El-Alfy *et al. Applied Biological Chemistry* (2024) 67:85
https://doi.org/10.1186/s13765-024-00916-4

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

Phytochemical composition and green insecticides from *Citrus aurantifolia* fruit peels against whitefly, *Bemisia tabaci*

Mariam S. El-Alfy[1*], Mohamed E. Mostafa[2], Abelaziz M. Dawidar[1] and Mamdouh Abdel-Mogib[1]

Abstract
Insecticidal potential of extracts of *Citrus aurantifolia*, family Rutaceae, was evaluated to control whiteflies, *Bemisia tabaci*. Biocidal activity directed chromatographic separation of chloroform and butanol fractions, with spectral identification (1D-NMR, 2D-NMR, ESIMS) of the active fractions have been resulted in separation and structural elucidation of for previously described coumarins (bergapten **1**, limettin **2**, isopimpinellin **3**, oxypeucedanin hydrate **4**) in addition to a new dimeric coumarin (12R, 12'R)-aurantifolin **5**, two known limonoids; 21,23-dihydro-23-methoxy-21-oxolimonin **6**, 21,23-dihydro-23-methoxy-21-oxonomilin **7**, and two known flavonoid glycosides; scoparin **8**, and narcissin **9**. Amongst these compounds, narcissin **9** was the most effective after 24 h. of treatment while, (12R, 12'R)-aurantifolin **5** was the most potent against *B. tabaci*, 3rd instar nymphs after 72 h. of treatment and under laboratory conditions, with LC_{50} values of 33.31and 15.92 ppm, respectively comparing with the positive control azadirachtin.

Keywords *Citrus aurantifolia*, Phytochemical investigation, Insecticidal activity, *Bemisia tabaci*. Coumarins, Limonoids, Flavonoid glycosides

Introduction

Whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of the most important cotton sucking pests. This sucking pest causes direct and indirect losses in productivity by sucking sap from plants and transferring several viruses. It produces honeydew on their leaves, which promotes the formation of sooty mould and lowers the nutritional value as well as the harvested crops' viability for the market. Honeydew dropping on open bolls, the lint becomes sticky, that causes difficulties while ginning [1].

Doubtlessly insecticides are frequently used to manage and regulate pests in quality crop preservation and can play a significant role in guaranteeing food security and agricultural productivity [2]. The excessive usage of several synthetic insecticides causes significant threats to others such as humans, domestic birds, essential terrestrial insects, animals, wild and aquatic life and the ecosystem as a whole [3, 4].

The secondary metabolites can serve as a viable substitute for synthetic pesticides because of their easy biodegradability, minimal residuals, and low negative impacts to other non-target organisms, and mammals [5].

Citrus aurantifolia Christm. Swingle (Rutaceae) from the genus *Citrus* that consists of over 160 genera and 1700 species [6]. *C. aurantifolia* is well-known as acid

*Correspondence:
Mariam S. El-Alfy
mariamsamir@mans.edu.eg
[1]Chemistry Department, Faculty of Science, Mansoura University, Mansoura 35516, Egypt
[2]Plant Protection Research Institute, Agriculture Research Center, Giza 12618, Egypt

lime, Key lime [7], and especially is called Banzhair lime in Egypt [8]. It has spread all over the world, from Southeast Asia to Brazil [9] and can thrive widely in hot tropical and subtropical areas [10]. This plant grow with smooth brown-to-gray barks and with numerous branches and irregular thorns and reach to a height of 3–6 m [9, 11].

A number of studies have discovered that *C. aurantifolia* has biological activities include insecticide [12], anticancer, antidiabetic [13], antioxidant, antimicrobial [14, 15], anti-inflammation and analgesic effects [16], besides, anti-hypertensive, antibacterial, antifungal [17], in addition to anthelminthic, anti-obesity [18, 19], and hepatoprotective properties [20]. Moreover, it can prevent urinary infections and protect bone, liver and heart diseases [17]. Also, it is beneficial in the treatment of Alzheimer's disease and colds, flu-like symptoms, with potential virucidal activity against HIV [21–23]. The previous secondary metabolites studies of *C. aurantifolia* exposed the presence of Alkaloids, coumarins [24], carotenoids, flavonoids, triterpenoids, essential oils [17], phenolic acids, and limonoids [25]. Also, steroids, tannins, saponins, cardiac glycosides were screened in this species [26].

Many literatures indicated that the chemical composition of compounds found in any plants can be influenced by various factors, such as the environment in which the plant is grown [27, 28], year of harvest [29], cultivar [30], and geographical area of cultivation [27, 31, 32].

Due to its variously distinct biological properties and chemical profiling, *C. aurantifolia* is possibly considered a miracle fruit and its peel extract could be a remarkable alternative for synthetic insecticides to reduce the risks associated with its application thereof [33].

Finding out new leads from *C. aurantifolia* fruit-peels' that could be utilized as natural insecticides against *Bemisia tabaci* (Genn.) was the main goal of the presented study, as well as conducting an in-depth phytochemical analysis to figure out the major active principles using spectral and chromatographic techniques.

Experimental

Instruments

$[\alpha]_D$ was measured on WXG-4 polarimeter. Ati-Unicam-UV/Visible Vision was employed for measuring UV spectra. NMR spectra were recorded on 500 MHz JEOL in CD_3OD or $CDCl_3$. Chemical shifts were represented in δ (ppm) considering the residual solvent peak as internal standard substance at Mansoura University's Faculty of Science. ESI mass spectra were obtained using an UPLC MS-MS "H_2O" 3100 "USA" with TQ detector and Bruker micro OTOF.

Chemicals

F_{254} (230–400 mesh) silica gel or polyamide 6 were used in performing columns chromatography (CC). Thin layer chromatography and preparative TLC were carried out on 0.25 mm thickness silica gel (Kieselgel 60, GF 254). Hexane, chloroform ($CHCl_3$), methylene chloride (CH_2Cl_2), butanol, ethyl acetate (EtOAc), methanol (MeOH) and anhydrous sodium sulphate were acquired from Loba Company, India.

Plant material

Citrus aurantifolia was collected from Mansoura University, faculty of agriculture Garden, Egypt in September 2021. Identification of the plant was made by Dr. Mahmoud Makram, Associate Professor, Ornamental Department, Faculty of Agriculture, Mansoura University.

Extraction and isolation

The fresh peels material (5.82 kg) was cut into small pieces and extracted by dist. hot water (1×15 L) for 15 min. The water extract was filtered, and the Marc was re-extracted again by dist. hot water (1×15 L) for 15 min. Filtration was performed and the filtrate was partitioned successively *via* separating funnel with chloroform and butanol to furnish chloroform (2.28 g) and butanol (11.36 g) fractions.

Chloroform fraction (2.28 g) was exposed to CC over silica gel and eluted using hexane: EtOAc and CH_2Cl_2: MeOH of raising polarity. Two fractions **I** and **II** were obtained, fraction **I** was also exposed on silica gel PTLC eluted by $CHCl_3$/ hexane (4: 1) to give compound **1** (30 mg, R*f* 0.68), **2** (40 mg, R*f* 0.55), and **3** (34 mg, R*f* 0.42), while fraction **II** was also further purified on silica gel PTLC eluted by $CHCl_3$/ MeOH (97: 3) to give compound **4** (30 mg, R*f* 0.24), **5** (33 mg, R*f* 0.31), **6** (37 mg, R*f* 0.44) and **7** (35 mg, R*f* 0.60).

