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Bioactive metabolites from two local cultivars of *Ricinus communis* and their free radical scavenging and acaricidal activities

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ABSTRACT

The two cultivars of Ricinus communis L. (Euphorbiaceae); red Hendi 21(RH21) and green Hendi 12 (GH12) were investigation of their phytoconstituents using standard methods. Eighteen and thirteen volatile compounds were identified from the n*-hexane extract of RH21 and GH12, respectively by using gas chromatography-mass spectrometry (GC/MS). The monoterpenoids; 1,8-cineole, camphene and α–thujone and the sesquiterpenoids; β-caryophyllene and longifolene were detected in both cultivars. Whereas, the monoterpenoids; camphor and α -pinene were found only in RH21. In addition the biochemical composition of minerals, protein, lipids, carbohydrates and the quantitative evaluation of the total content of phenolic, flavonoids, anthocyanins, tannins, and alkaloids were studied. Gallic acid, epicatechin, quercetin and kaempferol are the major phenolic compounds detected in the aerial parts of each cultivar in variable amount. The stable free radical scavenging activity (FRSA) of both cultivars was determined using the 1.1-diphenyl-2-picryl-hydrazil. Variable primary metabolites content in both cultivars was reported. The cultivar RH21 had the superiority in the contents of all the investigated secondary metabolites and FRSA than the GH12 cultivar. The potential of the cultivars crude extracts were bioassayed to determine their acaricidal activities against the mite Tetranychus urticae Koch using dipping and spray methods. The cultivar RH21 had high mortality on the larvae and adult females of T. urticae than GH12. The results of this study suggest that the natural product derived from the red cultivar RH21 of R. communis may has potential use as acaricidal agent. This activity of RH21 might be attributed to the presence of various bioactive phytochemicals.*

Keywords: *Ricinus communis*, metabolites, GC/MS, DPPH, *Tetranychus urticae*.

INTRODUCTION

The need for alternative, non chemical, control strategies in crop protection systems has increased in the last decade due to development of resistant strains of mites [1]. Herbal therapies are natural products, environmental friendly and cheap. The use of plant extracts with insecticidal activity provides a potential biodegradable alternative to synthetic pesticides. Plant-based acaricides are often ecologically friendly and safe alternative in managing mites with low frequency to cause mite resistance [2]. The use of new natural acaricides with different mode of action as

biodegradable and mammalian and environmentally safe mite control agents had increased [1]. The deleterious effects of crude plant extracts on mites are manifested in several ways, including toxicity, antifeedants and may affect some biological parameters such as growth rate, life span and reproduction [3, 4].

Metabolite is usually restricted to small molecules. Metabolites have various functions and medicinal plants are valuable source of a wide range of primary and secondary type, which are used as pharmaceuticals, agrochemicals and biopesticides. Crude plant extracts often consist of complex mixtures of active primary and secondary metabolites, they may show greater overall bioactivity compared to the individual constituents [5, 6].

The lipids, proteins and carbohydrates are sometimes referred to as proximate principles while minerals play an important role in the regulation of the metabolic activity in the body [4].

Secondary metabolites produced by plant are considered one of the most important defenses against pests and serious mites and have crucial role in the interaction of a plant with its biotic and a biotic environment. Many secondary plant metabolites are known for their acaricidal properties [7].

Ricinus communis, known as wonder tree, is an important traditional medicine plant in Egypt. It is a perennial scrub probably originates from Africa and was used in ancient Egypt and by the Romans and Greeks [8, 9]. It has been used to control insect pests in several crops [10-13]. It showed larvicidal [10] and insecticidal [14] activities against the adult of *Haemaphysalis bispinosa* Neumann and the hematophagous fly.

Tetranychus urticae Koch (Acari: Tetranychidae), also called two-spotted spider mite (TSSM), is serious pest worldwide, causing serious damage to vegetables, flowers, and fruit crops [15]. It has been reported to attack about 1200 species of plants, of which more than 150 are economically important [16] and are able to transmit many of plants viruses. In Egypt, *T. urticae* is a major economic pest of many host plant species, including cotton, corn, soybeans, many orchard crops, and ornamentals [17]. It can produce 10-20 generations per year and develop from eggs to adults in about one week. It can significantly weaken their host plant vigor and consequently crop reduction or total loss worldwide [18]. This spider mite has developed serious resistance to many chemical pesticides and when the population of it exceeds economic threshold, the use of plant insecticides are desirable in this pest control.

To our knowledge previous reports showed that no works have been published on the chemical composition, radical scavenging or acaricidal activity of the two local cultivars under investigation. In the current study, the biochemical composition of both cultivars was studied including the primary and secondary metabolites. Moreover, an evaluation of the free radical scavenging and acaricidal efficacy of them with emphasis for the possible future use of the promising extract as an alternative to chemical acaroids.

MATERIALS AND METHODS

1. Plant samples

The aerial parts [stems and leaves] of the two cultivars; Red Hendi 21 and GH12 of *Ricinus communis* L. (Euphorbiaceae) was collected during the beginning flowering stage in April 2013 from a field in Berma, Tanta District, El-Gharbia Governorate, Egypt. The plant materials were confirmed by Dr. S.S. El-Khanagry, Department of Flora and Phytotaxonomy Research, Horticultural Research Institute, Egypt. The dried plants were ground into fine powder followed by filtrating through a 300x100 screen. Subsequently, the cultivars powder was, separately, collected and sealed in black plastic bags at room temperature (25±2 °C and 70±5% relative humidity (RH) for later study.

2. Mite strain

The local strain of *Tetranychus urticae* Koch (*Arachnida*: Acari: Tetranychidae) used in this study originated from infested leaves of castor bean, *R. communis* L. The mite stocks were grown under controlled conditions in a climate room at 25±2 °C, 70±5% RH and a photoperiod of 16:8 h (L:D). Individual female mites were collected and transferred for bioassay tests.

