### Activity of Plant Extracts/Essential Oils Against Three Plant Pathogenic Fungi and Mosquito Larvae: GC/MS Analysis of Bioactive Compounds

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Certain natural products extracted from different parts of medicinal and aromatic plants were examined for their antifungal activity against three plant pathogenic fungi, Fusarium oxysporum, Rhizoctonia solani, and Alternaria solani, and insecticidal activity against mosquito larvae (Culex pipiens). Acetone extract of Tectona grandis showed the highest antifungal activity against R. solani and A. solani with EC<sub>50</sub> values of 118 and 294 µg/mL, respectively. The highest larvicidal activity was displayed by the essential oils of Ocimum basilicum and Eucalyptus gomphocephala with LC<sub>50</sub> value of 22, and 30 mg/L, respectively. By gas chromatographymass spectrometry (GC/MS) analysis 3-allylguaiacol (65.8%) and eugenol acetate (46.6%) were the main compounds in Syzygium aromaticum methanolic extract and essential oil, respectively. The main compound in T. grandis acetone extract was cyclohexylpentyl oxalate (8.7%); its water extract contained (E)-4,4-dimethyl-2-pentene (51.1%); E. gomphocephala branch oil contained p-cymene (28.8%); Euphorbia paralias leaf extract contained 1 $\beta$ H-romneine (26.3%); the seed extract contained  $\alpha$ -linolenic acid, TMS (15.2%); Punica granatum extract contained furfural (32.1%); and O. basilicum essential oil contained estragole (65.9%). Thus, extracts from the tested plants can be used as natural biofungicides to manage diseases caused by F. oxysporum, R. solani, and A. solani. Additionally, these extracts show potential la rvicide activities against mosquito larvae.

Keywords: Antifungal activity; Larvicidal activity; Pathogenic fungi Natural extract; Essential oils

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### INTRODUCTION

In crop plants, fungi cause more economic damage than any other group of microorganisms, with annual losses estimated at more than \$200 billion (Horbach *et al.* 2011). Chemical control of phytopathogenic fungi efficiently reduces the negative consequences resulting from these organisms; however, field application of these chemicals is not desirable. Extensive and improper use of synthetic fungicides can lead to serious consequences on the environment and on the health of humans and animals. Moreover, the development of fungicide-resistance in phytopathogenic fungi has been

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reported (Hahn 2014). Therefore, the search for novel antifungal agents is needed. Plantderived products may be an alternative approach against phytopathogenic fungi (Amadi *et al.* 2010; Badawy and Abdelgaleil 2014).

*p*-cymene and crypton were found to be the main compounds of leaf essential oil (EO) from *Eucalyptus gomphocephala* growing in Egypt (Salem *et al.* 2015). 1,8-cineole, pinene, viridiflorol, terpineol, aromadendrene and *trans*-pinocarveol are the abundant compounds in the leaf EO of *E. procera* cultivated in central Iran (Rahimi-Nasrabadi *et al.* 2012), while 1,8-cineole, cryptone, 4-allyloxyimino-2-carene, and 4-terpineol were found in leaf EO from *E. largiflorens* from the same area (Rahimi-Nasrabadi *et al.* 2013) with high antimicrobial activities. Also, 1,8-cineole,  $\alpha$ -pinene, and  $\alpha$ -terpineol are the main constituents of *E. oleosa* leaf EO with high antibacterial activity (Rahimi-Nasrabadi *et al.* 2013).

Different bioactive compounds of naphtoquinone, anthraquinone, 2-methyl anthraquinone (techtoquinone), lapachol, and deoxylapachol have been isolated from teak wood extracts (Windeisen *et al.* 2003; Thulasidas and Bhat 2007). Several studies have shown antifungal efficacy of plant extracts and EOs against plant and human pathogenic fungi (Mahboubi and Bidgoli 2010; Salem *et al.* 2016a,b; Sales *et al.* 2016). For example, the antifungal activity of natural extracts and EOs derived from *Tectona grandis*, *Syzygium aromaticum*, and *Eucalyptus gomphocephala* were tested against *Fusarium moniliforme*, *Fusarium oxysporum*, *Aspergillus* sp., *Mucor* sp., and *Arthrinium phaeospermum*. These compounds inhibit both the mycelial growth and sporulation of fungi (Astiti and Suprapta 2012). Moreover, the EO obtained from *E. camaldulensis* completely inhibits the mycelial growth of the five isolates of *Fusarium* spp. at a concentration range between 7 and 8  $\mu$ L/mL after five days of incubation (Gakuubi *et al.* 2017).

Mosquitoes are insects that cause great concern for public health, as they are vectors for numerous tropical and subtropical diseases. They are an important threat for over two billion people in the world (Odalo *et al.* 2005). The intensive use of conventional insecticides to control mosquitoes is causing many problems such as environmental pollution, toxic hazards to mammals and non-target organisms, and the development of insecticide resistance (Sutthanont *et al.* 2010). These complications have become the driving force for an expeditious search for alternatives: compounds offering protection against mosquitoes suitable for both public health and the environment. Among the present alternative approaches aimed at reducing mosquito populations, the use of biopesticides based on natural plant products is now one of the most promising (Rajamma *et al.* 2011).

The present study investigated the antifungal activity of natural extracts and EOs obtained from six aromatic plants, *T. grandis*, *E. gomphocephala*, *S. aromaticum*, *Euphorbia paralias*, *Ocimum basilicum*, and *Punica granatum* against three plant pathogenic fungi, *F. oxysporum*, *Rhizoctonia solani*, and *Alternaria solani*. These extracts also were tested for their efficacy against mosquitoes. Lastly, the chemical constituents of the extracts/EOs were identified using GC/MS analysis.