Butanol fraction (11.36 g) was subjected to polyamide-S6 column chromatography and eluted using mixture of distilled H_2O/ dist.H_2O: MeOH / MeOH / MeOH: Acetone/ Acetone/ Acetone: Ammonia/ Ammonia solvent system. Four fractions were obtained. Fraction I was further chromatographed on silica gel PTLC eluted by EMW (EtOAc/ MeOH/ H_2O) (41: 6: 3) to give compound **8** (42 mg, R*f* 0.45), and **9** (32 mg, R*f* 0.61).

Bergapten 1 White crystals, ^{1}H NMR (400 MHz, $CDCl_3$), δ value in ppm, (J value in Hz): 8.16 (1H, d, 9.8, H-4), 7.59 (1H, d, 2.4, H-2′), 7.14 (1H, s, H-8), 7.02 (1H, d, 2.4, H-3′), 6.28 (1H, d, 9.8, H-3), 4.27 (3 H, s, 5-OCH_3).

Limettin (citropten) 2 Pale-yellow Crystals, ^{1}H NMR (400 MHz, $CDCl_3$), δ value in ppm, (J value in Hz): 7.96 (1H, d, 9.6, H-4), 6.40 (1H, d, 2.2, H-8), 6.27 (1H, d, 2.2, H-6), 6.14 (1H, d, 9.6, H-3), 3.88 (3 H, s, 5-OCH_3), 3.84 (3 H, s,7-OCH_3).

Isopimpinellin 3 White Crystals, ^{1}H NMR (400 MHz, $CDCl_3$), δ value in ppm, (J value in Hz): 8.13 (1H, d, 9.8, H-4), 7.63 (1H, d, 2.4, H-2`), 7.00 (1H, d, 2.4, H-3`), 6.29 (1H, d, 9.8, H-3), 4.17 (3 H, s, 5-OCH_3), 4.16 (3 H, s, 8-OCH_3). ^{13}C NMR (100 MHz, δ, ppm): 160.66 (C-2), 113.04 (C-3), 139.57 (C-4), 144.50 (C-5), 114.82 (C-6), 150.02 (C-7), 128.08 (C-8), 143.82 (C-8a), 107.66 (C-4a), 145.28 (C-2`), 105.24 (C-3′), 61.89 (5-OCH_3), 61.00 (8-OCH_3).

Oxypeucedanin hydrate 4 Pale-yellow residue, ^{1}H NMR (500 MHz, CD_3OD), δ value in ppm, (J value in Hz): 8.43 (1H, d, 9.8, H-4), 7.80 (1H, d, 2.4, H-2`), 7.23 (1H, d, 2.4, H-3`), 7.20 (1H, br s, H-8), 6.29 (1H, d, 9.8, H-3), 4.39 (1H, dd, 9.8, 8.5, H-1"$_a$), 4.80 (1H, dd, 9.8, 2.4, H-1"$_b$), 3.82 (1H, dd, 8.5, 2.4, H-2"), 1.30 (3 H, s, H-4"), 1.24 (3 H, s, H-5").

Table 1 ^{1}H, ^{13}C and HMBC data of 5

No	^{1}H (multiplicity, J Hz)	^{13}C	Long range HMBC protons
2	-	162.79	H-3, H-4
3	6.30, d, 9.8	114.95	-----
4	8.26, d, 9.8	141.51	-----
4a	-	108.53	H-3
5	-	146.08	-OCH_3
6	-	116.18	H-9, H-10
7	-	151.64	H-9, H-10
8	-	128.27	H-9, H-10, H-11
8a	-	144.84	H-4, 5-OCH3
9	7.84, d, 2.3	146.78	H-10
10	7.24, d, 2.3	106.44	H-9
11$_a$	4.57, dd, 10.3, 2.8	76.79	H-12
11$_b$	4.29, dd, 10.3, 8.1		
12	3.84, dd, 8.0, 2.8	78.26	H-11, H-14, H-15
13	-	72.73	H-12, H-14, H-15
14	1.28, s	26.71	H-12, H-15
15	1.22, s	25.12	H-12, H-14
2′	-	162.71	H-3′, H-4′
3′	6.39, d, 9.6	113.05	-------
4′	8.04, d, 9.6	146.78	H-5′
4a′	-	117.97	H-3′, H-4′
5′	7.58, s	114.92	H-4′, H-9′
6′	-	127.95	-------
7′	-	149.17	H-9′, H-10′
8′	-	133.07	H-4′, H-5′, H-11′
8a′	-	144.25	H-4′, H-9′
9′	7.89, d, 2.2	148.52	H-10′, H-5′
10′	6.97, d, 2.2	107.97	H-9′, H-5′
11′$_a$	4.75, dd, 10.3, 2.7	76.46	H-12′
11′$_b$	4.45, dd, 10.3, 8.1		
12′	3.86, dd, 8.1, 2.7	78.26	H-11′, H-14′, H-15′
13′	-	72.73	H-12′, H-14′, H-15′
14′	1.26, s	26.65	H-12′, H-15′
15′	1.23, s	25.12	H-12′, H-14′
5-OCH_3	4.22, s	61.41	-------

(12R, 12'R)-aurantifolin 5 Yellow crystals, $[\alpha]^{21}_{D}$ +80° (c=0.01, MeOH), The UV (MeOH) λmax (log ε): 399 (4.46), sh 329 (4.53), sh 290 (4.61), and 252 (4.69) nm. The ESI-MS (positive mode) m/z 691$[M+CH_3OH+K]^+$ and m/z 662 $[M+CH_3CN+H]^+$, (negative mode) m/z 669 $[M+CH_3OH+H_2O-H]^-$, (Calcd for $C_{33}H_{32}O_{12}$, 620). ^{1}H NMR (500 MHz, CD_3OD); ^{13}C NMR (125 MHz, CD_3OD) (see Table 1).

21,23-dihydro-23-methoxy-21-oxolimonin 6 White residue, ^{1}H NMR (500 MHz, CD_3OD), δ value in ppm, (J value in Hz): 7.40 (1H, t, 1.3, H-22), 5.92(1H, t, 1.4, H-23), 5.36 (1H, t, 1.3, H-17), 4.95 (1H, d, 13.2, H-19*β*), 4.58 (1H, d, 13.2, H-19*α*), 4.18 (1H, d, 4.0, H-1*β*), 4.11 (1H, s, H-15), 3.08 (1H, dd, 14.7, 15.7, H-6*β*), 2.88 (1H, dd, 1.5, 16.6, H-2*α*), 2.78 (1H, dd, 4, 16.6, H-2*β*), 2.35 (1H, dd, 3.4, 14.7, H-6*α*), 1.37 (1H, m, H-12*α*) and 2.06 (1H, m, H-12*β*). (3.53(s), 1.08 (s), 1.24(s), 1.18(s), 1.12(s)).