3. Quantitative determination of bioactive metabolites

3.1. General

The volatiles were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). GC-MS was carried out using total ion monitoring mode on a Finnigan mass Spectrometer model SSQ 7000 equipped with library software Wiley 138 and NBS. Capillary DB-5 (methyl polysiloxane containing 5% phenyl groups) column 25 m x 0.25 mm i.d. was used. The initial column temperature was started at 60˚C for 2 min., programmed at 60-100˚C (2˚C/min) and 100-250˚C (5˚C/min). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Injection voltage 70 eV was used. Molecular ions (scan mass range: 40-450 mz^{-1}) were monitored for identification. UV–visible spectrophotometer, Shimadzu UV 240 (PIN 2041-5800), was used for recording UV spectra and measuring the absorbance in UV and visible range. An automatic Kjeldahl-Foss apparatus, model 16210 (Foss America Inc., Fishkill, NY), and a Markham distillation apparatus (Markham, Ontario, CA) for determination of total protein content were used. An atomic absorption spectrophotometer (Lambda II apparatus, P.46) and flame emission spectrometry (Eppendorof, DR Lang), were used for elements detection. The presence of major phenolic compounds; quercetin, ellagic, rutin, kaemferol-3-glucopyranoside, gallic acid, epicatechin and kaempferol, and ricinine tested in each extract by using previously isolated or standard compounds (purchased from Merck Company, Darmstadt, Germany), available in our laboratory of Natural Compounds Chemistry. Folin-Ciocalteu reagent, sodium carbonate, glucose and aluminum chloride were purchased from Merck Company (Darmstadt, Germany). 1.1-Diphenyl-2-picryl-hydrazil (DPPH) was purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA). All other reagents and solvents were of analytical grade.

3.2. Preliminary phytochemical screening

Phytochemical screening was performed to test the cultivar RH21 and GH12 extract [19, 20] for its volatile constituents, carbohydrates and/or glycosides, tannins, free and combined flavonoids, sterols (and/or terpenoids), alkaloids, anthraquinones and cardiac glycosides content. Alkaloids, tannins, anthraquinones, saponosides, sterols and triterpenes are chemical groups that are likely to have pesticidal properties (insecticides, acaricides, vermicides, molluscicides and fungicides) [4].

3.3. Gas chromatography–mass spectrometry (GC-MS) analysis of RH21 and GH12 extracts

3.3.1.Preparation of samples

500 g of fresh aerial parts of each cultivar RH21 and GH12 were extracted with CH_2Cl_2 (2 L) then evaporated till dryness. The residues (0.8 g of R1 and R2) were extracted with *n*-hexane then evaporated till dryness to get oily residues. The distilled essential oils were dried over anhydrous sodium sulfate, filtered, and stored at +4°C. Each residue was dried over anhydrous sodium sulfate filtered, and stored at 4 °C in a sealed vial for further analysis [21].

3.3.2.GC–MS analysis

The volatiles were analyzed by GC–MS. The relative percentage of the oil constituents was expressed as percentage by peak area normalization. The identification of the separated compounds was based on their retention indices, relative to a homologous series of *n*-alkane (C_8–C_{20}) on the DB-5 capillary column under the same operating conditions and computer matching with the GC–MS spectra from the library software Wiley 138 and NBS data and those reported in literature [22, 23].

3.4. Determination of primary metabolites of the two local cultivars

3.4.1.Micro- and macroelements

They were determined in the digested solution by acid mixture adapting flame emission spectrometry for Na^+, Ca^{2+}, Mg^{2+}, P^{3+}, and K^+ assay and atomic absorption for Hg^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Li^+ [24].

3.4.2.Total protein content

The percentage of total protein was measured by determining the nitrogen content by the Kjeldahl's method [21, 25] using a Markham distillation apparatus. The method is based on the principle of digestion of the substance with concentrated hydrochloric acid, a process in which the nitrogen is converted to ammonia. The total protein content was calculated in mg/g of the dried matter in the sample (% Protein = % Nitrogen x 6.25).

The dried ground sample 0.5 g was taken in a Kjeldahl flask. Added to it 18 ml of H_2SO_4 and 1g of $CuSO_4$ and 20-25 ml of conc. H_2SO_4. It was digested in Kjeldahl digestion unit for 6 hours. The mixture was cooled down to room temperature. It was transfer red about 50 ml of 4% boric acid solution in a receiving flask and added to it 3-5 drops of mixed indicator and placed it under the condenser of Kjeldahl. Distillation unit making sure that the condenser

tube extends beneath the surface of the acid, in the flask, now added to the Kjeldahl flask 50 ml water and 60 ml of 32% NaOH solution. Distilled so that a volume of 200°C is collected in the receiving flask, remove the flask for titration. Take 0.1 N HCl in burette and titrate the content of the flask against it. The percentage of protein is determined as follows: % Protein = % Nitrogen x 6.25.

3.4.3.Total lipid content

Fifty grams from each air-dried aerial parts under investigation was separately extracted with petroleum ether 40-60 ºC for ~5 hrs in a closed system consisting of a Soxhlet extractor equipped with a chilled condenser [20, 21]. Each of the resulted extract was concentrated *in vacuo* by rotary evaporator. The residues of crude lipids were weighed gravimetrically and kept in desiccators.

3.4.4.Total carbohydrate content

Total carbohydrates were determined after complete acid hydrolysis [26] and the absorbance of characteristic yellow colour measured at 490 nm. The percentages of total carbohydrates present in the aerial parts of each cultivar under investigation were calculated using standard calibration curve of glucose.

3.5. Quantitative determination of secondary metabolites of the two local cultivars

3.5.1. Total phenolic content

The total phenolic content of ethanol extract of two cultivars was determined according to the method described by Makkar et al., [27]. Aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded by colorimetry at 725 nm (using UV/VIS, 2041 spectrophotometer) against the reagent blank. The amount of total phenolics was calculated as gallic acid equivalents from a calibration curve. The standard curve was prepared using 500, 400, 350, 325, 300, 250, 225, 200, 150, 125, 100 and 50 mg/L solutions of gallic acid in methanol (y = 0.0009x; R2 = 0.9875). Total phenol values were expressed as gallic acid equivalents (mg GAE/g of dry mass), which is a common reference for phenolic compound. The test was conducted in triplicate.

3.5.2. Total flavonoid content

Total flavonoid content was estimated using the method of Ordoñez et al., [28]. To 0.5 ml of each cultivar ethanolic extracts, 0.5 ml of 2% AlCl ethanol 3 solution was added. Filtration after 1 h at room temperature was carried out and the absorbance was measured at 420 nm. Quercetin was used as standard (1mg/ml) in methanol (y= 0.0071x; R2= 0.9979). Total flavonoid values were expressed as quercetin equivalents (mg QE/g of dry mass), which is a common reference compound for flavonoids. All the tests were performed in triplicates.

