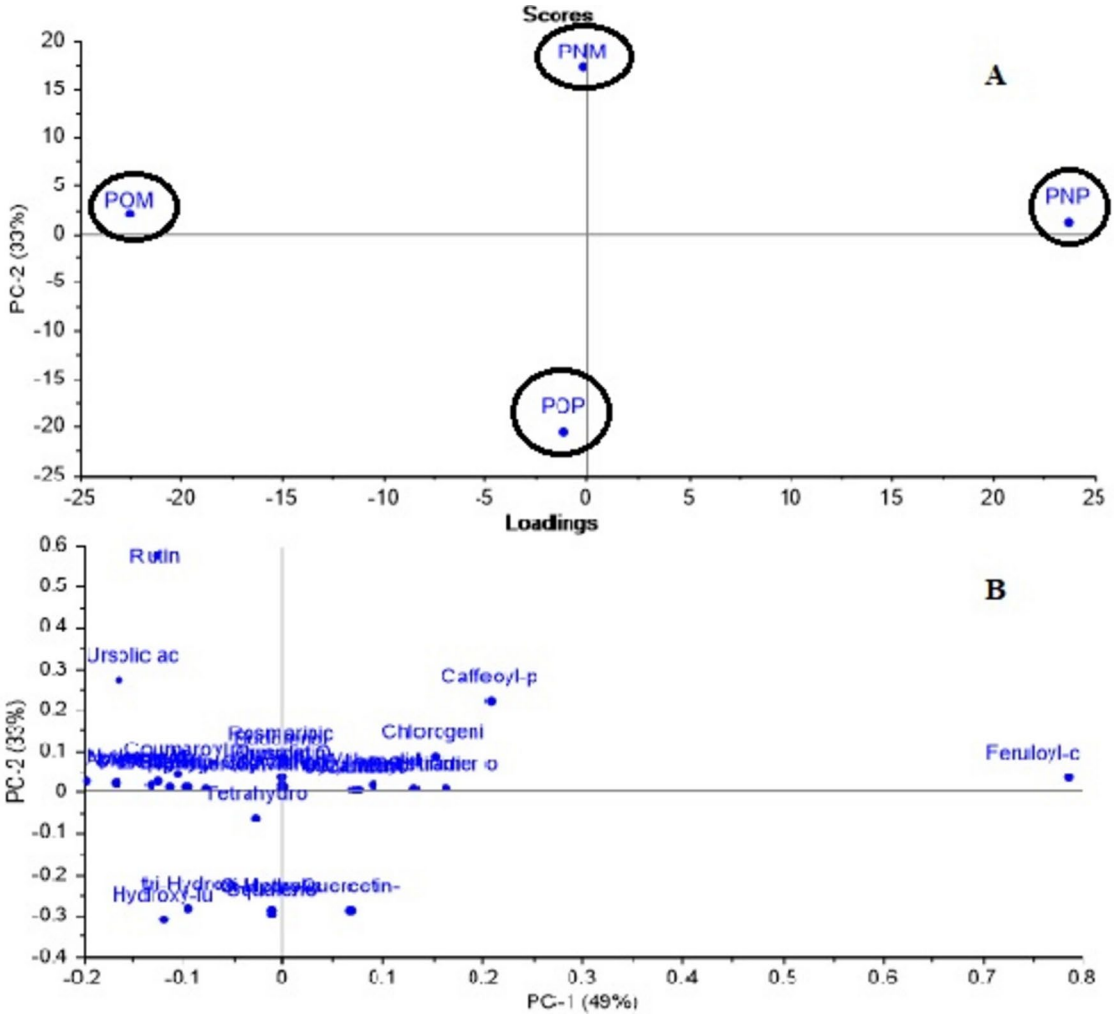

Fig. 4. (**A**) Score plot of PC1 *versus* PC2 of the identified secondary metabolites (−ve ion mode) from *P. peruviana* (area % as a variable). (**B**) Loading plot for PC1 and PC2 contributing metabolites and their assignments (area % as variable). POM; *Physalis* old season 70% methanol extract, PNM; *Physalis* new season 70% methanol extract, POP; *Physalis* old season petroleum ether extract, PNP; *Physalis* new season petroleum ether extract.

extracts of *P. peruviana* new (PNM) and old strains (POM) was 93.33 and 100% (MO%) (Table 2) with LC_{50} (50%, median lethal concentration) = 12.36 and 8.18 mg/ml, respectively (Table 3); whereas those of petroleum ether extracts new (PNP) and old (POP) were 90.67 and 96 (MO%) with LC_{50} values = 14.84 and 10.42 mg/ml. After 48 h of PT, the old strain of *P. peruviana*, either in methanol or petroleum ether extracts, had the highest larval mortality, reaching 100%, while the fresh strain, at 50 mg/ml, showed 100 and 97.33% mortality, respectively.

In terms of lethal concentrations, LC_{50} (50%, median lethal concentration) *P. peruviana* methanol old extract (POM) appeared to be most effective against *Cx. pipiens* larvae (LC_{50} = 6.63 mg/ml), followed by petroleum ether old extract (POP) (LC_{50} = 8.07 mg/ml), while *P. peruviana* methanol (PNM) and petroleum ether (PNP) new extracts appeared to be less effective against *Cx. pipiens* larvae (LC_{50} = 10.46 and 12.24 ppm), respectively (Table 3, Fig. 9a). Using the dipping technique, relative efficacy (RE) LC_{50} values were 1.8, 1.5, and 1.2 times for POM, POP, and PNM, respectively, i.e., higher than that of the *P. peruviana* petroleum ether new extract (PNP) (1.0) (Table 3).

Housefly larvicidal activity

All tested plant extracts had significantly higher mortality rates than the controls. The percentage of dead larvae in plant extract-treated contact medium at a high concentration of 60 mg/ml was 100, 96, 92, and 92% for POM, POP, PNM, and PNP, respectively, compared to 0.0% in control groups 24 h after treatment (Table 4). After 48 h of PT, the old strain of *P. peruviana*, either in methanol or petroleum ether extracts and PNM, had the highest larval mortality, reaching 100%, while the petroleum ether fresh extract mortality reached 96%.

