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# Phytochemical Profiling and Isolation of Bioactive Polyphenols from *Ipomoea carnea*

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**Abstract**

Four solvent fractions were obtained by successive extraction of *Ipomoea carnea* aerial parts. Five polyphenols were isolated and identified by spectroscopic methods from the ethyl acetate (EA) fraction named tiliroside (2) and from butanol (Bu) fraction named, apigenin-7*β*-*O*-glucoside (3), 3,5- di-*O*-caffeoylquinic acid (4) and two stereoisomers of 3,4- di-*O*-caffeoylquinic acid (5, 6) in addition to scopoletin (1) from methylene chloride (MC) fraction. GC/MS analysis of petroleum ether (PT) and MC fractions showed thirty-three compounds as well as, thirteen alkaloids were identified by using UPLC-ESI/MS/MS from alkaloid sub fraction (AK-sF) of MC. Insecticidal potentials of different fractions and the pure isolated compounds were evaluated for the first time on *Aphis craccivora* and *Bemisia tabaci*. The obtained results indicated that alkaloid sub fraction was the most potent against the two insects. Biochemical investigations of different six enzymes elucidated mode of action of the most potent fraction. Antioxidant activity of *I. carnea* fractions and pure compounds was performed against DPPH radical. The present study clarified that caffeoylquinic acid (**4-6**) are mostly responsible for the antioxidant activity of *I. carnea* Bu fraction while the strong insecticidal activity of MC fraction was due to the ergoline alkaloids found in its AK-sF.

Keywords: Ipomoea carnea; Insecticide; antioxidant activity; Alkaloids; Aphis craccivora; Bemisia tabaci.

## 1. Introduction

Plant sources have been used for many targets since ancient times as they have an extensive variation of biological activities [1]. Their biological activities are permanently related to the bioactive secondary metabolites accumulated in plants mostly as a defensive behavior against biotic or abiotic stresses [2]. In this respect, the shrub *I. carnea* family "Convolvulaceae" was used in India as antiseptic, laxative and to heal hypertension, venereal, skin and rheumatic diseases [3].

Seven different polyhydroxylated alkaloids identified from *I. carnea* showed an inhibitory activity toward *β*-glucosidase and *β*-mannosidase of rat lysosomes [4]. Likewise, the synergistically role of six polyphenols identified by HPLC-DAD analysis in *I. carnea* extracts has been reported to heal tumors and oxidative stress [5]. Umbelliferon, kaempferol 3-*O*-glucoside and kaempferol were separated from EA fraction of *I. carnea* flowers. Also, extracts from different parts were evaluated as antioxidant agents [6]. A new biflavonoid compound named Ipomoeflavoside was purified from *I. carnea* leaves [7]. Alcoholic extracts of different parts of *I. carnea* showed antioxidant and antitumor activities [7]. Moreover, hypolipidemic and antidiabetic activities of *I. carnea* alcoholic extract has been described using rat model [8]. Flowers methanolic extract had a high antioxidant activity, while leaves Bu fraction exhibited strong antibacterial and cell line cytotoxic activity [9]. Similarly, anticancer activity of hyroalcoholic extract of *I. carnea* has been studied *in-vivo* and *in-vitro* [10]. Interestingly, the brilliant anticancer property of octadecyl *p*-coumarates isomers purified from *I. carnea* has been recently stated [11]. Despite therapeutic and pharmacological properties of *I. carnea* described above, application of the plant extracts in pest and disease control still lack. However, essential oil of *I. carnea* stem and root had a repellent effect against Odoiporus longicollis [12]. Overall, plant different preparations such as volatile oils and solvent extracts have developed as a brilliant substitute to common synthetic insecticides for the control of agricultural

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pests in last decades. Mode of action of these preparations against insects may interfere with their role as repellents, antifeedants, respiration inhibitors or hinder the host plant identification [13]. Likewise, the larvicidal effects of plant secondary metabolites strongly affect the rate of oviposition and adult emergence.

*Aphis craccivora* is a very dangerous insect to a vast number of agricultural crops, especially legumes [14]. *A. craccivora* is a piercing sucking pest cause stunting and distorting in the growing crop with the possibility to secrete a honey dew stimulating the growth of some molds undesirably diminishing photosynthesis. The aphid is the vector of number of plant viruses counting peanut mottle virus and groundnut rosette virus [15]. White flies, (*Bemisia tabaci*) are a piercing sucking pest like *A. craccivora* which feed on the lower parts of plant leaves of many crops such as; cotton, potatoes, tomatoes and cucurbits. Also, this insect causes direct and indirect damage for plants by sucking sap and as a vector of virus diseases [16]. For those reasons, there was an urgent need to combat these pests by using control methods other than synthetic pesticides, as they cause many harms to humans, plants, animals and pollution of environment such as plant extracts. The obtainable literature has no informations about using solvent extracts or pure compounds of *I. carnea* to limit *A. craccivora* and *B. tabaci* populations. So, this work aims to obtain solvent fractions with potential insecticidal activity and to identify its active ingredients as well as studying the possibility of using these ingredients as natural antioxidants.

## 2. Materials and methods

### 2.1. Chemicals

Chitin from shrimp shells, acetyl choline bromide, guaiacol, 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), dinitro salicylic acid (DNSA), 1,1-Diphenyl-2picrylhydrzyl (DPPH), sodium lauryl sulphate and diazoblue B were purchased from Sigma-Aldrich (St. Louis, MO, USA). Preparative TLC silica gel Merck $GF_{254}$ precoated plates (20×20 cm) on aluminum sheets, silica gel (60-120 µm) for column chromatography India, polyamide S6 (Merck) for column chromatography, organic solvents, ammonia solution 25%, hydroxyl amine hydrochloride, $\alpha$-naphthyl acetate and $\beta$-naphthyl acetate were obtained from Edwic Company.

### 2.2. Plant material

*Ipomoea carnea* "*Convolvulaceae*" aerial parts were collected from the road between Jadela and Sandob in the city of Mansoura (31.04283ºN - 31.40627º E), Egypt in August 2020, the collected plant was identified by prof. Dr. Ibrahim Mashaly and Dr. Maha El-Shamy, Botany department, Faculty of science, Mansoura University according to Boulos [17]. A herbarium specimen was deposited in Herbarium of Botany department, faculty of science, Mansoura University under a special collection of A. El-Rokh.

### 2.3. Phytochemistry of *I. carnea*

#### 2.3.1. Extraction and liquid-liquid partitioning

The dried powder of *Ipomoea carnea* (8 kg) was extracted by methanol (16 L x 5). Crude methanol extract was concentrated using rotatory evaporator to volume of 1.5 L, then it was completely transferred to a separatory funnel containing 4.5 L of distilled $H_2O$. Successive extraction was done for the resultant extract using organic solvents with different polarities to obtain PT (235 g) which defatted with cold MeOH to give 50 gm, MC (20.7 g), EA (78.6 g) and Bu (23.2 g) fractions.

#### 2.3.2. Column Chromatography (CC)

##### 2.3.2.1. Silica Gel CC for MC fraction

MC fraction (15 g) was separated over silica gel column chromatography using PT/ EA as eluent system with increasing polarities till 50% ethyl acetate. Then, 100% MC was used as eluent and methanol was gradually added to give six sub fractions. Sub fraction **III** (90 mg) that obtained when the eluent solvent system consisted of 100% MC gave compound **1** after further purification using PTLC (silica gel, EA/PT, 1:4, $R_f$ = 0.44, 7 mg). Sub fraction V (Alkaloid sub fraction, AK-sF) which was obtained at eluent system MC/methanol (90:10) gave orange color on TLC with dragendorff's reagent.

##### 2.3.2.2. Polyamide CC for EA and Bu fractions

EA fraction (30g) was fractionated by polyamide column using distilled $H_2O$ (2L) followed by $H_2O$-MeOH gradually till 100% MeOH (1.5L) and then added acetone gradually as eluent. Six fractions were collected according to their TLC pattern. Fraction III (250 mg), which eluted from the polyamide CC by MeOH (100%), was purified by preparative TLC (silica gel, EtOAC-MeOH-$H_2O$, 14:0.5:0.1) to give compound (**2**) ($R_f$ 0.48, 15 mg). As well as, Polyamide column of Bu fraction (15 g) was eluted by distilled $H_2O$ (3.5L) as initial mobile phase followed by 1.5L mixture of $H_2O$-MeOH (1:1) and pure MeOH (1L). Finally, acetone was added gradually as eluent. The resultant effluents were combined to five sub fractions affording to their TLC pattern. Sub fraction II (200 mg), resulted from the existing column by $H_2O$:MeOH (50:50), gave by preparative TLC (silica gel, EA-MeOH-$H_2O$, 12:1:0.4) compound (**3**) ($R_f$ 0.55, 11 mg). Sub fraction IV which was obtained at eluent system acetone/MeOH (30:70) led to compound (**4**) and a binary mixture of compounds (**5**) and (**6**) after purification through TLC (silica gel, EA-MeOH-$H_2O$, 8.0:1.3:0.2), ($R_f$ 0.36, 13 mg and $R_f$ 0.59, 15 mg respectively).

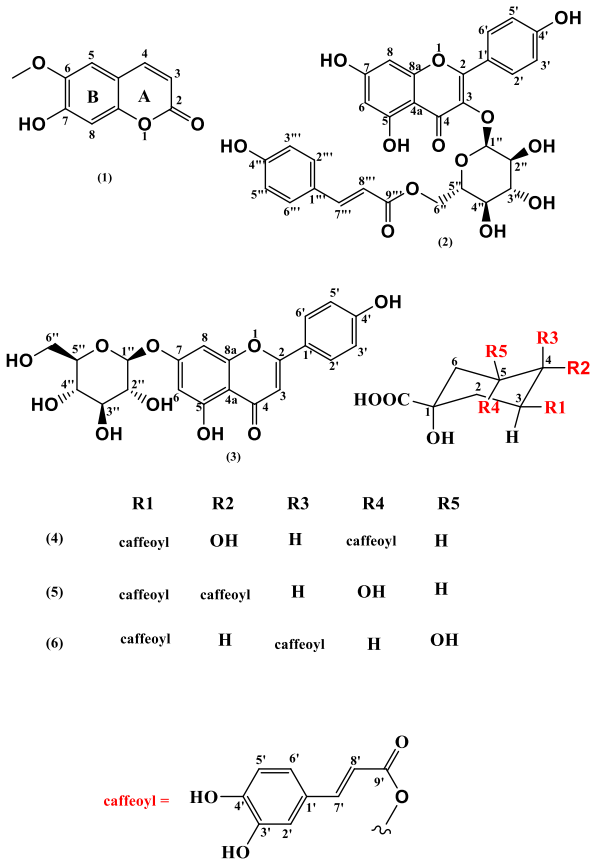


Figure 1: Chemical structures of compounds (1-6)

### 2.3.3. Characterization of separated compounds

The structural characterization of the bioactive compounds **(1-6)** (Figure 1) was carried out by proton nuclear magnetic resonance ($^1$H NMR) using Bruker 400 and JEOL 500 MHz spectrometers in $CD_3OD$, DMSO and $CDCl_3$ with tetramethylsilane (TMS) as an internal standard.