### EXPERIMENTAL

### **Plant Materials and Their Solvent Extraction**

The plant materials used in this study are presented in Table 1. The plant materials used for solvent extraction were previously air-dried under room temperature for one week and then ground to fine particles (40- to 60-mesh). Approximately 100 g of ground material

was soaked in solvent (200 mL) for 3 days, filtered, and concentrated to dryness using a rotary evaporator. The essential oils (EOs) were extracted by hydrodistillation using a Clevenger-type apparatus, where 100 g of green materials was hydrodistillated for 3 h. The collected oil was stored at 4 °C prior to analysis (Salem *et al.* 2013).

Plant	Part used	Extract/EO
Tectona grandis	Air dried wood	Acetone extract
T. grandis	Air dried wood	Water extract
Eucalyptus gomphocephala	Green branches	EO
Syzygium aromaticum	Air dried flower buds	Methanolic extract
S. aromaticum	Air dried flower buds	EO
Euphorbia paralias	Air dried leaves	Acetone extract
E. paralias	Air dried seeds	Acetone extract
Ocimum basilicum	Green leaves	EO
Punica granatum	Air dried peels	Acetone extract

Table 1	Plant	Materials	and	Their	Parts	Used f	or the	Extractions
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### Antifungal Assay

The antifungal activities of the plant extracts and/or EOs obtained from T. grandis, E. gomphocephala, S. aromaticum, E. paralias, O. basilicum, and P. granatum were evaluated on the mycelial growth of plant pathogenic fungi (Fusarium oxysporum, Rhizoctonia solani, and Alternaria solani) using the radial growth technique method (Zambonelli et al. 1996). The fungi were obtained from the Microbiological Laboratory, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University. The plant extracts dissolved in dimethyl sulfoxide (DMSO) were added to warm PDA medium (40 to 45 °C) at different concentrations, ranging from 50 to 1000 mg/L, before immediately pouring into 9-cm Petri dishes. Each concentration was tested in triplicate. Parallel controls contained PDA mixed only with DMSO. Mycelial discs (0.5 cm in diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA dishes, were transferred to the center of PDA dishes supplemented with either plant extracts or only DMSO. Inoculated dishes were incubated at 25 °C in the dark. The colony growth diameter was measured when the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated using the following equation (Pandey et al. 1982),

Mycelial growth inhibition (%) =  $[(DC-DT)/DC] \times 100$  (1)

where DC and DT are average diameters of the fungal colony of control and treatment, respectively. The concentrations of plant extract or oil that inhibited the fungi mycelial growth by 50% ( $EC_{50}$ ) with an equivalent confidence limit of 95% were estimated by the probit analysis method (Finney 1971).

### Mosquitoes

A laboratory colony of *Culex pipiens* L. (Diptera: Culicidae) obtained from the College of Food and Agriculture Sciences, King Saud University was used. Mosquitoes were maintained at  $25 \pm 2$  °C,  $60 \pm 5\%$  RH, and a 12-h photoperiod. Adult mosquitoes were provided with a 10% sucrose solution as food. A blood meal was introduced twice per week to feed the females. Larvae were reared in Cl-free water and fed daily with rabbit feed.

### Larvicidal Bioassay

The larvicidal activity of the extracts or oils was evaluated on the early fourth instar of *C. pipiens* larvae using the standard method described by the World Health Organization (WHO 1981), with slight modification. Stock solutions of the extracts or oils were prepared in ethanol, and Tween-80 (10 ppm) was used as an emulsifier. A series of six concentrations of each extract or EO were prepared in dechlorinated tap water. Twenty mosquito larvae were put into 200 mL cups containing 100 mL of test solution. The control treatment was prepared with water containing the same concentration of ethanol and Tween-80. Three replicates were used for each concentration. Larval mortalities were recorded after 24 h of exposure. Larvae were considered dead when they did not respond to probing with a needle. Mortality data were subjected to probit analysis to calculate the median lethal concentration values (LC<sub>50</sub>) of the extracts or oils (Finney 1971). Abbot's formula (Abbott 1925) was used when necessary to correct percentage mortality in the treatments.

### GC/MS Analysis of Extracts/Essential Oils

Samples of T. grandis (wood acetone and water extracts) and E. paralias (leaf and seed extracts) were analyzed using a derivatization process. For the derivatization process, 10 µL from the samples was added to the bottom of a 2 mL tube and dried with a gentle stream of pure nitrogen gas (99.999%). Immediately, 50 µL of N,O-Bis (trimethylsilyl, TMS) trifluoroacetamide (BSTFA) was added and vortexed. The reaction was incubated for 3 h at 70 °C. The products were dried by nitrogen blowing. After drying, hexane was added for dilution and injection into the GC-MS instrument. The injection volume was 2  $\mu$ L in splitless mode. The analysis of the alteration products and external standards was carried out by GC-MS on a Hewlett- Packard 6890 GC coupled to a 5973 Mass Selective Detector using a DB-5 (J and W Scientific, Agilent, Palo Alto, CA, USA) fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness) and helium as a carrier gas. The GC was temperature programmed from 65 °C (2 min initial time) to 300 °C at 6 °C/min (isothermal for 20 min final time). The MS was operated in the electron impact mode at 70 eV ion source energy. Data were acquired and processed with a Hewlett-Packard Chemstation and compounds were identified by comparison of mass spectra with those of authentic standards, literature and library data, and characterized mixtures. Unknown compounds were characterized by interpretation of the fragmentation pattern of their mass spectra.

The chemical constituents of *S. aromaticum* (methanolic extract and EO), *E. gomphocephala* (branch oil), *P. granatum* (extract), and *O. basilicum* (essential oil) were analyzed for their chemical compositions using the previous published methods (Salem *et al.* 2016a,b, 2019). Identification of the chemical composition of extracts/EOs was done based on MS library searches (NIST and Wiley), as well as by comparing with the MS literature data (NIST 11. 2011; Oberacher 2011).

