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Original article

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

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ABSTRACT

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.

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-Madriñán 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,

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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 larvicidal 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.

Ocimum tenuiflorum from Sana'a

Artemisia absinthium from Ibb

Fig. 1. Pictures of selected traditional plants analyzed in this study.

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 has been 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 pushed through the film layer for exposure to the headspace of the extract for 15 min at 40 °C. The fiber was then inserted immediately into the injection port of the GC-MS for desorption of the adsorbed volatile compounds for analysis.

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Table 1
List of screened plants against *Ae. aegypti* and their traditional uses.

Plant speciesl/local name	VN	Family	Collection sites & time	Traditional uses & Ailments treated
Acacia nilotica Linn. "Arabic Sant"	Sana-30	Fabaceae	Hajjah/Yemen August 2014	Gastrointestinal disorders (Ali et al., 2012)
Acalypha fruticosa Forssk "Anshat"	Sana-56	Euphorbiaceae	Mahwit/Yemen August 2013	Malaria, bacterial infections, nasal bleeding, stomachache and skin diseases (Duraipandiyan et al., 2006; Mothana et al., 2010)
Albizia lebbeck L. Benth "Labak"	KSU-15101	Leguminosae	Riyadh/SA June 2014	Asthma, arthritis, epilepsy, diabetes and burns (Migahid 1989)
Aloe perryi Baker "Teif"	Sana-4469	Xanthorrhoeaceae Asphodelaceae	Socotra/Yemen June 2014	Eye infections, hemorrhoids, wound healing, burns and skin diseases (Al-Fatimi et al., 2005)
Anagallis arvensis L. = *Scarlet pimpernel* "Ein alkot"	KSU-15866	Myrsinaceae	Wadi Al-Ghaat/ SA February 2013	Fungal infections, skin diseases, leprosy, epilepsy (Lopez et al., 2011)
Artemisia absinthium L. "Shoukr or Sheeh"	Sana-34	Asteraceae	Sana'a/Yemen June 2014	Fever, urinary tract diseases, fragmentation of kidney stones, expulsion of worms and snakes (Jaradat 2005; Lachenmeier 2010)
Caralluma quadrangula Forssk "Ghalf"	KSU-15450	Asclepiadaceae	Jizan/SA March/ 2013	Diabetes, general tonic (Adnan et al., 2014)
Caralluma wissmanii Schwartz = *Angolluma wissmanii* Plowes " Atba'a alkalbah"	KSU-16009	Asclepiadaceae	Agabat Alabna/ SA March 2013	Stomachache, diabetes (Adnan et al., 2014)
Citrullus colocynthis L. Schrad. "Hanzal"	Sana-32	Cucurbitaceae	Sabwa/Yemen January 2014	Diabetes, constipation, hair-growth-promoting (Rahimi et al., 2012)
Commiphora gileadensis L. = *C. opobalsamum* L. Engl. "Basham"	Sana-29	Burseraceae	Hadramout/ Yemen August 2014	Headache, urinary retention (Abbas et al., 2007)
Costus spicatus Jacq. "Qist"	Sana-5	Costaceae	Taiz /Yemen March 2013	Complaints of the bladder and urethra, expel kidney stones (Quintans Júnior et al., 2010)
Cyperus rotundus Linn. "Alsaad"	KSU-15182	Cyperaceae	Najd area/ SA March 2016	Wounds, bruises, carbuncles, uterine disorders (Al-Massarani et al., 2016)
Dracaena cinnabari Balf.f. "Dam alakhwain"	Sana-9 SP-D225	Asparagaceae	Socotra/Yemen March 2014	Dysentery, diarrhea, hemorrhage (Al-Fatimi et al., 2005)
Eucalyptus tereticornis Sm. "Kafoor"	Sana-41	Myrtaceae	Ibb/Yemen July 2014	Insecticidal, anesthetic, antiseptic (Maurya et al., 2016)
Foeniculum vulgare Mill. "Shomar"	Sana-19	Apiaceae	Ibb/Yemen July 2014	Abdominal pain (Oktay et al., 2003)
Hibiscus *sabdariffa* L. "Karkadeh"	Sana-18	Malvaceae.	Ibb/Yemen May 2014	Hypertension (Wahabi et al., 2010)
Hyssopus officinalis L. "Zofa"	Sana-4	Lamiaceae	Dhale/Yemen April 2013	Coughing and sore throat (Ortiz de Elguea-Culebras et al., 2018)
Indigofera spinosa Forssk. "Shabrak"	KSU-15794	Fabaceae	Jabal Shatha/SA February 2013	Kidney stones, cough, cold (Al-Fatimi et al., 2007)
Jasminum grandiflorum L. "Alganah"	Sana-45	Oleaceae	Taiz/Yemen June 2014	Toothache, ulcers (Arun et al., 2016)
Jasminum sambac L. "Fol or Yasmeen Arabi"	Sana-6	Oleaceae	Taiz/Yemen June 2014	Fragrance (Sengar et al., 2015)
Lavandula angustifolia Miller "Dharm"	Sana-13	Lamiaceae	Aden/Yemen June 2014	Diuretic (Cavanagh and Wilkinson 2002)
Nigella sativa L. "Habba soda"	KSU-15132	Ranunculaceae	LM/Riyadh/SA June 2014	Asthma, cough, bronchitis, rheumatoid arthritis (Al-Fatimi et al., 2005)
Ocimum *tenuiflorum* L. "Reehan"	Sana-48	Lamiaceae	Dalih/Yemen March 2014	Gastroenteritis, dysentery, diarrhea, wound healing, acne, vitiligo (Chhetri et al., 2015)
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. "Ajwa seed"	Sana-11	Arecaceae	Hadramout/ Yemen July 2013	Diabetes, febrile fever (Al-daihan and Bhat, 2012)
Propolis (bee glue) "Okbor"	Sana-28	Apidae	Mahwit/Yemen July 2013	Gastroenteritis, infectious diseases (Fernandes et al., 2005)
Psidium guajava Linn. "Guava"	Sana-20	Myrtaceae	Lahj/Yemen June 2013	Cough, inflammation, diabetes, hypertension, diarrhea (Gutierrez et al., 2008)
Punica granatum L. "Romman"	KSU-15156	Punicaceae	Ibb/Yemen May 2014	Diabetes, gum bleeding (Jurenka 2008)
Rosmarinus officinalis L. "Ekleel aljabal"	Sana-23	Lamiaceae	Taiz/Yemen May 2014	Renal colic, dysmenorrhea (al-Sereiti et al., 1999)
Ruta chalepensis L. "Shathab"	Sana-30	Rutaceae	Sana'a/Yemen May 2014	Pain, fever, rheumatism, epilepsy (Al-Said et al., 1990)
Salvia spinosa L. "Lesan Althor"	Sana-52	Labiatae	Dhamar/Yemen August 2014	Diarrhea, urinary disorders, piles, chest and stomach pains (Bahadori et al., 2015)
Saussurea lappa C.B. Clarke "Qist Hindi"	KSU-16323	Asteraceae	LM/Riyadh/SA March 2014	Asthma, gastric ulcer, inflammation and liver diseases (Zahara et al., 2014)
Sisymbrium irio L. "Howerna"	KSU-14380	Brassicaceae	Najd area/ SA February 2015	Cough, chest congestion, clean wounds (Al-Massarani et al., 2017)
Sisymbrium officinale L. "Samara"	Sana-17	Brassicaceae	Dhamar/Yemen April 2013	Sore throat, bronchitis, poison and venom antidote (Blazević et al. 2010)