**Scopoletin (1):**

A pale yellow residue (7 mg), $^1$H NMR (500 MHz) in ($CDCl_3$), $\delta_H$ 6.27 (d, *J* 9.5 Hz, H-3), 7.60 (d, *J* 9.5 Hz, H-4), 6.85 (s, H-5), 6.92 (s, H-8), 3.96 (s, $OCH_3$). U.V cm $^{-1}$ : 280, 344 and after adding shift reagent (Sodium acetate) 278, 344 and 390.

**Tiliroside (2):**

Amorphous yellow powder, (15 mg), $^1$H NMR (400 MHz) in DMSO-*d6*, $\delta_H$ 5.72 (d, *J* 1.0 Hz, H-6), 5.85 (d, *J* 1.0 Hz, H-8), 7.90 (d, J 8.7 Hz, 2H-2',6'), 6.78 (d, *J* 8.7 Hz, 2H-3',5'), 5.28 (d, *J* 7.4 Hz, H-1"), 3.40 (m, H-2"), 3.26 (m, H-3"), 3.17 (m, H-4"), 3.23 (m, H-5"), 4.30 (dd, *J* 1.3, 11.9 Hz, 1H-6a), 4.00 (dd, *J* 6.3, 11.9 Hz, 1H-6b), 7.36 (d, *J* 8.6 Hz, 2H-2"', 6"'), 6.79 (d, *J* 8.6 Hz, 2H-3"', 5"'), 7.36 (d, *J* 16.0 Hz, 1H-7"') and 6.14 (d, *J* 16.0 Hz, 1H-8"'); $^{13}$C NMR (100 MHz, DMSO-*d6*) $\delta_C$ 154.3 (C-2), 132.6 (C-3), 175.1 (C-4), 161.3 (C-5), 102.4 (C-6), 161.3 (C-7), 96.0 (C-8), 157.4 (C-8a), 104.5 (C-4a), 120.8 (C-1'), 130.4 (C-2',6'), 115.1 (C-3', 5'), 160.4 (C-4'), 102.5 (C-1"), 74.2 (C-2",5"), 76.5 (C-3"), 70.0 (C-4"), 63.7 (C-6"), 113.1 (C-8"'$\alpha$), 145.1 (C-7"'$\beta$), 124.1 (C-1"'), 130.3 (C-2"', 6"'), 116.2 (C-3"', 5"'), 160.7 (C-4"'), 166.5 (C-9"').

**Apigenin-7$\beta$-*O*-glycoside (3)**:

Amorphous yellow powder, (11 mg), $^1$H NMR (400 MHz) in ($CD_3OD$), $\delta_H$ 6.65 (s, H-3), 6.50 (d, *J* 2.0 Hz, H-6), 6.82 (d, *J* 2.0 Hz, H-8), 7.88 (d, *J* 8.7 Hz, 2H-2', 6'), 6.92 (d, *J* 8.7 Hz, 2H-3', 5') and 5.07 (d, *J* 7.0 Hz, H-1"), 3.50 (m, H-2"), 3.50 (m, H-3"), 3.56 (m, H-4"), 3.42 (m, H-5"), 3.94 (dd, *J* 1.8, 12.4 Hz, 1H-6a) and 3.72 (dd, *J* 5.6, 12.4Hz, 1H-6b).

**3,5- di-*O*-caffeoylquinic acid (4):**

Yellow powder, (13 mg), $^1$H NMR (400 MHz) in ($CD_3OD$), $\delta_H$ 1.86-2.15 (m, H-2a), 2.27 (dd, *J* 3.3. 15.5 Hz, H-2b), 5.53 (td, *J* 10.8, 10.8, 5.0 Hz, H-3), 3.91 (dd, *J* 3.3, 10.0 Hz, H-4), 5.39 (td, *J* 6.0, 6.0, 3.2 Hz, H-5), 1.86-2.15 (m, 2H-6), 7.07 (d, *J* 1.8 Hz, H-2'), 6.79 (d, *J* 8.2 Hz, H-5'), 6.95 (dd, *J* 1.8, 7.9 Hz, H-6'), 7.61 (d, *J* 15.8 Hz, H-7'), 6.42 (d, *J* 15.8 Hz, H-8'), 7.09 (d, *J* 1.9 Hz, H-2"), 6.79 (d, *J* 8.2 Hz, H-5"), 6.97 (dd, *J* 1.9, 8.0 Hz, H-6"), 7.60 (d, *J* 15.8 Hz, H-7"), 6.31 (d, *J* 15.8 Hz, H-8").

**3,4- di-*O*-caffeoylquinic acid isomers (5 and 6):**

Yellow powder, (15 mg), $^1$H NMR (400 MHz) in ($CD_3OD$), compound (**5**) $\delta_H$ 1.85-2.35 (m, 2H-2), 5.69 (td, *J* 10.4, 10.4, 5.2 Hz, H-3), 5.11 (dd, *J* 2.7, 10.2 Hz, H-4), 4.35 (d, *J* 1.9 Hz, H-5), 1.85-2.55 (m, 2H-6), 7.03 (d, *J* 1.9 Hz, H-2'), 6.75 (d, *J* 8.2 Hz, H-5'), 6.87 (dd, *J* 1.9, 8.2 Hz, H-6'), 7.58 (d, *J* 15.8 Hz, H-7'), 6.27 (d, *J* 15.8 Hz, H-8'), 7.00 (d, *J* 1.9 Hz, H-2"), 6.75 (d, *J* 8.2 Hz, H-5"), 6.89 (dd, *J* 1.9, 8.2 Hz, H-6"), 7.50 (d, *J* 15.8 Hz, H-7"), 6.19 (d, *J* 15.8 Hz, H-8") and compound (**6**) 1.85-2.35 (m, 2H-2), 5.62 (dt, *J* 3.0,3.0, 8.0 Hz, H-3), 5.19 (m, H-4), 4.15 (m, H-5), 1.85-2.55 (m, 2H-6), 7.08 (d, *J* 1.8 Hz, H-2'), 6.76 (d, *J* 8.2 Hz, H-5'), 6.89 (dd, *J* 1.8, 8.2 Hz, H-6'), 7.59 (d, *J* 15.8 Hz, H-7'), 6.31 (d, *J* 15.8 Hz, H-8'), 7.04 (d, *J* 1.8 Hz, H-2"), 6.78 (d, *J* 8.2 Hz, H-5"), 6.93 (dd, *J* 1.8, 8.2 Hz, H-6"), 7.52 (d, *J* 15.8 Hz, H-7"), 6.25 (d, *J* 15.8 Hz, H-8").

### 2.3.4. GC/MS analysis

GC/MS analysis was performed in Central Laboratory of Pesticides, Agricultural Research Center, using Finnegan SSQ 7000 GC to identify the compounds in PT and MC fractions (**7-41**) according to the details previously documented [18,19].

### 2.3.5. UPLC/MS/MS analysis

Compounds **(42-54)** found in AK-sF (table 2) were separated and quantified in Center for Drug Discovery Research and development, Faculty of Pharmacy, Ain Shams University, using Waters Acquity UPLC H-Class (Milford, MA, USA) and Xevo TQ-D micro MS system (Milford, MA, USA) with an ESI source operating in positive and negative ion acquisition mode according the details by Elsherbiny *et al* [20].

### 2.4. Antioxidant activity

The radical scavenging activities of crude extract , its fractions and sub fractions (12.5-500 µg $ml^{-1}$) as well as the compounds (**1-6**) at concentrations ranged between 5-50 µg $ml^{-1}$ were evaluated in three replicates by using the DPPH assay according the details of Darwish *et al* [21].

### 2.5. Evaluation of insecticidal activity

#### 2.5.1. Rearing of tested pests

The strains of *A. craccivora* and *B. tabaci* were separately collected from the farms of Mansoura University and were known to be free from contamination of insecticides. The colony of aphids was maintained on cowpea leaves under natural conditions in plastic greenhouse (3 × 2 × 2 m). The colony of whiteflies was maintained on cucumber leaves in entomological cages (1.5 × 1.5 × 1m) under natural conditions.

#### 2.5.2. Bioassay on *A. craccivora* by spray method

The tested plant samples were examined as insecticides by spray method on cowpea leaves. Plant extracts and their isolated components were individually dissolved in Tween 80 in water to prepare stock solutions. A series of diluted five concentrations was prepared from each stock (Four replicates per each concentration). The discs of cowpea were made in a diameter of 3.5 cm and placed over agar medium (1.5%) in Petri dishes. Ten aphids in each replicate were transferred and maintained in a petri dish for 30 minutes before treatment. Finally, each petri dish holding aphids and cowpea discs was sprayed by the tested sample in a total volume of 2 mL, then covered and kept at room temperature. Whereas, leaf discs of control were sprayed with the same volume of distilled water containing Tween 80. Mortality numbers were recorded after one day. The mortality percentages were corrected by Abbott's formula [22]. The corrected mortality percentages of each extract and compound were subjected to probit analysis according to Finney [23] for obtaining the LC50's, LC90's and their confidence limits, as well as slope of regression lines (LC-P line). Also, toxicity index was measured by using sun's equation [24].

#### 2.5.3. Bioassay on *Bemisia tabaci* nymphs

This trial was conducted on the third instar nymphs of *B. tabaci*. So, adults of *B. tabaci* were placed in 2 cm diameter clip cages (15 insects/cage) containing cucumber plant leaves [25]. After a period of 15 days, leaves surrounded by about 30 young nymphs were rounded into sections (~2 cm in diameter). Then , discs retaining the suitable number of nymphs were carefully transferred over agar medium (2%) in Petri dishes. A series of diluted five concentrations in ppm of *I. carnea* tested samples were applied on the third instar nymphs in a total volume of 2 ml. After one day of application, mortality was noticed [26]. Also, toxicity index was determined by using Sun's equation [24].

#### 2.5.4. Assay of insect enzymes

##### 2.5.4.1. Extraction of enzymes from insects

A number of 50 individuals per each replicate was homogenized in 5 mL of ice-cold phosphate buffer (50 mM, pH 7.2) using a glass type homogenizer as stated by Abdelaal *et al* [27]. The resultant homogenate was then centrifuged at 10000 rpm for 10 min at 4 ºC. Insoluble cell debris were discarded, and the resultant supernatant was obtained and kept at −20 ◦C as a source of the enzymes to be tested**.**

##### 2.5.4.2. The activity of insect enzymes

The activity of $\alpha$-EST and $\beta$-EST [28], AChE [29], (POD) [30], GST [31-32] and CTase [33] were evaluated in each supernatant in the presence of related substrates. Total protein contents were determined by the method of Bradford [34].

### 2.6. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA). The analysis was conducted using CoStat software (version 6.400, 798 lighthouse Ave, PMB 320, Montere, CA, 93940, USA). The significant difference of the means was determined by Fishers' least significant difference (LSD) test at $P < .05$. The $LC_{50}$ and $LC_{90}$ values of all extracts were determined using probit analysis.

