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Research Article

CHEMICAL CONSTITUENTS OF RADISH, RAPHANUS SATIVUS LINN.(BRASSICACEAE) LEAVES EXTRACTS AND THEIR INSECTICIDAL ACTIVITY AGAINST MEALYBUG, PHENACOCCUS SOLENOPSIS TINSLEY (HEMIPTERA: PSEUDOCOCCIDAE)

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ABSTRACT

The insecticidal activity of *Raphanus sativus* Linn. leaves extracts were investigated against third instar nymphs of *Phenacoccus solenopsis* Tinsley under laboratory conditions. In addition, the major chemical constituents of the leaves extracts were isolated, characterized and identified. Chromatographic separation of the extracts gave two phytosterols from petroleum ether fraction, which were identified as stigmast-5-en-3-ol (β-sitosterol) **(1)** and stigmasta-5,22E-dien-3β-ol (stigmasterol) **(2)**. In addition, two flavonoid glycosides were isolated from the ethyl acetate fraction. These flavonoid glycosides were identified as quercetin 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranosid **(3)**, a new compound, wasn't isolated form any natural source before, and kaempferol 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside**(4)**. The insecticidal activity of different fractions of *R. sativus* leaves were studied. The most effective fraction was ethyl acetate followed by methylene chloride then petroleum ether fraction with LC_{50} values of 421.455 ppm, 674.960 ppm and 875.563 ppm, at 72 hours post treatment, respectively.

INTRODUCTION

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a polyphagous insect pest infesting more than 154 plant species including field crops, vegetables, ornamentals, weeds, bushes and trees (Arif *et al*. 2009 and Saini *et al*. 2009).The infested leaves become crinkled, turn yellow and eventually drop off (Dhawan *et al*. 1980, Culik and Gullan 2005). Moreover, this insect excretes honey dew resulting in sooty mold growth, which hinders photosynthesis and reduces the marketability of the product (Saeed *et al*. 2007). Infested plants become weak, stunted, and produce fewer small flowers or fruit (Kousar *et al*. 2016, Saddiq *et al*. 2014 and Ahmad and Akhtar, 2016). The overuse of synthetic chemical insecticide led to serious impact to humans and environment, besides development of insect resistance with subsequent population outbreaks (Palumbo *et al*., 2001 and Irfan Ullah *et al*. 2017). Therefore, secure alternatives to the widest spread synthetic insecticides became a great demand. One of the most important alternatives is natural pesticides which depend on plant derived compounds.

*R. sativus*is an annual herb, consumed as vegetable. Different parts of *R. sativus*, including roots, seeds, and leaves, are known to possess a variety of medicinal properties (Nadkarni, 1976).

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It is used as anthelmintic, antifungal, antibacterial, antiscorbutic, diuretic, laxative, tonic, carminative, antiscorbutic, stimulant, stomachic, cholagogue, lithotripsic and emmenagogue. The fresh leaves juices are diuretic and laxative. Roots are used for urinary complaints and syphilitic disease; they are a reputed medicine for piles and gastrodynic pains (Kritikarand Basu, 1987, Bin Sina, 1987, Chopra *et al*., 1986). Furthermore, radish sprouts extracts exhibited antioxidant properties and significantly induced bile flow in rats (Barillari *et al*. 2006)Also, *R. sativus* exhibited antihepatotoxic (Mohammed *et al*. 2008) and antibacterial activities against the pathogenic bacteria *Escherichia coli*, *Salmonella typhi*, *Pseudomonas pyocyaneus*, *Pneumococci listeria*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococci*, *Micrococcus*, *Enterococcus*, *Lactobacillus* and *Pedicoccus* (Abdou, 1972,Yeung, 1985, Rakhmawati *et al*. 2009, Rani *et al*., 2008 and Shukla *et al*., 2011).

The phytochemical investigation of *R. sativus* led to isolation of 4-methylthio-3-butenyl isothiocyanate which was isolated and reported as a principal antimutagen in radish (Nakamura *et al* 2001). Also, 4-methylthiobutanyl derivatives and phenylpropanoid sucrosides were isolated and exhibited significant cytotoxic activity against the human tumor cell lines, A549, SK-OV-3, SK-MEL-2, and HCT-15, in addition to anti-inflammatory activities inhibiting nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated BV-2 microglial cells. (Kim *et al*. 2014 and Kim *et al*. 2015).