21,23-dihydro-23-methoxy-21-oxonomilin 7 White residue, ^{1}H NMR (500 MHz, CD_3OD), δ value in ppm, (J value in Hz): 7.42 (1H, t, J 1.3 Hz, H-22), 5.94 (1H, t, J 1.3 Hz, H-23), 5.36 (1H, t, J 1.3 Hz, H-17), 5.02 (1H, d, J 7.3 Hz, H-1β), 3.88 (1H, s, H-15), 3.57 (3 H, s, 23-OCH_3), 3.53 (1H, t, J 14.1 Hz H-6*β*), 2.60 (1H, dd, J 3.6, 14.3 Hz, H-6*α*), 2.02 (3 H, s, CH_3CO), (1.14 (s), 1.42(s), 1.38(s), 1.60(s), 1.24(s)).

Chrysoeriol 8-C-glucoside (scoparin) 8 Yellow amorphous powder, ^{1}H NMR (500 MHz, CD_3OD), δ value in ppm, (J value in Hz): 7.64 (1H, dd, 8.1, 2.8, H-6`), 7.54 (1H, br s, H-2`), 7.05 (1H, d, 8.1, H-5`), 6.58 (1H, s, H-3), 6.27 (1H, s, H-6), 5.04 (1H, d, 8.0, H-1"), 4.11 (1H, t, 9.6, H-2"), 3.98 (1H, br d, 12.4, H-6"a), 3.95 (3 H, s, 3`- OCH_3), 3.88 (1H, br. m, H-6"b).

Isorhamnetin 3-O-*β*-D-rutinoside (narcissin) 9 Yellow solid residue, ^{1}H NMR (500 MHz, CD_3OD), δ value in ppm, (J value in Hz): 7.95 (1H, d, 1.8, H-2`), 7.63 (1H, dd, 8.4, 1.8, H-6`), 6.91 (1H, d, 8.4, H-5`), 6.35 (1H, d, 2.0, H-8), 6.15 (1H, d, 2.0, H-6), 5.17 (1H, d, 7.3, H-1"), 4.50 (1H, d, 1.3, H-1"'), 3.95 (3 H, s, 3'-OCH_3), 1.1 (3 H, d, 6.3, H-6"').

Insect collection and rearing

By using aspirator, the whiteflies (*Bemisia tabaci*) adults were collected from the Mansoura University Faculty of Agriculture's farm. *B. tabaci* colony was kept alive on untreated cotton plants put in cages made of muslin material (1.5×1.5×1.5 m) in the greenhouse and were kept at 25–35 °C, 55–75% RH, and day light.

Bioassays

By using spray method technique, biological tests of the fractions and isolated compounds along with the positive control Okios 3.2% EC (azadirachtin) within lab setting were performed on *B. tabaci* 3rd instar nymphs following the method of Mostafa et al., 2019 [1].

Statistical analysis

Mortality percentages were adjusted using Abbott's formula [34]. Finney, 1971, states the values of LC_{50}, LC_{90}, and slope [35]. The toxicity index of *C. aurantifolia* fractions and its isolated compounds was determined according to Sun's equation [36].

Results and discussion

Identification of compounds (1–9)

The use of botanical insecticides was considered to be one of the most effective and less harmful biological method in controlling insect pests [37]. Our interest in detecting new leads that could be served as eco-friendly acceptable botanical insecticides has inspired us to evaluate the insecticidal potential of *C. aurantifolia* secondary metabolites on *B. tabaci* 3rd instar nymphs.

Bioassay-guided fractionation of chloroform and butanol fractions led to isolate and identify of nine secondary metabolites (Fig. 1) using chromatographic (CC and PTLC) and spectrophotometric analyses (^{1}H, ^{13}C NMR, HSQC, HMBC, NOESY and ESI-MS).

Compounds **1–4** were isolated from the $CHCl_3$ fraction. The ^{1}H NMR spectrum of **1** clearly confirmed the existence of *α*,*β*-unsaturated lactone of the pyrone ring of coumarins. Comparing with authentic spectra indicated that compound **1** is the 5-substituted linear furanocoumarin, **bergapten** [38] that was reported previously by Ramírez-Pelayo C from lime *C. aurantifolia* peels [39]. The ^{1}H NMR spectrum of compound **2** exhibited a simple coumarin pattern, which was verified by matching its spectra with those previously published [40] as **limettin (citropten)** from lime *C. aurantifolia* peels [39]. The ^{1}H NMR spectra of **3** revealed signals comparable to the pattern of **1** with the replacement of the aryl proton singlet by an additional methoxyl group in agreement with 5,8-dimethoxy linear furanocoumarin, that was confirmed by ^{13}C NMR and HMBC as **isopimpinellin** [41, 42] which was previously published from lime *C. aurantifolia* peels [39]. The ^{1}H NMR spectrum of compound **4** exhibited a furocoumarin pattern with H-5 has substituted with a prenyl side chain, which was identified as **oxypeucedanin hydrate** [43] that was reported previously from Kabosu (*C. sphaerocarpa* Hort. ex Tanaka) fruits [44] and from West Indian lime (*C. aurantifolia*) oil [45].

Compound **5** was isolated as yellow crystals also from the $CHCl_3$ fraction. (R_f 0.31). The UV spectrum showed absorption maxima at 399, sh 329, sh 290 and 252 nm. The positive mode ESI MS spectra of **5** exhibited a *quasi*-molecular ion peaks at m/z 691 and m/z 662 due to the