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Table 1 (*continued*)

Plant speciesl/local name	VN	Family	Collection sites & time	Traditional uses & Ailments treated
Taraxacum officinale L. "Tarkhashkon"	Sana- 8	Asteraceae	Ibb/Yemen May 2013	Digestive disorders, bile and liver problems, diuretic (Bhatia et al., 2014)
Teucrium polium L. "Ja'adah"	KSU-15788	Lamiaceae	Akabat Alabna/ SA April 2013	Diabetes, hypertension, inflammation, rheumatism (Tariq et al., 1989)
Tribulus terrestris L. "Qutiba"	Sana-17	Zygophyllaceae	Taiz/Yemen August 2013	Kidney stone, infertility, erectile dysfunction (El-Shaibany et al., 2015)
Vitis vinifera L. "Karm"	Sana-9	Vitaceae	Saada/Yemen June 2013	Diabetes, anti-cholesterol, and anti-platelet functions (Feei Ma and Zhang 2017)
Zea mays L. (silk corn fiber) "Zorah"	Sana-7	Poaceae	Sana/Yemen August 2014	Urinary tract disorders, kidney stones, asthma, hypertension (Žilić et al., 2016)

VN: Voucher No. of the Plant, Ht: height.
SA: Saudi Arabia, LM: local market.

2.8. GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 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 "Başer 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 screening 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. tenuifolium* was 70% against 1st instar *Ae. aegypti* larvae. Ethanol 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 $[M\text{-}H]^-$ then fragmented ion at *m*/*z* 179 $[M\text{-}H]^-$ and a base peak ion at *m*/*z* 135 $[M\text{-}H]^-$, 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 $[M\text{-}H]^-$ and product ions at *m*/*z* 197 $[M\text{-}H]^-$ and 161 $[M\text{-}H]^-$ 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 collogues (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 $[M\text{-}H]^-$ 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 glucouronide 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*

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Table 2
Adulticidal activities of tested plant extracts against *Ae. aegypti*.