### **RESULTS AND DISCUSSION**

### Antifungal Activity

The antifungal activities of plant extracts/EO obtained from different plants against *R. solani*, *A. solani*, and *F. oxysporum* in terms of radial growth inhibition are summarized in Tables 2, 3 and 4, respectively. The acetone extract of *T. grandis* showed the highest antifungal activity against *R. solani* and *A. solani* with EC<sub>50</sub> values of 118 and 294  $\mu$ g/mL,

respectively. However, the acetone extract of *T. grandis* showed the lowest antifungal activity against *F. oxysporum*. *S. aromaticum* extract exhibited moderate antifungal activities against the three tested fungi. Generally, *F. oxysporum* was less sensitive to *T. grandis* and *E. gomphocephala* extracts compared with the other two fungi. *E. gomphocephala* branch EO showed weaker antifungal activity than *T. grandis* and *S. aromaticum* extracts. Extracts of *E. paralias* (leaves), *E. paralias* (fruits), *O. basilicum*, *T. grandis* (obtained from wood), and *P. granatum* exhibited no antifungal activity against the three tested fungi.

					<b>a</b>	1
Extract/EO	$EC_{50}$	95% Confid	dence limits	Slope ±SE	Chi <sup>2</sup>	r
	(µg/mL)	Lower	Upper			
T. grandis wood acetone	118.39	32.57	206.41	0.69 ± 0.18	0.17	0.99
extract						
<i>E. gomphocephala</i> branch	446.17	355.40	518.16	$3.36 \pm 0.62$	1.6	0.98
EO						
S. aromaticum methanolic	491.22	467.95	520.37	8.53 ± 1.14	0.57	0.99
flower buds' extract						
S. aromaticum flower buds'	< 1000					
EO						
E. paralias acetone leaf	< 1000					
extract						
E. paralias seeds acetone	< 1000					
extract						
O. basilicum leaf EO	< 1000					
T. grandis wood water	< 1000					
extract						
P. granatum peels acetone	< 1000					
extract						

**Table 2.** In vitro Antifungal Activity of Plant Extracts/Oils against R. solani using the Mycelial Growth Inhibition Method

Table 3. In vitro Antifungal Activity of Plant Extracts/Oils against A. solani using
the Mycelial Growth Inhibition Method

Extract/EO	EC₅₀ (µg/mL)	95% Confidence limits		Slope ±SE	Chi <sup>2</sup>	r
		Lower	Upper			
<i>T. grandis</i> wood acetone extract	294.12	168.74	457.58	0.78 ± 0.18	0.22	0.99
<i>E. gomphocephala</i> branch EO	991.31	847.49	1262.4	2.9 ± 0.50	0.07	0.99
S. aromaticum methanolic flower buds' extract	635.57	561.11	713.25	3.7 ± 0.52	0.02	0.99
S. aromaticum flower buds' EO	< 1000					
<i>E. paralias</i> acetone leaf extract	< 1000					
<i>E. paralias</i> acetone seeds extract	< 1000					
O. basilicum leaf EO	< 1000					
T. grandis wood water extract	< 1000					
P. granatum peels acetone extract	< 1000					

# **Table 4.** In vitro Antifungal Activity of Plant Extracts/Oils against F. oxysporum using the Mycelial Growth Inhibition Method

Extract/EO	EC <sub>50</sub>	95% Confidence		Slope ±SE	Chi <sup>2</sup>	r
	(µg/mL)	limi	ts			
		Lower	Upper			
T. grandis wood acetone	965.31	641.18	2011.2	0.93 ± 0.19	0.18	0.99
extract						
<i>E. gomphocephala</i> branch EO	922.04	690.71	1416.2	1.36 ± 0.21	2.95	0.97
S. aromaticum flower buds'	439.17	416.77	460.02	9.1 ± 1.65	0.04	0.98
extract						
S. aromaticum flower buds'	< 1000					
EO						
E. paralias acetone leaf	< 1000					
extract						
E. paralias acetone seeds	< 1000					
extract						
O. basilicum leaf EO	< 1000					
T. grandis wood water extract	< 1000					
P. granatum peels acetone	< 1000					
extract						

### Larvicidal Activity

The larvicidal activity results of the test plant extracts/EO against *C. pipiens* L. are summarized in Table 5.

Plant	Extract/EO LC <sub>50</sub> (mg/L				Slope ± SE	Chi <sup>2</sup>	r
		(119/ )	Lower	Upper	0L		
<i>T. grandi</i> s wood	Acetone extract	251.39	138.65	329.57	3.50± 1.08	1.22	0.95
<i>T. grandi</i> s wood	Water extract	696.74	639.36	755.41	8.13 ± 1.26	0.88	0.99
<i>E. gomphocephala</i> branches	EO	30.07	29.04	31.15	9.63 ± 0.70	2.48	0.99
S. aromaticum flower buds	Methanolic extract	58.73	48.36	70.47	1.67 ± 0.19	2.50	0.98
S. aromaticum flower buds	EO	128.92	116.46	140.07	4.16 ± 0.63	0.42	0.99
<i>E. paralias</i> leaves	Acetone Extract	786.44	713.09	880.48	6.02 ± 1.07	2.07	0.98
<i>E. paralias</i> seeds	Acetone Extract	295.45	182.41	387.39	3.38± 1.03	2.11	0.92
O. basilicum leaves	EO	22.00	20.59	23.41	6.49 ± 0.59	1.32	0.99
P. granatum peels	Acetone Extract	955.35	807.33	1136.1	6.18± 1.78	1.78	0.95

**Table 5.** Larvicidal Activity of Plant Extracts/Oils against Culex pipiens L.

The EOs of basil *O. basilicum* and *E. gomphocephala* displayed the highest larvicidal activity with LC<sub>50</sub> values of 22 and 30.1 mg/L, respectively. Moreover, the methanolic extract of *S. aromaticum* showed high larvicidal activity against *C. pipiens* (LC<sub>50</sub> = 58.7 mg/L), while the essential oil of the same plant showed relatively lower

larvicidal activity (LC<sub>50</sub> = 129 mg/L). The acetone extract of *T. grandis* was more toxic to the larvae than the water extract of the same plant with LC<sub>50</sub> values of 251 and 697, respectively. The fruit extract of *E. paralias* exhibited more larvicidal activity to the larvae (LC<sub>50</sub> = 295 mg/L) than the leaves extract (LC<sub>50</sub> = 786 mg/L). Low larvicidal activity was observed for *P. granatum* extract with an LC<sub>50</sub> value of 955 mg/L.

