Comparative study of selected *Rosa* varieties' metabolites through UPLC-ESI-MS/MS, chemometrics and investigation of their insecticidal activity against *Culex pipiens* L.

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ABSTRACT

The 70 % methanolic extracts of three *Rosa* varieties (aerial parts & flowers); *Rosa banksiae* var. *banksiae* Ait. (RBW); *Rosa polyantha* Thunb. 'orange fairy' (RPO) and *Rosa polyantha* Thunb. 'white fairy' (RPW) were quantified using UPLC-ESI-MS/MS. Sixty one compounds were identified where flavonoid glycosides were the most abundant. Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) discriminated the six samples into four clusters where RBW-F & RPW-A formed one cluster, RPW-F & RPO-F formed another and RPO-A and RBW-A were in two separate clusters. The six *Rosa* extracts were tested as insecticidal agents against *Culex pipiens* L. larvae and pupae and their fecundity reducing activity was evaluated for the emerging adult mosquitoes. RPW flowers and aerial parts' extracts demonstrated powerful larvicidal and pupicidal activities with LC_{50} 373.3 and 383.2 ppm, respectively. Sterility indices reached 51.4% at highest concentrations used. All flower extracts possessed significantly high mortality and sterility activities ($P < 0.001$) compared to the aerial parts ($P < 0.01$).The UPLC-MS/MS metabolic profile of the extracts showed their richness in polyphenolic compounds. The 70% methanolic extracts of RBW, RPO and RPW aerial parts and flowers can be utilized as natural and safe plant-based insecticides for *C. pipiens* (filariasis vector) control.

Keywords: *Rosa*, UPLC-MS/MS, metabolomics, *Culex pipiens*, filariasis.

INTRODUCTION

Genus *Rosa* is among the largest genera belonging to family Rosaceae with more than 250 species mostly located in China and South-West Asia. *Rosa banksiae var. banksiae* Ait. is known as bankasian rose or lady banks, after Sir Joseph Banks's wife, usually seen as part of parks decoration and as ornamental flower in tombstones [1]. Bankasian rose is a climber shrub with white, yellow and rose colored flowers which blooms heavily during Spring in fascinating way. Polyantha flowers are part of modern roses, they are named *R. polyantha* Thunb. (*R. multiflora* Thunb.) due to their heavy flowering nature and the fairy roses are a new hybrid roses from this class since 1930s [2].

Family Rosaceae is rich in flavonoids [3], terpenoids [4], tannins [5] and fatty acids [6]. Most of the previous secondary metabolites studies were done using targeted approaches, focusing on certain class of compounds [7]. Nowadays, metabolomics play a pivotal role in the identification and quantification of a large set of metabolites through their molecular ion peaks and characteristic fragmentation pattern, taking advantage of the development of hyphenated techniques *viz.* tandem mass spectrometry (LC-MS/MS) [8]. Nevertheless, it has the advantage of saving time, money and efforts in isolating the secondary metabolites [9].

Early reports showed that essential oil of *R.*

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damascena Mill exerted a powerful contact, repellent and ovicidal activity against certain spider [10]. Plant derived insecticides have emerged as safe, affordable and effective alternative agents for mosquito vector control [11]. They are mostly target specific and readily biodegradable compared to chemical counterparties. The first known bio-insecticide was isolated from pyrethrum affected the insect nervous system. Regarding chemical insecticides, they are rapid, effective and broad spectrum killing agents, but they carry a lot of drawbacks being toxic to non-target organisms like fishes leading to direct and indirect toxic effects to the environment and human, as well as being highly resisted by many mosquito disease vectors [11].

Culex mosquito is the main vector transmitting *Wuchereria bancrofti* parasite which causes human lymphatic filariasis which is considered one of the neglected tropical diseases (NTDs). World health organization (WHO) has launched a worldwide program in 2000 with a goal of eliminating filariasis by 2020 which will never be possible unless immense efforts are to be done in order to control the main transmitting vector side by side to other management strategies [12].

The aim of this study was to unravel the metabolome composition of some selected species of genus *Rosa* cultivated in Egypt using ultra performance liquid chromatography – electrospray ionization - tandem mass spectrometry (UPLC-ESI-MS/MS) coupled to chemometrics *viz.* PCA and HCA for differentiating between three *Rosa* varieties with two different organs and evaluate their insecticidal potency against filariasis vector *Culex*; thus providing a safe natural source of plant derived insecticide to combat one of highly dangerous diseases in Africa.

Results

1. Identification of *Rosa* species metabolites through tandem mass spectrometry

Ultra performance liquid chromatography - tandem mass spectrometry (LC-MS/MS) has been approved for long as standard analysis and authentication tool for characterization and identification of plant metabolomics and profiling of different plant active constituents. Moreover, LC-MS/MS plays a fundamental role in the quantification of different constituents; compounds identification and confirmation relied upon both retention time and mass fragmentation [13]. Sixty one compounds, were tentatively identified and quantified in Table A. 1, Figures A. 1 and A. 2 and arranged in descending manner according to their retention times. The identified compounds can be classified into: 30 flavonoids, 12 phenolic acids, 8 tannins, 5 terpenoids, 2 alkaloids, 2 cyclic polyols, 1 anthocyanin and 1 chromone (Figure A. 3).

The highest number of identified compounds (Table A.1) were in RBW-A (51 compounds) followed by RPW-A (29 compounds) and RBW-F (26 compounds). The percentage identification ranged *ca.*70.41 % to 96.49% in ESI negative mode and *ca.* 16.70% to 43.99% in ESI positive mode. This confirmed that the negative mode can be used better for identification of polyphenolic compounds.

For the aerial parts, RBW-A was rich in chlorogenic acid **(M65)**, 20.96%; apigenin-6-*C*-pentoside **(M39)**, 15.75% and quercetin-7-*O*-hexoside-3-*O*-(malonyl)-hexoside **(M27)**, 10.97%. For RPO-A, quinic acid **(M1)**, 70.11%; chlorogenic acid **(M65)**, 18.24% and pibtocarphin B **(M74)**,10.95% were among the abundant compounds, while for RPW-A, they were chlorogenic acid **(M65)**, 16.89%;quinic acid **(M1)**, 15.28%; quercetin-3-*O*-glucouronide **(M17)**, 13.85% and quercetin aglycone **(M18)**, 13.55%.

The flower extracts major identified compounds were; quinic acid **(M1)**, 17.13%; chlorogenic acid **(M65)**, 13.80%; kaempferol-3-*O*-glucouronide **(M22)**, 11.95% and quercetin-7-*O*-hexoside-3-*O*-(malonyl)-hexoside **(M27)**, 11.79% for RBW-F; quinic acid **(M1)**, 26.03%, kaempferol-3-*O*-rhamnoside **(M26)**, 24.69%; gallic acid **(M4)**, 15.02% and quercetin-3-*O*-rhamnoside **(M21)**, 13.44% for RPO-F and kaempferol-3-*O*-rhamnoside **(M26)**, 25.29%, quinic acid **(M1)**, 17.88% and quercetin-3-*O*-rhamnoside **(M21)**, 13.31% for RPW-F (Figure A. 3).