Table 1: Chemical constituents identified by GC/MS technique from PE and MC fractions of *I. carnea*

| Compound Name | R.T | MF | Mol. Wt. | % of compounds | | m/z (ret. int. %) |
|---|---|---|---|---|---|---|
| | | | | PE | MC | |
| ***p*-Isopropyl benzaldehyde (7)** | 6.83 | $C_{10}H_{12}O$ | 148 | 1.06 | 1.14 | 148 (55%) $[M^{+\cdot}]$, 133 (95%) $[M^{+}\text{-}CH_3]$, 119 (38%) $[C_9H_{11}]^{+}$,105 (100%) $[C_7H_5O]^{+}$, 91 (41%) $[C_7H_7]^{+}$, 77 (55%) $[C_6H_5]^{+}$. |
| ***p*-Cymen-7-ol (8)** | 7.86 | $C_{10}H_{14}O$ | 150 | - | 1.39 | 150(40%) $[M^{+\cdot}]$, 135(100%) $[M - CH_3]^{+}$, 105 (67%) $[C_8H_9]^{+}$, 91 (57%) $[C_7H_7]^{+}$, 79 (72%) $[C_6H_7]^{+}$. |
| **Thymol (9)** | 8.05 | $C_{10}H_{14}O$ | 150 | 2.93 | - | 150 (33%) $[M^{+\cdot}]$, 135 (100%) $[M^{+} - CH_3]$, 107 (24%) $[C_9H_{11}]^{+}$, 91 (29%) $[C_7H_7]^{+}$, 77 (15%) $[C_6H_5]^{+}$. |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Dodecanoic acid, methyl ester (10)** | 12.29 | $C_{13}H_{26}O_2$ | 214 | 0.57 | - | 214 (1%) $[M^{+\cdot}]$, 171(7%) $[M - C_3H_7]^+$, 143 (11%) $[C_8H_{15}O_2]^+$, 83 (57%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{\cdot+}$ |
| **5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-2(4H)-Benzofuranone (11)** | 12.42 | $C_{11}H_{16}O_2$ | 180 | - | 0.92 | 180 (9%) $[M^{+\cdot}]$, 165(3%) $[M - CH_3]^+$, 152 (9%) $[M\text{-}CO]^+$, 137 (43%) $[C_{10}H_{17}]^+$, 111 (100%) $[C_6H_7O_2]^+$, 67 (54%) $[C_5H_7]^+$. |
| **Caryophyllene oxide (12)** | 13.45 | $C_{15}H_{24}O$ | 220 | 2.95 | 1.59 | 220 (0.5%) $[M^{+\cdot}]$, 205(2%) $[M - CH_3]^+$, 109 (38%) $[C_8H_{13}]^+$, 93 (74%) $[C_7H_9]^+$, 91 (79%) $[C_7H_7]^+$, 79 (100%) $[C_6H_7]^+$, 69 (53%) $[C_4H_5O]^+$. |
| **Ar-Tumerone (13)** | 14.99 | $C_{15}H_{20}O$ | 216 | 1.47 | - | 216 (7%) $[M^{+\cdot}]$, 201(9%) $[M - CH_3]^+$, 132 (17%) $[C_{10}H_{12}]^+$, 119 (52%) $[C_9H_{11}]^+$, 83 (100%) $[C_5H_7O]^+$ |
| ***β*-Tumerone (14)** | 15.66 | $C_{15}H_{22}O$ | 218 | 0.39 | - | 218 (2%) $[M^{+\cdot}]$, 120(100%) $[C_9H_{12}]^+$, 105 (41%) $[C_8H_9]^+$, 91 (28%) $[C_7H_7]^+$ , 83 (40%) $[C_5H_7O]^+$, 55 (20%) $[C_4H_7]^+$. |
| **Methyl tetradecanoate (15)** | 16.06 | $C_{15}H_{30}O_2$ | 242 | 1.67 | 0.93 | 242 (2%) $[M^{+\cdot}]$, 199(8%) $[M - C_3H_7]^+$, 157 (5%) $[C_9H_{17}O_2]^+$, 143 (16%) $[C_8H_{15}O_2]^+$, 87 (55%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{\cdot+}$. |
| **6-Hydroxy-4,4,7a-trimethyl-5,6,7,7atetrahydrobenzofuran-2(4H)-one (16)** | 16.96 | $C_{11}H_{16}O_3$ | 196 | - | 2.66 | 196 (3%) $[M^{+\cdot}]$, 178(40%) $[M - H_2O]^+$, 163 (34%) $[C_{10}H_{11}O_2]^+$, 135 (48%) $[C_{10}H_{15}]^+$, 111 (100%) $[C_6H_7O_2]^+$, 95 (55%) $[C_5H_3O_2]^+$, 79 (50%) $[C_6H_7]^+$, 67 (54%) $[C_5H_7]^+$. |
| **(1R,4R,6R,10R)-4,9,12,12-tetramethyl-5-oxatricyclo[8.2.0.04,6]dodecane (17)** | 17.17 | $C_{15}H_{26}O$ | 222 | - | 1.14 | 222 (2%) $[M^{+\cdot}]$, 193(3%) $[C_{13}H_{21}O]^+$, 179 (31%) $[C_{12}H_{19}O]^+$, 161 (14%) $[C_{11}H_{13}O]^+$, 91 (67%) $[C_7H_7]^+$, 79 (100%) $[C_6H_7]^+$, 67 (74%) $[C_5H_7]^+$. |
| **Z-*α*-Bisabolene epoxide (18)** | 17.60 | $C_{15}H_{24}O$ | 220 | - | 0.96 | 220 (3%) $[M^{+\cdot}]$, 179(16%) $[C_{12}H_{19}O]^+$, 147 (28%) $[C_{11}H_{15}]^+$, 123 (67%) $[C_9H_{15}]^+$, 109 (88%) $[C_8H_{13}]^+$, 81 (100%) $[C_6H_9]^+$, 69 (74%) $[C_5H_9]^+$. |
| **Pentadecanoic acid, methyl ester (19)** | 17.83 | $C_{16}H_{32}O_2$ | 256 | 0.68 | - | 256 (3%) $[M^{+\cdot}]$, 213 (6%) $[M - C_3H_7]^+$, 157 (5%) $[C_9H_{17}O_2]^+$, 185 (3%) $[C_9H_{17}O_2]^+$, 143 (14%) $[C_8H_{15}O_2]^+$, 87 (41%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{\cdot+}$ |
| **8-(4-octen-4-yl)-4-Cycloocten-1-one (20)** | 18.06 | $C_{16}H_{26}O$ | 234 | 0.63 | - | 234 (3%) $[M^{+\cdot}]$, 191 (29%) $[C_{13}H_{19}O]^+$, 123 (81%) $[C_8H_{11}O]^+$, 109 (28%) $[C_7H_9O_2]^+$, 95 (86%) $[C_6H_7O]^+$, 81 (100%) $[C_5H_5O_2]^+$, 69 (79%) $[C_5H_9]^+$ |
| **2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)propanal (21)** | 18.07 | $C_{15}H_{22}O_2$ | 234 | - | 1.39 | 234 (2%) $[M]^{+\cdot}$, 219 (4%) $[M - CH_3]^+$, 191 (22%) $[C_{13}H_{19}O]^+$, 123 (66%) $[C_8H_{11}O]^+$, 95 (100%) $[C_7H_{11}]^+$, 81 (95%) $[C_6H_9]^+$, 69 (88%) $[C_4H_5O]^+$, 57 (60%) $[C_3H_5O]^+$. |
| **6,10,14-trimethyl-2-Pentadecanone (22)** | 18.17 | $C_{18}H_{36}O$ | 268 | 1.86 | - | 268 (0.5%) $[M^{+\cdot}]$, 210 (1%) $[C_{15}H_{30}]^+$, 165 (7%) $[C_{11}H_{17}O]^+$, 124 (17%) $[C_8H_{12}O]^+$ , 109 (41%) $[C_8H_{13}]^+$, 95 (48%) $[C_6H_7O]^+$, 71 (76%) $[C_4H_7O]^+$, 58 (100%) $[C_3H_6O]^{\cdot+}$. |
| **Isoaromadendrene epoxide (23)** | 18.44 | $C_{15}H_{24}O$ | 220 | - | 2.22 | 220 (9%) $[M^{+\cdot}]$, 177(24%) $[C_{12}H_{17}O]^+$, 159 (29%) $[C_{12}H_{15}]^+$, 121 (55%) $[C_9H_{13}]^+$, 107 (74%) $[C_7H_7O]^+$, 93 (78%) $[C_7H_9]^+$, 81 (100%) $[C_6H_9]^+$. |
| **1-Hexadecanol (24)** | 18.78 | $C_{16}H_{34}O$ | 242 | 0.50 | - | 242 (0.5%) $[M^{+\cdot}]$, 224 (1%) $[M\text{-}H_2O]^{+\cdot}$, 196 (1%) $[C_{14}H_{28}]^+$, 125 (10%) $[C_9H_{17}]^+$ , 111 (31%) $[C_8H_{15}]^+$, 97 (60%) $[C_7H_{13}]^+$, 83 (67%) $[C_6H_{11}]^+$, 69 (100%) $[C_5H_9]^+$. |
| **(E)-octadec-9-en-12-ynoic acid (25)** | 18.78 | $C_{18}H_{30}O_2$ | 278 | - | 0.85 | 278 (4%) $[M^{+\cdot}]$, 234 (0.5%) $[C_{15}H_{22}O_2]^{+\cdot}$, 195 (3%) $[C_{12}H_{19}O_2]^+$, 123 (29%) $[C_9H_{15}]^+$, 111 (38%) $[C_8H_{15}]^+$, 95 (69%) $[C_7H_{11}]^+$, 83 (78%) $[C_6H_{11}]^+$, 69 (100%) $[C_5H_9]^+$, 55 (92%) $[C_4H_7]^+$. |
| **9(z)-hexadecenoic acid, methyl ester (26)** | 19.17 | $C_{17}H_{32}O_2$ | 268 | 0.65 | - | 268 (2%) $[M^{+\cdot}]$, 236 (10%) $[C_{16}H_{30}O]^{+\cdot}$, 194 (9%) $[C_{13}H_{22}O]^+$, 152 (14%) $[C_{10}H_{16}O]^+$, 123 (22%) $[C_9H_{15}]^+$, 96 (45%) $[C_7H_{12}]^+$, 83 (57%) $[C_6H_{11}]^+$, 69 (79%) $[C_5H_9]^+$, 55 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | (100%) $[C_4H_7]^+$. |
| **Hexadecanoic acid, methyl ester (27)** | 19.65 | $C_{17}H_{34}O_2$ | 270 | 23.00 | 20.43 | 270 (4%) $[M^{+\cdot}]$, 227 (9%) $[M - C_3H_7]^+$, 199 (7%) $[C_{12}H_{23}O_2]^+$, 171 (9%) $[C_{10}H_{19}O_2]^+$, 143 (20%) $[C_8H_{15}O_2]^+$, 87 (71%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{\cdot+}$. |
| ***n*-Hexadecanoic acid (28)** | 20.42 | $C_{16}H_{32}O_2$ | 256 | 5.44 | 6.55 | 256 (10%) $[M^{+\cdot}]$, 227 (3%) $[C_{14}H_{27}O_2]^+$, 213 (11%) $[C_{13}H_{25}O_2]^+$, 199 (7%) $[C_{12}H_{23}O_2]^+$, 157 (16%) $[C_9H_{17}O_2]^+$, 129 (34%) $[C_7H_{13}O_2]^+$, 115 (14%) $[C_6H_{11}O_2]^+$, 73 (100%) $[C_3H_5O_2]^+$. |
| **Heptadecanoic acid, methyl ester (29)** | 21.16 | $C_{18}H_{36}O_2$ | 284 | 0.68 | - | 284 (3%) $[M^{+\cdot}]$, 241 (6%)$[C_{15}H_{29}O_2]^+$, 185 (6%) $[C_{11}H_{21}O_2]^+$, 171 (9%) $[C_{10}H_{19}O_2]^+$, 143 (15%) $[C_8H_{15}O_2]^+$, 129 (6%) $[C_7H_{13}O_2]^+$, 87 (68%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{\cdot+}$. |
| **2-Methyl-1-hexadecanol (30)** | 22.07 | $C_{17}H_{36}O$ | 256 | - | 0.67 | 256 (0.5%) $[M^{+\cdot}]$, 139 (7%) $[C_{10}H_{19}]^+$, 125 (16%) $[C_9H_{17}]^+$, 111 (33%) $[C_8H_{15}]^+$, 97 (74%) $[C_7H_{13}]^+$, 83 (91%) $[C_6H_{11}]^+$, 69 (100%) $[C_5H_9]^+$, 55 (88%) $[C_4H_7]^+$. |
| **Linoleic acid, methyl ester (31)** | 22.28 | $C_{19}H_{34}O_2$ | 294 | 17.42 | 10.64 | 294 (3%) $[M^{+\cdot}]$, 262 (7%) $[C_{18}H_{30}O]^{+\cdot}$, 220 (1%) $[C_{15}H_{24}O]^+$, 164 (3%) $[C_{12}H_{20}]^+$, 135 (10%) $[C_{10}H_{15}]^+$, 109 (22%) $[C_8H_{13}]^+$, 95 (52%) $[C_7H_{11}]^+$, 81 (81%) $[C_6H_9]^+$, 67 (100%) $[C_5H_7]^+$. |
| **Oleic acid, methyl ester (32)** | 22.34 | $C_{19}H_{36}O_2$ | 296 | - | 21.63 | 296 (2%) $[M^{+\cdot}]$, 264 (15%) $[C_{18}H_{32}O]^{+\cdot}$, 222 (6%) $[C_{15}H_{26}O]^+$, 180 (7%) $[C_{12}H_{20}O]^+$, 97 (48%) $[C_7H_{13}]^+$, 69 (74%) $[C_5H_9]^+$, 55 (100%) $[C_4H_7]^+$. |
| **11-Octadecenoic acid, methyl ester (33)** | 22.44 | $C_{19}H_{36}O_2$ | 296 | 22.92 | - | 296 (2%) $[M^{+\cdot}]$, 264 (17%) $[C_{18}H_{32}O]^{+\cdot}$, 222 (8%) $[C_{16}H_{31}]^+$, 180 (9%) $[C_{13}H_{24}]^+$, 97 (60%) $[C_7H_{13}]^+$, 83 (68%) $[C_6H_{11}]^+$, 69 (79%) $[C_5H_9]^+$, 55 (100%) $[C_4H_7]^+$. |
| **Phytol (34)** | 22.55 | $C_{20}H_{40}O$ | 296 | 0.89 | 0.60 | 296 (0.5%) $[M^{+\cdot}]$, 264 (0.5%) $[C_{19}H_{36}]^{+\cdot}$, 123 (20%) $[C_9H_{15}]^+$, 95 (17%) $[C_7H_{11}]^+$, 81 (27%) $[C_6H_9]^+$, 71 (100%) $[C_4H_7O]^+$. |
| **Methyl stearate (35)** | 22.77 | $C_{19}H_{38}O_2$ | 298 | 10.21 | 7.17 | 298 (6%) $[M^{+\cdot}]$, 255 (9%)$[C_{16}H_{31}O_2]^+$, 199 (8%) $[C_{12}H_{23}O_2]^+$, 143 (19%) $[C_8H_{15}O_2]^+$ , 87 (68%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{\cdot+}$. |
| **Linoleic acid (36)** | 22.97 | $C_{18}H_{32}O_2$ | 280 | - | 6.05 | 280 (4%) $[M^{+\cdot}]$, 150 (6%) $[C_{11}H_{18}]^{+\cdot}$, 123 (9%) $[C_9H_{15}]^+$, 109 (21%) $[C_8H_{13}]^+$, 95 (31%) $[C_7H_{11}]^+$, 81 (76%) $[C_6H_9]^+$, 67 (100%) $[C_5H_7]^+$, 55 (60%) $[C_4H_7]^+$. |
| **Oleic acid (37)** | 23.06 | $C_{18}H_{34}O_2$ | 282 | - | 7.39 | 282 (1%) $[M^{+\cdot}]$, 264 (6%) $[C_{18}H_{32}O]^{+\cdot}$, 222 (2%) $[C_{16}H_{31}]^+$, 180 (9%) $[C_{13}H_{24}]^+$, 97 (60%) $[C_7H_{13}]^+$, 83 (68%) $[C_6H_{11}]^+$, 69 (79%) $[C_5H_9]^+$, 55 (100%) $[C_4H_7]^+$. |
| **Octadecanoic acid (38)** | 23.38 | $C_{18}H_{36}O_2$ | 284 | 0.80 | 1.14 | 284 (10%) $[M^{+\cdot}]$, 255 (2%) $[C_{16}H_{31}O_2]^+$, 241 (12%) $[C_{15}H_{29}O_2]^+$, 199 (7%) $[C_{12}H_{23}O_2]^+$, 185 (16%) $[C_{11}H_{21}O_2]^+$ , 129 (12%) $[C_7H_{13}O_2]^+$, 97 (43%) $[C_7H_{13}]^+$, 73 (100%) $[C_3H_5O_2]^+$. |
| **Eicosanoic acid, methylester (39)** | 25.64 | $C_{21}H_{42}O_2$ | 326 | 2.34 | 1.09 | 326 (3%) $[M^{+\cdot}]$, 283 (7%)$[C_{18}H_{35}O_2]^+$, 227 (3%) $[C_{14}H_{27}O_2]^+$, 143 (16%) $[C_8H_{15}O_2]^+$, 129 (6%) $[C_7H_{13}O_2]^+$, 87 (60%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{\cdot+}$. |
| **Docosanoic acid, methyl ester (40)** | 28.33 | $C_{23}H_{46}O_2$ | 354 | 0.94 | - | 354 (4%) $[M^{+\cdot}]$, 311 (4%) $[C_{20}H_{39}O_2]^+$, 255 (4%) $[C_{16}H_{31}O_2]^+$, 199 (6%) $[C_{12}H_{23}O_2]^+$, 143 (17%) $[C_8H_{15}O_2]^+$, 87 (66%) $[C_4H_7O_2]^+$, 74 (100%) $[C_3H_6O_2]^{+\cdot}$. |
| **9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (41)** | 30.38 | $C_{21}H_{40}O_4$ | 356 | - | 1.45 | 356 (0.5%) $[M^{+\cdot}]$, 264 (14%) $[C_{17}H_{28}O_2]^+$, 235 (3%) $[C_{16}H_{27}O]^+$, 207 (6%) $[C_{13}H_{19}O_2]^+$, 151 (8%) $[C_{10}H_{15}O]^+$, 137 (14%) $[C_{10}H_{17}]^+$, 98 (67%) $[C_7H_{14}]^+$, 81 (68%) $[C_6H_9]^+$, 67 (79%) $[C_5H_7]^+$, 55 (100%) $[C_4H_7]^+$. |