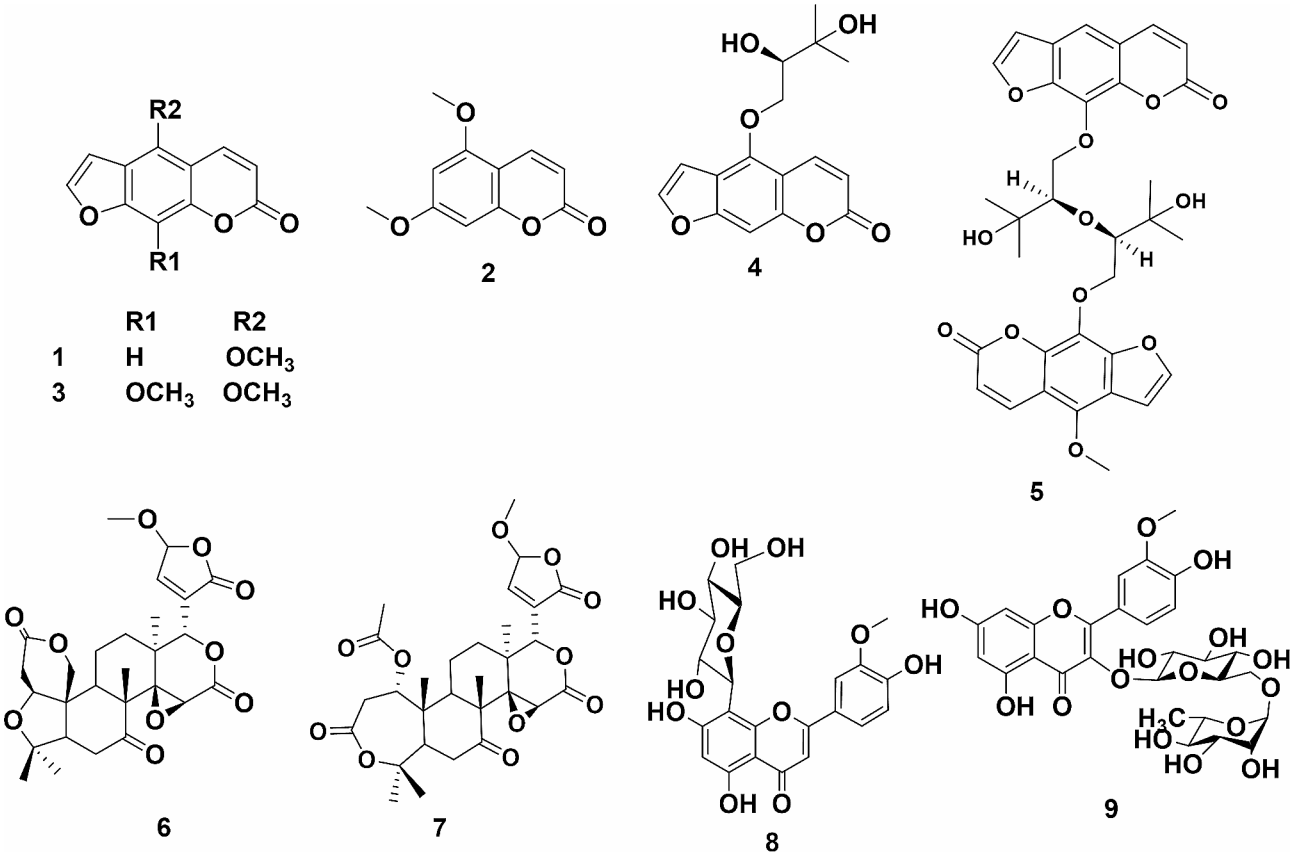

Fig. 1 Isolated compounds from *C. aurantifolia* fruit peels

adducts $[M+CH_3OH+K]^+$ and $[M+CH_3CN+H]^+$, respectively, while the negative mode spectrum displayed a *quasi*-molecular ion peaks at m/z 669 $[M+CH_3OH+H_2O\text{-}H]^-$ all typically with the molecular formula $C_{33}H_{32}O_{12}$. The ^1H, ^{13}C NMR and HSQC spectra of **5** (Table 1) indicated the existence of two sets of protons for two C-8-substituted linear furanocoumarin units at [δ_H (6.30 ppm (1H, d, J 9.8 Hz, H-3)/δ_C 114.95, (δ_H 8.26 (1H, d, J 9.8 Hz, H-4)/δ_C 141.51, (δ_H 7.24 (1H, d, J 2.3 Hz, H-9)/ δ_C 106.44 and δ_H 7.84 (1H, d, J 2.3 Hz, H-10)/δ_C 146.78], while the second mono-substituted furanocoumarin unit showed signals at [δ_H 6.39 ppm (1H, d, J 9.6 Hz, H-3')/δ_C 113.05, δ_H 8.04 (1H, d, J 9.6 Hz, H-4')/δ_C 146.78, δ_H 7.58 (1H, s, H-5')/δ_C 114.92, δ_H 6.97 (1H, d, J 2.2 Hz, H-9')/ δ_C 107.97, δ_H 7.89 (1H, d, J 2.2 Hz, H-10')/δ_C 148.52]. Furthermore, two pairs of oxymethylene protons appeared at δ_H 4.57 (1H, dd, J 10.3, 2.8 Hz, H-11_a), δ_H 4.29 (1H, dd, J 10.3, 8.1 Hz, H-11_b)/δ_C 76.79] and δ_H 4.75 (1H, dd, J 10.3, 2.7 Hz, H-11'$_a$), (δ_H 4.45 (1H, dd, J 10.3, 8.1 Hz, H-11'$_b$)/δ_C 76.46, two oxymethines protons at δ_H 3.84 (1H, dd, J 8, 2.7 Hz, H-12)/δ_C 78.26 and δ_H 3.86 ppm (1H, dd, J 8.1, 2.6 Hz, H-12')/δ_C 78.26, beside four methyl groups at δ_H 1.28 (3H, s, H-14)/δ_C 26.71, δ_H 1.22 (3H, s, H-15)/δ_C 25.12; δ_H 1.26 (3H, s, H-14')/δ_C 26.65 and δ_H 1.23 (3H, s, H-15')/δ_C 25.12. In addition, one methoxyl group appeared at δ_H 4.22 (3H, s)/δ_C 61.41 ppm. Thus compound **5** was suggested to contain two unites of prenylfurocoumarins, comprising (R)-heraclenol [46] and (R)-byakangelicin [47]. Careful examination of the long-range couplings in HMBC spectrum (Fig. 2) has indicated cross peaks of δ_H 4.22 (-OCH_3) to C-5 (146.08) and of the oxymethylene proton signals at δ_H 4.29 (H-11_b) and 4.57 (H-11_a) to C-8, C-12 and C-12' which has confirmed the location of 5-OCH_3 and the side chain at C-8 in the (R)-byakangelicin unit. Also, the observed cross peaks of δ_H 4.45 (H-11'$_b$) and 4.75 (H-11'$_a$) with C-8', C-12 and C-12' which has established the location of the other side chain at C-8' in the (R)-heraclenol. This has confirmed the ether linkage between the oxymethine carbons in both units. In the NOESY spectrum of **5** (Fig. 2), the correlation of both H-12' and H-12 with CH_3-14, CH_3-14', CH_3-15 and CH_3-15' as well as the correlation to each other indicated the relative stereochemistry of both protons to be α-oriented. The absolute configuration of C-12 and C-12' was assigned as R for both based on comparing the NMR data with those of (R)-heraclenol [46] and (R)-byakangelicin [47]. Thus, compound **5** was identified as a new compound named **(12R, 12'R)-aurantifolin** and to the best of our knowledge it wasn't reported previously from any natural source.

^1H NMR data of compound **6**, which was isolated from $CHCl_3$ fraction, displayed limonin-like signals, with the exception of the furan ring being absent and instead the existence of α-substituted γ-methoxybutenolide group [48]. Thus **6** was defined as **21,23-dihydro-23-methoxy-21-oxolimonin**, which was isolated previously from Satsuma Orange (*Citrus reticulata*) peels [48]. Compound **7** showed signals in the ^1H NMR spectrum almost similar to nomilin [48] with the exception of the furan ring being absent and instead the existence of same group as