The objective of this study was to investigate the phytochemistry and the insecticidal activity of *R. sativus* leaves extracts against the cotton mealybug, *P.solenopsis*.

MATERIALS AND METHOD

Instruments

GC/MS analysis was performed on a GC perkinelmerClaruss 500 equipped with PerkinElmer Claruss 500 Mass Spectrometer with Turbomass data system for MS identification of the GC components. NMR spectra were recorded on a Bruker AMX 400 and 500 instrument standard pulse sequences operating at 400 and 500 MHz in ^{1}H-NMR and ^{13}C-NMR were recorded at 125 MHz. Chemical shifts are obtained in δ (ppm) relative to TMS as internal standard material and the coupling constants (J) are in Hz. HSQC was recorded at 500 MHz. GC/MS analysis was performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components.

Chemicals

Columns chromatography (CC) was performed using silica gel (100-200 mesh), LOPA chemie. Thin layer chromatography and preparative TLC were performed on silica gel (Fluka analytical silica on TLC aluminum foils, (20×20 cm) with fluorescent indicator; Sigma Aldrich). Solvents of petroleum ether, methylene chloride, ethyl acetate and methanol were obtained from Adwic Company.

Plant material

Insecticides free *R. sativus* Linn. Leaves were collected from the farm of faculty of Agriculture, Mansoura University. The collected leaves were dried at room temperature and grounded to a powder.

Extraction and Isolation

The powder of *R. sativus* Leaves were extracted by a Soxhlet apparatus using methanol. Methanolic extract was evaporated to its 1/3 volume, then diluted with water and successively extracted by using different organic solvents of different polarities (pet. ether, methylene chloride and ethyl acetate) using separating funnel. All fractions were dried over anhydrous sodium sulphate and evaporated to dryness, then a sample of each fraction was analyzed by GC/MS technique for characterization and identification of the volatile components.
Pet. ether fraction was subjected to preparative thin layer chromatography using the eluent pet. ether/ ethyl acetate (17:3) yielding a binary mixture of compound (1&2) as colorless needles crystals (R_f:0.26).

Stigmast-5-en-3-ol(β-sitosterol), (1): colorless crystalline needles; GC/MS, m/z (rel. int): 414 (1%)[M+], 381(12.5%)[M+-CH_3-H_2O], 303 (20.83%) [$C_{21}H_{35}O$], 213 (37.5%) [$C_{16}H_{21}$]; ^{1}HNMR (400MHz, $CDCl_3$, □, ppm, J, Hz): □$_H$3.53 (1H, m, H-3), 5.35 (1H, br.s, H-6), 0.68 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.92 (3H, d, J 6.7 Hz, Me-21), 0.84 (3H, d, J 7.3 Hz, Me-26), 0.81 (3H, d, J 7 Hz, Me-27), 0.88 (3H, t, J 6.1 Hz, Me-29).

Stigmasta-5,22E-dien-3β-ol (stigmasterol), (2): colorless crystalline needles; 412 (100%)[M+], 255 (36.36%) [$C_{19}H_{27}$], 213 (45.45%) [$C_{16}H_{21}$], 55 (95.45%) [C_4H_7]; ^{1}HNMR(400MHz, $CDCl_3$, □, ppm, J, Hz):□$_H$ 3.53 (1H, m, H-3), 5.35 (1H, br. s, H-6),0.68 (3H, s, Me-18), 1.01(3H, s, Me-19), 0.92 (3H, d, J 6.7 Hz, Me-21), 5.02 (1H, dd, J 8.6, 15.2 Hz, H-22), 5.14 (^{1}H, dd, J 8.6, 15.2 Hz, H-23), 0.84 (3H, d, J 7.3 Hz, Me-26), 0.81 (3H, d, J 7 Hz, Me-27), 0.88 (3H, t, J 6.1 Hz, Me-29).

Also, when ethyl acetate fraction chromatographed over silica gel column chromatography, a sub-fraction was obtained by the eluent methylene chloride/methanol (17:3). This sub-fraction was further purified on silica gel TLC plates using methylene chloride/methanol (93: 07) to afford a pure binary crystalline mixture of compounds (3) and (4).