#	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	$CHCl_3$	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 wissmannii*	L, S	EtOH	3.3 ± 5.8
20	*Citrullus colocynthis*	L, S	MeOH	3.3 ± 5.8
21	*Commiphora gileadensis*	B	MeOH	56.7 ± 15.3
22	Commiphora *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 spinose*	L, S	EtOH	23.3 ± 15.3
31	*Jasminum grandiflorum*	L, S	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 tenuiflorum*	L, S	MeOH	96.7 ± 5.8
37	*Pandanus odoratissimus*	L	MeOH	46.7 ± 15.3
38	*Pandanus odoratissimus*	Peduncle	MeOH	23.1 ± 32.1
39	*Pandanus odoratissimus*	F	MeOH	3.3 ± 5.8
40	*Pandanus odoratissimus*	F	Pet. ether	50 ± 20
41	*Pandanus odoratissimus*	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 ± 23.1
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	*Saussurea lappa*	R	EtOH	100
51	*Sisymbrium irio*	L, S	Water	73.3 ± 5.8
52	*Sisymbrium officinale*	L, S	MeOH	10 ± 17.3
53	*Taraxacum officinale*	L, S	EtOH	93.3 ± 5.8
54	*Teucrium polium*	L	EtOH	16.7 ± 15.3
55	*Tribulus terrestis*	L, S	MeOH	36.7 ± 5.8
56	*Vitis vinifera* (Red grape)	Se	MeOH	23.3 ± 15.3
57	*Zea mays* (silk corn fiber)	Fiber	MeOH	3.3 ± 5.8
	Acetone			6.7 ± 5.8
	Untreated			0
	Permethrin (0.86 ng/mosquito)			100
	Permethrin (0.19 ng/mosquito)			60.0 ± 10.0

B: Bark; L: Leaves; F: Flowers; R: Roots or rhizomes; Re: resin; S: Stems; Se: Seeds; Fr: Fruits.

and *H. officinalis* extracts. *T. 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. officinale*, its glucoside derivative was found in *S. lappa*, whereas glucuronide derivative was found in *H. officinalis* (Table 3).

3.3. HS-SPME-GC/MS analysis

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

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

RT	$[M-H]^-$ m/z	Fragments	Identified compounds
H. officinalis leaves & stems EtOH extract # 29			
8.0	353	191, 179	3-caffeoylquinic acid
9.7	637	351, 285	luteolin diglucuronide
9.9	447	285	luteolin glucoside
9.9	609	301, 271, 255, 179	quercetin 7-rutinoside
10.5	637	461, 285	leukoseptoside A
11.1	621	487, 351, 269	apigenin derivative
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, 429, 284	luteolin / Kaempferol-sophoroside-glucoside
O. tenuiflorum leaves & stems MeOH extract # 36			
7.7	341	179	caffeoyl glucoside
9.4	609	300, 271, 179, 151	rutin
10.4	463	300, 271, 179, 151	quercetin glucuronide
13.2	539	471, 377, 307, 275	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	369, 351, 215, 191	feruloyl quinic acid derivative
14.3	313	298, 283, 265	dihydroxy-dimethoxyflavone similar to cirsimaritin
15.1	431	268	apigenin glucoside
T. officinale leaves & stems EtOH extract # 53			
6.0	341	179, 161	caffeoyl glucoside
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

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 μg/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 LC_{50} 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 LC_{50} 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.

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Table 4
The volatile composition of the petroleum ether extract of *Aloe perryi* flowers.

RRI	Constituents	Conc. %	IM
1348	6-Methyl-5-hepten-2-one	22.4	MS
1399	Methyl octanoate	1.2	MS
1400	Tetradecane	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
1521	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
1647	Butanedioic acid diethylester*	0.4	MS
1684	Isovaleric acid	2.9	RRI, MS
1703	6-Oxo-Isophorone	6.5	MS
1747	3,4-Dimethyl-2,5-furandione	0.8	MS
1762	Pentanoic acid	0.4	RRI, MS
1800	Ethyl phenylacetate	0.6	MS
1815	Methyl dodecanoate	0.3	MS
1870	Hexanoic acid	0.7	RRI, MS
1853	Ethyl dodecanoate	0.2	MS
1868	(*E*)-Geranyl acetone	0.2	MS
1882	1-Isobutyl-4-isopropyl-3-isopropyl-2,2-Dimethyl succinate	1.1	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; Czerniewicz et al., 2016). A study conducted by Mitchell and his coworkers, found that many plant flavonoids such as quercetin, chrysin, apigenin, kaempferol and morin, can inhibit, in a dose-dependent manner, the cytochrome P-450 dependent ecdysone 20-monooxygenase activity associated with adult female *Ae. aegypti* which results in direct cellular toxicity (Mitchell et al., 1993).

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, H. 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.jsps.2019.07.001.

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