The LC_{50} values for *P. peruviana* (POM) were 9.87 and 7.46 mg/ml, 24 and 48 PT, respectively. These values showed that POM was more toxic to house fly larvae than other of plant extracts (Table 5, Fig. 9b). According

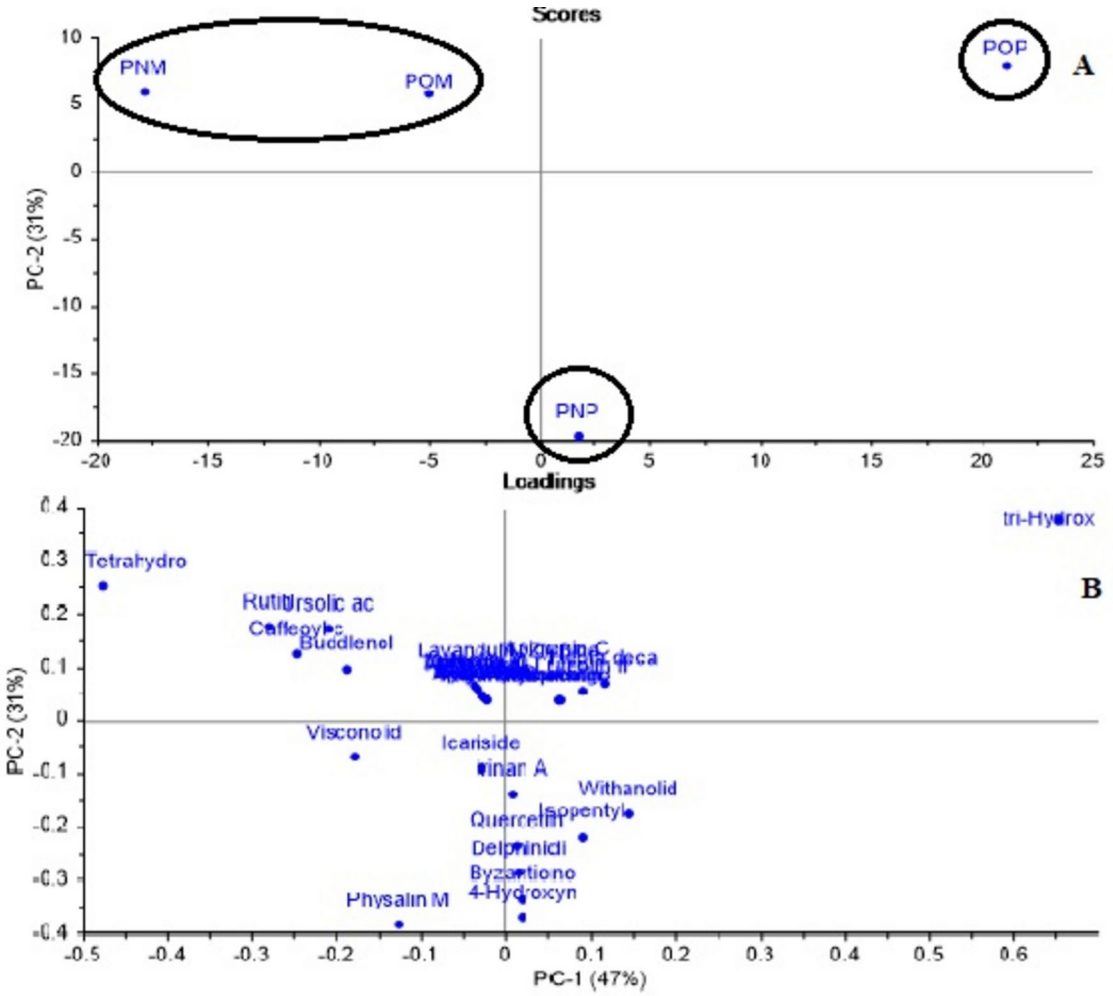

Fig. 5. (**A**) Score plot of PC1 *versus* PC2 of the identified secondary metabolites (+ve ion mode) from *Physalis peruviana* (area % as a variable). (**B**) Loading plot for PC1 and PC2 contributing metabolites and their assignments (area % as variable). POM; *Physalis* old season 70% methanol extract, PNM; *Physalis* new season 70% methanol extract, POP; *Physalis* old season petroleum ether extract, PNP; *Physalis* new season petroleum ether extract.

to LC_{50}, RE of *P. peruviana* extracts (POM, POP, and PNM) were 2.4, 3.5, 2.9, and 1.2, 1.9, and 1.5 times, respectively, i.e., higher than that of *P. peruviana* extracts (PNP) (1.00) (Table 5).

Discussion

Plant extracts have a number of significant natural components that are safe to employ in pest and disease management because they can effectively eradicate dangerous pests and then naturally decompose in the environment[77]. Just 5% of pesticides worldwide are biopesticides, despite their advantages as insecticides[78]. However, due to their special qualities that promote their application, such as their non-toxicity to the environment, biopesticides are growing quickly and are predicted to surpass chemical pesticides in the near future at an average annual growth rate of 9–20%[79].

Prior to discussing the results of our study, plants contain a wide range of phytochemicals, which are classified into primary and secondary metabolites according to their function in plant metabolism. Primary metabolites are essential for plant life and include carbohydrates, amino acids, proteins, and lipids. On the other hand, cells synthesize secondary metabolites (residual plant compounds) through metabolic pathways that originate from primary metabolism[80]. These chemicals act as antiviral, antifungal, and antibiotic agents, protecting plants from infection. Traditional medicine has used plant secondary metabolites for their biological activity in the treatment of many diseases[81,82]. They also interfere with industries, including pharmaceuticals, cosmetics, and specialty chemicals like pesticides. Agricultural waste is an integral part of plants and is no less important. Due to its richness in biologically active compounds, agricultural or agro-industrial waste serves as an alternative source for many products, including the production of biopesticides[83]. Therefore, many organizations and researchers are seeking to recycle agricultural waste to explore the valuable benefits it contains.