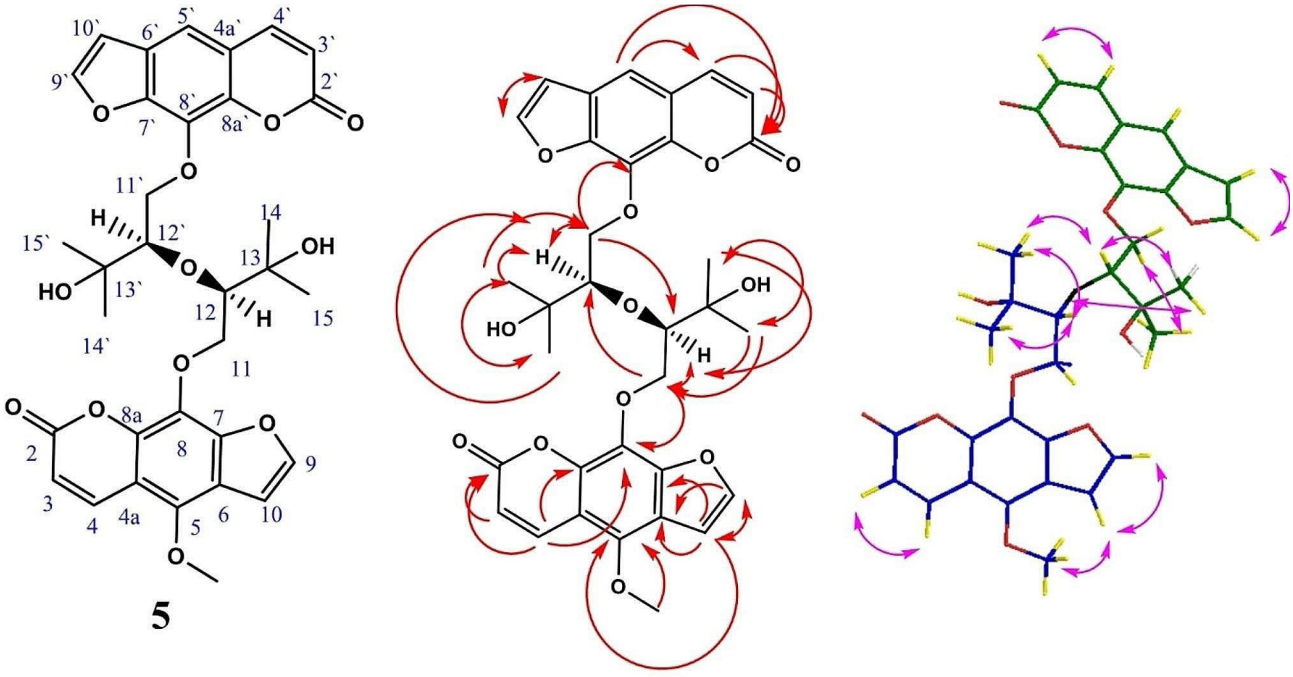

Fig. 2 HMBC and NOESY correlations of compound 5

Table 2 Toxic effect of *C. aurantifolia* fractions' after 24 h of exposure against *B. tabaci* 3rd instar nymphs

Fraction	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope ± SE	Toxicity index
		Lower	Upper		Lower	Upper		
Chloroform	37.1	13.5	56.9	298.7	167.9	1565.3	1.415 ± 0.385	100.00
Butanol	41.7	13.4	66.8	526.2	267.5	3735.7	1.164 ± 0.312	88.94
Okios 3.2% EC	45.5	33.8	66.6	203.8	119.5	575.7	1.968 ± 0.352	81.54

Table 3 Toxic effect of *C. aurantifolia* fractions' after 72 h of exposure against *B. tabaci* 3rd instar nymphs

Fraction	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope ± SE	Toxicity index
		Lower	Upper		Lower	Upper		
Chloroform	9.6	0.6	19.6	58.7	38.7	101.9	1.636 ± 0.513	100.00
Butanol	18.6	2.4	32.1	98.9	68.3	261.7	1.770 ± 0.562	51.80
Okios 3.2% EC	27.4	20.4	41.8	128.9	71.1	489.0	1.907 ± 0.396	35.04

Table 4 Toxic effect of *C. Aurantifolia* isolated compounds after 24 h of exposure against *B. tabaci* 3rd instar nymphs

Fraction	Isolated compounds	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope ± SE	Toxicity index
			Lower	Upper		Lower	Upper		
Chloroform	**(1)**	514.7	280.1	2719.9	9764.2	2101.8	1145866.6	1.003 ± 0.266	6.47
	(2)	214.0	131.5	845.9	2432.2	690.3	330618.9	1.214 ± 0.373	15.56
	(3)	305.7	186.3	1005.4	9955.6	2096.6	1428585.7	0.847 ± 0.229	10.90
	(4)	165.3	102.1	409.4	3206.8	863.4	552879.3	0.995 ± 0.307	20.15
	(5)	55.1	23.2	85.6	617.6	272.8	10755.6	1.221 ± 0.362	60.45
	(6)	121.5	64.6	217.3	3302.0	908.4	558156.0	0.894 ± 0.278	27.40
	(7)	73.9	20.6	121.2	2736.8	809.2	438783.9	0.817 ± 0.261	45.05
Butanol	**(8)**	109.6	75.3	177.9	791.7	368.7	6329.8	1.493 ± 0.368	30.38
	(9)	33.3	7.0	57.6	498.9	247.6	4694.7	1.090 ± 0.315	100.00
Okios 3.2% EC		45.5	33.8	66.6	203.8	119.5	575.7	1.968 ± 0.352	73.19

6. Thus, **7** was identified as **21,23-dihydro-23-methoxy-21-oxonomilin**, previously identified from Satsuma Orange (*Citrus reticulata*) peels [48].

Careful examination of ^{1}H NMR data of **8** and **9**, which were isolated from butanol fraction, showed proton signals pattern characterized for flavone and flavonol glycoside moieties respectively. After comparing these spectral data to those found in the literature, **8** was identified as **chrysoeriol 8-C-glucoside (scoparin)**, which was identified previously from *C. aurantifolia* peels by using LC-UV, LC-MS and MS/MS techniques [49, 50] and identified **9** as **narcissin** that was detected previously from *C. aurantifolia* peels [50, 51].

Insecticidal efficacy of *C. aurantifolia* fractions to whiteflies *B. tabaci* 3rd instar nymphs

According to several previously studies, *C. aurantifolia* has been studied as ecofriendly natural insecticides, larvicide, and repellent and the potential of replacing the high risky synthetic chemical insecticides to manage crop pests was established [12].

The chloroform and butanol fractions of *C. aurantifolia* were assessed for their toxic effect against *B. tabaci* 3rd -instar nymphs after 24 h of exposure using laboratory conditions in comparison with the positive control azadirachtin (Okios 3.2% EC) (Table 2). The chloroform fraction was the most potent at LC_{50} value followed by butanol fraction and Okios 3.2% EC. The recorded LC_{50} (Toxicity index) were 37.1 (100%), 41.7 (88.94) and 45.5 ppm (81.54), respectively. Also, after 72 h of treatment (Table 3), chloroform was the most potent followed by butanol fractions and Okios 3.2% EC. The recorded LC_{50} (Toxicity index) were 9.6 (100%) 18.6 (51.8) and 27.4 ppm (35.04), respectively.

Insecticidal efficacy of *C. Aurantifolia* isolated compounds to whiteflies *B. tabaci* 3rd instar nymphs

Nine pure isolated metabolites were isolated and identified from *C. aurantifolia* chloroform and butanol fractions using spectral techniques, these metabolites were evaluated for their toxicity against *B. tabaci* 3rd instar nymphs to find out the active principles of each fraction individually.