1.1. Flavonoid glycosides and their derivatives

The studied *Rosa* species herein were heavily enriched with quercetin and kaempferol either as aglycones or different forms of glycosides (Table A. 2) and detailed as

follows; compound **(M14)** with $[M-H]^-$ *m/z* 433 and $[M+H]^+$ *m/z* 435 was defined as quercetin-3-*O*-arabinoside and it gave daughter molecular fragment at *m/z* 301 for quercetin aglycone [14]. Another quercetin pentoside was detected at $[M-H]^-$*m/z* 447 and $[M+H]^+$ *m/z* 449 and was tentatively assigned to quercetin-3-*O*-rhamnoside **(M21)**. Similarly, quercetin-3-*O*-glucouronide **(M17)** was identified at $[M-H]^-$ *m/z* 477 with one major fragment at *m/z* 301 for quercetin aglycone. In both ESI positive and negative ion modes, quercetin aglycone **(M18)** was clear at *m/z* 301 and 303, respectively [15]. A set of compounds showing same $[M-H]^-$ *m/z* 711 were detected at different retention times as so were considered sugar isomers and tentatively defined as quercetin-7-*O*-hexoside-3-*O*-(malonyl) hexoside **(M27)** and its two isomers **(M30)** and **(M34)**[16]. Two commonly found compounds in citrus fruits were seen at $[M-H]^-$ *m/z* 757 and they were tentatively identified as eriodictyl-4'-*O*-neohesperidoside-7-*O*-glucoside **(M8)** with its daughter moieties at *m/z* 595 and 449 due to loss of hexoside moiety as glucose and neohesperidoside group, respectively and isorhamnetin-3-*O*-rutinoside **(M12)** at $[M-H]^-$ *m/z* 623[15].

On the other hand, kaempferol aglycone **(M32)** was detected in ESI –ve mode at *m/z* 285 and at *m/z* 287 in ESI +ve mode, while kaempferol-3-*O*-glucouronide **(M22)** was identified at $[M-H]^-$ *m/z* 461 [14] and it was only found in RBW-F. Another glucouronide was shown at $[M-H]^-$ *m/z* 593 and at $[M+H+Na]^+$ *m/z* 617 was defined as kaempferol-*O*-pentose-*O*-glucouronic acid **(M25)** with its fragments at *m/z* 441 and 417 for the successive loss of pentose and glucouronic acid, respectively. A deprotonated molecular ion was detected at $[M-H]^-$ *m/z* 417 and $[M+H+Na]^+$ *m/z* 441 and was agreed to be kaempferol-3-*O*-arabinoside **(M24)**. With rhamnose sugar moiety, kaempferol-3-*O*-rhamnoside **(M26)** was identified with $[M-H]^-$ *m/z* 431 and $[M+H+Na]^+$ *m/z* 455 [17]. Compound **(M29)**, identified as kaempferol-3-*O*-rutinoside showed molecular ion peak also at *m/z* 593 only in negative mode but with one fragment at *m/z* 285 for kaempferol aglycone [14].

Apigenin-6-*C*-pentoside **(M39)** was traced at $[M-H]^-$ *m/z* 503 and $[M+H+Na]^+$ *m/z* 527 [18]. Naringenin aglycone **(M63)** was found at *m/z* 271 in negative mode [15] with traces of its galloylated, acetylated and methylated derivatives such as quercetin-*O*-(2"-*O*-galloyl)-hexoside **(M13)** and Kaempferol-galloylhexoside **(M19)** which were identified in negative mode at $[M-H]^-$ *m/z* 615 and *m/z* 599, respectively. Another galloylated flavonoid glycoside was traced at $[M-H]^-$ *m/z* 601 which was recognized as myricetin-*O*-(*O*-galloyl)-pentoside **(M69)** and was further confirmed by a fragment at *m/z* 449 for the loss of pentose sugar. One acetylated flavonoid glycoside was assigned to (iso) rhamnetin-3-*O*-6"-*O*-acetyl-glucoside **(M36)** with a deprotonated peak at *m/z* 519 in negative mode [14]. 7-*O*-methylated flavonoid was detected at $[M+H]^+$ *m/z* 301 for compound **(M52)** which was identified tentatively as rhamnocitrin.

1.2. Phenolic Acids

Electrospray ionization negative ion mode revealed a deprotonated molecular ion peak for gallic acid **(M4)** at $[M-H]^-$ *m/z* 169 [14], gallic acid was traced only in RBW-F and RPO-F, it was also identified in other *Rosa* species[19]. Different caffeic acid derivatives were quantified (Table A. 2) where a molecular ion peak was shown at $[M-H]^-$ *m/z* 487 in negative mode and $[M+H+Na]^+$ *m/z* 511 in positive mode and it was assigned to caffeoylhexose deoxyhexoside **(M48)** with two major fragments at *m/z* 308 and 179 for loss of caffeoyl and deoxy hexose hexoside moieties, respectively. Compounds **(M49)** and **(M54)** were at the same molecular ion peak as compound **(M48)** which was attributed to presence of isomerism in the hexose sugar moiety so they appeared at different retention times with the same *m/z* value and fragmentation pattern. A dimer form of caffeic acid was detected at $[M-H]^-$ *m/z* 683 which was identified as caffeic acid-*O*-hexoside dimer **(M85)** and its identity was confirmed by its fragment ions at *m/z* 341 and 179 for the splitting of the dimer molecule to two monomers and

the loss of hexose molecule, respectively. In addition to that, an *O*-methylated phenolic acid known as syringic acid **(M89)** was detected at $[M-H]^-$ *m/z* 197. Another phenolic acid was identified as *p*-coumaric acid hexoside **(M11)** at *m/z* 325 in negative mode [18]. It is worthy noted that, the bankasian rose aerial parts were rich by caffeic and ferulic acid derivatives while gallic acid was not traced in it. Three cinnamic acid derivatives were observed namely; 5-*O*-*p*-coumaroyl-4-*O*-caffeoyl-4-methylpentanoic acid-5-hydroxy-3-quinate **(M37)**, chlorogenic acid (caffeoylquinic acid) **(M65)** and its isomer **(M67)** at $[M-H]^-$ *m/z* 693 and $[M+H]^+$ *m/z* 353, respectively.

1.3. Terpenoids

A lanostane type triterpenoid was spotted at $[M-H]^-$ *m/z* 501 for ganolucidic acid B **(M42)** together with daughter peaks at *m/z* 483, 394 and 377 due to $[M-H-OH]^-$, $[M-H-C_5H_6O_3]^-$, $[M-H-C_6H_6O_3]^-$ fragments [20]. Alisol C **(M47)**, a protostane type triterpenoid, was discovered at *m/z* 485 in negative mode with one fragment at *m/z* 468 due to loss of hydroxy group [21]. Oleanolic acid **(M61)** and its isomer, ursolic acid **(M64)**, were tentatively identified at the same $[M-H]^-$ *m/z* 455 due to isomerism but at different retention times [22]. Only one sesquiterpenoid named piptocarphin B **(M74)** was distinguished at $[M+H]^+$ *m/z* 437 and its side chain fragment appeared at *m/z* 169 [23].

1.4. Tannins

Different forms of hydrolysable tannins had been tentatively determined such as hexahydroxydiphenic acid hexoside **(M2)** which appeared at $[M-H]^-$ *m/z* 481, galloylquinic acid **(M3)** at *m/z* 343 deprotonated molecular ion peak in negative mode with fragmentation peak at *m/z* 191 for quinic acid moiety [18]. At $[M-H]^-$ *m/z* 633, HHDP-galloylglucopyranoside **(M6)** was defined and confirmed by HHDP and quinic acid moieties at *m/z* 301 and 191, respectively. A trigalloyl hexose **(M28)** was pointed at $[M-H]^-$ *m/z* 635 [24] as well as digalloylhexose tannin **(M60)** at *m/z* 483 in negative mode with fragment at *m/z* 331 due to loss of hexoside. Condensed tannins were less represented; 3-methyl-epigallocatechin gallate and its isomer were shown at $[M-H]^-$ *m/z* 471 as compounds **(M56 & M57)** with fragment at *m/z* 269 due to breaking of methyl gallate moiety. A fragment of proanthocyanidin **(M23)** was found at $[M+H]^+$ *m/z* 287 [25] .