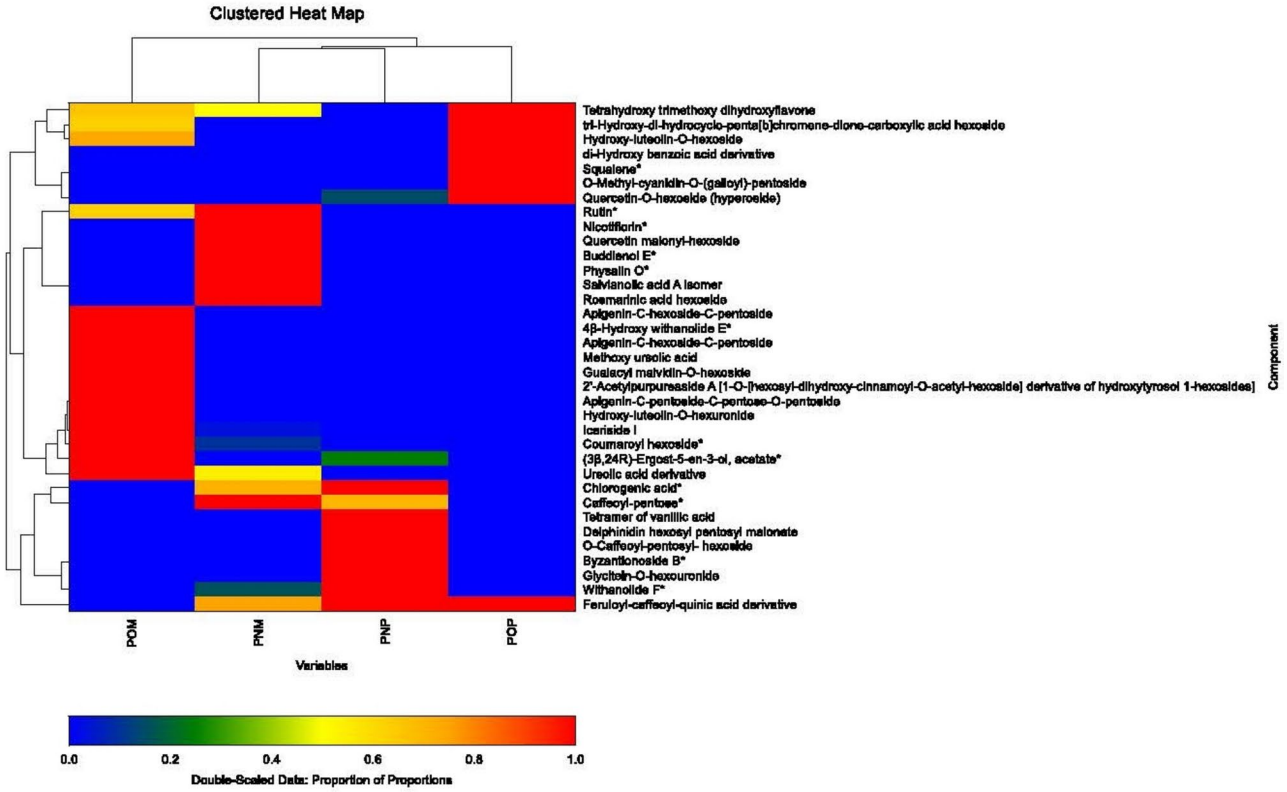

Fig. 6. Clustered heat map showing the identified components (−ve mode) from *Physalis peruviana*. A heat map was constructed using Euclidean distance and the unweighted group method. POM; *Physalis* old season 70% methanol extract, PNM; *Physalis* new season 70% methanol extract, POP; *Physalis* old season petroleum ether extract, PNP; *Physalis* new season petroleum ether extract.

In this current study, four samples from *Physalis peruviana* were prepared using calyces separated from the *Physalis* fruits through two consecutive fruiting seasons (2022–2023) and (2023–2024). Fifty-four secondary metabolites were tentatively identified through UPLC/MS analysis followed by multivariate data analysis for the resulting metabolites in order to discriminate the samples. The samples were denoted as *Physalis* old season 70% methanol extract (POM), *Physalis* new season 70% methanol (PNM), *Physalis* old season petroleum ether (POP), *Physalis* new season petroleum ether (PNP) according to their dissolving solvent and collection season.

Flavonoids, phenolic acids, withanolides and triterpenoids constituted the main classes of identified components where flavonoids and phenolic acids were mainly detected in the negative ion mode the triterpenoids and withanolides on the other hand were the main components from the positive ion mode.

The number and classes of major components identified from the two studied fruiting seasons were comparatively different, and this may be related to several factors concerning the temperature, humidity, availability of water, and nutrients, which finally lead to these changes in the plant's secondary metabolite composition, which in turn affects the biological activity resulting from each plant[84–86].

Our results showed that a slight increase in temperature and rainfall between the two seasons leads to a significant increase in the number and proportion of plant components in one season compared to the other, which further confirms the effect of interseasonal climate changes on the phytochemical composition and biological activities of the plant. Research concerning the composition of *P. peruviana* L. in relation to temperature indicates that its sugar content responds to stress[87], and exposure to low temperatures results in sugar accumulation[88], which subsequently enhances the fructan previously stored as sucrose[89]. Researchers conducted a study on *Crithmum maritimum* to investigate the impact of drought and high temperatures on its nutritional and antioxidant properties. While there were slight differences between regions, different populations of sea fennel showed high nutritional qualities suitable for consumption. Drought and high temperature caused an unexpected decrease in phenolic content, challenging the assumption that antioxidants increase in response to water scarcity[90]. Şahin, et al.[91] observed substantial alterations in withanolide content, with increases of 7.86 fold and 12.5 fold for 2-h and 5-h high-temperature exposure durations, respectively. Exposure to UV-B resulted in a 7.22-fold increase in withanolide content after 15 min and a sevenfold increase after 3 h.

Physalis peruviana L. typically necessitates ample water during its growth phase but requires reduced water during fruit maturation. It possesses high organic material content and can adequately withstand rainfall between 1000 and 2000 mm, as well as pH levels from 5.5 to 7.3. While it thrives in slightly acidic soil, it is intolerant of clay soils due to its shallow root system[92,93]. In addition to the climatic conditions, Monroy-Velandia and Coy-Barrera[94] demonstrated that the root is the first plant organ to experience an imbalance of ion stores in the