## 3. Results and discussion

### 3.1. Identification of compounds (1-6)

Compound **1** was isolated from MC fraction by CC and purified by PTLC (*c.f.* Materials and methods) as a pale-yellow residue (7 mg). Compound (1) was identified as **scopoletin** by $^1$H-NMR and U.V spectra which in agreement with that isolated before from *Zygophyllum coccineum* [35] and from the hydro-ethanolic extract of *Canarium schweinfurthii* [36]. Besides, scopoletin has been recently identified from other plants related to the same genus as *Ipomoea cairica* ethanolic extract as a major compound [37].

Compound (**2**) was isolated from EA fraction as amorphous yellow powder and appeared as a dark yellow colour with *p*-anisaldehyde spray reagent. $^1$H NMR and APT $^{13}$C NMR spectra showed the presence of flavonoid nucleus at $\delta_H$ 5.72 (d, *J* 1.0 Hz, H-6), $\delta_C$ 102.4 and $\delta_H$ 5.85 (d, *J* 1.0 Hz, H-8), $\delta_C$ 96.0 for ring A, in addition to AA'BB' system at $\delta_H$ 6.78 (d, *J* 8.7 Hz, 2H-3',5'), $\delta_C$ 115.1 and $\delta_H$ 7.90 (d, *J* 8.7 Hz, 2H-2',6'), $\delta_C$ 130.4. The above results mentioned of compound (**2**), **tiliroside** were in agreement with Liu *et al* [38].

Compound (**3**) was purified from Bu fraction as amorphous yellow powder and appeared as a pale yellow colour with *p*-anisaldehyde spray reagent. $^1$H NMR spectrum showed that compound (3) was identified as **Apigenin-7*β*-*O*-glycoside** which its NMR data agreed with those of isolated before [39-40].

Compound (**4**) was isolated as yellow powder and appeared as a dark yellow colour with *p*-anisaldehyde spray reagent. $^1$H NMR spectrum evaluated that compound (**4**) was identified as **3,5- di-*O*-caffeoylquinic acid** which described by Lee *et al.* [41]. Compounds (**5**) and (**6**) were isolated as isomeric binary mixture with ratio of (2:1), respectively as yellow powder, and dark yellow colour emerged up on treating with *p*-anisaldehyde spray reagent. $^1$H NMR spectrum of compound (**5**) reinforced that compound (**5**) was identified as **3,4-di-*O*-caffeoylquinic acid** [42,43]. As well as, $^1$H NMR spectrum of compound (**6**) revealed that compound (**6**) is similar to compound (**5**) with different conformation at C4 and C5 which oxygenated protons signals of quinic acid appeared at $\delta_H$ 4.15 (m, H-5), 5.19 (m, H-4) 5.62 (dt, *J* 3.0, 3.0, 8.0 Hz, H-3) [42,43].

