Original article

Assessment of selected Saudi and Yemeni plants for mosquitocidal activities against the yellow fever mosquito *Aedes aegypti*

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**A B S T R A C T**

As part of our continuing investigation for interesting biological activities of native medicinal plants, thirty-nine plants, obtained from diverse areas in Saudi Arabia and Yemen, were screened for insecticidal activity against yellow fever mosquito *Aedes aegypti* (L.). Out of the 57 organic extracts, *Saussurea lappa*, *Ocimum tenuiflorum*, *Taraxacum officinale*, *Nigella sativa*, and *Hyssopus officinalis* exhibited over 80% mortality against adult female *Ae. aegypti* at 5 μg/mosquito. In the larvicidal bioassay, the petroleum ether extract of *Aloe perryi* flowers showed 100% mortality at 31.25 ppm against 1st instar *Ae. aegypti* larvae. The ethanol extract of *Saussurea lappa* roots was the second most active displaying 100% mortality at 125 and 62.5 ppm. Polar active extracts were processed using LC-MS/MS to identify bioactive compounds. The apolar *A. perryi* flower extract was analyzed by headspace SPME-GC/MS analysis. Careful examination of the mass spectra and detailed interpretation of the fragmentation pattern allowed the identification of various biologically active secondary metabolites. Some compounds such as caffeic and quinic acid and their glycosides were detected in most of the analyzed fractions. Additionally, luteolin, luteolin glucoside, luteolin glucuronide and diglucuronide were also identified as bioactive compounds in several HPLC fractions. The volatile ketone, 6-methyl-5-hepten-2-one was identified from *A. perryi* petroleum ether fraction as a major compound.

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1. Introduction

An infected mosquito is the primary vector of numerous mosquito-borne illnesses, caused by bacteria, viruses or parasites. In fact, a mosquito bite can spread dangerous diseases, such as Japanese encephalitis, malaria, West Nile fever, Zika, dengue, yellow fever and chikungunya (Moreno-Madriñan and Turell, 2018). Severe cases of mosquito-borne diseases can lead to death. In 2016, the outbreak of dengue fever, one of the most disparaging diseases, in Jeddah and Jizan cities located in the west of Saudi Arabia, was triggered by the early season heat and humidity which caused mosquitoes to venture inside homes for shade (Alhaeli et al., 2016). The efforts of the Saudi Ministry of Health, which focus on fighting the mosquitoes and control their spread, led to the diminution of mosquito breeding areas and reduction in the number of infected people. However, exposure to conventional synthetic pesticides such as organochlorines, organophosphates and carbamates has raised serious concerns regarding toxic effects on human health, contamination of agricultural products, and the development of resistance to commonly used insecticides (Nicolopoulou-Stamati et al., 2016). On the other hand,
plant-based organic pesticides offer an effective, degradable, safe, environmentally friendly and cheaper alternative to conventional synthetic pesticides (Dinesh et al., 2014).

As a result, great efforts have been taken, over the last years, to improve the insecticidal properties of plant extracts and their isolated secondary metabolites. Indeed, a number of insecticidal agents have been reported from herbs including volatiles such as oils of Bifora, Satureja, Coridothymus, Thymbra, Coriandrum and Pimpinella (Sampson et al., 2005; Benelli et al., 2017; Vivekanandhan et al., 2018) and defense proteins such as lectins and proteinase and amylase inhibitors found in a wide variety of plants (Vandenborre et al., 2010). Saudi Arabia and Yemen are both characterized by wide diversity of flora due to climate and height differences among different areas. As part of our continuing investigation of native medicinal plants for interesting biological activities, fifty-seven extracts, prepared from thirty nine plants collected from different areas in Saudi Arabia and Yemen, were screened for larvalcidal and insecticidal activities against the mosquito vector Ae. Aegypti (see Fig. 1).

2. Materials and methods

2.1. Plant material

Based on local knowledge and use, thirty nine plants were selected from several areas in Saudi Arabia and Yemen during various periods and through several field trips, in the years 2013–2016. Some of the plant samples were purchased from the local market. Taxonomic identification of the plants was made by referring to published references at the Pharmacognosy Departments, Colleges of Pharmacy, King Saud and Sana’a Universities, Saudi Arabia and Yemen (Migahid, 1989; Chaudhary, 2001). Part of the identification was also done by the taxonomist, Dr. Ali Al-Ajami. A voucher specimen of each plant was deposited at the corresponding departments. The botanical and local Arabic names, families, collection places and common traditional uses of the inspected species are presented in Table 1.

2.2. Extraction of plant material

The air-dried and powdered plant materials (10 g of each) were extracted with different organic solvents (300 mL × 2) at room temperature by cold maceration. The combined extracts were filtered and concentrated under reduced pressure using a rotary evaporator (Buchi, Flawil, Switzerland) to obtain the crude dried residues; stored at −10 °C until use.

2.3. Mosquitoes

Aedes aegypti (L. (Orlando 1952 strain) used in larvicidal bioassays were supplied from a laboratory colony maintained at the Mosquito and Fly Research Unit at the USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), Gainesville, FL. The detailed mosquito rearing was previously reported (Pridgeon et al., 2008).