The relative susceptibility of the *B. tabaci* 3rd instar nymphs after 24 h of exposure using laboratory settings to *C. aurantifolia* isolated compounds were assessed (Table 4). Compound **9** was the most effective at LC_{50} level followed Okios 3.2% EC, **5**, **7**, **8**, **6**, **4**, **2**, **3** and the

Table 5 Toxic effect of *C. Aurantifolia* isolated compounds after 72 h of exposure against *B. tabaci* 3rd instar nymphs

Fraction	Isolated compounds	LC_{50} (ppm)	Confidence limit at 95%		LC_{90} (ppm)	Confidence limit at 95%		Slope ± SE	Toxicity index
			Lower	Upper		Lower	Upper		
Chloroform	**(1)**	67.7	32.0	109.4	853.2	334.4	29307.1	1.165 ± 0.357	23.51
	(2)	41.3	6.9	73.1	1090.98	430.8	36915.9	0.902 ± 0.279	38.53
	(3)	88.3	60.6	128.8	547.19	290.6	2600.5	1.619 ± 0.372	18.01
	(4)	27.9	3.7	52.9	565.09	279.2	5602.8	0.982 ± 0.287	56.96
	(5)	15.9	2.3	31.3	142.19	93.4	306.5	1.348 ± 0.354	100.00
	(6)	29.3	8.5	46.8	223.20	133.3	947.7	1.454 ± 0.403	54.29
	(7)	21.9	2.6	39.3	204.39	116.8	1463.0	1.321 ± 0.425	72.68
Butanol	**(8)**	27.3	12.2	41.4	141.20	102.4	231.7	1.798 ± 0.350	58.22
	(9)	19.8	4.0	33.1	101.38	72.9	197.6	1.810 ± 0.506	80.22
Okios 3.2% EC		27.4	20.4	41.8	128.9	71.1	489.0	1.907 ± 0.396	58.03

least one **1.** The recorded LC_{50} (Toxicity index) were 33.3 (100%), 45.5 (73.19), 55.1 (60.45), 73.9 (45.05), 109.6 (30.38), 121.5 (27.40), 165.3 (20.15), 214.0 (20.15), 305.7 (10.90) and 514.7 ppm (6.47), respectively. After 72 h of exposure (Table 5), the potency of arrangement started by the most effective compound **5** followed by **9**, **7**, **8**, Okios 3.2% EC, **4**, **6**, **2**, **1 and 3**, respectively. The recorded LC_{50} (Toxicity index) were 15.9 (100%), 19.8 (80.22), 21.9 (72.68), 27.3 (58.22), 27.4 (58.03), 27.9 (56.96), 29.3 (56.96), 41.3 (38.53), 67.7 (23.51), 88.3 (18.01), respectively. The slopes of the toxicity lines were calculated to be fluctuated and increased from (0.817) in **7** to (1.907) in Okios 3.2% EC and the other slopes came between these two fractions.

As shown by the toxicity index, the study clarified those compounds **(12R**, **12'R)-aurantifolin** and **narcissin** were the most effective principles amongst the tested metabolites. The nine isolated compounds' structure-activity connection showed that both class type and substitutions of the natural product were important. Whitefly *B. tabaci* third instar nymphs were significantly impacted by **glycosylated flavonoids (8**, **9)** and **prenylated coumarin** (**4**, **5**) classes, then **limonoids** (**6**, **7**) and **coumarin** (**1–3**) **classes**.

Another conclusion should keep in mind that isoprene substituted coumarin (**4**, **5**) classes is more potent than the unsubstituted ones (**1–3**). Also, the activity of methyl ester limonoids (**7**) is more toxic than other derivatives (**6**).

The synergistic effect could be concluded and observed from the assessment experiment results of chloroform and butanol fractions as the value of LC_{50} after 72 h of treatment were 9.6 and 18.6 ppm, respectively while, the isolated metabolites recorded toxic effect around 15.9 to 88.3 ppm after 72 h of treatment. So, the presence of multi-bioactive components with a diversity in the structural composition in a fraction could the potential for synergistic interactions and play an important role to reach this toxic effect [52].

The results obtained were in agreement with Mansour (2011) who reported the insecticidal effectiveness of the ethanolic extract of *C. aurantifolia* against 4th larval instars of *Musca domestica*, after 24 h of exposure [53]. In addition, the crude aqueous extracts of lemon peels was the most toxic among the tested extracts to *Culex pipiens* larvae [54]. Also, the lemon fruit peels aqueous extract showed a remarkable toxic effect against rose aphids, *Macrosiphum roseiformis* under both laboratory and field conditions with no toxicity towards the insect predator, *Coccinella septempunctata* [55].

A new identified constituent, (12R, 12'R)-aurantifolin as well as eight metabolites were isolated and identified by chromatographic and spectral analyses from *C. aurantifolia* fruit peels. The insecticidal potency of both isolated fractions and separated compounds was carried out against whiteflies (*B. tabaci*). The results showed that (12R, 12'R)-aurantifolin and narcissin were the most effective principles amongst the tested metabolites. So, plant natural sources could be used as a valuable source for the production of the natural green insecticides.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00916-4.

Supplementary Material 1

Acknowledgements
All authors express their deep appreciation to all stuff members in the Department of Chemistry, Faculty of Science, Mansoura University.

Author contributions
Mariam S. El-Alfy performed all the literature search, material preparation, chemistry experiments, Mamdouh Abdel-Mogib, Abelaziz M. Dawidar and, Mohamed E. Mostafa performed experimental planning and data analysis. Mamdouh Abdel-Mogib and Mohamed E. Mostafa reviewed and edited the manuscript after due conceptualization of the article. All authors wrote, read and approved the final manuscript.

Funding
The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability
All the datasets were presented in the main manuscript and additional supporting file.

Declarations

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

Received: 28 March 2024 / Accepted: 29 June 2024
Published online: 23 September 2024