3.5.3. Total tannin content

Tannins of each cultivar were determined using the modified vanillin hydrochloric acid (MV-HCl) as reported by Maxson and Rooney [29]. Sample of 1 gram dried cultivar was extracted with 1% concentrated hydrochloric acid in methanol. The mixture then were shaken for 24 hours and let to settle. A 5 ml of vanillin-HCl reagent (50:50 mixtures of 4% vanillin/ 8% HCl in methanol) was quickly added to 1 ml extract. The developed color was measured at 500 nm using spectrophotometer. The standard curve was obtained and tannins were calculated.

3.5.4. Total anthocyanin content

Anthocyanins were extracted overnight from fresh aerial parts of each cultivar, separately, with ethanol and 1% HCl (85:15) at 4°C. The optical density of each cultivar extract solution was measured at 535 nm. The total anthocyanins concentration was calculated according to Francis, [30] using the extinction coefficient:

$$\left(E_{1\text{cm}}^{1\%} = 98.2 \text{ at } 535 \text{ nm}\right)$$

3.5.5. Total alkaloid content

The total alkaloid content was extracted according to Lee and Waller [31] by homogenization of 2gm each cultivar dry aerial parts with 100 ml chloroform. The residue was further extracted with 75% methanol until the residue was free of soluble pigments. The chloroform and methanol-water extracts were combined and concentrated under reduced pressure to about 25 ml, cooled and the aqueous solution decanted. The residue was rinsed twice with water. The aqueous fraction was evaporated to dryness. The residue was then extracted with hot methanol. The collected

methanol extracts were concentrated and used for colorimetric estimation of alkaloids using calibration curve. Alkaloid content was expressed as ricinine equivalent (mg R/g of dry mass). The standard curve was prepared using 750, 500, 400, 250, 200, 150 and 100 mg/L solutions of ricinine in HCl 0.1N (y=0.0012x0.1044; R2 = 0.9851). Alkaloid contents were expressed ricinine equivalents. This test was performed in duplicate.

4. Detection of bioactive phenolic compounds

Aliquot of 5 µl of each cultivar extract were applied to 2 thin-layer chromatography (TLC) with the available authentic samples of phenolic compounds. TLC was performed on silica gel GF_{254} precoated plates (Merck, Darmstadt, Germany). The chromatograms were visualized under UV light at 254 and 366 nm before and after exposure to ammonia vapour, as well as spraying with $AlCl_3$ or *p*-anisaldehyde/sulfuric acid reagent using the solvent systems; S_1, benzene/ethyl acetate (9:2, v/v) and S_2, hexane/ethyl acetate (1.5:8.5, v/v). Paper chromatography (descending) Whatman No. 1 MM papers, using solvent systems (v/v): S_3, acetic acid/H_2O (1.5:8.5, v/v) and S_4, *n*-butanol/acetic acid/H_2O (4:1:5, upper layer) were applied [20, 21].

5. Biological evaluation of the two local cultivars RH21 and GH12

5.1. DPPH free radical scavenging assay

The free radical scavenging activity using DPPH reagent was determined according to Brand-Williams et al., [32]. The extract was soluble with 50% methanol: water. To 0.75 ml of the cultivar sample, 1.5 ml of freshly prepared methanolic DPPH solution (20 µg ml) was added and stirred. The decolorizing processes was recorded after 5 min of reaction at 517 nm and compared with a blank control. The absorbance decreased with increasing free radical scavenging activity. Radical scavenging activity = [(control absorbance - sample absorbance)/control absorbance] × 100%

5.2. Contact and absorption toxicity of RH21 and GH12 cultivar extract on larvae and adults of *T. urticae* Koch

5.2.1.Preparation of cultivars extract

The fresh aerial parts of each cultivar of *R. communis* (500 g) were, separately, exhaustively extracted with methanol (90%) using a Soxhlet apparatus. The methanolic extract of each sample was distilled *in vacuo* at 50 °C. The yields were 12.34 and 16.25 for RH21 and GH12, respectively. Each crude extract was diluted with distilled water at a concentration of 0.5g/50ml for biological evaluation.

5.2.2. Bioassay methods

Crude extract of two plant cultivars; RH21 and GH12 of *R. communis* was tested for acaricidal activity against *T. urticae*. The following bioassay methods were used to assess contact and absorption toxicity on mites:

5.2.2.1. Leaf-dipping bioassay

Leaf discs were collected from castor bean leaves (*R. communis*) and dipped separately of the crude extract solution in water for 10 sec. The treated discs were air-dried for about 5 min to evaporate the water. Control discs were sprayed with distilled water. Five adult females of *T. urticae* were placed on a single leaf disc prepared before and kept on moist cotton wool in a *Petri* dish (12cm in diameter by 2 cm in depth). Each dish contained five discs as replicates, the edges of each disc were covered with wet cotton to prevent mite escaping [33].

5.2.2.2. Leaf-spraying bioassay

Five adult females of *T. urticae* were placed on a single leaf disc of castor bean leaves and kept on moist cotton wool in *Petri* dish. There were five replicates for each treatment. Then *Petri*-dish was sprayed with 1 ml of the extract dilution or distilled water. The treated discs were air dried for about 5 min to evaporate the water. All the edges of each disc were covered with wet cotton to avoid escape of mites.

Treated mites were maintained at 25°C±2 and relative humidity of 70±5%. Mortality was checked at 24 h after treatment for 14 days. Mites were considered dead if they did not respond to a gentle probe with a fine brush [33].

5.3. Data analysis

Each experiment was run in triplicate, and mean values were calculated. Mortality data of the bioassays were corrected for control mortality employing Abbott's formula [34].