2.4. Adulticidal activity

The toxicity of each plant sample, against adult female Ae. aegypti, was measured using the procedure described by Chang et al. (2014). Initial adult screening was performed at 5 μg/mosq on three to six-day post-emergence females. Stock permethrin (0.1 μg/μL in DMSO) was a technical grade mixture of 46.1% cis and 53.2% trans isomers (Chemservice, West Chester, PA) and used to prepare controls of 0.38 and 1.72 ng/μL. These dilutions, along with acetone were included as positive and negative controls, respectively for each assay. Acetone was used to dilute the stock solutions and produce a 200 mM DMSO/acetone treatment solution that was used to prepare three serial dilutions (1:1) in acetone. Mortality was scored at 24 h and assays were repeated at least three times.

2.5. Larvicidal activity

Bioassays were conducted using the system described by Ali et al. (2013) to determine the larvicidal activity of the selected plants against 1st instar Ae. aegypti. This method uses 1st instar larvae, rather than the 3rd instars of the WHO larval bioassay, to take advantage of small quantities of isolated extracts which are often not available in amounts adequate for the WHO assay. Permethrin and acetone were used as positive and negative controls, respectively for each assay. Permethrin at 0.025 ppm gave 100% mortality and acetone had 0% mortality in the screening bioassays. Larval mortality was recorded 24 h post treatment.

2.6. LC-MS/MS analysis

LC-MS/MS analysis was carried out using an AB Sciex 3200 Q TRAP MS/MS detector. Experiments were performed with a Shimadzu 20A HPLC system coupled to an Applied Biosystems 3200 Q-Trap LC-MS/MS instrument equipped with an ESI source operating in negative ion mode. For the chromatographic separation, a GL Science Intersil ODS 250 × 4.6 mm, i.d., 5 μm particle size, octadecyl silica gel analytical column operating at 40 °C was used. The solvent flow rate was maintained at 0.7 mL/min (0.5 mL/min for O. tenuiflorum and N. sativa extracts). Detection was carried out with PDA detector. The elution gradient consisted of mobile phases (A) acetonitrile: water: formic acid (10:89:1, v/v/v) and (B) acetonitrile: water: formic acid (89:10:1, v/v/v). The composition of B was increased from 10% to 100% in 40 min. LC-ESI-MS/MS data were collected and processed by Analyst 1.6 software.

2.7. Headspace-SPME

The manual SPME device (Supelco, Bellafonte, PA, USA) with a fiber-precoated 65 μm thick layer of polydimethylsiloxane/divinyl benzene (PDMS/DVB-blue) was used for extraction of Aloe perryi volatiles. The vial containing the petroleum ether extract was sealed with parafilm. The fiber was then inserted immediately into the injection port of the GC-MS for desorption of the adsorbed volatile compounds for analysis.
List of screened plants against *Ae. aegypti* and their traditional uses.