References

1. Mostafa ME, Fathy A, Alorfi HS, Negm A, Abdel-Mogib M (2019) Naturally occurring insecticides from the marine sponge Xestospongia testudinaria to control the whitefly Bemicia Tabaci (Genn.) And the aphid Aphis Gossypii (Glover). Int J Entomol Nematology 5(1):121–127
2. Parven A, Khan MSI, Prodhan MDH, Venkateswarlu K, Megharaj M, Meftaul IM (2021) Human health risk assessment through quantitative screening of insecticide residues in two green beans to ensure food safety. J Food Compos Anal 103:104121. https://doi.org/10.1016/j.jfca.2021.104121
3. Mostafa ME (2021) Acaricidal potential and phytochemical analysis of the indigenous Centaurea Aegyptiaca L. against Tetranychus Urticae Koch (Acari: Tetranychidae). Int J Entomol Res 6(1):40–45
4. Razo-Belman R, Ozuna C (2023) Volatile organic compounds: a review of their current applications as pest biocontrol and disease management. Horticulturae 9(4):441. https://doi.org/10.3390/horticulturae9040441
5. Wang D, Xie N, Yi S, Liu C, Jiang H, Ma Z, Feng J, Yan H, Zhang X (2018) Bioassay-guided isolation of potent aphicidal Erythrina alkaloids against Aphis gossypii from the seed of Erythrina crista-galli L. Pest Manag Sci 74(1):210–218. https://doi.org/10.1002/ps.4698
6. Haokip SW, Sheikh KHA, Das S, Devi OB, Singh YD, Wangchu L, Heisnam P (2023) Unraveling physicochemical profiles and bioactivities of Citrus peel essential oils: a comprehensive review. Eur Food Res Technol 249(11):2821–2834. https://doi.org/10.1007/s00217-023-04330-w
7. Ennab HA, Mohamed AH, El-Hoseiny HM, Omar AA, Hassan IF, Gaballah MS, Khalil SE, Mira AM, Abd El-Khalek AF, Alam-Eldein SM (2023) Humic acid improves the resilience to salinity stress of drip-irrigated Mexican lime trees in saline clay soils. Agronomy 13(7):1680. https://doi.org/10.3390/agronomy13071680
8. Khalid KA, Ahmed AMA (2023) Exogenous trans-cinnamic acid applications affect leaf, flower and peel essential oils of Banzhair lime cultivated on arid region. Vegetos 1–9. https://doi.org/10.1007/s42535-023-00642-0
9. Weimer P, Moura JGL, Mossmann V, Immig ML, de Castilhos J, Rossi RC (2021) Citrus aurantiifolia (Christm) Swingle: Biological potential and safety profile of essential oils from leaves and fruit peels. Food Bioscience 40:100905. https://doi.org/10.1016/j.fbio.2021.100905
10. Fagodia SK, Singh HP, Batish DR, Kohli RK (2017) Phytotoxicity and cytotoxicity of Citrus aurantiifolia essential oil and its major constituents: Limonene and citral. Ind Crops Prod 108:708–715. https://doi.org/10.1016/j.indcrop.2017.07.005
11. EL-Tanany MM, Sourour MSM, Tayel EA (2011) Effect of fasting and foliar application with urea on Egyptian lime trees productivity a- leaf ammonium content, flowering, fruit setting and yield of lime trees. Alexandria Sci Exch J 32(2):215–226. https://doi.org/10.21608/asejaiqjsae.2011.2420
12. Galovičová L, Borotová P, Vukovic NL, Vukic M, Kunová S, Hanus P, Kowalczewski PŁ, Bakay L, Kaˇcániová M (2022) The potential use of Citrus aurantifolia L. essential oils for decay control, quality preservation of agricultural products, and anti-insect activity. Agronomy 12(3):735. https://doi.org/10.3390/agronomy12030735
13. Ademosun AO (2022) Citrus peels odyssey: from the waste bin to the lab bench to the dining table. Appl Food Res 2(1):100083. https://doi.org/10.1016/j.afres.2022.100083
14. Asmah N, Suniarti DF, Margono A, Mas'ud ZA, Bachtiar EW (2020) Identification of active compounds in ethyl acetate, chloroform, and N-hexane extracts from peels of Citrus aurantifolia from Maribaya, West Java, Indonesia. J Adv Pharm Tech Res 11(3):107–112. https://doi.org/10.4103/japtr.JAPTR_177_19
15. Julaeha E, Nurzaman M, Wahyudi T, Nurjanah S, Permadi N, Anshori JA (2022) The development of the antibacterial microcapsules of citrus essential oil for the cosmetotextile application: a review. Molecules 27(22):8090. https://doi.org/10.3390/molecules27228090
16. Shchérazade OSF, Pétronille AZ, Joseph FKY, Georges A (2021) Study of the analgesic effect of the aqueous extract of the leaves of Citrus aurantifolia (Rutaceae) in mice. GSC Biol Pharm Sci 14(3):207–214. https://doi.org/10.30574/gscbps.2021.14.3.0072
17. Narang N, Jiraungkoorskul W (2016) Anticancer activity of key lime, Citrus aurantifolia. Pharmacogn Rev 10(20):118–122. https://doi.org/10.4103/0973-7847.194043
18. Karatoprak GŞ, Aşık ÇY, Çakır A, Şafak EK (2021) In vitro pharmacological screening of antioxidant, cytotoxic and enzyme inhibitory activities of Citrus aurantifolia Linn. Dried fruit extract. Int J Environ Health Res 31(8):991–1000. https://doi.org/10.1080/09603123.2020.1714558
19. Ibrahim FA, Usman LA, Akolade JO, Idowu OA, Abdulazeez AT, Amuzat AO (2019) Antidiabetic potentials of Citrus aurantifolia leaf essential oil. Drug Res 69(04):201–206. https://doi.org/10.1055/a-0662-5607
20. Jain S, Arora P, Popli H (2020) A comprehensive review on Citrus aurantifolia essential oil: its phytochemistry and pharmacological aspects. Brazilian J Nat Sci 3(2):354–364. https://doi.org/10.31415/bjns.v3i2.101
21. Tundis R, Loizzo MR, Bonesi M, Menichini F, Mastellone V, Colica C et al (2012) Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of Citrus aurantifolia Swingle, C. Aurantium L., and C. Bergamia Risso and Poit. Peel essential oils. J Food Sci 77(1):40–46. https://doi.org/10.1111/j.1750-3841.2011.02511.x
22. Fokou PVT, Fokouo RDY (2020) Exploring the indigenous knowledge systems to respond to coronavirus infection 2019 in Cameroon. Ethnobotany Res Appl 20:1–27. https://ethnobotanyjournal.org/index.php/era/article/view/2141
23. Cruz-Valenzuela MR, Tapia-Rodríguez MR, Vazquez-Armenta FJ, Silva-Espinoza BA, Ayala-Zavala JF (2016) Lime (Citrus aurantifolia) oils. In: Essential oils in food preservation, flavor and safety. Elsevier pp.531–537. https://doi.org/10.1016/B978-0-12-416641-7.00061-4
24. Al Namani J, Baqir E, Al Abri A, Al Hubaishi T, Husain A, Khan SA (2018) Phytochemical screening, phenolic content and antioxidant activity of Citrus aurantifolia L. leaves grown in two regions of Oman. Iran J Pharm Sci 14(1):27–34. https://doi.org/10.22037/ijps.v14.40669
25. Kazeem MI, Bankole HA, Oladokun TI, Bello AO, Maliki MA (2020) Citrus aurantifolia (Christm.) Swingle (lime) fruit extract inhibits the activities of polyol pathway enzymes. eFood 1(4):310–315. https://doi.org/10.2991/efood.k.200824.001
26. Keihanian F, Moohebati M, Saeidinia A, Mohajeri SA, Madaeni S (2021) Therapeutic effects of medicinal plants on isoproterenol-induced heart failure in rats. Biomed Pharmacother 134:111101. https://doi.org/10.1016/j.biopha.2020.111101
27. Galovi˘cová L, Borotová P, Vukovic NL, Vukic M, Kunová S, Hanus P, Kowalczewski PŁ, Bakay L, Ka˘cániová M (2022) The potential use of Citrus aurantifolia L. essential oils for Decay Control, Quality Preservation of Agricultural Products, and Anti-insect Activity. Agronomy 12:735. https://doi.org/10.3390/agronomy12030735
28. Luro F, Garcia Neves C, Costantino G, da Silva Gesteira A, Paoli M, Ollitrault P, Tomi F, Micheli F, Gibernau M (2020) Effect of environmental conditions on the yield of Peel and Composition of essential oils from Citrus Cultivated in Bahia (Brazil) and Corsica (France). Agronomy 10:1256. https://doi.org/10.3390/agronomy10091256
29. Gioffrè G, Ursino D, Labate MLC, Giuffrè AM (2020) The Peel Essential Oil Composition of Bergamot Fruit (Citrus bergamia, Risso) of Reggio Calabria (Italy): a review. Emir. J Food Agric 32:835–845
30. Maria GA, Riccardo N, Citrus bergamia (2020) Risso: the Peel, the Juice and the seed oil of the Bergamot Fruit of Reggio Calabria (South Italy). Emir J Food Agric 32:522–532. https://doi.org/10.9755/ejfa.2020.v32.i7.2128
31. BenHsouna A, Ben Halima N, Smaoui S, Hamdi N (2017) Citrus lemon essential oil: Chemical composition, antioxidant and antimicrobial activities with its Preservative Effect against Listeria Monocytogenes inoculated in Minced Beef Meat. Lipids Health Dis 16:146. https://doi.org/10.1186/s12944-017-0487-5
32. AL-Jabri NN, Hossain MA (2018) Chemical Composition and Antimicrobial Potency of locally grown Lemon essential oil against selected bacterial strains. J King Saud Univ -Sci 30:14–20. https://doi.org/10.1016/j.jksus.2016.08.008
33. Arani Y, Nithiyagowry R (2021) Study on bioactivity of lime, Citrus aurantifolia (Christm.) Against larvae of diamond back moth, Plutella xylostella (L.) on cabbage crop under laboratory. Vingnanam J Sci 16(1):1–5