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using standard methods [19]. The present results of the phytochemical screening showed that both cultivars under investigation are free from coumarins and cardiac glycosides. Other constituents such as anthraquinones are very scarce. Volatile constituents, carbohydrates and/or glycosides, unsaturated sterols and/or triterpenes, tannins, saponins, anthraquinones and flavonoides [both aglycones and glycosides], alkaloids, and/or nitrogenous compounds in the studied RH21 and GH12 cultivars in considerable amount. These detected compounds have valuables effect as acaricidal and antioxidant agents [4]. *Ricinus communis* L. belongs to Euphorbiaceae family that consists of species develop a wide battery range of defensive secondary metabolites and diverse medicinal importance [35, 36]. *Ricinus* species were reported as rich in phenolic compounds, glycosides, volatile phytochemicals, alkaloids and steroids [37], the compounds now been known to affect insect behavior, growth reproduction and survival [38].

Chemical composition of the essential oil identified by GC/MS analysis

Eighteen and thirteen compounds were identified by GC/MS of *n*-hexane extract of RH21 and GH12, representing 90.51 and 90.00% of their total content. The identified compounds are listed in Table 1, according to their elution order on a DB-5 capillary column.

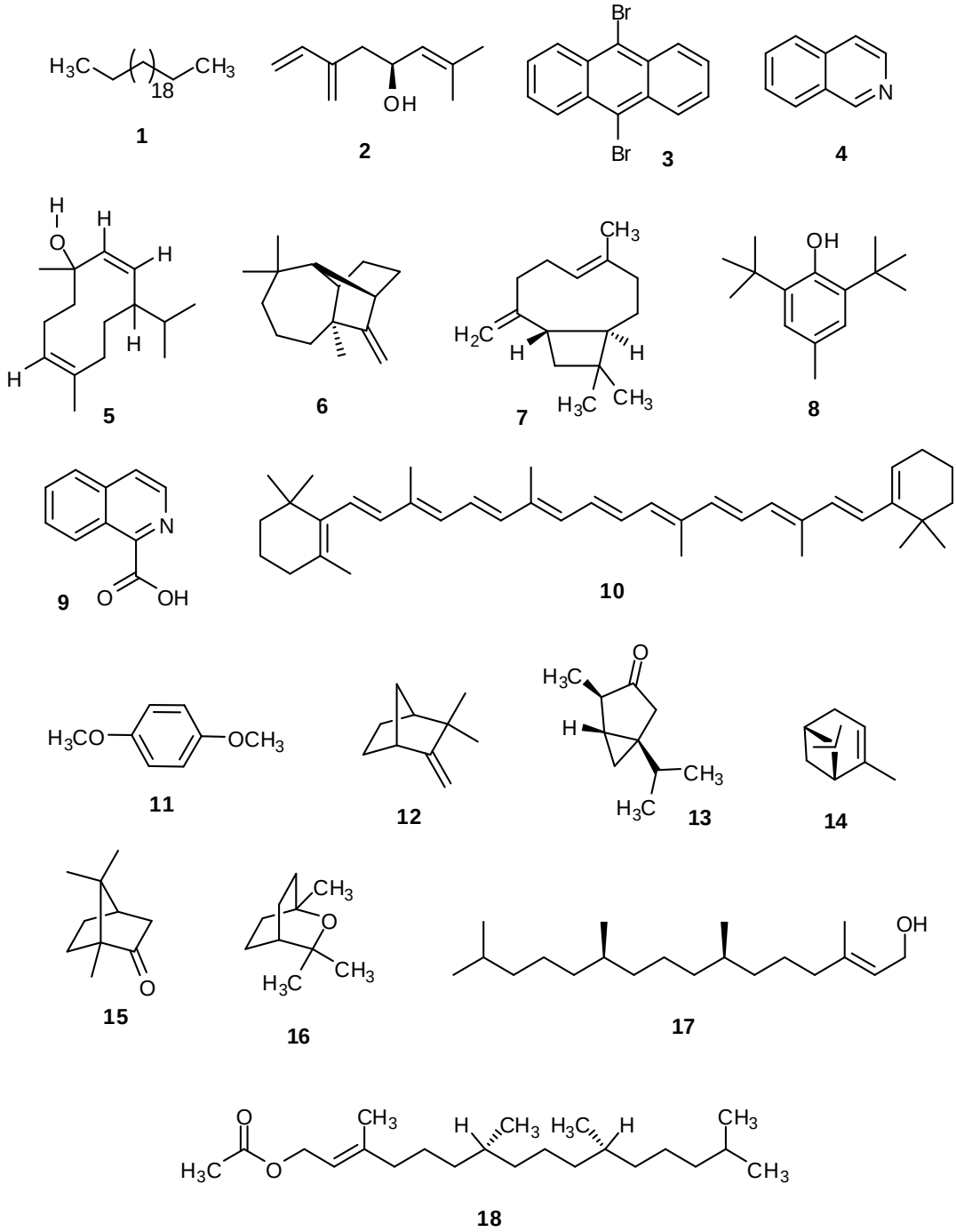
Table 1: The volatile compounds identified in the *n*-hexane extract of the two cultivars; RH21and GH12 of *Ricinus communis* L. by using GC/MS

Compound No.	RT	Identified Compound	Conc. (%)		Type	Identification method
			RH21	GH12		
1	6.38	Docosane	0.33	3.23	HC	MS
2	6.43	Ipsdienol	0.29	0.51	LOC	MS& KI
3	7.58	9,10–Dibromo anthracene	0.45	---------	AHC	MS
4	20.18	Isoquinoline	0.53	0.95	AHC	MS
5	21.49	Germacra-1(10),5-dien-4-ol	2.03	1.73	ST	MS& KI
6	22.65	Longifolene	0.98	----------	ST	MS& KI
7	24.26	*β-Caryophyllene	10.50	----------	ST	MS& KI
8	26.51	2,6-*bis*-(1,1-Dimethyl-propyl)-4-methyl-phenol (DPMP)	13.55	17.07	LOC	MS
9	27.15	1-Isoquinoline carbonitrile	0.49	2.19	AHC	MS
10	29.76	β-Carotene	1.30	1.38	TT	MS& KI
11	30.60	2-Bromo-1,4-dimethoxybenzene	0.33	0.56	HOC	MS
12	30.95	*Camphene	3.40	12.93	MT	MS& KI
13	32.16	*α– Thujone	12.62	10.74	MT	MS& KI
14	32.45	*α-Pinene	11.20	----------	MT	MS& KI
15	33.68	*Camphor	4.52	----------	MT	MS& KI
16	36.84	*1,8-Cineole	10.48	10.53	MT	MS& KI
17	39.01	Phytol	17.00	27.67	DTA	MS
18	39.70	Phytol acetate	0.51	0.51	DTA	MS