### **Chemical Composition of the Extracts/Essential Oils**

3-Allylguaiacol (65.8%) and eugenol acetate (23.4%) were the main compounds in *S. aromaticum* methanolic extract (Table 6), while eugenol acetate (46.6%), isoeugenol (21.5%), *trans*-caryophyllene (15.8%) and  $\alpha$ -humulene (9.0%) were the main compounds in the EO (Table 7).

Compound name	RT <sup>a</sup> (min)	Area %	SIÞ	RSI⁰
3-Allylguaiacol	13.98	65.79	884	922
trans-Caryophyllene	15.16	6.5	687	846
1,4,7-Cycloundecatriene	15.77	3.25	708	904
Eugenol acetate	16.69	23.36	701	874
Octadecanoic acid	23.25	0.68	664	751
trans-9-octadecenoic acid	24.86	0.42	856	868

### Table 6. Chemical Constituents of S. aromaticum Methanolic Extract

<sup>a</sup>RT, Retention Time (min.).

<sup>b</sup>SI, Standard Index.

<sup>c</sup>RSI, Reverse Standard index.

Compound Name	RT <sup>a</sup> (min)	Area %	SIb	RSI⁰
2-Heptyl acetate	8.99	0.43	862	933
2-Nonanone	9.84	0.24	858	924
linalool	10.01	0.06	813	866
Benzyl acetate	10.97	0.12	858	922
3-Cyclohexen-1-ol	11.32	0.15	810	905
Cryptone	11.41	0.12	809	860
Chavicol	12.34	0.12	895	936
Eugenol acetate	14.12	46.62	894	911
α-Copaene	14.31	0.78	823	937
trans-Caryophyllene	15.32	15.81	823	929
α-Humulene	15.87	8.96	875	918
α-Amorphene	16.10	0.26	866	903
Germacrene-D	16.26	0.29	875	918
Valencene 1	16.41	0.05	821	924
<i>E,E</i> -α-Farnesene	16.48	0.71	816	923
Isoeugenol	16.76	21.46	815	900
α-Cadinene	16.83	0.58	913	951
Benzeneacetic acid	17.27	0.06	909	941
[2,2-Dimethyl-4-(3-methylbut-2-enyl)-6-	17.94	1.24	896	925
methylidene-cyclohexyl]methanol				
Caryophylladienol I	18.71	0.12	885	927

#### Table 7. Chemical Constituents of S. aromaticum Essential Oil

<sup>a</sup>RT, Retention Time (min.).

<sup>b</sup>SI, Standard Index.

<sup>c</sup>RSI, Reverse Standard index.

Compound Name	RT <sup>a</sup> (min)	Area (%)	SI♭	RSI⁰
Vinylather	5.84	2.84	773	790
2,2-Dimethyl-3-propyloxirane	6.29	1.05	714	745
Cyclohexylpentyl oxalate	6.78	8.66	735	772
1-Methyl-2,3-dihydro-1H-pyrrole	7.87	0.67	734	771
3-[(2-methoxyethoxy)methoxy]-2- methyl-tricyclo[5.2.2.0(2,6)]undec-8- en-11-one	7.95	0.97	678	804
Glycerol, TMS derivative	14.34	0.84	747	787
Eudesma-3,7(11)-diene	21.84	0.62	693	823
2-Isopropyl-5-methyl-9-methylene- bicyclo[4.4.0]dec-1-ene	22.11	0.58	734	777
α-D-Arabinofuranose, 4TMS derivative	22.31	0.48	834	863
Phthalic acid, 2TMS derivative	22.89	1.13	836	867
Vanillic acid, 2TMS derivative	24.13	0.80	838	865
Protocatechoic acid, 3TMS derivative	25.17	0.75	776	814
2-Thioxo- <i>cis</i> -perhydro-3,1- benzoxazine	25.57	0.39	776	813
Methyl-α-D-glucofuranoside, 4TMS derivative	25.86	0.52	765	805
2-Thiobarbituric acid, TMS	26.20	6.58	825	865
3-Hydroxy-3-methylglutaric acid, tri- TMS	26.27	3.21	752	798
D-Galactose, 5TMS derivative	26.74	0.48	778	820
Palmitic acid, TMS derivative	28.65	1.08	902	934
Techtoquinone	29.72	8.46	903	948
2-Methylbenzenethiol, TMS derivative	30.63	2.34	890	933
Stearic acid, TMS derivative	31.59	0.53	888	924
3-(1,3-Benzodioxol-5-yl)-5-hydroxy-4- nitrocyclohexanone	34.93	7.23	886	910
1,2-bis(TMS-oxy)-cyclooctene	35.36	8.58	913	915
Cyclohexyl isobutyl phthalate	35.73	0.71	924	945
trans-Crotyl alcohol, TMS derivative	35.97	2.06	913	925
5-(4-methylphenoxy)-6-(4-nitrophenyl)- 4-phenyl-1H-pyrimidin-2-one	38.70	0.42	834	819
Squalene	39.12	0.47	799	805

<sup>b</sup>SI, Standard Index.