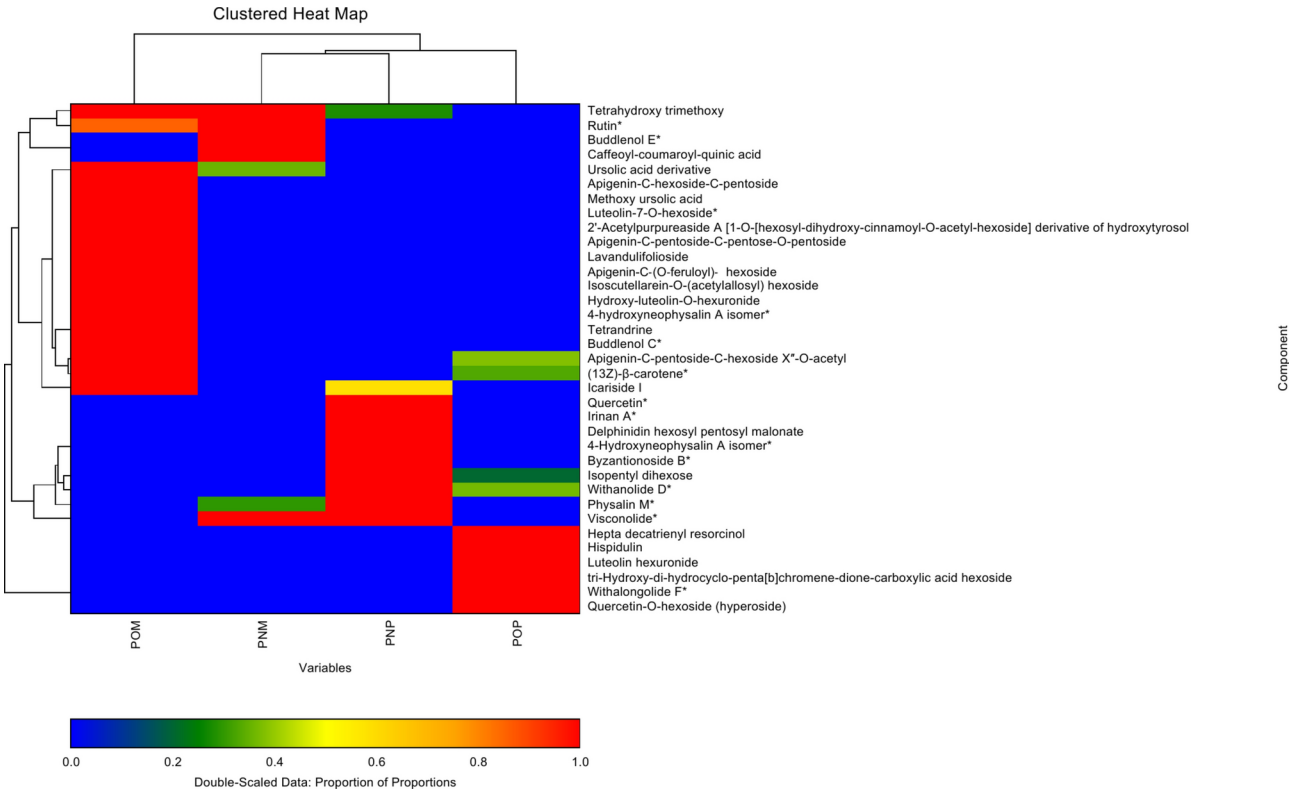

Fig. 7. Clustered heat map showing the identified components (+ve mode) from *Physalis peruviana*. A heat map was constructed using Euclidean distance and the unweighted group method. POM; *Physalis* old season 70% methanol extract, PNM; *Physalis* new season 70% methanol extract, POP; *Physalis* old season petroleum ether extract, PNP; *Physalis* new season petroleum ether extract.

substrate. Stress from osmotic pressure and ionic toxicity from salinity may slow the growth of adult plants, as shown by studies of different Physalis species[95]. These effects get worse as the salt concentration rises.

Fischer and Melgarejo[96] pointed out that unfavorable climatic conditions may affect crops and cause stress, such as extreme heat, drought, intense UV radiation, and wind; their impact can be very detrimental to the plant. No single factor independently affects plant performance functions or physiological status. Some variables, such as location, including climate, soil, and crop management, affect plant size, duration of phenological stages, and timing and size of harvest[97]. Therefore, growing a crop in a location that is not suitable for its environment and biology leads to higher production costs, lower economic performance, and lower plant nutritional value[98].

Withanolides are a group of modified C28 ergostane-type steroids with a C-22, C-26 d-lactone side chain or a C-23, C-26 c-lactone side chain. They enjoy a limited distribution in the plant kingdom and predominantly occur in several genera of the family *Solanaceae*. Of which, the genus *Physalis* is an important resource for this type of natural molecules[8]. The tentatively identified withanolides in this study viz. 4β-hydroxy withanolide E, irinan A, physalin O, visconolide, physalin M, 4-hydroxyneophysalin A isomer, withalongolide F and withanolide F together with the detected flavonoids and phenolic acids may be responsible for the reported insecticidal activities against *Culex pipiens* and *Musca domestica*. The insecticidal activity was found to be more potent for the methanol extracts which can be linked to their richness with flavonoids, phenolic acids and their derivatives together with some of the withanolides. It is worthy noted that, the old season extracts, either the methanol or the petroleum ether, showed higher activity represented by higher mortality rates compared to the old season extracts which may be attributed to the changes in phytochemical composition of the plant during each season.

Upon reviewing literature on genus *Physalis*, many other studies showed interesting phytochemical components and biological activities that were worth mentioning as detailed below.

Physalis peruviana juice helped in lowering the blood sugar levels in rats (dose: 1 ml/200 g BW/day and 5 ml/200 g BW/day of GB juice) (−79.15; −110.44; −108.20) and HOMA-IR (−2.40; −2.92; −3.02). In addition, it was also able to increase insulin level (0.26; 1.99; 1.42) ($p < 0.05$)[99]. *Physalis alkekengi* L. hydroalcoholic extraction and decoction were compared by LC-ESI/LTQOrbitrap/MS followed by LC-ESI/LTQOrbitrap/MS/MS to identify 58 phytocompounds using the two different extraction techniques. The antioxidant activity of the different *Physalis alkekengi* L. extracts were evaluated. It was found that *Physalis alkekengi* L. extracts are a good source of metabolites such as flavonoids, organic acids, phenylpropanoids, physalins and carotenoids, with various biological activities, in particular, antioxidant activity capable of reducing the production of free radicals in intestinal Caco-2 cells[52].

Physalis angulata AH-ZE1 extract was evaluated through Gas Chromatography–Mass Spectrometry (GC–MS) analysis where it showed twenty-four effective compounds that have a lot of medicinal efficacies. Antioxidant