1.5. Anthocyanins

Compound **(M77)** with $[M+H]^+$ *m/z* 503 and MS/MS fragments at *m/z* 327, 281, 265, 249 and 205 was identified as pelargonidin-succinyl-arabinoside or pelargonidin-malonyl rhamnoside [25].

1.6. Alkaloids

Ergocristine **(M78)** and its dehydrate form **(M87)** were detected at $[M+H]^+$ *m/z* 610 and 592 [26], respectively with daughter fragments at *m/z* (436, 221, 43) and (330, 289, 237, 69), respectively.

1.7. Miscellaneous

A deprotonated molecular ion peak was traced at $[M-H]^-$ with *m/z* 191 in negative mode representing quinic acid **(M1)**, with daughter fragment at *m/z* 145 due to loss of terminal COOH group [18]. Another peak appeared **(M66)** at *m/z* 279 in negative mode for quinic acid derivative [27].

2. Chemometrics analysis

Metabolic profiling (61 components, Table A. 1) were subjected to both PCA and HCA to reveal the chemical variability, and the inter-relationships between the extracts of the three studied *Rosa* varieties (aerial parts and flowers).

PCA score plot explained 79% of the variance of the data for the three varieties *viz.* RBW, RPO, RPW aerial and flowers, as shown in Figure A. 4 (A & B). Four clusters had been constructed where RPO-A cluster lies in the lower right quadrant; RPW-F and RPO-F lie in the upper left quadrant. Nevertheless, RPW-A and RBW-F were superimposed over each other in the left lower quadrant together with RBW-A. Loading plots Figure A. 4B, showed that the main discriminating marker was quinic acid for RPO-A. However, quercetin-7-*O*-hexoside-3-*O*-(malonyl)-hexoside was the leading metabolite discriminating RPW-F and RPO-F. Loading

plots displayed ganolucidic acid as the main discriminating markers for RBW-F and RPW-A, while trigalloyl hexose and tricin aglycone were specific to RBW-A. Additionally, HCA was applied as unsupervised pattern recognition method in order to confirm results obtained by PCA. The dendrograms obtained for all *Rosa* varieties endorsed the results of PCA as shown in Figure A. 4. The main identified compounds in the six samples were represented in clustered heatmap (Figure A. 6) where the highest concentrations were in red and the lowest in blue shades and between them lies areas in yellow and green for medium concentrations.

3. Insecticidal activity of *Rosa* extracts against *Culex pipiens*

3.1. Effect of different *Rosa* extracts on different stages of *Cx. pipiens*

The biological activity of the tested RBW, RPO and RPW extracts varied according to plant part used and the concentration of the extracts. Both larval and pupal mortality percentages were increased linearly by increasing the concentration of the tested extracts. Flower extracts were more effective against *Cx. pipiens* larvae and pupae than the aerial parts for all *Rosa* extracts (Table A.2). Complete larval mortality percent (100.0%) occurred at the highest concentration (700ppm) of RPW aerial parts and flowers. Also, the highest pupal mortality percent (38.9%) was recorded by RPW-A extract at 600ppm, respectively. According to LC_{50} values for different flower extracts , RPW-F (373.3ppm) was the most potent followed by RBW-F (479.3ppm) and RPO-F (554.1ppm), while the LC_{50} values for aerial parts were 383.2, 491.4 and 862.3ppm for RPW, RPO and RBW, respectively (Figure A. 5).

Regarding the larval and pupal periods, all tested extracts prolonged these periods at the highest concentrations significantly ($P<0.05$), while RBW-F insignificantly ($P>0.05$) prolonged larval and pupal periods at all concentrations used as compared with untreated group (Table A. 2).

3.2. Effect of *Rosa* extracts on the reproductive potential of resulted females

RBW, RPO and RPW extracts significantly ($P<0.001$) reduced the fecundity and increased the sterility % of females developed from treated larvae as compared with the untreated groups, the fecundity and sterility percentages were varied according to plant part and concentration of the extract (Table A. 3). In addition, a noticeable decrease in the hatchability % of eggs laid by females resulted from treated larvae with *Rosa* extracts was observed in a dose dependent manner. RPW aerial parts and flowers extracts recorded the highest sterility index percentages (56.1 and 55.5) at the same concentration (600ppm), respectively, compared with 10.4 and 9.1% sterility in the untreated groups.

Discussion

LC-ESI-MS/MS plays fundamental role in the metabolomic profiling of plant extracts. Different plant extracts can be compared side by side both qualitatively and quantitatively through LC-MS/MS analysis. Different components can be identified and quantified with their daughter fragments which helps in profiling and fingerprinting plant species and varieties with close chemical composition. Herein three varieties, *Rosa banksiae var. banksiae* Ait., *R. polyantha* Thunb. 'orange fairy' and *R. polyantha* Thunb. 'white fairy' belonging to genus *Rosa* were analysed through tandem mass and their components were revealed and compared.Flavonoids and their derivatives are the most abundant class of phytoconstituents identified through LC-ESI-MS/MS which is in accordance with most of the other studied *Rosa* extracts. Different studies utilized LC-MS analysis for the analysis of different *Rosa* species where phenolic acids, flavonols and anthocyanins were identified from the rosehips extract of *R. canina* [28]. Phenolic acids, flavonols and hydrolysable tannins were the main components of *R. rugosa* petal extract analysed using UPLC-PDA-Q/TOF-MS [29]. The stem extracts of *R. moschata*, *R. canina* and *R. sempervirens* were compared through LC-ESI-

MS and flavonoids were abundant in the three of them [30]. Flavonoids and their aglycones were the major classes identified from *R. rugosa* leaves and achenes [19].

Principal component analysis (PCA) and Hierarchical clustering analysis (HCA) chemometric tools further discriminated the metabolomics data arising from the LC-MS/MS analysis resulting in four main clusters that were separated according to their spatial composition with one or two discriminating component for each cluster. The main identified compounds were colour coded in the clustered heatmap in which they were arranged in line with the PCA and HCA results. Here chemometrics played added role in comparing and differentiating the six *Rosa* samples and helped in their differentiation by showing one or more main differentiating component for each.

The obvious existence of flavonoids in the three *Rosa* varieties had played an important role in their insecticidal activity *viz.* larvicidal, pupicidal and fecundity activities [31]. The flower extracts were more potent as an insecticide than the aerial parts extracts. *Rosa polyantha* Thunb. white fairy (RPW) aerial parts and flower extracts showed the strongest larvicidal, pupicidal and fecundity activities against *Cx. pipiens* compared to others. Flavonoids had previously proven their role in regulating fecundity and feeding activities of insects exposed to them [32] *e.g.* quercetin-3-*O*-rutinoside showed oviposition deterrence against cabbage butterflies[33]. Different quercetin and kaempferol glycosides identified form *Kalanchoe beharensis* and *K. longiflora* leaves showed their strong insecticidal activity for the cotton worm [34]. Plant flavonoids usually provoke their insecticidal activity through their neuroactivity on the insects' central nervous system by inhibition of acetylcholine esterase [32].