Quercetin 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside(3) and Kaempferol 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside(4): yellow amorphous binary crystalline mixture, (R_f 0.57); ^{1}H NMR (DMSO-d_6, 500 MHz, J in Hz): 6.43 (1H, br. s, H-6), 6.81 (1H, br. s, H-8), 6.87 (1H, d, J 8.5 Hz, H-3', 5′), 8.10 (1H, d, J 8.5 Hz, H-2', 6′), 5.29 (1H, d, J 3.9 Hz, H-1″), 3.56 (1H, dd, J11.5, 5 Hz, H-5"ax), 3.20 (1H, br. d, J5 Hz, H-5"eq), 5.53 (1H, brs, H-1‴), 1.09 (3H, d, J 5.7 Hz, H-6‴); ^{13}C NMR (DMSO-d_6,125 MHz): 156.78 (C-2), 133.85 (C-3), 177.75 (C-4), 160.87 (C-5), 99.43 (C-6), 161.63 (C-7), 94.60 (C-8), 155.95 (C-9), 105.62 (C-10), 120.54 (C-1′), 131.17 (C-2′), 115.34 (C-3′), 160.27 (C-4′), 115.34 (C-5′), 131.17 (C-6′), 101.19 (C-1″), 71.57 (C-2″), 70.79 (C-3″), 66.05 (C-4″), 64.30 (C-5″),98.33 (C-1‴), 69.85 (C-2‴), 70.26 (C-3‴), 71.61 (C-4‴), 70.11 (C-5‴), 17.96 (C-6‴).

The tested insect pest

The mealy bug was collected from infested okra plants, *Abelmoschus esculentus* at the farm of faculty of Agriculture, Mansoura University, and it has been confirmed that it is free from pesticides. Then, it was identified at Scale Insect Department, Plant Protection Research Institute, Agric. Res. Center, Giza, Egypt as *P. solenopsis*. The mealybug was transferred to the laboratory and was reared on okra (3-4 weeks old) planted in small pots (15 cm^3) and kept under plastic greenhouse conditions of 27± 5˚C, 70±5 RH and 14:10 hours L:D.

Bioassay

Ten newly molted third instar nymphs of *P. solenopsis* were randomly selected and carefully transferred to the okra leaf using camel hair brush and placed in a plastic Petri dish (9cm in diameter) to be considered as one replicate. Then, sprayed with aqueous solution of the tested extracts containing 0.3% Tween 80. Each treatment was replicated three times in addition to control (sprayed only with water containing 0.3% Tween 80). Finally, the lids of Petri-dishes, bearing the ventilation holes were sealed.

Statistical analysis

Mortality percentages of the third instar nymphs of *P. Solenopsis* were determined after 24h and 72h of initial application and corrected by using Abotts' formula (1925). Also, the LC_{50}, LC_{90} and slope values were estimated according to Finney (1971). Toxicity index of each tested plant extract fraction was computed by comparing it with the most effective one using Sun's equation (1950).

RESULT AND DISCUSSION

All different fractions of *R. sativus* leaves were analyzed by GC/MS technique (Table 1). Pet ether fraction revealed presence of 11 peaks corresponding to 11 compounds, while methylene chloride fraction contained19 compounds and ethyl acetate fraction contained seven compounds. These compounds were identified by comparing their mass spectra with those of their analogous spectra reported by Wiley, NIST and Pfleger libraries.