<sup>c</sup>RSI, Reverse Standard index.

Cyclohexylpentyl oxalate (8.7%), 1,2-bis(TMS-oxy)-cyclooctene (8.6%), techtoquinone (8.5%), 3-(1,3-benzodioxol-5-yl)-5-hydroxy-4-nitrocyclohexanone (7.2%), and 2-thiobarbituric acid, TMS (6.6%) were the main compounds in *Tectona grandis* wood acetone extract (Table 8). The main compounds isolated from water extract of *T. grandis* were (*E*)-4,4-dimethyl-2-pentene (51.14%), vinylather (15.7%), and divinyl carbinol (7.6%) (Table 9).

Compound Name	RT <sup>a</sup>	Area (%)	SIÞ	RSI⁰
2-Methyl-2-(1-methylethyl)-oxirane	3.61	2.38	741	742
3-Heptene	4.36	0.97	730	761
(E)-3-Heptene	4.45	0.81	776	778
Hexane	5.49	0.28	741	743
Vinylather	5.84	15.73	740	742
2,2-Dimethyl-3-propyloxirane	6.29	2.20	789	796
2-Methyl-hexane	6.49	5.11	788	796
(E)-4,4-Dimethyl-2-pentene	6.78	51.14	748	748
Divinyl carbinol	7.36	7.63	727	727
Morpholine, 4-α-D-Mannopyranose, 5TMS derivative	22.34	0.27	762	801
Vanillic acid, 2TMS derivative	24.13	0.42	757	797
Cyanuric acid, 3TMS derivative	26.22	2.08	752	793
L-Rhamnose, 4TMS derivative	26.75	0.27	904	937
(E)-3-Stilbenol	30.64	2.18	901	908
1-Methyl-2-pentamethyldisilanyloxycyclohexane	35.36	5.92	899	907
1-Ethyl-2-pentamethyldisilanyloxycyclohexane	35.97	2.26	881	889
S-(+)-Reticuline	36.45	0.26	812	824
4-(3-chlorophenyl)-2-methylsulfonyl-thiophene-3- carbonitrile	36.64	0.36	785	846
DT Detention Time (min )				

Table 9. Chemical Constituents of	T. grandis Wood Water Extract
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<sup>b</sup>SI, Standard Index.

<sup>c</sup>RSI, Reverse Standard index.

Table 10 presents the chemical compounds of *Eucalyptus gomphocephala* branch EO where the main compounds were *p*-cymene (28.8%), (+)spathulenol (13.0%),  $\Delta$ 3-carene (7.5%), 2-methyl-3-phenylpropanal (3.9%), and 1,8-cineole (3%).

Table 11 presents the chemical constituents of *E. paralias* leaf extract where the main compounds were 1 $\beta$ H-romneine (26.3%),  $\beta$ -amyrin, TMS derivative (8.3%), 8-bromo-neoisolongifolene (10.0%), 3,5,6,7,8,8 $\alpha$ -hexahydro-4,8 $\alpha$ -dimethyl-6-(1-methyl-ethenyl)-2(1H)-naphthalenone (5.7%) and 24-methylene-cycloartenol, acetylated (4.7%).

			I	
Compound Name	RTª	Area (%)	SI⁵	RSI⁰
∆3-Carene	7.34	7.46	781	785
Sabinene	7.98	0.98	807	811
β-Pinene	8.09	0.61	796	807
β-Myrcene	8.23	0.47	786	835
L-Phellandrene	8.54	0.56	793	797
α-Terpinolene	8.72	0.15	781	807
<i>p</i> -Cymene	8.89	28.82	780	789
1,8-Cineole	9.02	3	821	837
cis-Linalool oxide	9.59	0.26	807	811
trans-Linaloloxide	9.84	0.25	802	815
Linalool	10.04	1.25	787	798
trans-Caryophyllene	10.35	0.17	832	835
2-Cyclohexen-1-ol	10.48	1.61	823	839
trans-Pinene hydrate	10.78	1.87	806	810
Pinocarvone	11.06	0.26	794	806
Cryptone	11.55	17	814	832

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cis-Carveol	11.90	0.56	804	809
2-Methyl-3-phenylpropanal	12.28	3.91	799	812
Cyclohexadienemethanol	12.67	0.57	779	808
Phellandral	12.78	2.06	819	839
Cuminol	12.94	1	805	812
Carvacrol	13.02	2.97	802	817
Orivone	13.21	0.48	786	816
trans-Caryophyllene	15.12	0.32	824	845
Diosphenol I	15.57	0.16	813	819
Aromadendrene 2	15.88	0.2	806	821
(-)-Caryophyllene oxide	17.42	0.43	822	842
(+) Spathulenol	18.01	13.05	816	822
Humulene oxide	18.37	0.98	789	843
Isospathulenol	18.65	0.44	789	843
Ledene	19.23	0.11	820	841
3,5,14,19-Card-20(22)-enolide	19.86	0.17	814	821
γ-Cadinen-15-al	20.00	0.19	804	820

<sup>a</sup>RT, Retention Time (min.).

<sup>b</sup>SI, Standard Index.

<sup>c</sup>RSI, Reverse Standard index.