General Experimental

1. Plant Material and Extraction Procedure

Aerial parts (A) and flowers (F) of *Rosa banksiae* var. *banksiae* Ait. (RBW) known as Banksian rose were collected from Merryland Botanical Garden, Cairo, Egypt ($30^0$05'37"N$31^0$18'51"E), while *Rosa polyantha* Thunb. orange fairy (RPO) and *Rosa polyantha* Thunb. white fairy (RPW) were collected from a private garden, Al-Mariouteya Road, Giza, Egypt ($30^0$01'13"N$31^0$04'42"E, during March-April 2016 (flowering season) and were authenticated by Eng. Terease Labib, Ministry of Agriculture, Giza, Egypt. Voucher specimens were kept under codes: (PHG-P-RB 165), (PHG-P-RP 205) and (PHG-P-RP 204) for RBW, RPO and RPW, respectively at Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Aerial parts and flowers (500g for aerial parts and 200g for flowers) of RBW, RPO and RPW were collected, dried in shade then percolated, separately in 2L 70% (v/v) HPLC grade methanol. The filtered extracts were dried *in vacuo* at low temperature (45^0C) till dryness then lyophilized. The lyophilized extracts weighed; 45g, 43.2g and 42.5g for RBW-A, RPO-A and RPW-A, respectively and 20.3g, 19.8g and 15.4g for RBW-F, RPO-F and RPW-F, respectively.

2. Ultra Performance Liquid Chromatography – Electrospray Ionization -Tandem Mass Spectrometry (UPLC-ESI-MS/MS) Analysis

UPLC-ESI-MS/MS in both positive and negative ion acquisition modes were carried out on a XEVO TQD triple quadrupole instrument, Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer. Chromatographic separation of the sample was done by injecting 10µl into UPLC instrument equipped with reverse phase C-18 column (ACQUITY UPLC - BEH, 2.1 × 50 mm column; 1.7 µm particle size). The sample (100 µg/mL) solution was prepared using HPLC grade methanol, filtered using a membrane discfilter (0.2 µm) disc and degassed by sonication before injection then subjected to LC-ESI-MS/MS analysis. Gradient mobile phase comprising two eluents: eluent A is H_2O acidified with 0.1% formic acid and eluent B is MeOH acidified with 0.1% formic acid. Elution was made at flow rate 0.2 mL/min as follows: (10%B) from 0 to 5 min.; (30% B) from 5 to 15 min.; (70% B) from 15 to 22 min.; (90% B) from 22 to 25 min. and (100% B) 25-29 min. The analysis was accomplished using negative ion mode as follows: source

temperature 150°C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440°C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h.

2.1. Data Processing

Mass spectra were recorded in Electrospray ionization (ESI) (negative and positive ion modes) (*m/z* 100–1000). They were processed using Masslynx 4.1 software and tentative identification was done by comparing their retention times (R_t), mass spectra and fragmentation patterns with reported data.

2.2. Chemometric Analysis

LC-MS/MS metaolomic profile was subjected to chemometric analysis. Principal component analysis (PCA) acts as the first step in data analysis in order to provide an overview of all observations and samples to identify and evaluate groupings, trends and strong outliers. Hierarchal Cluster Analysis (HCA) was then applied to allow clustering of different *Rosa* species. The clustering pattern was conducted usingcomplete linkage method for group building and computed by Euclidean method. For PCA and HCA, Unscrambler® X 10.4 from CAMO (Computer Aided Modeling, AS, Norway) was applied. A clustered heatmap was constructed using NCSS. 12 software with Euclidean distance and the unweighed pair group method.

3. Determination of Insecticidal Activity

3.1. Larvicidal and Pupicidal Activities

Culex pipiens larvae were collected, during summer 2016, from Sadat City, Cairo-Alexandria desert road and were grown for several generations in Medical Entomology Insectary, Animal House, Department of Zoology, Faculty of Science, Al-Azhar University under standard experimental procedure adopted to provide third instar larvae for the bioassay.

Standard methods [35] for evaluating the larvicidal and pupicidal activity of plant extracts against *Cx. pipiens* larvae were followed with small modifications. *Rosa* extracts were dissolved in 0.1ml methanol to enhance the dissolving in 250ml dechlorinated tap water in 350ml plastic cups. Then, 3rd instar larvae (25 larvae) were placed in plastic cups containing the extracts at different concentrations. Three replicates were used for all tested concentration. All plastic cups were incubated under controlled conditions and subsequently mortality was recorded; control larvae received 0.1ml methanol in 250ml water. Mortality was calculated daily where dead larvae and pupae were removed until the emergence of adults. Larval mortality was confirmed when larvae showed no response to mechanical stimulation and estimated using equation of Briggs[36].

3.2. Reproductive Potential of Resulted Females

Females (3rd instar larvae) treated with tested concentrations of *Rosa* extracts were collected and kept with normal adult males in wooden cages and fed on10% (w/v) sucrose solution for 3 days. They were left for one day without sugar solution then at the 5th day, the starved females were allowed to take a blood meal from a pigeon and allowed to lay egg rafts on clean water (oviposition traps) in the cages. The number of egg/raft was counted using binocular and then mean values were taken. Under a dissecting microscope, the non-hatched embryonated eggs were identified by the apparent confirmation of the embryo presence [37]. Sterility percentages were calculated according to Toppozada formula [38].

3.3. Statistical Analysis

Data were presented as Mean ± SD. Student t-test and one way analysis of variance (ANOVA) was performed using Sigmaplot version 11.0. LC_{50} values were calculated through multiple linear regressions [39].

Conclusion

The metabolomic profiling for the tested species belonging to genus *Rosa* had showed their richness in phenolic secondary metabolites which played an important role in their larvicidal, pupicidal activities and in decreasing the reproductive power of the female *Cx. pipiens*. The identified metabolites were successfully grouped into four clusters through PCA and HCA tools and the individual components were constructed into colored clustered heatmap

depending on the area % values. Finally, the tested *Rosa* species can be regarded as a renewable source for new safe, effective and economic mosquitocidal agent. To further support the profiling method of the selected species, we may carry out isolation of the major components responsible for these biological activities in the future.

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Authors' Contributions

E.A.E. performed the experiments and wrote the article; **N.M.M.** analyzed the data and revised the manuscript, **A.Z.I.S.** performed the biological part**, R.M.L.** analyzed the data and revised the manuscript and **A.B.S.** supervised the whole work and revised the manuscript.