Table 1 The GC/MS analysis of different fractions of *R. sativus* leaves

Compound Name	R_t, min	Area%	M.F.	M.wt.
Pet. ether fraction of *R. sativus* leaves				
Octadecanoic acid methyl ester(5).	27.68	1.3	$C_{19}H_{38}O_2$	298
9,12,15-Octadecatrienoic acid methyl ester(6).	28.20	3.06	$C_{19}H_{32}O_2$	292
9,12,15-Octadecatrienoic acid ethyl ester(7).	30.87	0.86	$C_{20}H_{34}O_2$	306
(Z,Z,Z)-9,12,15-Octadecatrienoic acid(Linolenic acid,8).	31.90	5.21	$C_{18}H_{30}O_2$	278
9-Octadecenoic acid(Z-Oleic acid,9).	32.20	1.65	$C_{18}H_{34}O_2$	282
1-Octadecanol(Stearyl alcohol,10).	34.89	0.89	$C_{18}H_{38}O$	270
Nonadecane(11).	37.34	0.37	$C_{19}H_{40}$	268
Docosane(12).	40.24	0.73	$C_{22}H_{46}$	310
Octacosane(13).	42.96	2.37	$C_{28}H_{58}$	394
Pentatriacontane(14).	45.52	0.86	$C_{35}H_{72}$	492
Stigmast-5-en-3-ol(β-Sitosterol,1).	47.99	1.44	$C_{29}H_{50}O$	414
Methylene chloride fraction of *R. sativus* leaves:				
Tridecanal(15).	17.59	6.46	$C_{13}H_{26}O$	198
Octadecane(16).	20.29	3.19	$C_{18}H_{38}$	254
Tetradecanal(Myristic aldehyde,17).	20.65	3.23	$C_{14}H_{28}O$	212
Octadecanal(Stearaldehyde,18).	20.78	0.36	$C_{18}H_{36}O$	268
Heneicosane(19)	21.06	0.15	$C_{21}H_{44}$	296
2-Phyten-1-ol (3,7,11,15-Tetramethyl-2-hexadecen-1-ol or Phytol (2E,7R,11R)-form,20).	22.20	1.08	$C_{20}H_{40}O$	296
Pilocarpine (3-Ethyldihydro-4-(1-methyl-1H-imidazol-5-yl)methyl-2(3H)-furanone,21).	22.60	1.49	$C_{11}H_{16}N_2O_2$	208
11,12-Epoxide, 2,3,14-tri-Ac Derivative of: 8,17-Epoxy-2,3,9,14-tetrahydroxy-5,11-briaradien-18,7-olide(22).	22.8	1.57	$C_{26}H_{34}O_{11}$	522
1-Octadecanol(10)	23.19	2.3	$C_{18}H_{38}O$	270
2,6,10,14-Tetramethylheptadecane(23).	25.03	1.55	$C_{21}H_{44}$	296
Hexacosane(Cerane, 24).	25.98	0.70	$C_{26}H_{54}$	366
9-Octadecenoic acid (9).	26.77	2.33	$C_{18}H_{34}O_2$	282
Dotriacontane(25).	27.07	1.31	$C_{32}H_{66}$	450
1-Docosanol(26).	28.66	1.76	$C_{22}H_{46}O$	326
Pentatriacontane (27).	28.99	1.68	$C_{35}H_{72}$	492
1,30-Triacontanediol (28).	29.41	4.76	$C_{30}H_{62}O_2$	454
1-Hexacosanol (29).	35.50	1.54	$C_{26}H_{54}O$	382
11-Hexacosyne(30).	36.29	2.27	$C_{26}H_{50}$	362
1,3(20)-Phytadiene (7,11,15-Trimethyl-3-methylene-1-hexadecene orNeophytadiene,31).	37.85	2.41	$C_{20}H_{38}$	278
Dihydromahubynolide B(32).	44.86	1.83	$C_{21}H_{34}O_3$	334
Ethyl acetate fraction of *R. sativus* leaves:				
Tetrahydrothiophene 1,1-dioxide (Sulfolane orBondolane A,33).	10.75	9.04	$C_4H_8O_2S$	120
(Z)-9-Octadecenoic acid (9).	28.51	5.81	$C_{18}H_{34}O_2$	282
Acetyl tributyl citrate(34).	33.86	21.04	$C_{20}H_{34}O_8$	402
Tricosane(35).	40.39	11.71	$C_{23}H_{48}$	324
Nonacosane(36).	41.60	1.3	$C_{29}H_{60}$	408
4,12-Dihydroxy-2,7,10-cembratrien-6-one(37).	42.00	1.59	$C_{20}H_{32}O_3$	320
Pentatriacontane(14).	45.50	1.34	$C_{35}H_{72}$	492

Chromatographic separation using column and thin layer chromatography of the tested fractions resulted in isolation of four compounds (Fig 1). Two phytosterols were isolated from pet. ether fraction and were identified as stigmast-5-en-3-ol (β-Sitosterol) (1) andstigmasta-5,22E-dien-3β-ol (stigmasterol) (2). In addition, two flavonoid glycosides were isolated from the ethyl acetate fraction. These flavonoid glycosides were identified as quercetin 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranosid (3), which wasn't isolated before from any natural source and kaempferol 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside(4).