Table 2: Characterization of thirteen alkaloids by positive mode UPLC/MS/MS.

| Compounds | RT | Area% | Mol. Formula | Mol. Wt. | $[M+H]^+$ | $[M+Na]^+$ | $[M+K]^+$ |
|---|---|---|---|---|---|---|---|
| **Ergoline (42)** | 6.33 | 0.15 | $C_{14}H_{16}N_2$ | 212 | - | - | 251 |
| **Penniclavine (43)** | 6.42 | 0.25 | $C_{16}H_{18}N_2O_2$ | 270 | - | 293 | - |
| ***N*-*trans*-feruloyl tyramine (44)** | 6.98 | 13.32 | $C_{18}H_{19}NO_4$ | 313 | 314 | - | - |
| ***N*-*cis*-feruloyl tyramine (45)** | 7.30 | 5.93 | $C_{18}H_{19}NO_4$ | 313 | 314 | - | - |
| **Ergonovine (46)** | 9.17 | 0.28 | $C_{19}H_{23}N_3O_2$ | 325 | - | 348 | - |
| **Lysergic acid (47)** | 9.31 | 0.16 | $C_{16}H_{16}N_2O_2$ | 268 | | 291 | - |
| **Setoclavine (48)** | 10.96 | 0.49 | $C_{16}H_{18}N_2O$ | 254 | - | 277 | - |
| **Elymoclavine (49)** | 11.04 | 0.98 | $C_{16}H_{18}N_2O$ | 254 | - | 277 | - |
| **Isosetoclavine (50)** | 11.31 | 1.00 | $C_{16}H_{18}N_2O$ | 254 | - | 277 | - |
| **Fumigaclavine B (51)** | 11.65 | 0.36 | $C_{16}H_{20}N_2O$ | 256 | - | 279 | - |
| **Chanoclavine I (52)** | 11.78 | 0.49 | $C_{16}H_{20}N_2O$ | 256 | - | 279 | - |
| **Agroclavine (53)** | 13.93 | 0.22 | $C_{16}H_{18}N_2$ | 238 | - | 261 | - |
| **Ergocristine (54)** | 20.54 | 3.65 | $C_{35}H_{39}N_5O_5$ | 609 | 610 | - | - |

### 3.2. GC/MS analysis.

PT and MC fractions were analyzed by the GC/MS technique to identify their major compounds. Table (1) displayed the identification of a total of 35 compounds from the chromatograms of these fractions depending on comparing their MS with those of their analogous itemized by NIST library. The identified compounds can be classified as follows:- Five monoterpenes **(7)**, **(8)**, **(9)**, **(11)** and **(16)**, eight sesquiterpenes **(12)**, **(13)**, **(14)**, **(17)**, **(18)**, **(21)**, **(22)** and **(23)**, one diterpene **(34)**, two saturated fatty acids **(28)** and **(38)** eight saturated fatty acid methyl esters (**4**), **(14)**, **(18)**, **(26)**, **(28)**, **(34)**, **(39)** and **(40)**, three unsaturated fatty acids **(25)**, **(36)** and **(37)**, five unsaturated fatty acid esters (**26**), **(31)**, **(32)**, **(33)** and **(41)** and three oxygenated hydrocarbons **(20)**, **(24)** and **(30)**.

Twenty-two compounds were identified in PT fraction, hexadecanoic acid methyl ester **(27)** and 11-Octadecenoic acid methyl ester **(33)** were the predominant components with percentages of 23.00 and 22.92, respectively. Also, twenty-three compounds were identified in MC fraction, the main components were oleic acid methyl ester **(32)** (21.63 %), hexadecanoic acid methyl ester **(27)** (20.43 %) and linoleic acid methyl ester **(31)** (10.64 %). Remarkably, ten compounds partitioned themselves between PT and MC fractions (**7**, **12**, **15**, **27**, **28**, **31**, **34**, **35**, **38** and **39**).

### 3.3. Identification of alkaloid sub-fraction by UPLC/MS/MS positive mode

Alkaloid sub fraction (AK-sF) was analyzed by positive mode UPLC/MS/MS, thirteen alkaloids **(42-54)** were identified (Table 2). Identification of the bioactive metabolites was based on interpretation of MS data as well as the comparison with the available literature data. All the proposed compounds from the AK-sF were summarized in Table 6. Eleven ergoline alkaloids (**42, 43, 46, 47, 48, 49, 50, 51, 52, 53** and **54**) were identified in addition to, two phenolic compounds (**44** and **45**). Noticeably, the phenolic compounds of N-*trans*-feruloyl tyramine (**44**) and N-*cis*-feruloyl tyramine (**45**) in addition to the ergot alkaloid of ergocristine (**54**) represented the highest peak areas in AK-sF as 13.32%,5.93% and 3.65%, respectively. Overall, the literature data regarding the phytochemistry of the plants *Ipomoea* genus displayed that the leading common bioactive ingredients are ergoline alkaloids [44]. Remarkably, the ergot alkaloids of **42, 45, 48, 51** and **52** have previously isolated but from the seeds of four other species of *Ipomoea* genus [45]. To our knowledge, it is the first time to report the identification of these ergoline alkaloids from *I. carnea* **(41, 42, 45, 46, 47, 48, 49, 50, 51** and **52**).

### 3.4. The antioxidant activity

The antiradical activity of the crude extract of *I. carnea* and its solvent fractions and sub-fractions were compared using DPPH assay (Figure 2). Remarkably, Bu fraction had the highest scavenging activity against DPPH radical in comparing with other tested extract/fractions. It recorded inhibition percentages of 57.05 and 94.32 when tested at concentrations of 100 and 250 µg/ml, respectively (Figure 2). The lower the $IC_{50}$ value, the higher the antiradical potential of studied sample. The findings displayed in table (3), exhibited the high antioxidant potency of *I. carnea* Bu fraction ( $IC_{50}$ : 85.21 µg/ml) followed by AK-sF ( $IC_{50}$ : 93.27 µg/ml) , methanolic extract ( $IC_{50}$ : 122.67 µg/ml), EA fraction ( $IC_{50}$ : 186.92 µg/ml) , MC fraction ( $IC_{50}$ : 459.78 µg/ml) and lastly PT fraction ( $IC_{50}$ : 1582.16 µg/ml). The highest $IC_{50}$ of *I. carnea* petroleum ether fraction reflects the poor antioxidant potential of this fraction. The moderate antioxidant activity of *I. carnea* especially for Bu fraction largely agreed with the findings obtained by Abbasi *et al* [46]. They showed that all solvent fractions of *I. carnea* have notable antioxidant activity and *n*- butanol had the highest value of % scavenging of DPPH radical with $IC_{50}$ value of 74.65 which was comparable to BHT. Noticeably, the crude methanolic extract of *I. carnea* showed higher antiradical activity than all its fractions except for butanol fraction and AK-sF, reflecting the synergistic potential of their constituents as antioxidant agents.

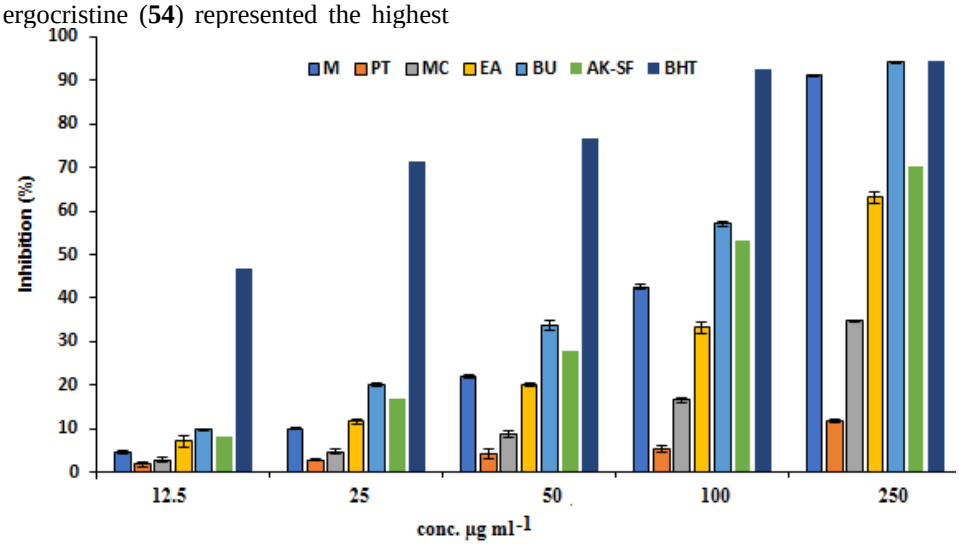


Figure 2: percentages of inhibition of DPPH radical by *I. carnea* extract/fractions

**Table 3: $IC_{50}$ values of DPPH radical scavenging activity**

| Extracts /pure compounds | $IC_{50}$ | Unit |
|---|---|---|
| Methanol extract (MeOH) | 122.67 | µg ml$^{-1}$ |
| Petroleum ether fraction (PT) | 1582.16 | |
| Methylene chloride fraction (MC) | 459.78 | |
| Alkaloid sub fraction (AK-sF) | 93.27 | |
| Ethyl acetate fraction (EA) | 186.92 | |
| Butanol fraction (Bu) | 85.21 | |
| Scopoletin **(1)** | 1766.75 | µM |
| Tiliroside **(2)** | 114.33 | |
| Apigenin-7*β*-*O*-glucoside **(3)** | 89.1 | |
| 3,5- di-*O*-caffeoylquinic acid **(4)** | 10.34 | |
| 3,4- di-*O*-caffeoylquinic acid **(5)(6)** | 10.41 | |
| BHT | 13.68 | µg ml$^{-1}$ |
| | 62.08 | µM |

The antioxidant property of ferulic acid and great number of its derivatives has been reported [47]. Remarkably, UPLC/MS/MS analysis in this study showed that AK-sF had considerable amount of feruloyl teramines (44) and (45) represented 19.25%. Likewise, the scavenging of the DPPH radical by feruloyl tyramine isomers isolated from MC extract of *Tinospora crispa* has been reported [48]. They proved that phenolic alkaloids were more potent than BHT as a reference antioxidant. So, it could be noticed that the considerable antioxidant activity of AK-sF might partially elucidated by the attendance of significant amount of feruloyl teramines owning antioxidant capacity.

Antioxidant activity of the purified compounds (**1-6**) were also examined using DPPH assay. The antioxidant ability of the above mentioned compounds was tested in concentrations ranged between 1-50 µg/ml and corresponding $IC_{50}$ values were calculated and the results are presented in Table (3). Among the compounds evaluated, the phenolic compounds of **(4)** and the isomer mixture of **(5+6)** which obtained from Bu fraction displayed potent activity with $IC_{50}$ of 10.34 and 10.41 µM, respectively. In other words, the previously mentioned polyphenols [49] possess antioxidant activity higher than that of BHT as a reference antioxidant ($IC_{50}$ = 62.08 µM). The glucosidic flavonoids of **(3)** and **(2)** obtained from *I. carnea* had considerable antiradical property with higher $IC_{50}$ values of 89.1 and 114.33 µM, respectively. Scopoletin **(1)** obtained from *I. carnea* methylene chloride fraction showed poor antioxidant potency with the highest $IC_{50}$ (1766.75 µM).

Caffeoylquinic acids are particular secondary metabolites biosynthesized via phenylpropanoid pathway. These compounds show a defensive property in plants against different types of stress [50]. They also have a widespread range of possible profits with curative properties. The antioxidant property of caffeoylquinic acids has been also described [51-53]. Obtained data revealed that, compounds of **(4-6)** obtained from Bu fraction as caffeoylquinic acids displayed the highest antiradical potential against DPPH radical which was more potent than BHT as noted by their lower $IC_{50}$ values.