*HC, hydrocarbon; LOC, lightly oxygenated compound; AHC, aromatic hydrocarbon; ST, sesquiterpene; TT, tetraterpene; HOC, heavily oxygenated compound; MT, monoterpene; DTA, diterpene alcohol; MS, confirmed by comparison with mass spectrum; KI, confirmed by comparison with Kovat's index on a B5 column; Conc., concentration based on peak area integration; and RT, retention time, * previously identified compound in R. communis L.*

The identified compounds comprise 1.80, 6.37% unoxygenated compounds, 14.17, 18.14% oxygenated compounds, 64.05, 35.19% terpenoidal compounds in RH21 and GH12, respectively. The oil contains a complex mixture consisting of mainly oxygenated mono- and sesquiterpenes, and mono- and sesquiterpene hydrocarbons. The results revealed the presence of monoterpenoids (1,8-cineole, camphene and α–thujone) in both of RH21 and GH12. The monoterpenoids (camphor and α-pinene) were found in the red cultivar RH21 only. Sesquiterpenoids (β-caryophyllene and longifolene) was found in both local cultivars. The percentage of terpenoidal compounds in the red cultivars RH21 (64.05%) is higher than the green one GH12 (35.19%). The oil of *Ricinus communis* cultivars has a high chemical potential offers a wide variety of terpenoids whose insecticidal activity has already been tested and confirmed [39]. Figure 1 showed the structure of the identified volatile compounds from the two cultivars RH21 and GH12 of *Ricinus communis* L. using GC/MS analysis.

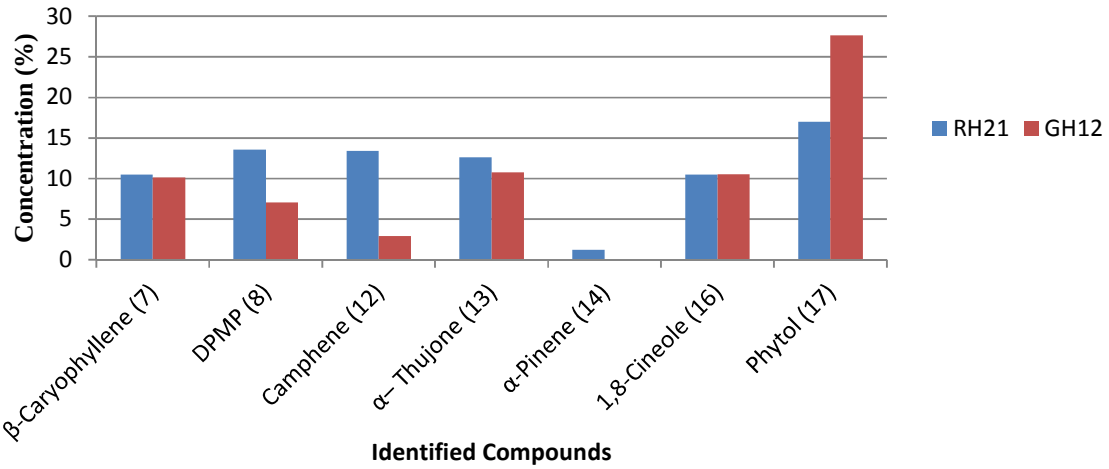
Figure 1. Structure of the identified volatile compounds from of the two cultivars; RH21and GH12 of *Ricinus communis* L. using GC/MS analysis



In the current study, good levels of specific secondary metabolites like camphor and β-caryophyllene were detected in the red cultivar RH21 by GC-MS analysis. No camphor was detected in the green cultivar GH12. The red cultivar RH21 showed remarkable acaricidal activity than the green one GH12. Recently, Singh et al., [39] reported that camphor and β-caryophyllene (known to be involved in defense) were significantly increased in leaves upon insect attack. In addition to, larvae fed on artificial diet supplemented with the plant extract of *Ocimum kilimandscharicum*, camphor and β-caryophyllene showed growth retardation, increased mortality rates and pupal deformities.

The diterpene alcohol; phytol (27.67 and 17.00%) and the low oxygenated compound; 2,6-*bis*-(1,1-dimethyl-propyl)-4-methyl-phenol (17.07, 13.55%) were the predominant compounds detected in the oil of GH12 and RH21, respectively. The monoterpenes; α-thujone, α-pinene and 1,8-cineole with nearly equivalent good contents (12.62, 11.20 and 10.48%, respectively), and a sesquiterpene; β-caryophyllene (10.50%) in RH21, as shown in Table 1 while, camphene (12.93%), thujone (10.74%) and 1,8-cineole (10.53%) were detected in GH12. The comparison of the major identified volatile compounds (**7**, **8**, **12**-**14**, **16** and **17**) in RH21 and GH12 cultivars was illustrated in Figure 2.

Figure 2. The major volatile compounds identified in the cultivars; GH12 and RH21 of *R. communis* L. by using GC/MS



A tetraterpene; β-carotene and the alcohol germacra-1(10),5-dien-4-ol, also called germacrene D-4-ol were also found to be the minor compounds of oil of both *Ricinus communis* cultivars in the present study. Most of the major compounds from the two cultivars extract are biologically active molecules. They are considered to be a part of plant defense systems, and as such have been included in a large group of protective molecules found in plants named "phytoanticipins" or "phytoprotectants [40]. Our results show that the variation in quantities of the main components *e.g.* monoterpenes and sesquiterpene, might be responsible for the different acaricidal activity. The red cultivar RH21 has higher percentage of monoterpenes (42.22%) than the green one GH12 (34.20%). This result was in agreement with many studies reported the intense insecticidal properties of plant extract might be associated with their high contents of oxygenated monoterpenes [41]. In the present study, camphor, α-thujone and 1,8-cineole were identified in the oil of RH21. In another study, *Artemisia herba-alba* oil rich in camphor, α-thujone and 1,8-cineole were demonstrated to have potent insecticidal activity against three stored product beetles [42]. Monoterpenes possess many pesticidal activities, including insecticidal [43]. A linear relationship between LD_{50} values for house fly toxicity and Mulliken populations in aromatic monoterpenoids was found.