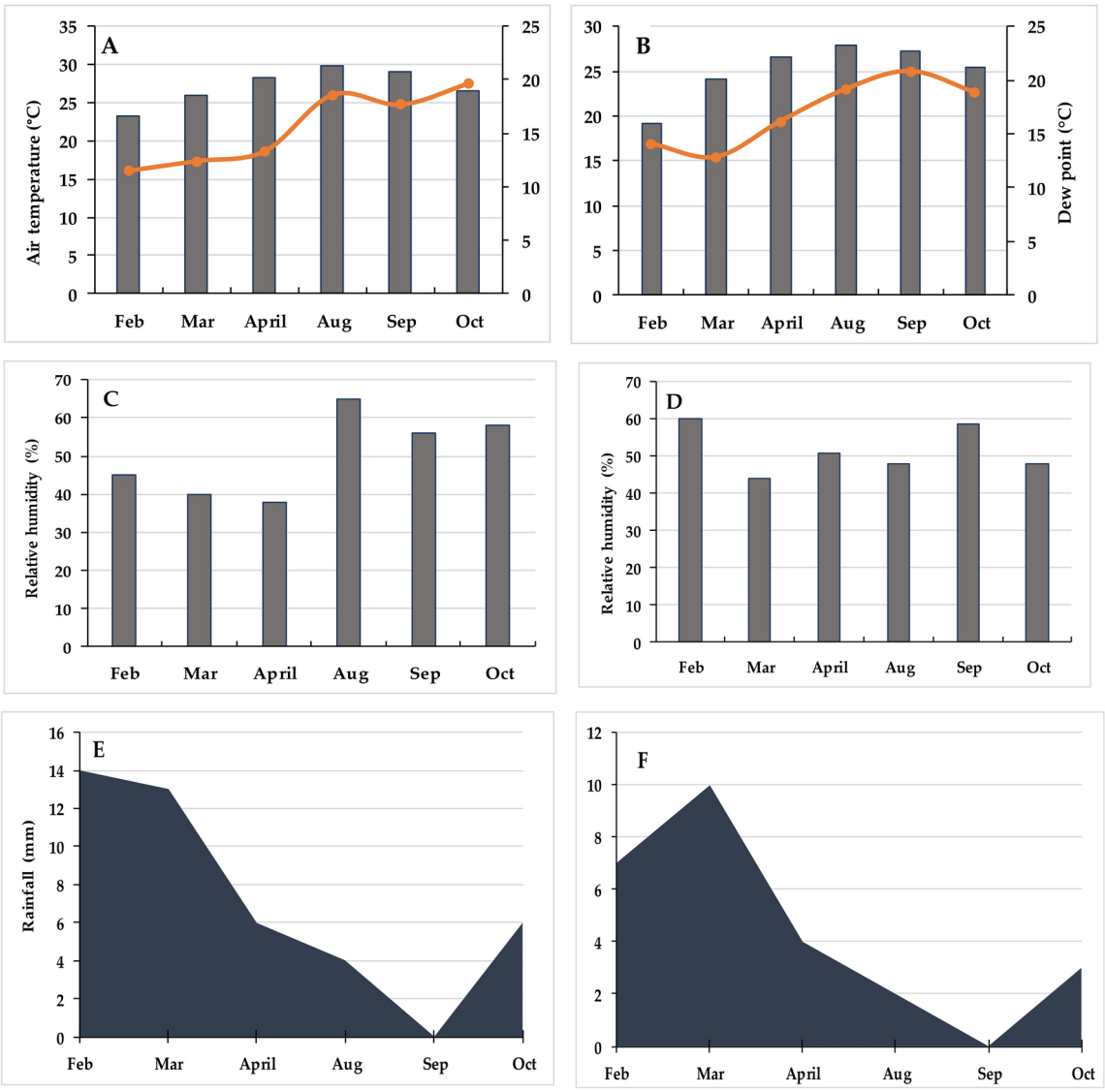

Fig. 8. Seasonal variation of monthly air temperature (**A**,**B**), relative humidity (**C**,**D**), and monthly rainfall (**E**,**F**) in the agricultural area of the *Physalis* plant.

content of 94% at the concentration of 140 μg/ml of the extract, and the lowest percentage of antioxidant 42% at the concentration of 20 µg/ml is as a result of the high percentage of phenolic compounds in the plant extract, which is directly proportional to the percentage of antioxidants[100].

Eight recognized chemicals and fourteen novel withanolides, designated withalongolides A–N, respectively, were extracted from the aerial sections of *Physalis longifolia*. Through the use of an MTS viability assay, eight withanolides (1, 2, 3, 7, 8, 15, 16, and 19) and four acetylated derivatives (1a, 1b, 2a, and 2b) demonstrated strong cytotoxicity against melanoma (B16F10 and SKMEL-28), human head and neck squamous cell carcinoma (JMAR and MDA-1986), and normal foetal fibroblast (MRC-5) cells, with IC_{50} values ranging from 0.067 to 9.3 μM[65].

Traditional uses of the pantropical *Physalis minima* include the prevention and treatment of a wide range of ailments, diseases, and malignancies. After the fruit extract was applied for 24 h, the colorectal cancer cell lines HCT116 and HT29 showed signs of programmed cell death, including cytoplasm shrinkage and nucleus condensation. Using phytochemical analysis, 71 potential metabolites were found. The association between the probable metabolites and the cytotoxicity against colorectal cancer cells shown in this study is further supported by the reports of some of these metabolites' variable ability to suppress malignancies[101].

In Egypt and several neighbouring countries, *Physalis peruviana* L. is a commonly consumed fruit. The ethyl acetate extract demonstrated preferential action towards the human pancreatic cancer cell line PANC-1, with an IC_{50} value of 5.23 ± 0.2 µg mL^{-1}. Following HR-LCMS-guided and biological activity-guided isolation, magnolin was found to be a powerful selective antiproliferative agent against PANC-1 with an IC_{50} of 0.51 ± 0.46 µM, equivalent to the positive control doxorubicin (IC_{50} of 0.17 ± 0.15 µM). Additionally, magnolin was far less

Time (h)	Conc. (mg/ml)	Mortality (%)			
		PNM	PNP	POM	POP
24	0	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}
	2.5	9.33 ± 1.33^{eC}	6.67 ± 1.33^{eD}	14.67 ± 1.33^{eA}	12.00 ± 2.31^{eB}
	5.0	22.67 ± 1.33^{dC}	18.67 ± 1.33^{dD}	32.00 ± 2.31^{dA}	25.33 ± 1.33^{dB}
	10.0	41.33 ± 3.53^{cC}	37.33 ± 3.53^{cD}	52.00 ± 2.31^{cA}	46.67 ± 3.53^{cB}
	25	66.67 ± 1.33^{bC}	58.67 ± 1.33^{bD}	84.00 ± 2.31^{bA}	73.33 ± 1.33^{bB}
	50	93.33 ± 1.33^{aC}	90.67 ± 1.33^{aD}	100.0 ± 0.00^{aA}	96.00 ± 2.31^{aB}
48	0	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}
	2.5	12.00 ± 2.31^{eC}	9.33 ± 1.33^{eD}	18.67 ± 1.33^{eA}	16.00 ± 2.31^{eB}
	5.0	25.33 ± 3.53^{dC}	21.33 ± 3.53^{dD}	34.67 ± 1.33^{dA}	32.00 ± 2.31^{dB}
	10.0	44.00 ± 4.00^{cC}	41.33 ± 3.53^{cD}	61.33 ± 1.33^{cA}	50.67 ± 1.33^{cB}
	25	70.67 ± 3.53^{bC}	64.00 ± 2.31^{bD}	94.67 ± 1.33^{bA}	85.33 ± 2.67^{bB}
	50	100.0 ± 0.00^{aA}	97.33 ± 2.67^{aD}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}

Table 2. Efficacy of *Physalis peruviana* extracts on *Culex pipiens* larval mortality, 24 and 48 h post-treatment. a, b & c: There is no significant difference ($P > 0.05$) between any two means for each time, within the same column have the same superscript letter. A & B: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter. PNM: *Physalis* new season 70% methanol extract; PNP: *Physalis* new season petroleum ether extract; POM: *Physalis* old season 70% methanol extract; POP: *Physalis* old season petroleum ether extract.