Table 4: Toxicity of *I. carnea* extracts against *A. craccivora* after 24 hrs of treatment.

| Plant species (Family) | Plant extract | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope | Toxicity index at $LC_{50}$ value |
|---|---|---|---|---|---|
| *Carnea* (Convolvulaceae) | Methanol extract | 522.00 (432.49 – 621.42) | 1404.71 (1064.32 – 2344.11) | 2.98 | 6.43 |
| | Pet. ether fraction | 496.57 (681.18 – 913.54) | 3575.52 (2273.20 – 7969.42) | 1.78 | 6.76 |
| | Methylene chloride fraction | 33.58 (26.33 – 42.14) | 117.99 (86.46 – 189.37) | 2.35 | 100 |
| | Ethyl acetate fraction | 169.07 (132.71 – 211.89) | 596.05 (437.88 – 949.94) | 2.34 | 19.86 |
| | Butanol fraction | 420.29 (322.71 – 549.98) | 1755.58 (1178.44 – 3416.14) | 2.06 | 7.99 |

Table 5 : Toxicity of *I. carnea* compounds against *A. craccivora* after 24 hrs of treatment.

| Plant species (Family) | Plant compounds | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope | Toxicity index at $LC_{50}$ value |
|---|---|---|---|---|---|
| *Carnea* (Convolvulaceae) | Scopoletin (1) | 59.72 (51.10 – 69.95) | 138.82 (108.60 – 216.06) | 3.50 | 22.67 |
| | Alkaloid sub fraction from $CHCl_3$ fraction | 13.53 (8.53 – 20.11) | 126.96 (70.45 – 357.61) | 1.32 | 100 |
| | Tiliroside (2) | 138.04 (117.04 – 161.61) | 329.85 (258.80 – 506.78) | 3.39 | 9.80 |
| | Apigenin-7*β*-*O*-glucoside (3) | 470.89 (391.13 – 551.87) | 1160.41 (920.71 – 1724.85) | 3.27 | 2.87 |
| | 3,5- di-*O*-caffeoylquinic acid (4) | 289.19 (244.36 – 342.56) | 725.19 (554.50 – 1187.96) | 3.21 | 4.68 |
| | 3,4- di-*O*-caffeoylquinic acid (5, 6) | 251.28 (210.13 – 294.81) | 621.58 (488.46 – 947.12) | 3.26 | 5.38 |

Table 6: Toxicity of *I. carnea* extracts against *B. tabaci* after 24hrs of treatment.

| Plant species (Family) | Plant extract | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope | Toxicity index at $LC_{50}$ value |
|---|---|---|---|---|---|
| *Carnea* (Convolvulaceae) | Methanol extract | 661.72<br>564.55 765.05 | 1476.05<br>1193.21<br>2115.19 | 3.68 | 12.80 |
| | Pet. ether fraction | 1341.15<br>1020.18 1825.87 | 6245.41<br>3916.20<br>14165.79 | 1.92 | 6.31 |
| | Methylene chloride fraction | 84.65<br>63.15<br>111.31 | 388.74<br>258.38<br>778.51 | 1.94 | 100 |
| | Ethyl acetate fraction | 422.09<br>312.96 573.56 | 2223.28<br>1375.49<br>5245.20 | 1.78 | 20.05 |
| | Butanol fraction | 487.10<br>368.79 641.56 | 2178.70<br>1440.76<br>4392.97 | 1.97 | 17.38 |

Table 7: Toxicity of *I. carnea* compounds against *B. tabaci* after 24 hrs of treatment.

| Plant species (Family) | Plant compounds | $LC_{50}$ (ppm) and confidence limits at 95% | $LC_{90}$ (ppm) and confidence limits at 95% | Slope | Toxicity index at $LC_{50}$ value |
|---|---|---|---|---|---|
| *Carnea* (Convolvulaceae) | Scopoletin (1) | 65.66<br>55.07<br>77.26 | 164.12<br>127.68 256.57 | 3.22 | 54.84 |
| | Alkaloid sub fraction from $CHCl_3$ fraction | 36.01<br>28.10<br>45.80 | 128.86<br>91.79<br>221.29 | 2.31 | 100 |
| | Tiliroside (2) | 296.49<br>250.37<br>353.08 | 755.21<br>571.30<br>1272.12 | 3.16 | 12.15 |
| | Apigenin-7*β*-*O*-glycoside (3) | 686.96<br>589.98<br>791.54 | 1490.21<br>1209.94<br>2121.44 | 3.81 | 5.24 |
| | 3,5- di-*O*-caffeoylquinic acid (4) | 381.28<br>316.13<br>451.73 | 1003.06<br>769.76<br>1619.65 | 3.05 | 9.44 |
| | 3,4- di-*O*-caffeoylquinic acid (5, 6) | 370.86<br>315.28<br>427.55 | 814.11<br>666.68<br>1129.81 | 3.75 | 9.71 |

The present findings concerning the isolated caffeoylquinic acids (**4-6**) from *I. carnea* agreed with those obtained by Hung *et al* [54] who found that the same compounds naturally isolated from *Dipsacus asper* had antiradical activity against DPPH higher than that of BHT. Remarkably, the $IC_{50}$ values obtained by Hung *et al* [54] for 3,4-di-*O*-caffeoylquinic and 3,5-di-*O*-caffeoylquinic acids (13.4 and 18.2 µM, respectively), which are slightly higher than those obtained in this study. The mechanism of the antiradical activity of caffeoylquinic acids esters of **(4)** and the mixture of **(5)** and **(6)** might be explained by the presence of two phenolic rings with catechol structure which are extremely powerful radical scavengers as a result of their ability to form a stable *ortho*-semiquinone in each phenolic ring [55].

### 3.5. Susceptibility of *A. craccivora* and *B. tabaci* to the probable insecticides

The toxicity of crude methanolic extract of *I. carnea* and its solvent fractions PT, MC, EA and Bu as well as, AK-sF and compounds (**1-6**) isolated against *A. craccivora* and *B. tabaci* was examined and compared. Data in (Tables 4 and 6) cleared that MC was the most effective fraction against *A.*

*craccivora* and *B. tabaci* with $LC_{50}$ of 33.58 and 84.65 µg $ml^{-1}$, respectively. The toxicity of Me-OH, PT, EA and Bu extract/fractions against *A. craccivora* showed $LC_{50}$ of 522.00, 496.57, 169.07 and 420.29 µg $ml^{-1}$, respectively (Table 8). Also, the toxicity of the same extract/fractions against *B. tabaci* established the same trend with higher $LC_{50}$ ranged between 422.09- 1341.15 µg $ml^{-1}$ (Table 6).

It is worthy to mentioned that, AK-sF and scopoletin **(1)** from MC fraction showed the maximum insecticidal activity against *A. craccivora* in comparing to other fractions and pure compounds with the lowest $LC_{50}$ of 13.53 and 59.72 µg $ml^{-1}$, respectively (Table 5). Also, they were showed the most effective as toxic against *B. tabaci* with $LC_{50}$ of 36.01 and 65.66 µg $ml^{-1}$, respectively (Table 7). The analysis of the most effective AK-sF by UPLC/MS/MS indicated the presence of ergot alkaloid compounds (**42-54**). Previous findings regarding AK-sF largely agreed with those recently stated [56]. They found that crude extracts derived from different morning glories *(Ipomoea* sp) related to the *Convolvulaceae* family as well as some pure ergot alkaloids related to these plants showed strong insecticidal activity against *Diaphorina citri* adults. In this context, several reports have documented the strong insecticidal potential of alkaloids extracted from different plants against larvae or adults of *A. craccivora* [17], *Mythimna separate* [57] and *Tripterygium wilfordii* [58].

### 3.6. Effect of Alkaloid sub fraction (Alk-sF) on Insect Enzymes Activity

The effect of AK-sF on the activities of insect enzymes (*α*-EST and *β*-EST, AChE, CTase, GST and POD ) were determined as shown in table (8). Activity of α-esterase (*α*-EST) was slightly increased with no significance after 24 hrs. of the treatment. While the *β*- esterases (*β*-EST), for the control and AK-sF aphid insects showed the activities of 64.03 and 76.21 µg *β*-naphthol/min/g b.wt, respectively. The non-significant increased activity of defensive enzymes (*α*-/*β*- esterases) regarding AK-sF -treated insects of *A. craccivora*, showed their likely role in metabolizing some constituents of AK-sF. Similar observations have been noted for different essential oils [59]. Likewise, the use of a sub-lethal concentration ($LC_{50}$) of lectins caused an elevation in *β*-EST activity [60]. Contrarily, some essential oils and their nano-emulsions were able to reduce the activity of detoxifying enzymes like *β*-EST and GST reflecting a potential role to control cowpea aphid [26].

The treated sample with AK-sF showed a significant reduction ($P \leq 0.001$) in the activity of acetylcholine esterase (AChE) compared to the control group with values of 509.5 and 643.8 µg AchBr /min/ g b wt., respectively. So, this sub fraction may depress the enzymatic hydrolysis of acetylcholine , the specific neurotransmitter at nerve synapses and neuromuscular junctions. In other words, AK-sF had the highest toxicity as pesticide. Similarly, Maazoun *et al* [61] declared that the insecticidal activity of *Urginea maritima* bulb crude extract rich in alkaloids was due to AChE inhibitory activity of these bioactive molecules. Another mode of action of alkaloids toxicity has documented by Maazoun *et al* [61] via increasing membrane fluidity permitting the entry of polyphenols into insect cells.

Sprayed aphid with AK-sF displayed a significant increase ($P \leq 0.05$) in the activity of chitinase (CTase) compared to the control group, where the CTase activity values of control and AK-sF were 10.5 and 30.4 µg NAGA/min/g b.wt., respectively (Table 8). Interestingly, different compounds with adhere property are able to penetrate the waxy layer and reach to chitinous procuticle, thus causing significant contact toxicity. For example, tea saponins initiating CTase activity have been found to display insecticidal activity against *Ectropis obliqua* by disruption not only the insect waxy layer of the surface epidermis but also, chitin layer of both outside and inside causing serious of damaging alternations [62]. Overall, insecticides that affect chitin metabolic cycles have been of special attention for the control of agricultural pests [63].