34. Abbott WS (1925) A method of computing the effectiveness of an insecticide. J econ Entomol 18(2):265–267
35. Finney DJ (1971) Probit analysis. A statistical treatment of the sigmoid response curve, 7th edn. Cambridge University Press. Cambridge. England.333p
36. Sun YP (1950) Toxicity index-an improved method of comparing the relative 378 toxicity of insecticides. J Econ Entomol 43(1):45–53. https://doi.org/10.1093/jee/43.1.45
37. Ismail M, Raza AB, Majeed MZ, Abbas U, Hussain R (2022) Biocidal activity of some selected phytoextracts and fruits of different Citrus cultivars against fruit fly Bactrocera dorsalis (Hendel) (Diptera: Tephritidae). Sarhad J Agric 38(3):800–811. https://doi.org/10.17582/journal.sja/2022/38.3.800.811
38. Wisetsai A, Schevenels FT, Lekphrom R (2021) Chemical constituents and their biological activities from the roots of Diospyros filipendula. Nat Prod Res 35(16):2739–2743. https://doi.org/10.1080/14786419.2019.1656630
39. Ramírez-Pelayo C, Martínez-Quiñones J, Gil J, Durango D (2019) Coumarins from the peel of Citrus grown in Colombia: composition, elicitation and antifungal activity. Heliyon 5(6):E01937. https://doi.org/10.1016/j.heliyon.2019.e01937
40. Sandoval-Montemayor NE, García A, Elizondo-Treviño E, Garza-González E, Alvarez L, Del Rayo Camacho-Corona M (2012) Chemical composition of hexane extract of Citrus aurantifolia and anti-mycobacterium tuberculosis activity of some of its constituents. Molecules 17(9):11173–11184. https://doi.org/10.3390/molecules170911173
41. Dehghan H, Sarrafi Y, Salehi P, Nejad Ebrahimi S (2017) α-Glucosidase inhibitory and antioxidant activity of furanocoumarins from Heracleum Persicum. Med Chem Res 26(4):849–855. https://doi.org/10.1007/s00044-017-1796-y
42. Widyawaruyanti A, Tanjung M, Permanasari AA, Saputri R, Tumewu L, Adianti M, Aoki-Utsubo C, Hotta H, Hafid AF, Wahyuni TS (2021) Alkaloid and benzopyran compounds of Melicope Latifolia fruit exhibit anti-hepatitis C virus activities. BMC Complement Med Ther 21(1):1–9. https://doi.org/10.1186/s12906-021-03202-8
43. Kiyonga AN, Hong G, Kim HS, Suh YG, Jung K (2021) Facile and rapid isolation of oxypeucedanin hydrate and byakangelicin from Angelica Dahurica by using [Bmim]Tf2N ionic liquid. Molecules 26(4):830. https://doi.org/10.3390/molecules26040830
44. Shimada A, Ueno H, Inagaki M, Yoshimitsu H (2022) Glutaminase inhibitory activity of umbelliferone isolated from kabosu (Citrus Sphaerocarpa Hort. Ex Tanaka). Nat Prod Res 36(2):605–609. https://doi.org/10.1080/14786419.2020.1788553
45. Stanley WL, Vannier SH (1967) Psoralens and substituted coumarins from expressed oil of lime. Phytochemistry 6(4):585–596. https://doi.org/10.1016/S0031-9422(00)82921-0
46. Fujioka T, Furumi K, Fujii H, Okabe H, Mihashi K, Nakano Y, Matsunaga H, Katano M, Mori M (1999) Antiproliferative constituents from umbelliferae plants. V. A new furanocoumarin and falcarindiol furanocoumarin ethers from the root of Angelica Japonica. Chem Pharm Bull 47(1):96–100. https://doi.org/10.1248/cpb.47.96
47. Numonov S, Bobakulov K, Numonova M, Sharopov F, Setzer WN, Khalilov Q, Begmatov N, Habasi M, Aisa HA (2018) New coumarin from the roots of Prangos pabularia. Nat Prod Res 32(19):2325–2332. https://doi.org/10.1080/14786419.2017.1413558
48. Kikuchi T, Ueno Y, Hamada Y, Furukawa C, Fujimoto T, Yamada T, Tanaka R (2017) Five new limonoids from peels of Satsuma orange (Citrus reticulata). Molecules 22(6):907. https://doi.org/10.3390/molecules22060907
49. El-Sayed MA, Al-Gendy AA, Hamdan DI, El-Shazly AM (2017) Phytoconstituents, LC-ESI-MS profile, antioxidant and antimicrobial activities of Citrus limon L. Burm. f. Cultivar Variegated Pink Lemon. J Pharm Sci Res 9(4):375–391
50. Brito A, Ramirez J, Areche C, Sepúlveda B, Simirgiotis M (2014) HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three Citrus species consumed in northern Chile. Molecules 19(11):17400–17421. https://doi.org/10.3390/molecules191117400
51. Phucharoenrak P, Muangnoi C, Trachootham D (2023) Metabolomic analysis of phytochemical compounds from ethanolic extract of lime (Citrus aurantifolia) peel and its anti-cancer effects against human hepatocellular carcinoma cells. Molecules 28(7):2965. https://doi.org/10.3390/molecules28072965
52. Claude B-B, Philogène BJR (1993) Insecticide synergists: role, importance, and perspectives. J Toxicol Environ Health: Curr Issues 38(2):199–223. https://doi.org/10.1080/15287399309531712
53. Mansour SA, Bakr RFA, Mohamed RI, Hasaneen NM (2011) Larvicidal activity of some Botanical extracts, commercial insecticides and their binary mixtures against the Housefly, Musca Domestica L. Open Toxinology J 4:1–13
54. Thomas CJ, Callaghan A (1999) The use of garlic (Allium sativa) and lemon peel (Citrus limon) extracts as culexpipiens larvacides: persistence and interaction with an organophosphate resistance mechanism Chemosphere. 39(14):2489–2496
55. Garima G, Uday A, Harneet K, Neelima RK, Pawan G (2017) Aphicidal effects of terpenoids present in Citrus limon on Macrosiphum Roseiformis and two generalist insect predators. J Asia Pac Entomol 20(4):1087–1095. https://doi.org/10.1016/j.aspen.2017.08.007

Publisher's Note