Time (h)	Plant extracts	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	RE	Slope ± SE	X^2 (sig.)
24	PNM	12.36	54.04	82.10	1.2	2.000 ± 0.155	4.408
		(10.67–14.37)	(42.22–74.45)	(61.18–120.95)			(0.220)
	PNP	14.84	65.67	100.10	1.0	1.984 ± 0.155	5.936
		(12.79–17.36)	(50.61–92.31)	(73.47–150.82)			(0.114)
	POM	8.18	29.67	42.75	1.8	2.290 ± 0.172	6.343
		(7.13–9.35)	(24.27–38.29)	(33.66–58.26)			(0.096)
	POP	10.42	43.61	65.43	1.4	2.061 ± 0.158	4.234
		(9.02–12.05)	(34.63–58.67)	(49.70–93.79)			(0.237)
48	PNM	10.46	41.02	60.44	1.2	2.158 ± 0.162	13.96
		(5.94–18.48)	(32.77–139.03)	(51.07–256.50)			(0.002)
	PNP	12.24	49.70	73.93	1.0	2.106 ± 0.159	12.202
		(7.36–21.26)	(38.72–158.38)	(59.63–290.97)			(0.006)
	POM	6.63	21.14	29.37	1.8	2.543 ± 0.197	5.528
		(5.81–7.51)	(17.61–26.66)	(23.64–38.95)			(0.137)
	POP	8.07	29.45	42.51	1.5	2.279 ± 0.172	7.450
		(7.03–9.23)	(24.08–38.04)	(33.44–58.02)			(0.058)

Table 3. Lethal concentrations (ppm) of *Physalis peruviana* extracts on *Culex pipiens* larval mortality, 24 and 48 h post-treatment. PNM: *Physalis* new season 70% methanol extract; PNP: *Physalis* new season petroleum ether extract; POM: *Physalis* old season 70% methanol extract; POP: *Physalis* old season petroleum ether extract.

harmful to normal human cells (dermal fibroblasts; HDFa) at 5 μg mL^{-1} than the positive control doxorubicin (6.96% and 30.48% growth inhibition, respectively).

Additionally, magnolin was able to suppress the formation of PANC-1 colonies in a concentration-dependent manner. This means that when the tumour cells were treated with 25 nM, 50 nM, and 100 nM concentrations of the compound, the number of PANC-1 colonies formed was reduced by 36%, 57, and 78%, respectively. Furthermore, magnolin was found to have a significant anti-migratory effect on the PANC-1 cell line by limiting the migration of PANC-1 tumour cells in the tumour cell wound healing assay. The molecular target that mediates these reported effects on PANC-1 cells may be matrix metalloproteinase-3 (MMP3), according to a later in silico-based analysis of this compound structure. Based on 100 ns long molecular dynamics simulation (MDS) tests and absolute binding free energy calculation (ΔGbinding), the magnolin structure may accomplish significantly stable binding inside the MMP3 active site and has strong affinity for it. Consequently, magnolin

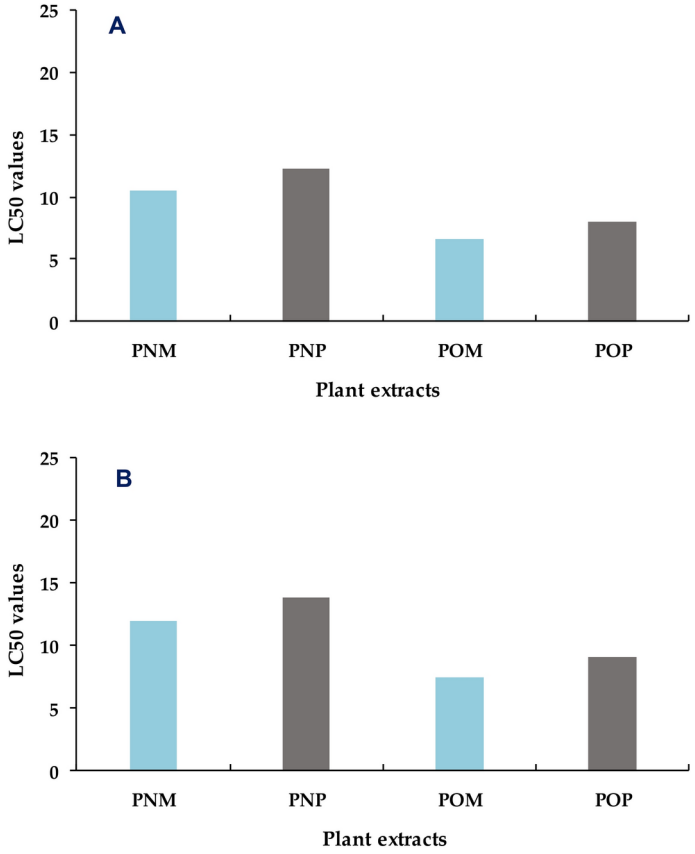

Fig. 9. The mean number of larval mortalities induced by the effects of *Physalis peruviana* extracts against *Culex pipiens* (**a**) and *Musca domestica* (**b**), 48 h post-exposure.

Time (h)	Conc. (mg/ml)	Mortality (%)			
		PNM	PNP	POM	POP
24	0	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}
	2.5	4.00 ± 0.00^{eC}	2.67 ± 1.33^{eD}	9.33 ± 1.33^{eA}	6.67 ± 1.33^{eB}
	5.0	13.33 ± 1.33^{dC}	12.00 ± 0.00^{dC}	22.67 ± 1.33^{dA}	18.67 ± 1.33^{dB}
	10.0	34.67 ± 1.33^{cC}	30.67 ± 1.33^{cD}	44.00 ± 2.31^{cA}	40.00 ± 2.31^{cB}
	25	68.00 ± 2.31^{bC}	58.67 ± 1.33^{bD}	82.67 ± 2.67^{bA}	76.00 ± 2.31^{bB}
	50	92.00 ± 2.31^{aC}	88.00 ± 2.31^{aD}	100 ± 0.00^{aA}	96.00 ± 0.00^{aB}
48	0	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}	00.0 ± 0.0^{fA}
	2.5	8.00 ± 2.31^{eC}	5.33 ± 2.67eD	14.67 ± 1.33^{eA}	12.00 ± 2.31^{eB}
	5.0	20.00 ± 2.31^{dC}	17.33 ± 3.53^{dD}	29.33 ± 1.33^{dA}	26.67 ± 1.33^{dB}
	10.0	41.33 ± 3.53^{cC}	34.67 ± 3.53cD	58.67 ± 1.33^{cA}	48.00 ± 2.31^{cB}
	25	76.00 ± 2.31^{bC}	64.00 ± 2.31^{bD}	92.00 ± 2.31^{bA}	82.67 ± 2.67^{bB}
	50	100 ± 0.00^{aA}	96.00 ± 2.31^{aB}	100 ± 0.00^{aA}	100 ± 0.00^{aA}

Table 4. Efficacy of *Physalis peruviana* extracts on housefly larval mortality, 24 and 48 h post-treatment. a, b & c: There is no significant difference ($P > 0.05$) between any two means for each time, within the same column have the same superscript letter. A & B: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter. PNM: *Physalis* new season 70% methanol extract; PNP: *Physalis* new season petroleum ether extract; POM: *Physalis* old season 70% methanol extract; POP: *Physalis* old season petroleum ether extract.

was found to inhibit MMP3's catalytic activity in a dose-dependent manner following experimental validation, with a nanomolar IC_{50} value of 185 nm ± 4.86 and a Ki of 112 nm ± 6.31[49].