| Plant species/local name                                      | VN     | Family      | Collection sites & time | Traditional uses & Ailments treated                                                                 |
|-------------------------------------------------------------|--------|-------------|-------------------------|-----------------------------------------------------------------------------------------------------|
| *Acacia nilotica* Linn.                                      | Sana-30| Fabaceae    | Hajjah/Yemen August 2014| Gastrointestinal disorders (Ali et al., 2012)                                                        |
| "Arabic Sant"                                                |        |             |                         |                                                                                                     |
| *Acalypha fruticosa* Forssk                                 | Sana-56| Euphorbiaceae| Mahwi/Yemen August 2013| Malaria, bacterial infections, nasal bleeding, stomachache and skin diseases (Durand-vynyan et al., 2006; Mohana et al., 2010) |
| "Anshalat"                                                   |        |             |                         | Asthma, arthritis, epilepsy, diabetes and burns (Migahid 1989)                                      |
| *Albizia lebbeck* L. Benth.                                  | KSU-1510| Leguminosae | Riyadh/YA June 2014     |                                                                                                     |
| "Labak"                                                      |        |             |                         |                                                                                                     |
| *Aloe perryi* Baker "Telif"                                  | Sana-4469| Xanthorrhoeaceae | Socotra/Yemen June 2014| Eye infections, hemorrhoids, wound healing, burns and skin diseases (Al-Fatimi et al., 2005)          |
| *Anagallis arvensis* L. = *Scarlet pimpernel* "Ein al'bot"    | KSU-15866| Myrsinaceae | Wadi Al-Ghaith/ SA     | Fungal infections, skin diseases, leprosy, epilepsy (Lopez et al., 2011)                            |
| *Artemisia absinthium* L.                                    | Sana-34| Asteraceae   | Sana'a/Yemen March 2013 | Fever, urinary tract diseases, fragmentation of kidney stones, expulsion of worms and snakes (Jaradat 2005; Lachenmeier 2010) |
| "Shoukr or Sheeh"                                            |        |             |                         |                                                                                                     |
| *Caralluma quadrangula* Forssk "Ghalf"                      | KSU-15450| Asclepiadaceae | Jizan/Sa March/2013    | Diabetes, general tonic (Adnan et al., 2014)                                                        |
| *Cyperus rotundus* Linn.                                     |        | Cyperaceae   |                         |                                                                                                     |
| *Sisymbrium officinale* L.                                   |        | Brassicaceae |                         |                                                                                                     |
| *Sisymbrium irio* L.                                         |        |             |                         |                                                                                                     |
| "Lesan Althor"                                               |        |             |                         |                                                                                                     |
| *Solanum lappa*                                              | KSU-16323| Asteraceae   |                         |                                                                                                     |
| C.B. Clarke "Qist Hindi"                                     |        |             |                         |                                                                                                     |
| *Sisymbrium irio* L.                                         | KSU-14380| Brassicaceae  | Najd area/ SA February 2015 |                                                                                                       |
| *Sistema officinale* L. "Samara"                             | Sana-17| Brassicaceae | Dharm/Yen April 2013    | Sore throat, bronchitis, poison and venom antidote (Blazevš et al. 2010)                             |
| *Costus spicatus* Jacq.                                      | Sana-5 | Costaceae    | Taiz/Yemen March 2013   | Complains of the bladder and urethra, expel kidney stones (Quintans Júnior et al., 2010)             |
| "Qist"                                                       |        |             |                         |                                                                                                     |
| *Cyperus rotundus* Linn.                                     | KSU-15182| Cyperaceae   | Najd area/ SA March 2013| Wounds, bruises, carbuncles, uterine disorders (Al-Massarani et al., 2016)                          |
| "Alsafa"                                                     |        |             |                         |                                                                                                     |
| *Dracaena cinnabari* Ballf.                                  | Sana-9 | Asparagaceae | Socotra/Yemen March 2016| Dysentery, diarrhea, hemorrhage (Al-Fatimi et al., 2005)                                            |
| "Dam alakhwain"                                              |        |             |                         |                                                                                                     |
| *Eucalyptus tereticorni* Sm. "Kafoor"                         | Sana-41| Myrtaceae    | ibb/Yemen July 2014     | Insecticidal, anesthetic, antiarctic (Maurya et al., 2016)                                          |
| *Foeniculum vulgare* Mill.                                   | Sana-19| Apiaceae     | ibb/Yemen July 2014     | Abdominal pain (Oktay et al., 2003)                                                                  |
| "Shomar"                                                     |        |             |                         |                                                                                                     |
| *Hibiscus subdariffa* L.                                     | Sana-18| Malvaceae    | ibb/Yemen May 2014      | Hypertension (Wahabi et al., 2010)                                                                   |
| "Karkadeh"                                                   |        |             |                         |                                                                                                     |
| *Hyssopus officinalis* L.                                    | Sana-4 | Lamiaceae    | Dhale/Yemen April 2013  | Coughing and sore throat (Ortiz de Elguea-Culebras et al., 2018)                                     |
| "Zofa"                                                       |        |             |                         |                                                                                                     |
| *Indigofera spinosa* Forssk.                                 | KSU-15794| Fabaceae    | Jabal Shatha/Sa February 2013 | Kidney stones, cough, cold (Al-Fatimi et al., 2007)                                               |
| "Shabak"                                                     |        |             |                         |                                                                                                     |
| *Jasminum grandiflorum* L. "Alghanah"                        | Sana-45| Oleaceae     | Taiz/Yemen June 2014    | Toothache, ulcers (Arun et al., 2016)                                                                |
| *Jasminum sambac* L.                                         | Sana-6 | Oleaceae     | Taiz/Yemen June 2014    | Fragrance (Sengar et al., 2015)                                                                     |
| "Fol or Yasmeen Arabi"                                       |        |             |                         |                                                                                                     |
| *Lavandula angustifolia* Miller "Dharm"                       | Sana-13| Lamiaceae    | Aden/Yemen June 2014    | Diuretic (Cavanagh and Wilkinson 2002)                                                               |
| *Nigella sativa* L.                                          | KSU-15132| Ranunculaceae| LM/Riyadh/SA June 2014  | Asthma, cough, bronchitis, rheumatoid arthritis (Al-Fatimi et al., 2005)                           |
| "Habba soda"                                                 |        |             |                         |                                                                                                     |
| *Ocimum tenuliforum* L.                                      | Sana-48| Lamiaceae    | Dalih/Yemen March 2014  | Gastroenteritis, dysentery, diarrhea, wound healing, acne, vitiligo (Chhetri et al., 2015)          |
| "Reeha"                                                      |        |             |                         |                                                                                                     |
| *Pandanus odoratissimus* Linn. "Kadi"                        | Sana-111| Pandanaceae  | Hodeida/ Yemen June 2014| Headache, rheumatism, epilepsy, urinary complains, enuresis (El-Shaibany et al., 2016)             |
| *Phoenix dactylifera* L.                                     | Sana-11| Arecaceae    | Hodeira/ Yemen July 2013| Diabetes, fever (Al-daihan and Bhat, 2012)                                                          |
| *"Ajwa seed"                                                 |        |             |                         |                                                                                                     |
| *Propolis* (bee glue)                                        | Sana-28| Apidae       | Mahwi/Yemen July 2013   | Gastroenteritis, infectious diseases (Fernandes et al., 2005)                                       |
| "Okbor"                                                      |        |             |                         |                                                                                                     |
| *Psidium guajava* Linn.                                      | Sana-20| Myrtaceae    | Lah/Yemen June 2013     | Cough, inflammation, diabetes, hypertension, diabetes (Gutierrez et al., 2008)                       |
| "Guava"                                                      |        |             |                         | Diabetes, gum bleeding (Jurenia 2008)                                                                |
| *Punica granatum* L.                                         | KSU-15156| Punicaceae  | ibb/Yemen May 2014      | Renal colic, dysmenorrhea (al-Sereiti et al., 2007)                                                |
| "Romanman"                                                   |        |             |                         |                                                                                                     |
| *Rosmarinus officinalis* L.                                  | Sana-23| Lamiaceae    | Taiz/Yemen May 2014     |                                                                                                     |
| "Ekleel aljabal"                                             |        |             |                         |                                                                                                     |
| *Ruta chalepensis* L.                                        | Sana-30| Rutaceae     | Sana'a/Yemen May 2014   | Pain, fever, rheumatism, epilepsy (Al-Said et al., 2004)                                           |
| "Shatbah"                                                    |        |             |                         |                                                                                                     |
| *Salvia spinosa* L.                                          | Sana-52| Labiatae     | Dharm/Yemen August 2014 | Diarrhea, urinary disorders, piles, chest and stomach pains (Bahadori et al., 2015)                  |
| "Lesan Althor"                                               |        |             |                         |                                                                                                     |
| *Sausurea lappa*                                             | KSU-16323| Asteraceae | LM/Riyadh/SA March 2014 | Asthma, gastric ulcer, inflammation and liver diseases (Zahara et al., 2014)                        |
| C.B. Clarke "Qist Hindi"                                     |        |             |                         |                                                                                                     |
| *Sisymbrium irio* L.                                         | KSU-14380| Brassicaceae | Najd area/ SA February 2015| Cough, chest congestion, clean wounds (Al-Massarani et al., 2017)                                   |
| "Hoverna"                                                    |        |             |                         |                                                                                                     |
| *Sisymbrium officinale* L. "Samara"                          | Sana-17| Brassicaceae | Dharm/Yen April 2013    |                                                                                                     |
| *Tulip radiata*                                               |        |             |                         |                                                                                                     |
2.8. GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450. Relative percentage amounts of the separated compounds were calculated from TIC chromatograms.

The identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0) (McLafferty and Stauffer, 1989; Hochmuth, 2008) and in-house “Basër Library of Essential Oil Constituents” built up by genuine compounds and components of known oils.

3. Results

3.1. Adulticidal and larvicidal activities against Ae. aegypti

The Saudi and Yemeni medicinal plants were evaluated in adulticidal and larvicidal bioassays against the yellow fever and dengue mosquitoes. In adult bioassays, extracts were tested at the screen-dose of 5 µg/mosquito against female Ae. aegypti. Out of the 57 screened extracts, the EtOH extract of H. officinalis aerial parts (#29), the MeOH extract of N. sativa seeds (#34), the MeOH extract of O. tenuiflorum aerial parts (#36), the EtOH extract of S. lappa roots (#50) and the EtOH extract of T. officinale aerial parts (#53) produced over 80% mortality at the tested concentration of 5 µg/mosquito (Table 2). Among which, S. lappa possessed the greatest mortality in the adulticidal activity. Average mortality in the lower dose permethrin control (0.19 ng/mosquito) was 60 ± 10% and at the higher dose of 0.86 ng/mosquito was 100%. The acetone had an average mortality of 6.7 ± 5.8% and untreated controls showed 0% mortality. Screening for larvicidal activity indicated 100% mortality at the dose of 31.25 ppm for petroleum ether extract of A. perryi flowers whereas methanol extract of P. dactylifera showed 100% mortality at 125 ppm and the mortality of methanol extract of O. tenuiflorum was 70% against 1st instar Ae. aegypti larvae. Ethanolic extract of S. lappa roots gave 100% mortality at 125 and 62.5 ppm whereas larval mortality at 31.25 ppm was 40%. Control mortality in these experiments was 0 for solvent only wells and 100% in permethrin treated wells (0.025 ppm).

3.2. LC-MS/MS analysis

Based on results, the active alcoholic extracts were processed for LC MS/MS analysis to identify the bioactive compounds. The results of the analyzed fractions (the MeOH extracts of H. officinalis and O. tenuiflorum and the EtOH extracts S. lappa, H. officinalis and T. officinale) are presented in Table 3.