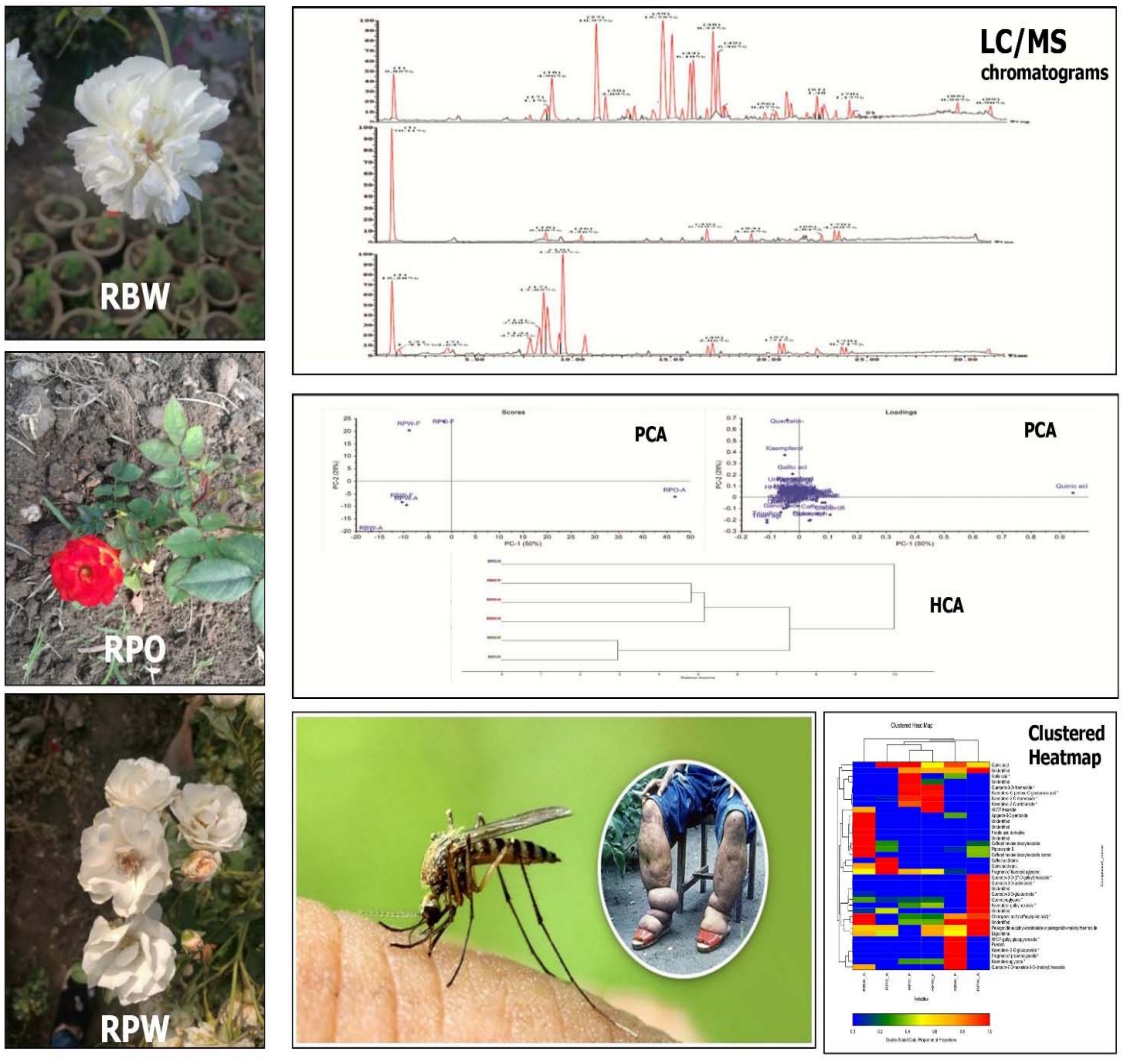
Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of interest

The authors declare no conflict of interest.

Figures



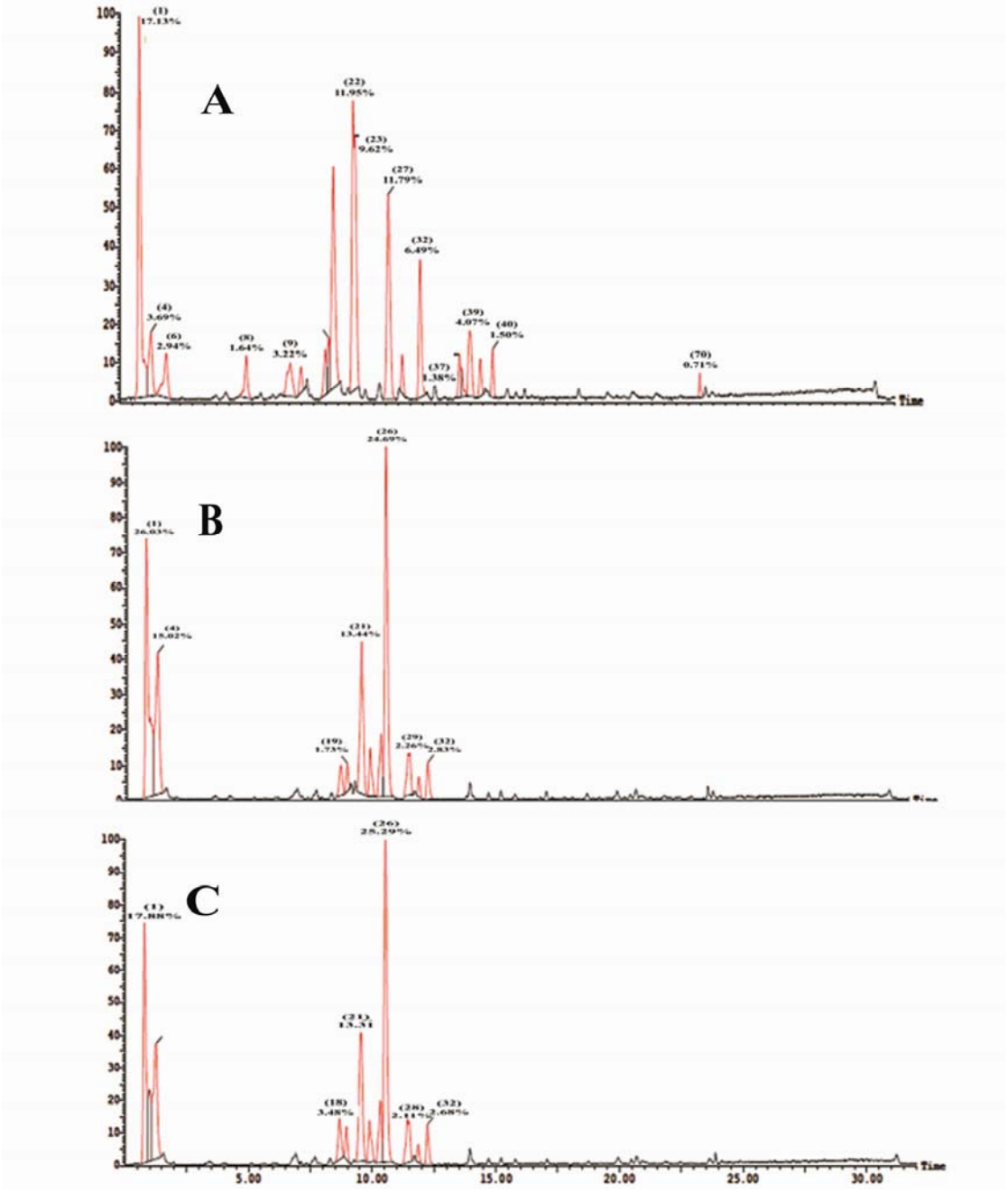


Fig. A. 1 LC/ESI/MS negative ion mode spectrum of (A) *R. banksiae var. banksiae* Ait. aerial parts (RBW-A) methanolic extract; (B) *R. polyantha* Thunb. orange fairy aerial parts (RPO-A) methanolic extract and (C) *R. polyantha* Thunb. white fairy aerial parts (RPW-A) methanolic extract