Table 8: Activity of defensive enzymes of *Aphis craccivora* adults treated with $LC_{50}$ of alkaloid sub fraction.

| Treatments | *α*-esterase activity (µg *α*-naphthol/min/g b wt.) | *β*-esterase activity (µg *β*-naphthol/min/g b wt.) | Chitinase Activity (µg *NAGA* /min/g b wt.) | Acetyl choline esterase activity (µg AchBr /min/gm b wt.) | GST activity ( mmol sub.conjugated /min/mg protein) | Peroxidase activity (Δ mO.D./min/ mg protein) |
|---|---|---|---|---|---|---|
| Alkaloid sub fraction | 37.33 ±1.56[a] | 76.21± 5.32[a] | 30.4±4.19[a] | 509.5±4.72[b] | 2.57±0.05[a] | 347.33±4.41[a] |
| control | 31.13 ±3.54[a] | 64.03±6.54[a] | 10.5±0.67[b] | 643.8±3.18[a] | 1.92±0.04[b] | 242.33±1.45[b] |
| LSD (0.05) | 10.76 | 23.41 | 11.78 | 15.81 | 0.35 | 12.89 |
| F | 2.56 | 2.09 | 21.82 | 556.54 | 109.26 | 511.47 |
| P | 0.18 | 0.22 | 0.01 | 0.00 | 0.0005 | 0.00 |

GST, glutathione-S-transferase; F, F-test; P, P-value

On the basis of this criterion, the CTase activity in our study was significantly increased after treatment with AK-sF representing that this alkaloidal sub fraction might be used in the biodegradation of chitin, which leads to aphid death. In this context, some reports have documented the strong insecticidal activity of some fungal metabolites or plant extracts through induction of CTase activity [63-65].
According to the presented data in (Table 8), AK-sF significantly increased glutathione-*S*-transferase (GST) and peroxidase (POD) activity in *A. craccivora* when compared with control group. Some previous studies showed that insects secrete these enzymes, perhaps as a response to being treated with some pesticides to increase their resistance [66].

## 4. Conclusion

Phytochemicals profiling of *Ipomoea carnea* solvent fractions were done in the present work as well as identification of bioactive compounds. A total of 35 compounds from PT and MC fractions, by GCMS analysis. Five phenolic compounds were isolated for the first time from the plant EA and Bu fractions. The strong antiradical activity of Bu fraction was due its abundance of phenolic antioxidants (**3-6**) particularly caffeoylquinic acids(**4-6**). The insecticidal activity of *I. carnea* solvent fractions/compounds were studied for the first time against *Aphis craccivora* and *Bemisia tabaci*, crude alkaloids sub fraction (AKsF) showed the highest insecticidal activity. The insecticidal activity of AKsF was mostly due to its inhibitory activity against AChE. UPLC/MS/MS analysis of AK-sF showed the presence of feruloyl tyramines (**44-45**) and the ergot alkaloid of ergocristine (**54**) in high peak areas.

## Declaration of Competing Interest

The authors declare no competing interests.

## Data availability

Data will be made available on request

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## 5. References

[1]. Cortés-Chitala, M.C.; Flores-Martínez, H.; Orozco-Ávila, I.; León-Campos, C.; Suárez-Jacobo, Á.; Estarrón-Espinosa, M. and López-Muraira, I. (2021): Identification and Quantification of Phenolic Compounds from Mexican Oregano (Lippia graveolens HBK) Hydroethanolic Extracts and Evaluation of Its Antioxidant Capacity. Molecules 29(3):702, doi:10.3390/molecules 2603 0702.

[2]. Opara, E.I. and Chohan, M. (2014): Culinary herbs and spices: Their bioactive properties, the contribution of polyphenols and the challenges in deducing their true health benefits. International Journal of Molecular Sciences, 15: 19183–19202.

[3]. Fatima, N.; Rahmana, M.M.; Khan, M.A. and Junjiang, F. (2014): A review on *Ipomoea carnea*: pharmacology, toxicology and phytochemistry. Journal of Complementary and Integrative Medicine, 11: 55.

[4]. Haraguchi, M.; Gorniak, S.L.; Ikeda, K., Minami, Y.; Kato, A.; Alison ,A.; Watson , A.A.; Nash, R.J.; Russel, J.; Molyneux, R.J. and Asano, N. (2003): Journal of Agriculture and Food Chemistry, 51: 4995-5000.

[5]. Khan, M.Z.; Zahra, S.S.; Ahmed, M.; Fatima, H.; Mirza, B.; Haq, I. and Khan, S.U. (2018): Polyphenolic profiling of *Ipomoea carnea* Jacq. by HPLC-DAD and its implications in oxidative stress and cancer. Natural Products Research, https://doi.org/10.1080/14786419.2018.1482551 .

[6]. Abdallah, W.E.; Osman, A.F.; El Gendy, A.G.; Khaled, A.; Abdelshafeek, K.A. and Omer, E.A. (2017): Different methods for extraction of some chemical constituents from different organs of *Ipomea carnea* and their antioxidant activity. Bioscience Research, 14(4): 1024-1041.

[7]. Amel, M.K.; Abdel-Shakour, Z.T.; AbdelAll, S.R.; Sleem, A.A. and Haggag, E.G. (2017): Phytochemical and Biological Investigation of *Ipomoea carnea* Jacq. Grown in Egypt. International Journal of Pharmacognosy and Phytochemical Research, 9(2): 266-281.

[8]. Khan, T.Y.; Raina, R.; Verma, P.K.; Sultana, M. and Jyoti, M.K. (2014): Phytochemical constituents and antidiabetic potential of *Ipomoea carnea* Jacq leaves extracts.Journal of Experimental and Integrative Medicine, 4(2): 137-142.

[9]. Hasan, M.; Ibrahim, S.; Abo El-Seoud, K. and El-Aasr, M. (2015): Cytotoxic, antioxidant and antimicrobial activities of *Ipomoea carnea* spp. Fistulosa (Mart. ex Choisy) D. Austin. World Journal of Pharmaceutical Sciences, 3(6): 1217-1231.

[10]. Anand, G.; Sumithira, G.; Chinna, R.R.; Muthukumar, A. and Vidhya, G. (2013): In vitro and in vivo anticancer activity of hydroalcoholic extract of *Ipomoea carnea* leaf against *Ehrlich Ascites* Carcinoma cell lines. International Journal of Advances in Pharmaceutical Genuine Research, 1(1): 39-54.

[11]. Nidiry, E.J. and Lokesha, A.N. (2020): Octadecyl p-Coumarates: Probable Anticancer Compounds of *Ipomoea carnea* subsp.

Fistulosa. Natural Product Communications, 15(8): 1–2.

[12]. Sahayaraj, K.; Kombiah, P.; Dikshit, A.K.. and Rathi. J.M. (2015): Chemical constituents of the essential oils of *Tephrosia purpurea* and *Ipomoea carnea* and their repellent activity against *Odoiporus longicollis*. Journal of the Serbian Chemical Society, 80(4): 465–473.

[13]. Magierowicz, K.; Górska-Drabik, E. and Golan, K. (2020): Effects of plant extracts and essential oils on the behavior of *Acrobasis advenella* (Zinck.) caterpillars and females. Journal of Plant Diseases and Protection,127: 63-71.

[14]. Abd-Ella, A.A. (2014): Toxicity and persistence of selected neonicotinoid insecticides on cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae). Arch Phytopathol Pflanzenschutz, 47 (3): 366–376.

[15]. Padgham, D.E.; Kimmins, F.M. and Ranga Rao G.V. (1990): Resistance in groundnut (*Arachis hypogaea* L.) to *Aphis craccivora* (Koch). Annals of Applied Biology, 177: 285-294.

[16]. Cohen, S. and Berlinger, M.J. (1986): Transmission and Cultural Control of Whitefly-borne Viruses. Agriculture, Ecosystems & Environment, 17: 89-97.

[17]. Boulos, L. (2000): Flora of Egypt. Al Hadara Publishing Cairo, Egypt 2: 288.

[18]. Taher, M.; El-Daly, N.M.; El-Khateeb, A.Y.; Hassan, S.M. and Elsherbiny, E.A. (2021): Chemical Composition, Antioxidant, Antitumor and Antifungal Activities of Methanolic Extracts of *Coleus blumei*, *Plectranthus amboinicus* and *Salvia splendens* (Lamiaceae). Journal of Agricultural Chemistry and Biotechnology, 12: 177–187.

[19]. El-Amier, Y.A.; Soliman, H.M.; ElHalawany, E.S.; El-Nabawy, B.S. (2022): Chemical Characterization of *Reichardia tingitana* Methanolic Extract and Evaluation of Its Antioxidant and Anticancer Activity, Egypt. J. Chem. Vol. 65, No. SI:13B pp. 933-940. doi: 10.21608/EJCHEM.2022.155720.6722

[20]. Elsherbiny A. E.; Dawood H. D.; Elsebai M.F.; Mira, A.; Taher, M.A. (2023): Control of dry rot and resistance induction in potato tubers against *Fusarium sambucinum* using red onion peel extract. Postharvest Biology and Technology, 195: 112-119, doi:10.1016/j.postharvbio.2022.112119

[21]. Darwish, M.S.; Qiu, L.; Taher, M.A.; Zaki, A.A.; Abou-Zeid, N.A.; Dawood, D.H.; Shalabi, O.M.A.K., Khojah, E. and Elawady, A.A. (2022): Health Benefits of Postbiotics Produced by *E. coli* Nissle 1917 in Functional Yogurt Enriched with Cape Gooseberry (*Physalis peruviana* L.). Fermentation,8(3): 128, https://doi.org/10.3390/ fermentation8030128

[22]. Abbott, W.S. (1925): Journal of Economic Entomology, 18 (2): 625-627.

[23]. Finney, D.J. (1971): Probit Analysis. A Statistical treatment of the sigmoid response curve. 7th Ed. Cambridge Univ. press, Cambridge, England.

[24]. Sun, Y.P.(1950): Journal of Economic Entomology,43:45-53, https://doi.org/10.1186/s1 2906-020-03100-5

[25]. Muñiz,M. and Nombela, G. (2001): Differential variation in development of the B- and Q-biotypes of *Bemisi tabaci* (Homoptera: Aleyrodidae) on sweet pepper at constant temperatures. Environmental Entomology, 30(4): 720-727.

[26]. Cruz-Estrada, A.; Gamboa-Angulo, M.; Borges-Argáez, R. and Ruiz-Sánchez, E. (2013): Insecticidal effects of plant extracts on immature whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyroideae). Electronic Journal of Biotechnology, 16(1): 1-9, doi:10.2225/vol16-issue1-fulltext-6

[27]. Abdelaal, K.; Essawy, M.; Quraytam, A.; Abdallah, F.; Mostafa, H.; Shoueir, K.; Fouad, H.; Hassan, F.S. and Hafez, Y. (2021): Toxicity of Essential Oils Nanoemulsion Against *Aphis craccivora* and Their Inhibitory Activity on Insect Enzymes, Processes, 9: 624, https://doi.org/10.33 90/pr9040624

[28]. Van Asperen, K. (1962): A study of housefly, esterases by means of sensitive colourimetric method. Journal of Insect Physiology ,8, 401-416.

[29]. Simpson, D.R., Bull, L.D., and Lindquist, A.D. (1964): A semi micro techniques for estimation of cholinesterase activity in boll Weevil. Annals of the Entomological Society of America, 57(3): 367- 377.

[30]. Taher, M.A. and Elsherbiny, E.A. (2023): Impact of Isonicotinic Acid Blending in Chitosan/Polyvinyl Alcohol on Ripening-Dependent Changes of Green Stage Tomato. Polymers, 15(4): 825; https://doi.org/10.3390/pol ym 15040825

[31]. Habig, W.H.; Pabst, M.J. and Jakoby, W.B. (1974): Glutathione-*S*-transferase the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249: 7130-7139.