3.2.1. Identification of caffeic and quinic acids derivatives

The EtOH extract of T. officinale revealed caffeoyl and hexose esterification of caffeic acid, indicated by a molecular ion peak at m/z 341 [M-H]- then fragmented ion at m/z 179 [M-H]- and a base peak ion at m/z 135 [M-H]-, characteristic for caffeic acid, in addition to another peak at 161. Loss of a –162 amu hexose unit allowed the determination of this compound as caffeoyl glucoside. Cichoric acid which contains two caffeic acid and tartaric acid units was also identified in the T. officinale extract (Table 3). Cichoric acid presented a pseudo molecular ion peak at m/z 473 and caffeic acid fragments at m/z 179 and 161 (Table 3). The EtOH extract H. officinalis showed a molecular ion peak at m/z 359 [M-H]- and product ions at m/z 197 [M-H]- and 161 [M-H]- which presents characteristic fragmentation behavior of rosmarinic acid (caffeic acid dimer). Rosmarinic acid was also the most abundant compound in O. tenuiflorum extract (Table 3).

A molecular ion peak at m/z 191 with fragmented ions at m/z 173 and 127 indicated the presence of quinic acid in T. officinale extract (Table 3). Caffeic acid and quinic acid esters were distinguished as caffeoylquinic acid. Identification of these compounds was done according to the identification key previously published by Clifford and colleagues (Clifford et al., 2003, 2005, 2008).

The 3-caffeoylquinic acid, presented by a pseudo molecular ion peak at m/z 353 and a base peak ion at m/z 191 was determined in the EtOH extract of H. officinalis. The high abundance ion at m/z 179 allowed the identification of this compound as 3-caffeoylquinic acid (Table 3).

3.2.2. Identification of luteolin and apigenin derivatives

The luteolin was determined in T. officinale and H. officinalis with its molecular ion peak at m/z 285 [M-H]- and product ions at m/z 151 and 133 amu. Luteolin glucuronide, determined in T. officinale and H. officinalis extracts, showed a pseudo molecular ion peak at m/z 461 and a base peak ion at m/z 285 (luteolin aglycon) due to the loss of a 176 amu glucuronide moiety (Kapp et al., 2013). The diglucuronide of luteolin was also suggested in H. officinalis extract at m/z 637 amu. Luteolin glucoside presenting a molecular ion peak at m/z 447 and an aglycon at m/z 285 (loss of a glucose moiety) was detected in O. tenuiflorum extract (Table 3).
and \( H. \text{ officinalis} \) extracts. \( T. \text{ officinale} \) extract had also showed the same molecular ion peak but with different product ions at \( m/z \) 357 and 327.90. The 30 amu difference from the molecular ion peak indicated that the compound is a C-glucoside (Taamalli et al., 2015). Subsequently, it was determined as luteolin-6-C-glucoside (isoorientin). Apigenin and its derivative were determined in the same way described above. As an aglycon, apigenin was only determined in \( T. \text{ officinale} \), its glucoside derivative was found in \( S. \text{ lappa} \), whereas glucuronide derivative was found in \( H. \text{ officinalis} \) (Table 3).

### 3.3. HS-SPME-GC/MS analysis

In order to identify the volatile constituents of the petroleum ether extract of \( Aloe \text{ perryi} \), HS-SPME-GC/MS analysis was conducted. Thirty-three compounds were identified which