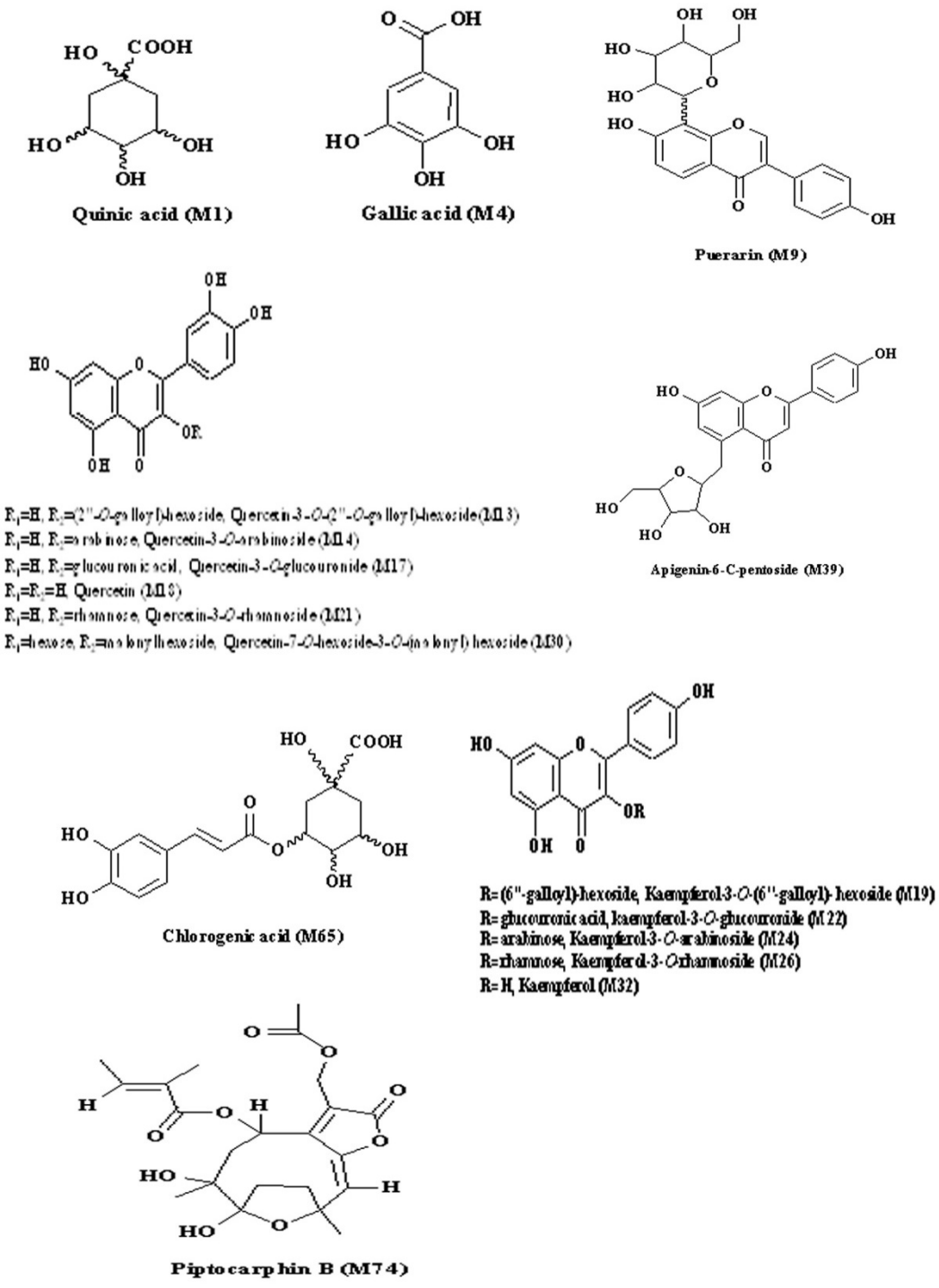


Fig. A. 2 LC/ESI/MS negative ion mode spectrum of (A) *R. banksiae var. banksiae* Ait. flower (RBW-F) methanolic extract, (B) *R. polyantha* Thunb. orange fairy flower (RPO-F) methanolic extract and (C) *R. polyantha* Thunb. white fairy flower (RPW-F) methanolic extract

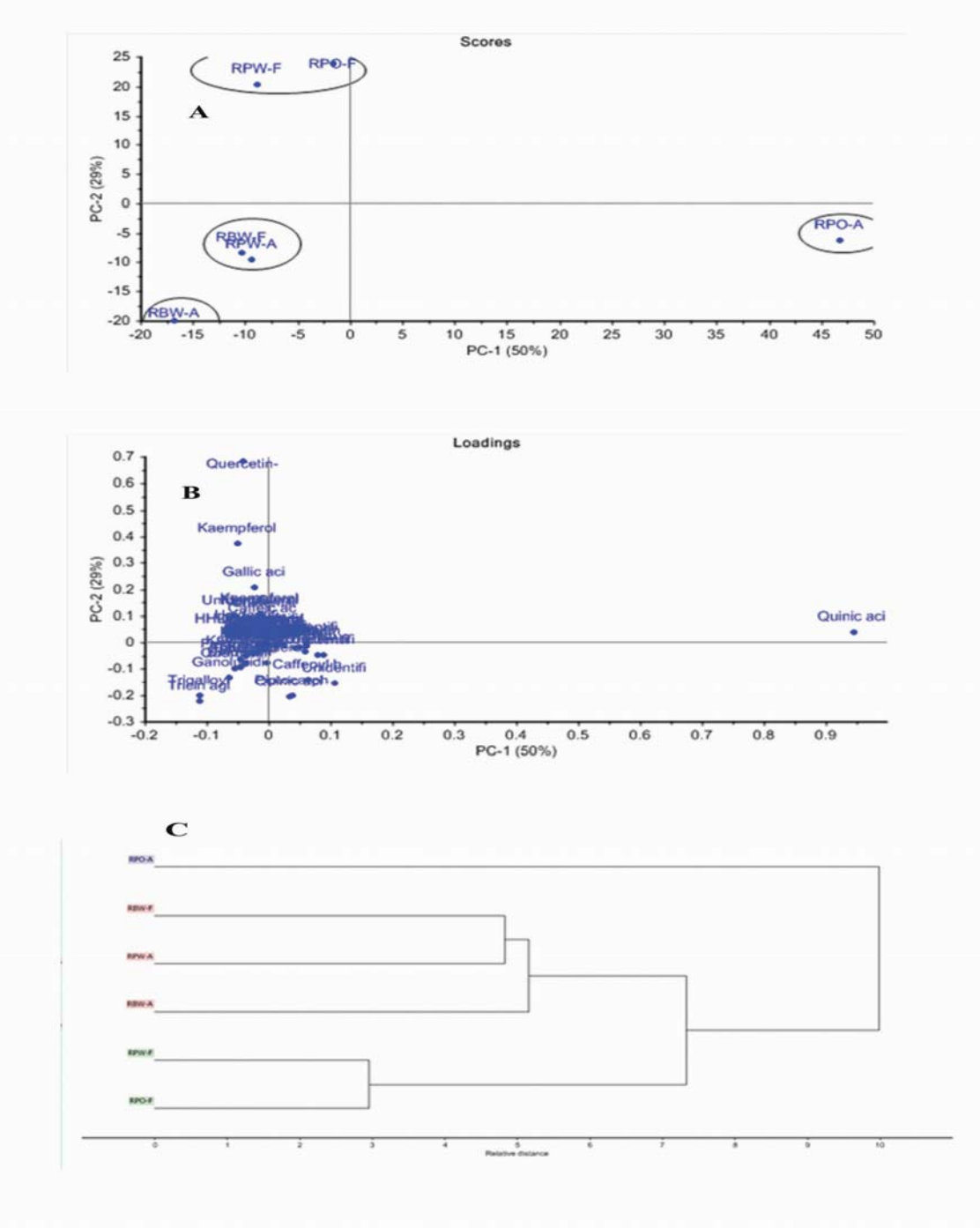


Fig. A. 3 Chemical structures of the major tentatively identified compounds from the 70% methanolic extracts of the tested *Rosa* varieties through LC/ESI/MS^n.