Therefore, the detected acaricidal properties of the red cultivar RH21 extract could be due to the relatively high concentration of monoterpenes, which is believed to actively inhibit the mite *Tetranychus urticae* Koch.

Volatiles from insect resistant rice cultivars repel feeding by the green rice leaf hopper [44]. Similarly, Volatile hydrocarbons emitted by the foliage of resistant plant may act to repel mites.

Determination of bioactive metabolites

Primary metabolites have functions that are essential to growth and development. In contrast, secondary metabolites functions are specific to the plants in which they are found.

Primary metabolites of the two local cultivars of *Ricinus communis* L

Macroelements (%), calculated with reference to air-dried aerial part of each cultivar and microelements in ppm were calculated (Table 2).

Table 2: Micro- and macroelements content of cultivars RH21 and GH12 of *Ricinus communis*

Element type	Element symbol	Local cultivar	
		RH21	GH12
Macroelements (%)	P^{3+}	1.33	1.06
	N	1.50	2.72
	K^{+}	1.04	1.18
	Mg^{2+}	18.29	11.59
	Ca^{2+}	2.36	4.67
	Na^{+}	0.70	1.10
Microelements (ppm)	Fe^{2+}	98	114
	Mn^{2+}	56	47
	Zn^{2+}	35	27
	Cu^{2+}	6	9

Minerals and trace elements play well-defined roles as components of metalloenzymes and medicinal plants belong to Euphorbiaceae family are good sources of them [45]. High content of Mg of red cultivar RH21 may affect the potential acaricidal activity [46].

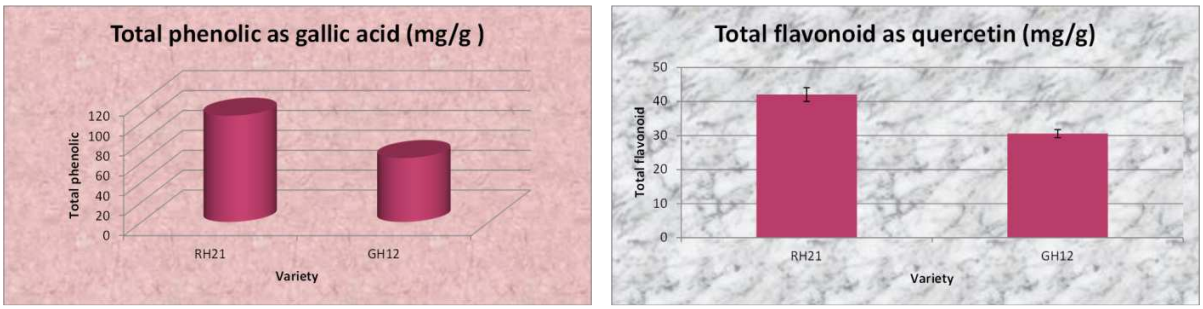
Many primary metabolites are acts as precursors or pharmacologically active metabolites in bioactive compounds [18]. Plant produces various primary metabolites such as lipid, protein, carbohydrates and phenolics. In the present study; 9.68, 9.85% on dry weight basis was the total protein, 4.2±0.12, 6.6±0.09 g the total lipid; 0.280±0.002, 0.288±0.002 (g/100g) the total carbohydrate content, for RH21 and GH12, respectively. Lipids are known to be insecticidal and their activity is maximized through saponification and esterification [4]. The activity of *R. communis* as insecticidal against *Zabrotes subfasciatus* (Coleoptera: Bruchidae) [12, 13] was suggested to be related to the defense-related metabolites lipophilic acyl chains, free hydroxyl groups, alkaline and esters content of oil.

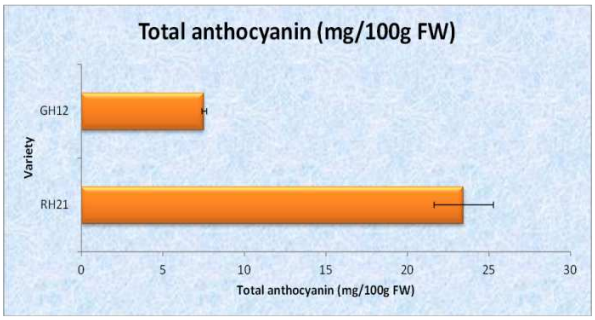
In the current study, considerable percentage of total protein content in both cultivars was reported. It War et al., [47] reported that an alteration in the protein's amino acid content or sequence influences the function of that protein. The anti-insect activity of a proteolysis-susceptible toxic protein can be improved by administration of protease inhibitors, which allows toxic protein to exert their defensive function [47]. There continues to be considerable interest in the potential benefits of phenolic compounds present in a number of botanical supplements [8].

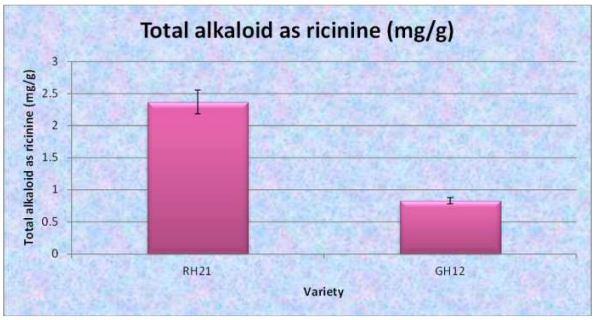
Secondary metabolites of the two local cultivars RH21 and GH12 of *Ricinus communis* L

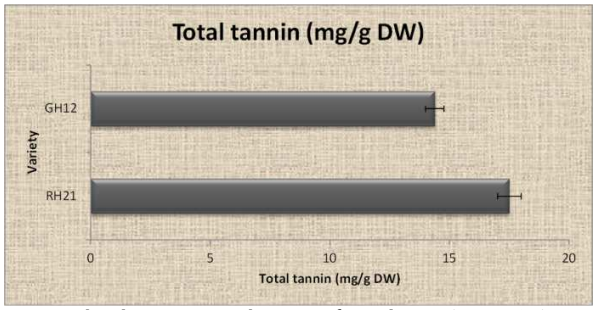
Phytochemical analyses of bioactive secondary metabolites in each plant cultivar were carried out using different assays. As illustrated in Figure 3, the extract of red cultivar of *R. communis* RH21 reported the higher quantities of total phenolic, flavonoids, anthocyanins, alkaloids content than the green cultivar GH12. The total phenolics (mg gallic/g dry weight (D.W.) were 106.71±2.85, 62.64±1.70; the flavonoids (mg quercetin/g D.W.) were 42.04±2.04, 30.54±1.185, the anthocyanins (mg/100g fresh weight (F.W.) were 23.45±1.82, 7.48±0.144; the tannins (mg/g D.W.) were 17.5±0.49, 14.38±0.37 and the alkaloids (mg/g ricinine) were 2.37±0.185, 0.83±0.05, in RH21 and GH12, respectively.