| # | Plant species | Plant part screened | Extraction solvent | % mortality ± SE 5 µg/mosquito |
|---|--------------|---------------------|-------------------|-------------------------------|
| 1 | Acacia nilotica | F | EtOH | 63.3 ± 15.3 |
| 2 | Acalypha fruticosa | L, S | MeOH | 30 ± 20 |
| 3 | Acalypha fruticosa | L, S | n-Hexane | 30 ± 17.3 |
| 4 | Acalypha fruticosa | L, S | CHCl, | 23.3 ± 5.8 |
| 5 | Acalypha fruticosa | L, S | EtOAc | 6.7 ± 11.5 |
| 6 | Acalypha fruticosa | L, S | n-BuOH | 3.3 ± 5.8 |
| 7 | Acalypha fruticosa | L, S | Water | 6.7 ± 5.8 |
| 8 | Albizia lebbeck | Fr | EtOH | 3.3 ± 5.8 |
| 9 | Albizia lebbeck | Se | EtOH | 10 ± 10 |
| 10 | Aloe perryi | F | MeOH | 53.3 ± 30.6 |
| 11 | Aloe perryi | F | Pet. ether | 60 ± 17.3 |
| 12 | Aloe perryi | F | Water | 50 ± 10 |
| 13 | Anagallis arvensis | L, S | MeOH | 10 ± 10 |
| 14 | Anagallis arvensis | L, S | EtOH | 6.7 ± 5.8 |
| 15 | Artemisia absinthium | L, S | MeOH | 10 |
| 16 | Artemisia absinthium | L, S | n-Hexane | 6.7 ± 5.8 |
| 17 | Artemisia absinthium | L, S | Water | 3.3 ± 5.8 |
| 18 | Caralluma quadrangula | L, S | EtOH | 13.3 ± 5.8 |
| 19 | Caralluma wisssmannii | L, S | EtOH | 3.3 ± 5.8 |
| 20 | Citrus colochothys | L, S | MeOH | 3.3 ± 5.8 |
| 21 | Commmopora gileadensis | B | MeOH | 56.7 ± 15.3 |
| 22 | Commmopora gileadensis | Gum – | 70 ± 26.5 |
| 23 | Costus spicatus | R | EtOH | 56.7 ± 28.9 |
| 24 | Cyperus rotundus | R | EtOH | 63.3 ± 20.8 |
| 25 | Dracaena cinnabari | Re | MeOH | 20 ± 10 |
| 26 | Eucalyptus tereticornis | L | MeOH | 23.3 ± 15.3 |
| 27 | Foeniculum vulgare | L | MeOH | 6.7 ± 5.8 |
| 28 | Hibiscus sabdariffa | L | MeOH | 16.7 ± 20.8 |
| 29 | Hyssopus officinalis | L, S | EtOH | 86.7 ± 15.3 |
| 30 | Indigofera spinoise | L, S | EtOH | 23.3 ± 15.3 |
| 31 | Jasminum grandiflorum | L | EtOH | 3.3 ± 5.8 |
| 32 | Jasminum sambac | L | MeOH | 3.3 ± 5.8 |
| 33 | Lavandula angustifolia | F | MeOH | 10 ± 10 |
| 34 | Nigella sativa | Se | MeOH | 93.3 ± 11.5 |
| 35 | Nigella sativa | Se | Water | 50 ± 17.3 |
| 36 | Ocimum tenuiforum | L, S | MeOH | 96.7 ± 5.8 |
| 37 | Pandalus odoratissimus | L | MeOH | 46.7 ± 15.3 |
| 38 | Pandalusodoratissimus | Peduncle | MeOH | 23.1 ± 32.1 |
| 39 | Pandalusodoratissimus | F | MeOH | 3.3 ± 5.8 |
| 40 | Pandalusodoratissimus | F | Pet. ether | 50 ± 20 |
| 41 | Pandalusodoratissimus | F | Water | 63.3 ± 15.3 |
| 42 | Phoenix dactylifera | Se | MeOH | 10 ± 10 |
| 43 | Phoenix dactylifera | Se | Pet. ether | 10 ± 10 |
| 44 | Propolis (bee glue) | Re | MeOH | 13.3 ± 5.8 |
| 45 | Psidium guajava | L | MeOH | 23.1 ± 32.1 |
| 46 | Punica granatum | F | MeOH | 73.3 ± 15.3 |
| 47 | Rosmarinis officinalis | L, S | MeOH | 6.7 ± 11.5 |
| 48 | Ruta chalepensis | L, S | MeOH | 16.7 ± 28.9 |
| 49 | Salvia spinosa | L, S | MeOH | 13.3 ± 5.8 |
| 50 | Sausurea lappa | R | EtOH | 100 |
| 51 | Sisymbrium trius | L, S | Water | 73.3 ± 5.8 |
| 52 | Sisymbrium officinal | L, S | MeOH | 10 ± 17.3 |
| 53 | Taraxacum officinalis | L, S | EtOH | 93.3 ± 5.8 |
| 54 | Teucrium polium | L | EtOH | 16.7 ± 15.3 |
| 55 | Tribulus terrestris | L, S | MeOH | 36.7 ± 5.8 |
| 56 | Vitis vinfera (Red grape) | Se | MeOH | 23.3 ± 15.3 |
| 57 | Zea mays (silk corn fiber) | Fiber | MeOH | 3.3 ± 5.8 |

**Table 2**

Adulticidal activities of tested plant extracts against \( A. \text{ aegypti} \).

B: Bark; L: Leaves; F: Flowers; R: Roots or rhizomes; Re: resin; S: Stems; Se: Seeds; Fr: Fruits.
represented 70.6% of A. perryi constitution. The ketone, 6-methyl-5-hepten-2-one (22.4%), menthol (8.9%) and the sesquiterpine 1,4-bis (1,1-dimethylethyl)-benzene (4.5%) were the main constituents (Table 4).