### Table 11. Chemical Constituents of E. paralias Leaf Extract

	RT <sup>a</sup>	Area	SIb	RSI⁰
Compound Name		(%)	51	NOI
2,5-dimethyl-1,5-Heptadiene-3,4-diol	5.84	0.63	789	843
n-Decanol tetrahydropyran ether	6.49	0.25	826	850
(E)-4,4-Dimethyl-2-pentene	6.78	2.18	810	817
N-Methylpyrrolin	7.87	0.75	786	822
Methyl cis-3-chloropropenoate	7.95	0.77	777	812
Vinylcyclohexyl ether	8.97	0.32	828	850
Glycerol, TMS derivative	14.34	0.51	808	814
Erythritol (4TMS) derivative	19.59	0.20	795	803
$\beta$ -Arabinose, TMS derivative	22.31	0.19	786	820
Shikimic acid, 4TMS derivative	25.14	1.03	824	846
1,2,4,5-Cyclohexanetetrol, 4TMS derivative	25.24	0.89	803	810
Tagatose, TMS derivative	25.38	0.84	783	818
Myristic acid, TMS derivative	25.45	0.33	767	802
Methyl- $\alpha$ -D-ribofuranoside, 3TMS derivative	25.87	0.20	820	841
α-D-Glucopyranose, 5-TMS	26.71	2.98	809	809
Gallic acid, 4TMS	27.58	3.27	788	801
Palmitic acid, TMS	28.65	0.96	824	846
Oleic Acid, (Z)-, TMS derivative	31.24	0.17	799	806
Eicosane	37.53	0.20	779	815
Dihydropseudoionone	40.48	0.38	817	818
9,9,10-Trimethyl-9,10-dihydroanthracene	44.01	0.18	792	806
4H-1-Benzopyran-4-one, 8-β-D-glucopyranosyl-5,7-dimethoxy-2-(4-	44.54	0.29	845	862
methoxyphenyl)-				
Lanosterol, TMS derivative	45.45	1.37	778	789
$\beta$ -Sitosterol, TMS derivative	45.55	0.48	755	759
3,5,6,7,8,8a-Hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H)- naphthalenone	45.79	5.66	740	750
$\beta$ -Amyrin, TMS derivative	46.37	8.34	819	827

1βH-Romneine	46.53	26.28	793	825
Moretenol	47.29	2.59	793	823
24-methylene-cycloartenol, acetylated	47.62	4.73	808	832
7-allyloxy-4-methylcoumarin	48.40	0.87	796	813
8-bromo-Neoisolongifolene	49.83	10.05	810	836
5-Isopropylidene-6-methyldeca-3,6,9-trien-2-one	50.14	1.43	800	808
3-Hydroxy-4-phenyl-5-isopropyl-1,2,4-triazole	50.84	2.27	793	811

<sup>b</sup>SI, Standard Index.

<sup>c</sup>RSI, Reverse Standard index.

In *E. paralias* seed extract (Table 12) the main compounds were  $\alpha$ -linolenic acid, TMS (15.2%), 2-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-*N*-(4-fluorophenyl) acetamide (8.3%), 8-bromo-neoisolongifolene (7.5%), gallic acid, 4TMS (5.7%),  $\alpha$ -*D*-glucopyranose, 5TMS derivative (5.4%), 24-methylene-cycloartenol, acetylated (5.3%), palmitic acid, TMS (4.0%), cyclohexylpentyl oxalate (3.3%), shikimic acid (4TMS) (3.2%) and  $\beta$ -amyrin-TMS (3.1%).

Table 12. Chemical Constituents of E. paralias Seeds Extract

Compound Name	RTª (min.)	Area (%)	SIÞ	RSI⁰
Allyl <i>n</i> -octyl ether	3.61	0.47	775	813
Vinylather	5.85	1.08	812	836
<i>n</i> -Decanol tetrahydropyran ether	6.49	0.35	800	817
Cyclohexylpentyl oxalate	6.78	3.32	798	806
Glycerol, TMS derivative	14.34	0.94	778	815
Theitol, 4TMS	19.43	0.23	838	845
Erythritol (4TMS)	19.58	0.81	810	837
1-Deoxypentitol, 4TMS derivative	20.24	0.22	806	815
D-Arabinose, 4TMS	22.31	0.34	805	814
L-Fucitol TMS	24.55	0.29	811	821
Shikimic acid (4TMS)	25.14	3.24	808	836
1,2,4,5-Cyclohexanetetrol, 4TMS derivative	25.24	2.30	792	812
Tagatose, TMS derivative	25.38	2.93	792	811
L-Sorbopyranose, (1S,2R,3S)-, 5TMS derivative	25.46	0.80	810	841
Mannonic acid, 1,5-lactone, TMS	25.86	0.22	808	820
β-Gulofuranose, TMS	25.92	0.40	800	823
α-D-Glucopyranose, 5TMS derivative	26.71	5.44	779	796
Gallic acid, 4TMS	27.58	5.66	821	823
Myo-Inositol (6TMS)	27.80	0.30	804	823
Palmitic acid, TMS	28.65	4.05	797	809
Esculetin, 2TMS derivative	29.66	0.45	840	847
Linoleic acid, TMS	31.15	2.01	806	811
$\alpha$ -Linolenic acid, TMS	31.26	15.18	807	840
Stearic acid, TMS derivative	31.59	0.74	798	812
Galactose 5TMS	34.14	0.30	774	822
2,3,4,5-Tetraphenylpyrrole	36.36	0.38	767	786
Monoolein TMS	38.42	2.12	810	840
Monostearin TMS	38.70	0.41	809	843

Propargyl methacrylate	40.48	0.77	796	811
2-Ethylacridine	40.81	0.29	792	808
Deoxyglucose, 4TMS derivative	41.00	0.25	779	793
N,N-Dimethyl-4-nitroso-3-aniline TMS	44.01	0.28	774	788
2-(1-Adamantyl)ethyl phenylacetate	44.85	1.43	809	845
(3β,5α)-Cholesta-8,24-dien-3-ol, TMS derivative	45.45	2.15	787	804
β-Sitosterol TMS	45.54	1.59	779	793
3,5,6,7,8,8α-Hexahydro-4,8α-dimethyl-6-(1-	45.78	2.19	795	811
methylethenyl)-2(1H)-naphthalenone				
$\beta$ -Amyrin, TMS derivative	46.36	3.11	779	792
2-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-	46.49	8.28	793	823
yl)-N-(4-fluorophenyl)acetamide				
Moretenol	47.28	2.65	788	806
24-methylene-cycloartenol, acetylated	47.62	5.34	784	797
9-tert-Butyl-10-ethyl-9,10-dihydroanthracene	49.45	1.65	768	770
8-bromo-Neoisolongifolene	49.81	7.51	815	835
8S,14-Cedran-diol	50.12	1.28	808	813

<sup>b</sup>SI, Standard Index.