Additionally, a study was planned to determine the variety of secondary metabolites present in *P. angulata* (ciplukan) plant tissue and callus in vitro. Using LC–MS analysis, the withanolide and physalin profiles of

Time (h)	Plant extracts	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	RE	Slope ± SE	X^2 (sig.)
24	PNM	14.59	50.39	71.60	2.4	2.381 ± 0.174	1.090
		(12.80–16.71)	(40.86–65.76)	(55.96–98.38)			(0.779)
	PNP	34.48	64.00	92.67	1.0	2.260 ± 0.171	2.088
		(15.12–20.06)	(50.74–86.37)	(70.51–132.51)			(0.554)
	POM	9.87	31.30	43.41	**3.5**	2.557 ± 0.183	7.152
		(8.71–11.19)	(26.01–39.47)	(34.92–57.30)			(0.067)
	POP	11.82	40.26	56.98	**2.9**	2.408 ± 0.171	2.122
		(10.39–13.47)	(33.06–51.67)	(45.18–76.62)			(0.547)
48	PNM	11.98	46.83	68.93	1.2	2.164 ± 0.174	0.460
		(10.38–13.90)	(36.82–64.18)	(51.81–100.77)			(0.927)
	PNP	13.88	49.78	71.50	1.0	2.310 ± 0.169	2.310
		(9.58–20.85)	(36.87–109.13)	(52.42–179.81)			(8.194)
	POM	7.46	23.10	31.83	**1.9**	2.609 ± 0.195	4.310
		(6.57–8.44)	(11.80–28.99)	(25.74–41.87)			(0.229)
	POP	9.09	31.05	43.98	**1.5**	2.402 ± 0.176	7.024
		(7.97–10.35)	(25.57–39.67)	(34.96–59.08)			(0.711)

Table 5. Lethal concentrations (ppm) of *Physalis peruviana* extracts on housefly larval mortality, 24 and 48 h post-treatment. PNM: *Physalis* new season 70% methanol extract; PNP: *Physalis* new season petroleum ether extract; POM: *Physalis* old season 70% methanol extract; POP: *Physalis* old season petroleum ether extract.

callus tissues, plantlets produced from cotyledonary shoots, and plants derived from germinated seeds were assessed. The variety in the quantity and kind of withanolide and physalins was revealed by the LC–MS analysis of the methanol extract. This work verified that the biosynthetic activity was not changed and the withanolide accumulation sites were not absent in undifferentiated callus cultures and in vitro produced *P. angulata* plantlets. On the other hand, more withanolides and physalins were created by the in vitro regenerated plant. Thus, it is a promising future prospect to modify the plant cell culture system to boost withanolide synthesis, including *P. angulata*'s production of physalin[50].

Using a quadrupole time-of-flight analyser (UPLC-ESI-QTOF MS) on extracts (n = 10) made under pressurised liquid extraction (PLE) conditions, the profile of compounds presented in the fruits and husks of *Physalis peruviana* from Costa Rica was ascertained through ultra-performance liquid chromatography combined with high-resolution mass spectrometry. Nine flavonoids, twenty-three sucrose ester derivatives, and thirty-four withanolides were among the sixty-six distinct chemicals that were found. Fruits were tested for β-carotene and flavonoids in all 10 samples using UPLC-DAD analysis; the results showed that samples from the Dota region had greater levels (58.6–60.1 µg/g of dry material versus 1.6–2.8 mg/g of dry material). Husk extracts from the Dota region had the highest values (4.3–5.1 mg GAE/g of dry material against IC_{50} = 1.6–2.3 mg of dry material/mL) for the Folin–Ciocalteau total polyphenolic content (FC) and antioxidant activity measured by the DPPH method. Furthermore, a noteworthy inverse relationship was discovered among the RU, FC, and DPPH values ($r = -0.902$, $p < 0.05$), which is consistent with earlier findings regarding the function of polyphenols in antioxidant activity. The HRMS results were subjected to principal correlation analysis (PCoA) and hierarchical clustering (HC) analysis. The results showed that the D1 and D2 fruit samples from the Dota region were grouped with husks associated with a higher presence of the metabolites under study. Principal component analysis (PCA) was then used to determine the flavonoid content and antioxidant activity. The results showed that the fruit samples and D1 and D2 husks from the Dota region had the maximum antioxidant activity and stood out significantly[102].

In this work, every *P. peruviana* extract tested had a notable insecticidal effect on house fly and mosquito larvae. The plant extracts, particularly the older strain, had lower toxicity than the new strain. Additionally, it was evident that *P. peruviana*'s methanolic extract poisoned mosquito and house fly larvae since, within 24 h, every single larval treated with it died (100 MO%). The investigation showed that the larval mortality rate rose with time, concentration, and solvent type in both species. Many studies have demonstrated that acetone, methanol, or plant extracts were superior to other solvents in terms of their ability to effectively kill mosquito or fly larvae[103]. This is consistent with the findings of Bosly[104] which seems to order the highly hazardous leaf extracts according to their toxicity to mosquito larvae: acetone, methanol, aqueous, and hexane. It was proposed that the plant extracts' ability to repel particular kinds of mosquitoes could be influenced by the solvent employed to extract the phytochemicals responsible for the reactions.

Physalis angulata L.'s leaves and fruits were used in a similar study on mosquito larvae to examine the larvicidal effects of the extracts on Anopheles mosquito larvae. Fruit extracts at the same concentrations produced 38%, 47%, 72%, and 83% mortality, but leaf extracts at concentrations of 5%, 10%, 15%, and 20% caused 61%, 80%, 91%, and 92% mortality. At the same dosages and timeframes, a mixture of fruit and leaf extracts produced greater mortality rates of 67%, 84%, 91%, and 95% and had synergistic effects. Additionally, the findings demonstrated that concentration-dependent increases in the larvicidal activities of fruit, leaf, and

synergistic extracts[38]. When Cirigliano, et al.[105] studied the effects of *P. peruviana* crude extracts and its main withanolides (withanolide E and 4-*ß*-hydroxywithanolide E) on fruit fly larvae and adults, they found that they had biological effects. While modest doses (1000 ppm) significantly affected larval mortality, development delay, and puparia length, high concentrations of crude extracts (10,000 and 35,000 ppm) in the larval diet resulted in 100% death.