As reported sitosterol is difficult to be obtained in a pure state (Pollock and Stevem, 1965, Pateh *et al*. 2008 and Kamboj and Saluja, 2011). Consequently, compound (1) and (2) were isolated and identified together, having the same R_f despite the use of several solvent systems. ^{1}HNMR spectrum revealed the presence of two angular methyl groups signals at δ_H 0.68 and δ_H 1.01 ppm (each s), an olefinic proton signal at δ_H 5.35 ppm (br. s) and a proton-multiplet of a hydroxylated methine at δ_H 3.53 ppm, due to H-3 of a Δ^5- 3β-hydroxysterol.

The side chain signals appeared at δ_H 0.92 (3H, d, J= 6.7 Hz, H-21), δ_H 1.83 (1H, m, H-25), δ_H 0.84 (3H, d, J7.3 Hz, H-26), δ_H 0.81 (3H, d, J 7 Hz, H-27), and δ_H 0.88 (3H, t, J 6.1 Hz, H-29), suggesting that the sterol was stigmast-5-en-3-ol **(1).** Moreover, ^{1}HNMR spectrum revealed the presence of two olefinic proton signals at δ_H 5.02 and δ_H 5.14 ppm (each of them dd, J 8.6, 15.2 Hz for H-22 & H-23), suggesting existence of stigmasta-5,22E-dien-3β-ol **(2)**. Both of compound (1) and (2) are phytosterols spread in many plants and were previously isolated and characterized from the leaves

of *Rubus suavissimus* (Chaturvedula and Prakash, 2012) and from the leaves of *Odontonem astrictum* (Pierre and Moses, 2015).

Compound (3, 4) were isolated together as yellow amorphous binary crystalline mixture. ^{1}H-NMR data of compound (3) displayed proton signals pattern of flavonol moiety. Ring A was 5,7-disubstituted, as shown by two meta-located protons at δ_H 6.43 ppm (1H, br. s, H-6) and δ 6.77 ppm (1H, br. s, H-8). On the other hand, the observation of ABX system at δ 7.53 ppm (1H, br. s, H-2'), 6.82 ppm (1H, d, J=8.1 Hz, H-5') and 7.68 ppm (1H, br. d, J=8.1 Hz, H-6') has suggested a 3',4'-disubstituted ring B, which was identified as quercetin. HSQC spectrum confirmed the presence of two *α*-anomeric protons signals appeared at δ_H 5.53 (1H, br. s), δ_C98.33 and δ_H 5.29 (1H, d, J3.9 Hz),δ_C101.37, respectively in the aliphatic region which are belonging to two sugar moieties.

^{13}C NMR spectrum of compound (3) showed twenty six signals, eleven in the aliphatic region for two sugar units and the remaining signals in the aromatic region for the quercetin unit. The presence of one 3-O-α-L-arabinopyranosyl moiety in compound (**3**) was confirmed by its characteristic chemical shifts (δ_C101.37, 71.65, 70.75, 66.11 and 64.35 ppm for C-1", C-2", C-3", C-4" and C-5") respectively, another 7-*O*-α-L-rhamnopyranoside moiety was also confirmed from its chemical shifts (δ_C98.33, 69.85, 70.26, 71.61, 70.11 and 17.96 ppm for C-1'", C-2'", C-3'", C-4'", C-5'" and C-6'") respectively. Comparison of all previous spectral data of compound (3)with partial structures of its components which correspond to quercetin-7-O-arabinoside-3-glucoside and quercetin-7-glucoside-3 rhamnoside, which were isolated from *R. raphanistrum* (Kamil and Kalina, 1977) has revealed its identity as quercetin 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside, which, to our knowledge, wasn't isolated before from any natural source.

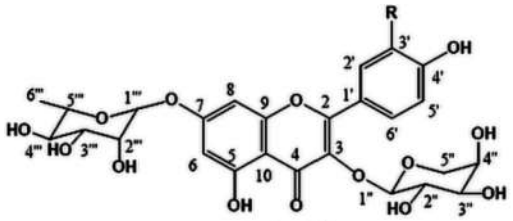


Fig 1 The isolated compounds

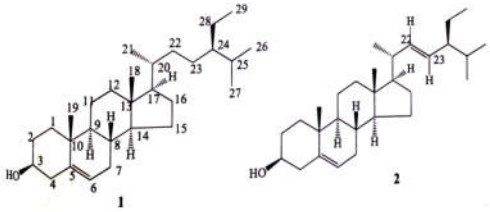
Insecticidal activity against mealybug, P.solenopsis