°RSI, Reverse Standard index.

Table 13 shows chemical composition of peels of *P. granatum* extract: furfural (32.1%), orotyl amide (11.0%), D-allose (9.2%), *n*-capric acid (6.4%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (6.1%), 2,5-dimethylfurane (4.6%), ethyl  $\alpha$ -D-gluco-pyranoside (4.3%), *cis*-isoeugenol (3.9%), and 1,6-anhydro- $\beta$ -D-glucofuranose (3.7%).

Table 13. Chemical Constituents of P. granatum Extract

Compound name	RT <sup>a</sup>	Area %	SI⁵	RSI⁰
2,5-Dimethylfurane	5.59	4.56	803	818
Orotyl amide	7.41	10.96	787	799
2-Nonanol	8.43	3.32	777	801
2,3-Dihydroxy-propanal	9.14	1.94	775	803
Bicyclo[2.2.1]heptane	9.74	2.44	797	810
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-	10.75	6.07	790	837
one				
Heptanal	11.54	0.77	775	829
Furfural	11.96	32.09	770	820
<i>cis</i> -Isoeugenol	13.78	3.87	762	885
Larixinic acid	14.34	1.26	810	830
Semiphorone	14.72	0.7	765	890
(+)-Endo-6-methyl-2-methylene-	15.12	0.31	755	770
Cytidine	15.45	1.48	749	776
D-Allose	16.36	9.15	824	835
1,6-Anhydro-β-d-glucofuranose	18.01	3.73	784	899
Ethyl α-D-glucopyranoside	18.38	4.34	783	805
cis-tetrahydroionol	19.37	1.18	783	795
n-Capric acid	22.44	6.42	791	821
Palmitic acid	22.73	1.32	786	806

<sup>a</sup>RT, Retention Time (min.).

<sup>b</sup>SI, Standard Index.

°RSI, Reverse Standard index

Table 14 presents the chemical composition of *O. basilicum* essential oil where the main compounds were estragole (65.9%), eucalyptol (5.1%), linalool (4.9%), *trans*-4-methoxycinnamaldehyde (3.8%), and fenchyl acetate (2.2%).

Name	RTª	Area %	SIÞ	RSI⁰
α-Pinene	6.01	0.84	782	817
β-Myrcene	7.82	0.82	780	826
Eucalyptol	9.08	5.10	802	838
3-Carene	9.73	0.34	772	798
L-Fenchone	11.04	2.46	791	826
Linalool	11.55	4.90	784	808
(+)-Fenchol	11.93	0.24	775	800
Octen-1-ol acetate	12.02	0.41	763	852
DL-Camphor	13.01	2.93	796	813
(-)-Terpinen-4-ol	14.23	0.21	795	823
Estragole	15.29	65.93	792	820
Acetic acid, octyl ester	15.63	0.43	792	815
Fenchyl acetate	15.88	2.23	795	810
<i>p</i> -Anisaldehyde	16.92	1.64	775	812
Bornyl acetate	18.07	1.13	772	815
<i>p</i> -Propyl anisole	18.50	0.18	807	818
trans-Benzylideneacetone	18.65	0.26	781	813
exo-2-Hydroxycineole acetate	19.98	0.22	778	802
Methyl eugenol	22.11	0.69	781	815
α-Bergamotene	23.11	2.11	781	804
<i>trans</i> -β-Farnesene	24.63	0.23	860	883
γ-Muurolene	25.52	0.55	855	884
trans-4-Methoxycinnamaldehyde	27.16	3.81	868	888
Spathulenol	27.41	0.30	853	876
Carotol	28.49	0.40	826	846
tauCadinol	29.25	2.02	833	853

Table 14	Chemical	Constituents	of	O. basilicum Essential Oil
	Chemical	Constituents	UI.	

<sup>a</sup>RT, Retention Time (min.).

<sup>b</sup>SI, Standard Index.

<sup>c</sup>RSI, Reverse Standard index

The results showed that the EOs extracted in acetone of *T. grandis*, *S. aromaticum*, and *E. gomphocephala* have remarkable antifungal activities against the three tested pathogenic fungi, *A. solani*, *F. oxysporum*, and *R. solani*. The acetone extract of *T. grandis* showed the highest antifungal activity against *R. solani* and *A. solani* with EC<sub>50</sub> values of 118 and 294  $\mu$ g/mL, respectively. However, *T. grandis* acetone extract showed lower antifungal activity against *F. oxysporum*. The other plant extracts from *S. aromaticum* and *E. gomphocephala* exhibited moderate antifungal effects against the three tested fungi. These results confirmed the antifungal activity of different plant extracts against three plant pathogenic fungi.

The major compounds (eugenol acetate, eugenol and caryophyllene (Nassar *et al.* 2007) of the EOs from *S. aromaticum* have been known to possess various antibacterial and antifungal properties (Fu *et al.* 2007; Singh 2018). Eugenol, eugenol acetate,

caryophellene, acetyle, eugenol, sesquiterpene ester, phenyl propanoid were the main compounds in the ethanolic extract from dried flower buds of *S. aromaticum* (Ghelardini *et al.* 2001; Miyazawa and Hisama 2003; Sumalatha *et al.* 2010). A 20% concentration of flower bud aqueous extract of *S. aromaticum* showed 100% inhibition of mycelial growth of *Aspergillus niger* (Avasthi *et al.* 2010). The antifungal effect of *S. aromaticum* was found on *Aspergillus* spp. and *Penicillium* spp. (Garg and Siddiqui 1992; Vazquez *et al.* 2001).