The *Solanaceae* family includes more than 2500 species of plants, many of which are indigenous to North America[106]. This family contains powerful poisons like mandrake, henbane, and deadly nightshade in addition to important crops like potatoes, tomatoes, and aubergines[107]. Most Solanaceae species are toxic to cattle and people alike. As we referred to *Physalis* (*Physalis pubescens* L.) is an important genus of the Solanaceae family.

Some studies were carried on Solanaceae plants as in work of Benhissen, et al.[107] who conducted studies on Solanaceae plants, using aqueous extracts of three plants: *Hyoscyamus albus, Solanum elaeagnifolium,* and *Solanum nigrum* to determine their effect on the death of *Culiseta longiareolata* larvae in their fourth growth stage. He demonstrated that the *S. nigrum* extract was the most effective, followed by the *H. albus* and *S. elaeagnifolium* extracts, which are *C. longiareolata* larvae, respectively.

The larvicidal activities of crude and solvent extracts of *Solanum nigrum* L. leaves were carried against *Culex quinquefasciatus*. After 24, 48, and 72 h of exposure, the results showed that the 0.5% concentration had the highest death rates of all the crude extracts tested on all stages of larvae[108]. Comparative efficacy of the aqueous and hexane extracts of dried fruit of *Solanum nigrum* was tested against five laboratory colonized strains of mosquito species, namely *Anopheles culicifacies* species A, *An. culicifacies* species C, *An. stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*, to assess the possibility for use of these extracts for their control. Also, in bioassays with larvae, all five species died completely at 1000 ppm with aqueous extract and at 100 ppm with hexane extract of dried fruit[109].

The fruit fly *Ceratitis capitata* was used to study the biological effects of *P. peruviana* crude extracts and its main withanolides, withanolide E and 4-*ß*-hydroxywithanolide E, on larvae and adults. There were significant changes in larval mortality, development delay, and puparia length when crude extracts at low concentrations (1000 ppm) were fed to larvae; 100% of the larvae died at high doses (10,000 and 35,000 ppm). On larvae, withanolide E and 4-*ß*-hydroxywithanolide E (500 ppm) similarly significantly reduced mortality. When crude extracts were added to adult drinking vessels, the effects were significantly fatal at 10,000 and 30,000 ppm[105].

The larvicidal effects of *P. angulata* L. leaves and fruits on *Anopheles* mosquito larvae were studied using ethanol extracts. Within 30 min of coming into touch with the plant extracts, larval fatalities were noted. Fruit extracts at the same concentrations caused 38%, 47%, 72%, and 83% of deaths, but leaf extracts at 5%, 10%, 15%, and 20% caused 61%, 80%, 91%, and 92% of deaths[38].

Research has proven the effectiveness of many plant extracts and essential oils in killing or repelling various medical, veterinary, and agricultural pests[110,111]. Therefore, numerous researchers and scientific bodies are keenly interested in conducting further research on these highly toxic and effective chemicals. The use of plant wastes as natural pesticides is gaining more attention and, along with its various industrial applications, is increasingly popular.

Many scientists are currently working on developing new tools to control insect populations, such as secondary plant metabolites like alkaloids, glycoalkaloids, terpenoids, organic acids, and alcohols, which show promise for use in plant protection. These chemicals can influence all levels of an insect's biology, but they usually mess up cellular and physiological processes. For example, they can change redox balance, hormonal regulation, neuronal signaling, or the ability of exposed individuals to reproduce. Repellence is the most important effect of secondary plant metabolites, which cause toxic effects at both lethal and sublethal levels. Plants from the Solanaceae family, which contains numerous economically and ecologically important species, produce a variety of substances that affect insects belonging to most orders, particularly herbivorous insects, and other pests. Furthermore, α-Chaconine, α-solanine, and various Solanum sp. extracts have been shown to be toxic to leaf-eating insects, pests of stored products (e.g., seeds, flour), mosquitoes that feed on animal tissues, termites or flies and cockroaches that feed on feces and garbage, and predatory species. Recently, we reported that dietary α-solanine treatments influence the biological fitness of wax moth, *Galleria mellonella* (L.), larvae[112,113].

Conclusion

The phytochemical profiling of four extracts of different polarities from *Physalis peruviana* calyces collected in two consecutive seasons was evaluated. Fifty-four secondary metabolites were qualitatively and quantitatively identified. The resulting phytochemical profiles were subjected to PCA and clustered heatmap, where the data was differentiated and discriminated into four clusters in the negative ion mode and three in the positive ion mode. The clustered heat maps produced for the two UPLC/MS analysis modes further confirmed and visually illustrated the collected data. The results revealed that the previous season's *P. peruviana* methanol extract (POM) and the petroleum ether extract (POP) were found to be the most effective in killing *Cx. pipiens* and *M. domestica* larvae, while the newer extracts (PNM and PNP) were less successful in killing larvae. In conclusion, this study had discussed, for the first time, the potential use of an important fruit waste (calyx) for biocontrol against mosquito and house fly larvae, thus paving the way for the new and effective development of green insecticides with less harmful effects on the environment. This indicates that plants adapt to the surrounding natural conditions by producing secondary metabolites from one season to another according to their vital and defensive requirements and environmental stresses. More future research is encouraged in order to isolate the main components responsible for the insecticidal properties of this fruit's calyx.

Data availability

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

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Conceptualization, E.A.E., M.M.B., H.S.G., M.H.A., R.M.M., A.M.S. and M.E.G.; methodology, E.A.E., M.M.B., R.M.M., and M.E.G.; validation, E.A.E., M.M.B., A.M.S. and M.E.G.; formal analysis, E.A.E., M.M.B., H.S.G., M.H.A., R.M.M., A.M.S. and M.E.G.; investigation, E.A.E., M.M.B., R.M.M., A.M.S. and M.E.G.; resources, E.A.E., M.M.B., and A.M.S.; data curation, E.A.E., M.M.B., A.M.S. and M.E.G.; writing—original draft preparation, E.A.E., H.S.G., M.H.A., M.M.B., and M.E.G.; writing—review and editing, E.A.E., M.M.B., H.S.G., M.H.A., R.M.M., A.M.S. and M.E.G.; supervision, E.A.E., M.M.B., A.M.S. and M.E.G. and; All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical statement

The protocol of work was approved by the Ethics Committee of the Faculty of Science, Benha University (Code: BUFS-REC-2024-271 Ent). The study was conducted in accordance with the local legislation and institutional requirements.

Additional information

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