R. sativus leaves were processed to give three different fractions of different polarities: petroleum ether, methylene chloride and ethyl acetate fractions. These fractions were screened for their insecticidal activity against the third instar nymphs of *P. solenopsis* at 24 and 72 hours of exposure to find out the most effective fractions. Table 2.revealed that the most effective fraction was ethyl acetate followed by methylene chloride then petroleum ether fraction at the LC_{50} levels at both 24 and 72 hours post-treatment. The insecticidal efficiency of any plant extract depends up on its chemical constituents. So, the insecticidal activity of the petroleum ether fraction may be due to the presence of some components such as fatty acids specially, linolenic (5.21%) which showed high significant toxic effect on the 2nd and 4th instar larvae of *Spodoptera littoralis* (Yousef *et al*. 2013) and oleic acid (1.65%) which showed high insecticidal activity against *Aedes aegypti* (Tare and Sharma, 1991).

Table 2 Susceptibility of *P. solenopsis* third instar nymphs to different extracts of *R.sativus* leaves using spray method under laboratory conditions

Tested fraction	After 24h of treatment					After 72h of treatment				
	LC50 (ppm) and confidence limits at 95%	LC90 (ppm) and confidence limits at 95%	Slope ± SE	X2	Toxicity index*	LC50 (ppm) and confidence limits at 95%	LC90 (ppm) and confidence limits at 95%	Slope ± SE	X2	Toxicity index*
Pet. ether	2337.671 1528.9085345.882	23739.821 8322.112 856127.729	1.273 ± 0.3647	0.518	38.703	875.563 512.992 1227.291	5260.497 3107.536 18623.1	1.646 ± 0.382	0.201	48.135
Methylene chllride	1416.799 913.392 2199.921	11377.129 5292.512 100863.613	1.4166 ± 0.364	0.487	63.858	674.690 290.396 1000.799	4655.102 2556.109 29791.829	1.528 ± 0.431	1.519	62.466
Ethyl acetate	904.739 628.71471463.424	6237.483 2954.716 45469.451	1.5285 ± 0.370	0.788	100	421.455 277.834 560.542	1802.521 1212.83 4015.364	2.0306 ± 0.409	2.416	100

^{1}H and ^{13}C NMR as well as HSQC spectra were clarified that compound (**4**) is kaempferol 3-*O*-α-L-arabinopyranosyl-7-*O*-α-L-rhamnopyranoside which was identified before from *Viciafaba* (Allam *et al*., 2018).



Both linolenic acid and linoleic acid, showed insecticidal activities on the larval development of *S.frugiperda* (Ramos-López *et al*., 2012). Also, stigmasterol has been reported to inhibit acetyl cholinesterase activity and thus responsible for the larvicidal and repellent properties of *Chromolaena odorata* (Gade *et al*., 2017).

Methylene chloride fraction showed moderate efficiency against the 3rd instar nymphs of *P. solenopsis*. This fraction contained phytol which had been used as chemical deterrent agent against sumac flea beetles (Vencl and Morton, 1998), and recently, it is considered to be the key bioactive compound in most botanical insecticides (McGinty *et al*. 2010). Also, this fraction contained alkaloidal compound (21)(1.49%) and diterpenes (22) (1.57%) & (23) (1.55%), in addition to oleic acid (9) (2.33%).

Ethyl acetate fraction revealed the highest mortality, this might be as a result of its chemical constituents such as oleic acid (9)(5.81%) and some compounds with high polarities such as the isolated flavonoids glycosides (3&4) (12.6%).We can`t confirm the exact active ingredient of each fraction because of the wide range of chemical constituents of it, in addition to the synergistic effect of many components to reveal the insecticidal activity of the fraction.

SUMMARY AND CONCLUSION

The insecticidal activity of different solvent fraction of *R. sativus* leaves were studied. The most active fraction was the ethyl acetate fraction followed by methylene chloride then petroleum ether fraction. The major components of the tested fractions were characterized by GC/MS and chromatographically isolated. Compound (3), quercetin 3-O-α-L-arabinopyranosyl-7-O-α-L-rhamnopyranoside, was isolated for the first time from natural sources. All previous data emphasized that *R. sativus* leaves extracts are promising factors, that can be used as green insecticides.

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