4. Discussion

In our continuous search to find novel bioactive compounds from plants, 39 plant were evaluated for their insecticidal potential activities. Our findings are in agreement with reports for folkloric use, in some communities, for several of the currently screened plants or related species, as insecticides (Tomczyk and Szymanska 1995; Singh et al., 2014; Benchouikh et al., 2015; Ortiz de Elguea-Culebras et al., 2018). The roots of S. lappa showing 100% mortality at 5 g/mosquito are traditionally used in the Himalaya region. In a previous study, a moderate larvicidal activity was observed for the essential oil of S. lappa against Ae. aegypti with LC50 value 141.43 (Manzoor et al., 2013). The sesquiterpene costunolide in S. lappa demonstrated significant insecticidal activity against Papilio demoleus butterflies (Vattikonda et al., 2015). The 20% alcohol solution of Nigella sativa showed a 100% mortality against the cattle tick, Rhipicephalus annulatus (Aboelhadid, et al., 2016). T. officinale was also effective against the insect, B. cockerelli, commonly found on potato and tomato crops. The ethanol extract of the plant dried leaves killed more than 50% of 5th and 3rd instars with concentration of 0.01 and 0.1 g/ml, respectively (Granados-Echegoyen et al., 2015). The leaf extract of Ocimum gratissimum had a potent larvicidal activity with an LC50 19.50 mg/ml against Ae. aegypti, while in the present study, the tested species, O. tenuiflorum, was inactive against the larvae and demonstrated remarkable potency as adulticidal agent (Ghosh et al., 2012). Results of a previous study, on the mosquito larvicidal activity of petroleum ether extract of A. vera leaf, showed 34% mortality against Ae. aegypti 1st instar larvae at 80 ppm, increased to 89% at 400 ppm of A. vera leaf extract treatment (Subramaniam et al., 2012). The results clearly reveals that the currently investigated species, A. perryi, exhibits a higher potency (100% mortality for petroleum ether extract at 31.25 ppm). This could be due to higher concentration of active larvicidal compounds in the flowers.

Table 3
Identified compounds by LC-MS/MS analysis of the active plant extracts.

| Compound                        | RT [min] | [M-H] m/z | Fragments | Identified compounds                  |
|---------------------------------|----------|-----------|-----------|--------------------------------------|
| H. officinalis leaves & stems    | 8.0      | 353       | 191, 179  | 3-caffeoylquinic acid                |
| EtoH extract # 29               | 9.7      | 637       | 351, 285  | luteolin diglucuronide               |
|                                 | 10.5     | 637       | 461, 285  | luteolin glucoside                   |
|                                 | 11.1     | 621       | 487, 351, 269 | quercetin 7-rutinoside               |
|                                 | 11.4     | 461       | 285       | luteolin glucuronide                 |
|                                 | 12.6     | 445       | 269, 175, 113 | apigenin glucuronide                |
|                                 | 13.1     | 359       | 197, 179, 161 | rosmarinic acid                  |
|                                 | 16.3     | 285       | 175, 133  | luteolin                           |
| N. sativa seeds MeOH extract # 34| 3.5      | 286       | 162       | unknown                              |
|                                 | 4.9      | 267       | 249       | unknown                              |
|                                 | 5.3      | 289       | 199, 169, 127 | unknown                     |
|                                 | 6.8      | 401       | 283, 269  | apigenin pentoside                   |
|                                 | 7.7      | 353       | 191, 179, 161 | 5-caffeoylquinic acid               |
|                                 | 7.9      | 771       | 609, 425, 284 | luteolin / Kaempferol-sophoroside-glucoside |
| O. tenuiflorum leaves & stems    | 7.7      | 341       | 179       | caffeoyl glucoside                   |
| MeOH extract # 36               | 9.4      | 609       | 300, 271, 179, 151 | quercetin glucuronide                |
|                                 | 10.4     | 463       | 300, 271, 179, 151 | unknown                     |
|                                 | 13.9     | 359       | 197, 179, 161 | rosmarinic acid                  |
|                                 | 18.0     | 581       | 461, 436  | unknown                              |
|                                 | 27.5     | 343       | 328, 313, 241 | nevadensin                      |
|                                 | 27.8     | 283       | 240, 215  | acacetin / wogonin                   |
|                                 | 30.6     | 555       | 487, 469, 425 | unknown                      |
|                                 | 33.6     | 293       | 275, 235, 231, 171 | unknown                      |
| S. lappa root EtoH extract # 50  | 9.2      | 353       | 191, 173  | 5-caffeoylquinic acid                |
|                                 | 12.3     | 515       | 352, 191, 179, 161 | 1,5-dicaffeoylquinic acid              |
|                                 | 12.4     | 515       | 353, 299, 203, 191, 179 | 4,5-dicaffeoylquinic acid                   |
|                                 | 13.2     | 561       | 309, 351, 215, 191 | feruloyl quinic acid derivative       |
|                                 | 14.3     | 313       | 298, 283, 265 | dihydroxy-dimethoxyflavone similar to cirsimarin - apigenin glucoside |
|                                 | 15.1     | 431       | 208       | unknown                              |
| T. officinale leaves & stems    | 6.0      | 341       | 179, 161  | caffeoyl glucoside                   |
| Etoh extract # 53               | 9.1      | 191       | 173, 127  | quinic acid                          |
|                                 | 9.9      | 447       | 357, 327  | luteolin-6-C-glucoside               |
|                                 | 10.0     | 179       | 135       | caffeic acid                         |
|                                 | 11.1     | 477       | 301, 179, 151 | quercetin glucuronide                |
|                                 | 11.3     | 198       | 163       | unknown                              |
|                                 | 12.1     | 461       | 285       | luteolin glucuronide                 |
|                                 | 14.5     | 473       | 293, 219, 179, 161 | cichoric acid          |
|                                 | 15.9     | 285       | 199, 151, 133 | luteolin                           |
|                                 | 17.9     | 269       | 201, 151, 117 | apigenin                           |
The ketone 6-methyl-5-hepten-2-one, identified as the major volatile constituent in the pet. ether extract of A. perryi, is also a volatile oil component of lemon-grass oil (Cympopogon citratus), and a known alarm pheromone constituent in many species of ants and other insects. Additionally, this compound is an acetylcholinesterase (AChE) and esterase enzyme inhibitor (Ganjewala, 2009). A former study indicated that AChE inhibitors decreases, in a dose-dependent way, the hatching of treated eggs, delay the development of larvae and deleteriously affect the behavior of insects (Emara, 2004). These results provide a rational explanation for the remarkable currently observed larvicidal activity of A. perryi. It is worth mentioning, that to the best of our knowledge, this is the first study assessing the mosquitocidal effects of most of the tested plants. Moreover, this is the first report investigating the volatile constituents of A. perryi collected in Yemen or elsewhere.