Figure 3: Secondary metabolites of two cultivars RH21 and GH12 of *Ricinus communis* L.









**Each value represents the mean of 3 replicates (Mean ±SD).*

It also contains a reservoir of diverse secondary metabolites including flavonoids, terpenoids, and alkaloids that can be poisonous, repellent, or trap insects and other organisms, thus forming a combination of structural and chemical defense [4, 8, 10]. Among the secondary metabolites, phenolics are phytochemicals constitute one of the most common and widespread group of defensive compounds against pathogens, herbivore attacks and combating stresses [47]. Flavonoids play a central role in various facets of plant life especially in plant-environment interactions. These defend plants against various biotic and abiotic stresses. Barbehenn and Peter-Constabel reported the role of tannins in plant defense and their induction in response to insect damage in many plants [48]. The allelochemical compounds found most frequently to cause deterrence are alkaloids, terpenes and phenols.

Plant secondary metabolites show great structural diversity and wide variability. A large number of secondary metabolites in plants have a role in direct plant defense [4, 17]. Many authors studied the relation between secondary metabolites and mite attack. The variability in the amount of secondary metabolites in plants is clearly linked with genetic characteristics, although biotic and abiotic factors can provoke changes in this variability [49, 50]. The resistance of many plants to mites is always relative. It is composed of the genetically inherited qualities that result in a plant of one cultivar or species being less damaged than is the susceptible one, which lacks these qualities. The degree of resistance is leased on comparison to susceptible plants that are more safely damaged under similar test conditions.

It is noteworthy that many plants have been used as botanical pesticides in the agricultural fields because of their acaricidal properties which are due to compounds synthesized in the secondary metabolism of the plant [4]. Therefore, the biological activities of *R. communis* aerial parts may be attributed to the presence of a group of compounds or more belongs to one or more of these classes of plant secondary metabolites.

I the present study, examination of the total extract of the two cultivars of *R. communis* using TLC was visualized by spraying reagents, revealed the plant aerial parts to contain a unique complex mixture of several components, including flavonoid aglycones and phenolic acids. The TLC profile under 254 nm showed presence of three flavonoid aglycones; epicatechin, quercetin and kaempferol and one phenolic acid; gallic acid, that is consistent with Singh et al., [51]. Catechin and gallocatechin in leaves of *Quercus robur* L., were reported to inhibit winter moth larvae, *Operophtera brumata* [47]. In a previous work, two kaempferol glycosides and tannins have been isolated

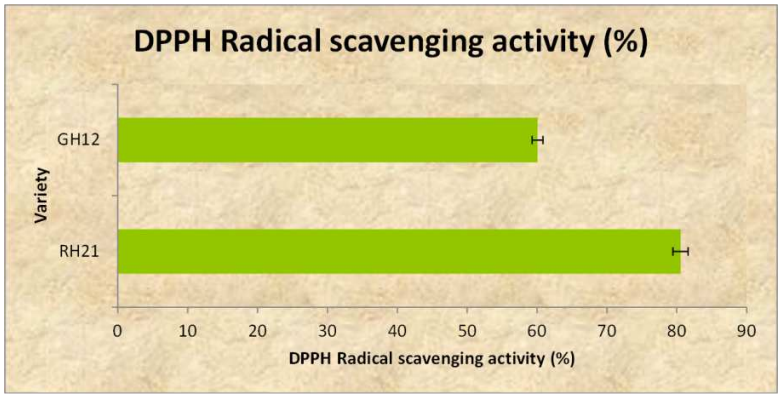
from the leaves part of *Ricinus communis* [4]. Role of phenolics in the resistance mechanisms of plants against phytophagous insects was reported [52].

There is a relationship between the level of plant metabolites plant extract and the degree of insect infestation [49]. Phytohaemagglutinin (ricin), alkaloids, polyphenolics, triterpenes, and oxalic acid are anti-nutrition factor. Cazal Cde et al., [53] reported the alkaloid ricinine; obtained from *Ricinus communis* as an insecticide for leaf-cutting ant (*Atta sexdens rubropilosa*).

The free radical scavenging (FRS) activity of both cultivars was determined using DPPH. The DPPH assay is often used to measure the ability of an agent to scavenge free radicals. Lower absorbance value of the red cultivar RH21 indicates higher FRS activity by spectrophotometric assay at 517 nm (Figure 4). The FRS was 80.53±1.09; 60.06±0.79 of RH21 and GH12, respectively. This higher activity may be attributed to the presence of bioactive metabolites including phenols, flavonoids, tannins, and alkaloids in good amounts in the extract. In addition to the major metabolites, also minor metabolites may make a significant contribution to the biological activity of these extracts. Figure 3 illustrated the superiority of RH21 in FRSA than the GH12 cultivar.

The obtained results are in agreement with Singh et al., [51] who found that the methanol: water (8:2) extract of castor leaves showed strong radical-scavenging activity. The current study could infer that the FRS activity effect of *R. communis* cultivars is the synergistic effect of their compositions. It is well known that in response to mite attack, qualitative and quantitative alterations in phenols and elevation in activities of oxidative enzyme is occurred [47].

Figure 4: Free radical scavenging activity of RH21 and GH12 cultivars of *Ricinus communis* L.