[32]. Bosch-Serra, D.; Rodríguez, M.A.; Avilla, J.; Sarasúa ,M.J. and Miarnau, X. (2021):.Esterase, Glutathione-S-transferase and NADPH-cytochrome P450 reductase activity evaluation in *Cacopsylla pyri* L. (Hemiptera: Psyllidae) individual adults. Insects, 12: 329, doi: 10.3390 /insects12040329

[33]. Ishaaya, I. and Casida, J.E. (1974): Dietary TH 6040 alters composition and enzyme activity of

housefly larval cuticle. Pesticides Biochemistry and Physiology, 4: 484-490.

[34]. Bradford, M.M. (I976): A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

[35]. El-Rokh, A.R. (2007): Chemical Studies on Some Natural Extracts and Their Constituents to Control Some Aphid Species. M. Sc. Thesis, Fac. of Sci. Mans. Unv. Egypt.

[36]. Sokoudjou, J.B.; Atolani, O.; Njateng, G.S. and Khan, A. (2020): Isolation, characterization and in vitro antisalmonellal activity of compounds from stem bark extract of *Canarium schweinfurthii*. BMC Complementary Medicine and Therapies, 20: 316.

[37]. Valverde, V.A.; Rodríguez, G.R. and Vargas, S.A. (2022). Bioguided Phytochemical Study of *Ipomoea cairica* Extracts with Larvicidal Activity against *Aedes aegypti*. Molecules, 27: 1348, doi: 10.3390/molecules27041348

[38]. Liu, W.J.; Hou, X.Q.; Chen, H.; Liang, J.Y. and Sun, J.B. (2016). Chemical constituents from *Agrimonia pilosa* Ledeb. and their chemotaxonomic significance. Natural Products Research, http://dx.doi.org/ 10.1080/14786419.2016.1198351

[39]. Erdenetsogt, U.; Nadmid, S.; Paulus, C.; Chanagsuren, G.; Dolgor, E.; Gotov, C.; Dahse, H.; Luzhetskyy, A. and Dagvadorj, E. (2020): Bioactive flavonoids from plant extract of *Pyrethrum pulchrum* and its acute toxicity, Natural Products Research, https://doi.org/10.1080/1478 6419.2020.1806271

[40]. Goodarzia ,S.; Tabatabaeib, M.J.; Razieh, M.; Jafarid, R.M.; Shemiranic, F.; Tavakolib, S.; Mofasseric, M. and Tofighi, Z. (2020): *Cuminum cyminum* fruits as source of luteolin-7-O-glucoside, potent cytotoxic flavonoid against breast cancer cell lines. Natural Products Research, 34(11): 1602–1606.

[41]. Lee, H.J.; Sim, M.O.; Woo, K.W.; Jeong, D., Jung, H.K.; An, B. and Cho, H.W. (2019): Antioxidant an Antimelanogenic Activities of Compounds Isolated from the Aerial Parts of *Achillea alpina* L. Chemistry and Biodiversity, 16: (1-10) e1900033.

[42]. Belaouira, R.; Marchionim E.; Benayache, F. and Benayache, S. (2020): On-line screening and identification of polyphenolic antioxidant compounds of *Convolvulus trabutianus*. Natural Products Research, 34(10): 1490–1493.

[43]. Chen, J.; Mangelinckxb, S.; Maa, L.; Wang, Z.; Li, W. and Kimpe, N.D. (2014): Caffeoylquinic acid derivatives isolated from the aerial parts of *Gynura divaricata* and their yeast α-glucosidase and PTP1B inhibitory activity. Fitoterapia 99, 1–6,

[44]. Diana, D.J.; Malysheva, S.V.; Sanders, M.; Larionova, D.; Robbens, J.; Dubruel, P.; Peteghem, C.V. and Saeger, S.D. (2012): Development and validation of a new LC-MS/MS method for the simultaneous determination of six major ergot alkaloids and their corresponding epimers. Application to some food and feed commodities. Food Chemistry, 135: 292–303.

[45]. Wilkinson, E.R.; Hardcastle, W.S. and McCormic, C.S. (1987): Seed ergot alkaloid contents of *Ipomoea hederifolia*, *I. quamoclit*, *I. coccinea* and *I. wrighdi*. Journal of the Science of Food and Agriculture, 39: 335-339.

[46]. Abbasi, M.A.; Zafar, A.; Riaz, T.; Aziz-ur-Rehman; Arshad, S.; Shahwar, D.; Jahangir, M.; Siddiqui, S.Z.; Shahzadi, T. and Ajaib, M. (2010): Evaluation of comparative antioxidant potential of aqueous and organic fractions of Ipomoea carnea. Journal of Medicinal Plants Research, 4(18): 1883-1887.

[47]. De Oliveira Silva, E. and Batista, R. (2017): Ferulic Acid and Naturally Occurring Compounds Bearing a Feruloyl Moiety: A Review on Their Structures, Occurrence, and Potential Health Benefits. Comprehensive Reviews in Food Science and Food Safety, 16(4): 580-616.

[48]. Cavin, A.; Hostettmann, K.; Dyatmyko, W. and Potterat, O. (1998): Antioxidant and lipophilic constituents of *Tinospora crispa*. Planta Medica, 64(5): 393-396.

[49]. Shalaby, E.A.; Sanaa M. Shanab, S.M.; Fayed, S.A.; Abde Gawad, H.M.; Nasr, M.F. and Gaballa, H.S. (2022): Chemical Constituents and Biological Activity of Successive Extracts and Silver Nanoparticles from Red Onion Peels, Egypt. J. Chem. Vol. 65, No. SI:13B pp. 593-604. doi: 10.21608/EJCHEM.2022.136419.6010

[50]. Uleberg, E.; Rohloff, J.; Jaakola, L.; Trost, K.; Junttila, O. and Haggman, H. (2012): Effects of temperature and photoperiod on yield and chemical composition of northern and southern clones of bilberry (*Vaccinium myrtillus* L.). Journal of Agriculture and Food Chemistry, 60: 10406– 10414.

[51]. Hamed, Y.S.; Abdin, M.; Chen, G.; Akhtar, H.M.S. and Zeng, X. (2020): Effects of impregnate temperature on extraction of caffeoylquinic acid derivatives from Moringa oleifera leaves and evaluation of inhibitory activity on digestive enzyme, antioxidant, anti-proliferative and antibacterial activities of the extract. International Journal of Food Science & Technology, 55: 3082– 3090.

[52]. Nzekoue, F.K.; Angeloni, C.; Vitali, L.A.; Sagratini, G.; Vittori, S. and Angeloni, S. (2020) Coffee silverskin extracts: quantification of 30 bioactive compounds by a new HPLC-MS/MS

method and evaluation of their antioxidant and antibacterial activities. Food Research International, 133: 109128.

[53]. Trendafilova, A.; Ivanova, V.; Rangelov, M.; Todorova, M., Ozek, G., Yur, S., Ozek , T., Aneva, I.; Veleva , R.; Moskova-Doumanova, V.; Doumanov, J. and Topouzova-Hristova, T. (2020): Caffeoylquinic acids, cytotoxic, antioxidant, acetylcholinesterase and tyrosinase enzyme inhibitory activities of six inula species from Bulgaria. Chemistry & Biodiversity, 17: e2000051.

[54]. Hung, T.M.; Na, M.; Thuong, P.T.; Su, N.D.; Sok, D.; Song, K.S.; Seong, Y.H. and Bae, K. (2006): Antioxidant activity of caffeoyl quinic acid derivatives from the roots of *Dipsacus asper* Wall. Journal of Ethnopharmacology, 108(2): 188-92.

[55]. Kumar, S. and Pandey, A.K. (2013): Chemistry and biological activities of flavonoids: an overview. Scientific World Journal, 29: 162750, doi:10.1155 /2013/162750

[56]. Chen ,X.; Kaur ,N.; Horton, D.R.; Cooper, W.R.; Jawwad, A.; Qureshi, J.A. and Stelinski, L.L.(2021): Crude Extracts and Alkaloids Derived from *Ipomoea-Periglandula* Symbiotic Association Cause Mortality of Asian Citrus *Psyllid Diaphorina citri Kuwayama* (Hemiptera: Psyllidae). Insects, 12: 929, doi:10.3390/insects 12100929

[57]. Zhou, L.; Feng, J.; Ma, Z.Q. and Zhang, X. (2006): Insecticidal activity of total alkaloid from *Tripterygium wilfordii* Hook against *Mythimna separate* (Walker) (Lepidoptera: Noctuidae). Acta Phytophylacica, Sinica, doi:10.1360/yc-006-1280

[58]. Ma, Z.; Li, Y.; Wu, L. and Zhang, X. (2014): Isolation and insecticidal activity of sesquiterpenes alkaloids from *Tripterygium wilfordii* Hook f. Industrial Crops and Products, 52: 642-648.

[59]. Intirach, J.; Junkum, A.; Lumjuan, N.; Chaithong, U.; Somboon, P.; Jitpakdi, A.; Riyong, D.; Champakaew, D.; Muangmoon, R.; Chansang, A. and Pitasawat, B. (2019): Biochemical Effects of *Petroselinum crispum* (Umbellifereae) Essential Oil on the Pyrethroid Resistant Strains of *Aedes aegypti* (Diptera: Culicidae), Insects 10:1, doi:10.3390/ insects10010001

[60]. Khoobdel, M.; Rahimi, V.; Ebadollahi, A. and Krutmuang, P. (2022): Evaluation of the Potential of a Lectin Extracted from *Polygonum persicaria* L. as a Biorational Agent against *Sitophilus oryzae* L. Molecules, 27:793, https://doi.org/10.3390/molecu les 27030793

[61]. Maazouna, A.M.; Hlela, T.B.; Hamdib, S.H.; Belhadja, F.; Ben Jemâab, J.M. and Marzouki, M.N. (2017): Screening for insecticidal potential and acetylcholinesterase activity inhibition of *Urginea maritima* bulbs extract for the control of *Sitophilus oryzae* (L.), Journal of Asia-Pacific Entomology, 20: 752–760.

[62]. Cui, C.; Yang, Y.; Zhao, T.; Zou, K.; Peng, C.; Cai, H.; Wan, X. and Hou, R. (2019): Insecticidal Activity and Insecticidal Mechanism of Total Saponins from *Camellia oleifera*, Molecules, 24: 4518, doi:10.3390/molecules24244518

[63]. Merzendorfer, H. and Zimoch, L. (2003): Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. Journal of Experimental Biology, 206: 4393-4412.

[64]. Elbanhawya, A.A.; Elsherbiny, E.A.; Abd El-Mageed, A.E. and Abdel-Fattah, G.M. (2019): Potential of fungal metabolites as a biocontrol agent against cotton aphid, *Aphis gossypii* Glover and the possible mechanisms of action. Pesticide Biochemistry and Physiology, 159: 34–40.

[65]. Narendrakumar, G. and Karthic, R. N. S. (2021): Surface-modified nanosilica–chitinase (SiNp-Chs)-doped nanoenzyme conjugate and its synergistic pesticidal activity with plant extracts against armyworm *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae), Nanobiotechnolog, 15: 117–134.

[66]. Enayati, A.A.; Ranson, H. and Hemingwa, J. (2005): Insect glutathione transferases and insecticide resistance. Insect Molecular Biology, 14(1): 3 - 8.