### 5. Conclusion

The present study rationalizes the use of some of the explored plants in ethno-agricultural practices. The results suggest that A. perryi, O. officinalis, N. sativa, O. tenuiflorum, S. lappa and T. officinale could be promising sources of new potential eco-friendly mosquitocidal agents. Based on the data, treatments with phenolic acids and flavonoids-rich plant extracts caused a significant insecticidal activity and decreased the number of Ae. aegypti adults. However, bio-guided fractionation must be carried out in order to isolate and identify the compounds that are responsible for these insecticidal activities.

### Declaration of Competing Interest

All the authors declare that they have no conflict of interest.

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### Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsp.2019.07.001.

### Table 4

| RRI | Constituents | Conc. | IM |
|-----|--------------|------|----|
| 1348 | 6-Methyl-5-hepten-2-one | 22.4 | MS |
| 1399 | Methyl octanoate | 1.2 | MS |
| 1400 | Tetradeacne | 1.7 | RRI, MS |
| 1440 | 1,4-Bis (1,1-dimethylethyl)-benzene | 4.5 | MS |
| 1443 | Ethyl octanoate | 1.7 | MS |
| 1452 | 1-Octen-3-ol | 3.5 | MS |
| 1475 | Acetic acid | 1.2 | RRI, MS |
| 1479 | Furfural | 1.3 | RRI, MS |
| 1496 | 2-Ethyl hexanol | 0.5 | MS |
| 1500 | Methyl nonanoate | 0.8 | MS |
| 1516 | 2-Acetyl furan | 0.4 | MS |
| 1523 | 2-Nonanol | 0.5 | MS |
| 1541 | Neomenthyl acetate | 0.7 | MS |
| 1541 | Benzaldehyde | 1.3 | RRI, MS |
| 1544 | Ethyl nonanoate | 0.8 | MS |
| 1589 | Ethyl malonate | 0.5 | MS |
| 1590 | 5-Methyl-2-furfural | 1.1 | MS |
| 1591 | 2-Methyl propanoic acid | 0.7 | MS |
| 1602 | 6-Methyl-3,5-heptadien-2-one | 1.7 | MS |
| 1625 | 4,4-Dimethyl but-2-enolide | 0.6 | MS |
| 1638 | Menthol | 8.9 | RRI, MS |
| 1643 | acetic acid | 1.2 | RRI, MS |
| 1747 | 3,4-Dimethyl-2,5-furandione | 0.8 | MS |
| 1762 | Pentanoic acid | 0.4 | RRI, MS |
| 1868 | 1-Isobutyl-4-isopropyl-3-isopropyl-2,2-Dimethyl-1,4-benzodioxane | 0.2 | MS |
| 1882 | 1-Isobutyl-4-isopropyl-3-isopropyl-2,2-Dimethyl-1,4-benzodioxane | 0.2 | MS |
| 1965 | 2-Ethyl hexanoic acid | 0.5 | MS |
| Total | 70.6 | |

RRI: Relative retention indices calculated against n-alkanes; %: calculated from TIC data; *: Tentative identification from Wiley; IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS; identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Careful interpretation of the LC-MS/MS data of the five active extracts allowed the identification of 28 compounds. Luteolin was detected in three of the analyzed extracts (H. officinalis, T. officinale and N. sativa). Caffeic acid and its caffeoylquinic acid derivatives were also common constituents among the five analyzed fractions. The significant activities of some of the extracts can be substantially justified by their rich contents of phenolic acids and phenolic compounds in general. Previous studies demonstrated that phenolics may reduce insect herbivory in several ways such as discouraging feeding and oviposition, reducing fertility and shortening the insects life span (Dawkar et al., 2013; Czerneckicz et al., 2016). A study conducted by Mitchell and his coworkers, found that many plant flavonoids such as quercetin, chrysos, api- genin, kaempferol and morin, can in inhibit, in a dose-dependent manner, the cytochrome P-450 dependent ecysdese 20-monooxygenase activity associated with adult female Ae. aegypti which results in direct cellular toxicity (Mitchell et al., 1993).

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