Contact and absorption toxicity of RH21 and GH12 cultivar extract on larvae and adults of *T. urticae Koch*

Table 3 shows the effect of the plant extract of RH21 and GH12 of *R. communis* aerial parts as acaricidal by using leaf-disc dipping and spray method. In dipping method, RH21 extract was more effective than that GH12. Along the 14 days of investigation, both the mean numbers of dead individuals of *T. urticae* Koch and their percentage were considerably higher when treated with the RH21 extract. The 3rd, 7th, and 13th days were chosen to confirm this trend; mortality percentage in the treatments of RH21 recorded 24, 44, and 96% clearly higher than 16, 20, and 88% for GH12, respectively during the three chosen days respectively. The mortality percentage during these above-mentioned days was 0, 16, and 84% in the control treatment. The Table 3 also showed that the death started after the 1st day in both RH21 and GH12 treatments while it started after the 3rd day in the control. All the investigated individuals were completely dead after 14 days.

In respect of the spraying technique, data of the same treatments showed more or less confirmed the results of dipping technique. Along the period of investigation, both the mean numbers of dead individuals and mortality percentage showed higher values, when treated with RH21 extract than those treated with GH12. During the three chosen days (3rd, 7th, and 13th), mortality percentage in the treatment of RH21 extract recorded 64, 84, 100% respectively against 44, 76, and 88% for GH12, respectively. These values in the control treatment recorded 0, 40, and 80%, respectively. All individuals treated with RH21 extract of *R. communis*, were completely dead after 11 days, earlier than those treated with the GH12 (14th day). It was also noticed that, in spray technique, a considerable numbers of dead individuals were achieved during the first two days in RH21 extract treatment, as they represented

60 and 64% against 12 and 24% in the dipping method during 1st and 2nd days, respectively. Results are clearly indicated that there are differences in the potential acaricidal activity between the two cultivars, due to the bioactive primary and secondary metabolites of the two cultivars of *Ricinus communis*. The leaves extract of *R. communis* was reported to possess molluscicidal activity against *Lymnaea acuminata* and larvicidal activity against *Anopheles arabiensis*, *Callosobruchus chinensis* and *Culex Quinquefasciatus* mosquitoes [4, 37]. Zahir et al., [14] reported that the dried seed extracts of *R. communis* exhibited acaricidal and insecticidal activities against the adult of *Haemaphysalis bispinosa* Neumann and *Hippobosca maculata* Leach. The activity of *R. communis* against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) was reported [7].

Table 3: Evaluation of the two local cultivars RH21 and GH12 *Ricinus communis* cultivars by dipping and spray bioassay

Treatments / Days	Dipping									Spray								
	GH12			RH21			Control			GH12			RH21			Control		
	¯x	S d	%	¯x	S d	%	¯x	S d	%	¯x	S d	%	¯x	S d	%	¯x	S d	%
1	0.4	0.89	8	0.6	0.89	12	0	0	0	2.2	1.48	44	3	1	60	0	0	0
2	0.8	1.79	16	1.2	0.45	24	0	0	0	2.2	1.48	44	3.2	0.84	64	0	0	0
3	0.8	1.79	16	1.2	0.45	24	0	0	0	2.2	1.48	44	3.2	0.84	64	0	0	0
4	0.8	1.79	16	1.2	0.45	24	0.6	0.55	12	3.0	1.73	60	3.6	0.89	72	0.2	0.45	4
5	0.8	1.79	16	1.2	0.45	24	0.6	0.55	12	3.0	1.73	60	3.6	0.89	72	1.4	0.89	28
6	0.8	1.79	16	1.4	0.55	28	0.6	0.55	12	3.2	1.79	64	3.6	0.89	72	1.4	0.89	28
7	1.0	1.73	20	2.2	0.45	44	0.8	0.45	16	3.8	1.64	76	4.2	0.89	84	2	0.71	40
8	1.4	1.52	28	3.0	1.0	60	1	0.71	20	4.0	1.22	80	4.4	0.89	88	2	0.71	40
9	1.6	1.34	32	3.0	1.0	60	1.2	0.84	24	4.0	1.22	80	4.4	0.89	88	2	0.71	40
10	1.8	1.30	36	3.2	0.84	64	1.2	0.84	24	4.0	1.22	80	4.4	0.89	88	2	0.71	40
11	2.0	1.41	40	3.8	0.45	76	1.8	0.45	36	4.0	1.22	80	4.6	0.55	92	2	0.71	40
12	4.0	1.22	80	4.4	0.89	88	3.2	0.45	64	4.2	1.30	84	5	0	100	3	0.71	60
13	4.4	0.89	88	4.8	0.45	96	4.2	0.84	84	4.4	0.89	88	5	0	100	4	0.71	80
14	5	0	100	5	0	100	5	0	100	5	0	100	5	0	100	5	0	100

The present work showed that *R. communis* aerial parts extract, which is non-edible and abundant in several countries including Egypt, may be very useful in agricultural programs. It can be a useful alternative due to its properties of low phytotoxicity to many plant species and to the fact that its residues break down rapidly through microbial action in the environment, translating into safety for humans and the environment [37]. The contact and absorption toxicity of RH21 and GH12 cultivar extract on larvae and adults of *T. urticae* Koch was evaluated in the current study. This mite *Tetranychus urticae* Koch affect several significant food, fiber crops and ornamentals plants, leading to serious damage and consequently crop reduction or total loss worldwide. The results are highly promising in view of its apparent efficiency in the control of *Tetranychus urticae*, its low toxicity and its low cost in Egypt. For the other hand, it could represent an excellent alternative to control a wide range of spider mites, since it is environmentally safe, biodegradable, non-resistible to botanical insecticides, and its cost is low.

The effect of *Ricinus communis* extract on *T. urticae* indicates that the extract showed contact and absorption toxicity on larvae and adults of *T. urticae* Koch

CONCLUSION

The results demonstrated that the red cultivars RH21 of *R. communis* possess some compounds with acaricidal and free radical scavenging properties, which can be used as acaricidal agent in new drugs for control of pests. It is quite difficult to attribute these effects of this cultivar to one or a few active principles, because extract always contain a mixture of different chemical compounds. The minor components may also make a significant contribution to the biological activity of extract. Following the results above, we could infer that the acaricidal and the free radical scavenging effects of red cultivar RH21 is the synergistic effect of their secondary metabolites. The natural acaricidal properties of the local red cultivar RH21 of *R. communis* may make it useful as potential alternative *T. urtica* control agents as well as good lead agents for the development of safe, effective, and fully biodegradable pesticides.

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