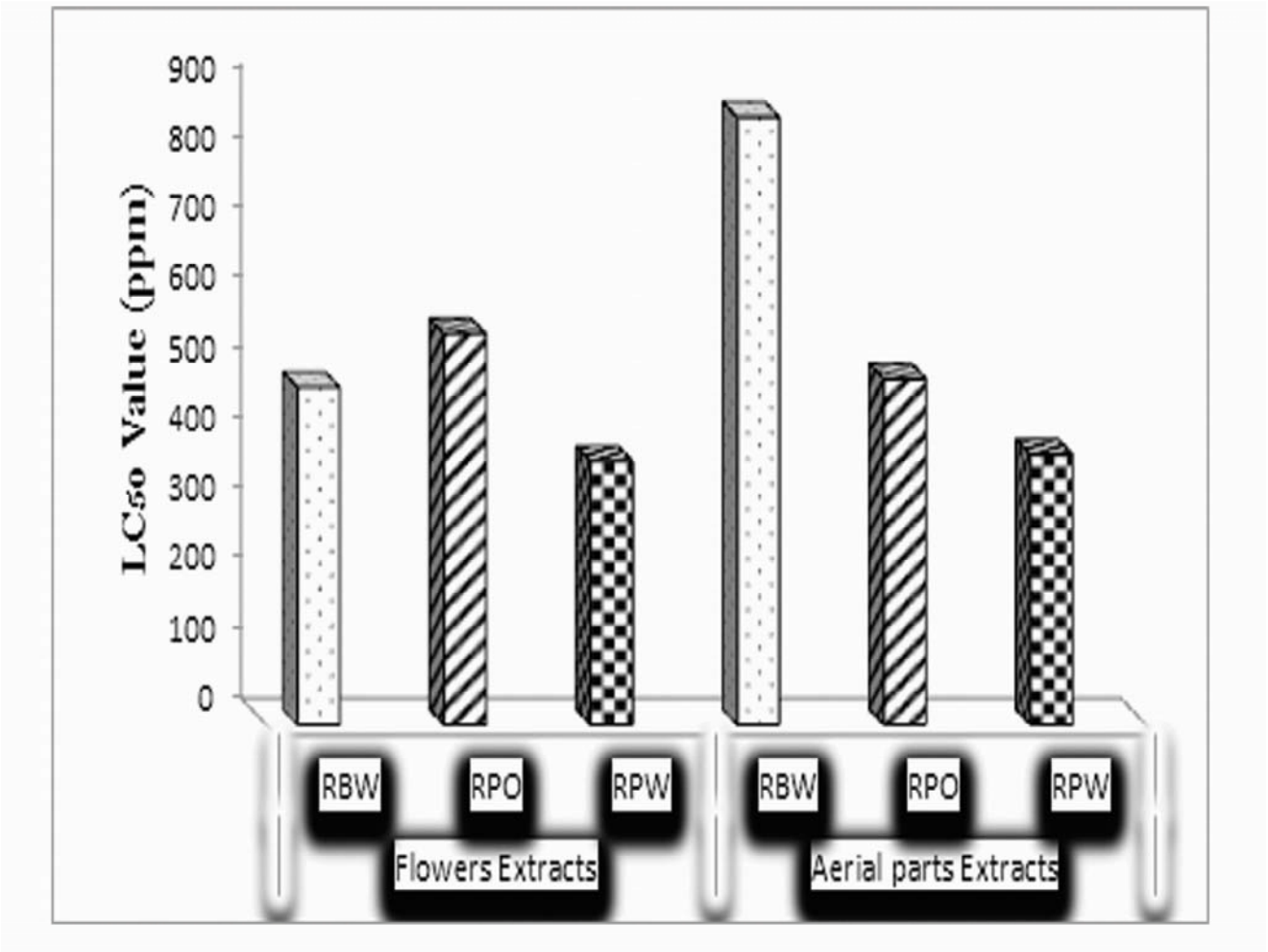


Fig. A. 4 (A, B) PCA score and loading plots of the tested *Rosa* varieties, (C) HCA dendogram of of the tested *Rosa* varieties.

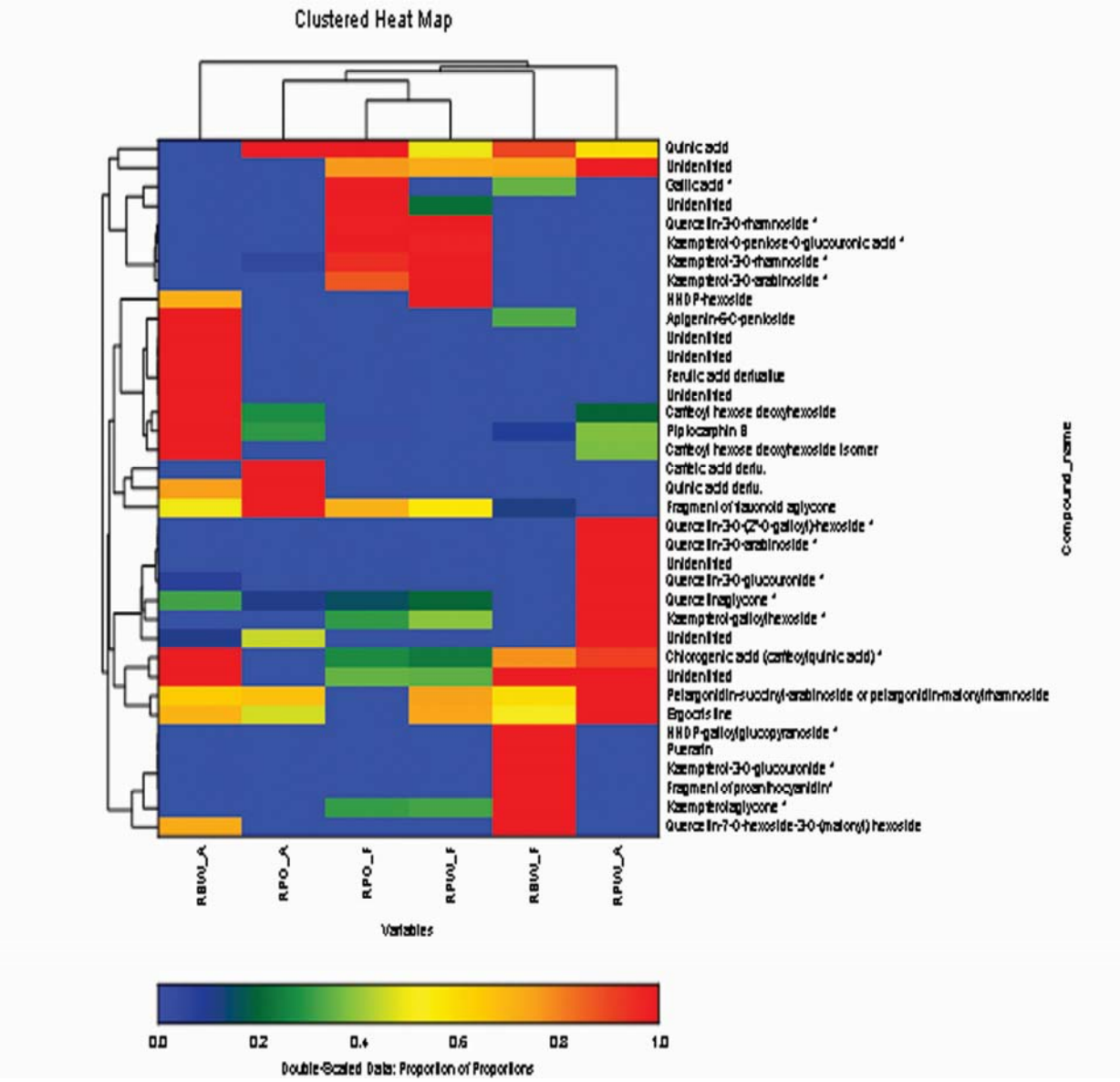


Fig. A. 5 Half Lethal Concentration (LC_{50}) values of methanol extracts of different *Rosa* species against 3rd instar larvae of *C. pipiens* (*R. banksiae var. banksiae* Ait. (RBW), *R. polyantha* Thunb. orange fairy (RPO) and *R. polyantha* Thunb. white fairy (RPW).

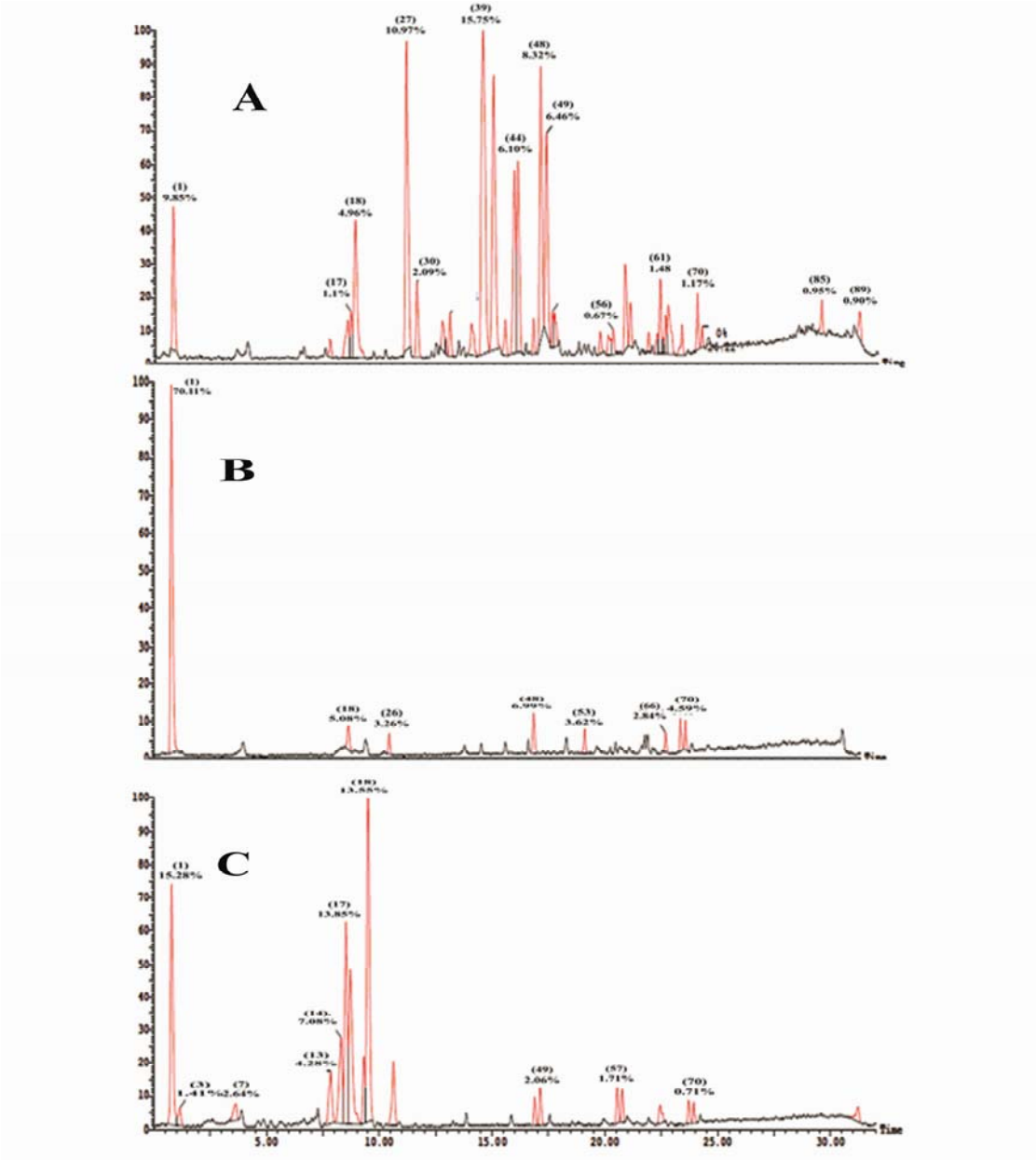


Fig. A. 6 Clustered heatmap showing different metabolites of the six studied *Rosa* samples (Heat map was constructed using Euclidean distance and the unweighed group method. Compounds with % composition of at least 3% were included).

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دراسة مقارنة بين نواتج الأيض لأنواع مختاره من روزا من خلال تحليل الكتلة عالي الكفاءة والكيموميترى وتقييم نشاطهم كمبيدات حشريه ضد كيولكس بيبيينز

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ملخص

باستخدام تحليل الكتلة عالي الكفاءة تم القياس الكمي لمستخلصات الميثانول بتركيز 70 % لثلاث تنوعات من روزا (الأجزاء الهوائية والورود) وهم روزا بانكزيا تنوع بانكزيا، روزا بوليانسا "الورد البرتقالى" وروزا بوليانسا "الورد الأبيض" وتم التعرف وتحديد نسبه 61 مركب أغلبهم من جلايكوسيدات الفلافونويد. تم تقسيم العينات الست إلى أربعه أقسام باستخدام تحليل المكون الأساسي وتحليل المكون الهرمى وهم RBW-F & RPW-A), (RPW-F & RPO-F), RPO-A and RBW-A وقد تم قياس نشاط الست عينات كمبيدات حشريه ضد كيولكس بيبيينز طورا اليرقات والشرانق وكذلك قدرتهم على تقليل الخصوبة للحشرات البالغة. تمكنت مستخلصات الأجزاء الهوائية والورود ل RPW من قتل اليرقات والشرانق بأعلى كفاءه LC50 373.3 and 383.2 ppm لكل منهما تباعا ووصل مؤشر العقم إلى 51.4% عند أعلى تركيز مستخدم كذلك كانت مستخلصات الورود أقوى من مستخلصات الأجزاء الهوائية. ومما سبق يمكن أن نستخدم مستخلصات RBW, RPO and RPW كمبيدات حشرية من أصل نباتي آمنه وفعاله ضد ناقل داء الفيل الكيولكس بيبيينز.

الكلمات الدالة: روزا، تحليل الكتله عالى الكفاءه، نواتج الأيض، كيولكس بيبيينز، داء الفيل .

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