There was an increase in mycelial growth over time except for 50  $\mu$ L/20 mL of PDA, where no mycelial growth was detected. In addition, the present results agree with other studies, where essential oils of *S. aromaticum* were used against various common fungal pathogens of plants namely, *F. moniliforme*, *F. oxysporum*, *Aspergillus* sp., *Mucor* sp., *Trichophyton rubrum* and *Microsporum gypseum* (Pinto *et al.* 2009; Rana *et al.* 2011; Sharma *et al.* 2016).

Extracts from almost every part of *T. grandis* were composed of chemical compounds from different classes such as flavonoids, steroidal compounds, glycosides, quinones, and phenolic acids (Ohmura *et al.* 2000), with remarkable antifungal and antitermitic effects (Healey and Gara 2003; Thulasidas and Bhat 2007; Shalini and Rachana 2009; Florence *et al.* 2012; Guerrero-Vásquez *et al.* 2013). Lapachol is a naphthoquinone and lapachonone, found in *T. grandis* wood and bark (Goel *et al.* 1987; Sumthong *et al.* 2006). Bis (2-ethylhexyl) phthalate has been isolated from wood extracts of *T. grandis* and is a good repellent to termites (Alabi and Oyeku 2007). Heartwood extract from *T. grandis* reduces the weight loss caused by white rots (*Pleurotus squarrosulus* and *Lentinus subnudus*) in two hardwood species (*Triplochiton scleroxylon* and *Gmelina arborea*) (Adegeye *et al.* 2009).

Naphtoquinone, anthraquinone (Thulasidas and Bhat 2007), and 2-methyl anthraquinone (techtoquinone) (Windeisen *et al.* 2003) that inhibited the growth of of *Coniophora puteana*, a brown rot fungus (Haupt et al., 2003). Lapachol and deoxylapachol are naphtoquinone derivatives which also reported as biologically active compound (Windeisen *et al.* 2003). Plant extracts containing naphtoquinones (chromatic pigments) have been used for cancer and rheumatoid arthritis treatment (Babula *et al.* 2006). Lapachol is a natural quinone has been isolated from heartwood of Asian and South American Bignoniaceae (*Tabebuia, Taigu*, and *Tecoma*) (Steinert *et al.* 1995).

The sawdust of *T. grandis* contains the active components deoxylapachol and tectoquinnone, which inhibited the growth of *A. niger* (Neamatallah *et al.* 2005; Sumthong *et al.* 2006; Hussain *et al.* 2007). Furthermore, its leaf extract significantly suppressed the growth of *Arthrinium phaeospermum* (Astiti and Suprapta 2012). Leaf and bark extracts of *T. grandis* also have antifungal activity against *A. niger*, *Trichoderma viride*, and *A. flavus* (Lanka and Parimala 2017).

Previous studies have shown that *n*-hentriacontane, *n*-nonacosane, *n*-triacontane, *n*-dotriacontane, *n*-tritriacontane, and *n*-pentatriacontane, hexacosanol, octacosanol, cycloartenol, methylenecycloartenol,  $\beta$ -sitosterol, stigmasterol, campesterol, cholesterol, oleanolic, betulin and  $\beta$ -amyrin were isolated from extracts of *E. paralias* (Rizk *et al.* 1974; Shi *et al.* 2008; Noori *et al.* 2009) and have potential antimicrobial activities (Jassbi 2006; Shi *et al.* 2008; Noori *et al.* 2009).

Many previous studies reported that the EO of *Eucalyptus* sp. completely inhibited mycelial growth of plant pathogens. *p*-cymene (17.2%) and crypton (8.9%) were the main compounds in *E. gomphocephala* leaf essential oil (Salem *et al.* 2015). The EOs of extracts from different parts of *E. gomphocephala* have been reported to have potential

antimicrobial activities (Salem *et al.* 2015; Elansary *et al.* 2017). EOs of *Eucalyptus* sp. have been shown to have antibacterial and antifungal activities (Bendaoud *et al.* 2009; Bachheti *et al.* 2011). The antimicrobial/antifungal activities of essential oils are generally due to the presence of components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, *p*-cymene, eucamalol, limonene, and linalool (Nezhad *et al.* 2009).

Alternaria alternata, Stemphylium botryosum, and Fusarium spp. were significantly inhibited by the aqueous extracts of pomegranate peels (Glazer *et al.* 2012). Methanolic extracts of pomegranate showed inhibitory activity against *A. niger*, *Penicillium citrinum, Rhizopus oryzae*, and *Trichoderma reesei* (Dahham *et al.* 2010).

Phenolic compounds such as punicalagin, punicalin, granatins A and B, gallagyldilacton, tellimagrandin I, pedunculagin, and corilagin were isolated from peel extract of *Punica granatum* were responsible for the antimicrobial activity (Fetrow and Avila 2000; Catão *et al.* 2006; Dudonné *et al.* 2009). Tannins, flavonoids, and alkaloids presented in peel aqueous extract of *P. granatum* were observed positive tests against diarrhea (Qnais *et al.* 2007). *P. granatum* peel methanolic extract showed antifungal activity against *Candidia albicans* (Höfling *et al.* 2010). Alcoholic and hot water extracts of *P. granatum* peel with high amount of gallotanic acid showed good antifungal activities against *C. albicans, C. tropicalis, A. fumegatus*, and *A. nigar* (Shaokat *et al.* 2007). Many tactics have been developed to control the threat of mosquito-borne disease. One such tactic is to kill mosquito larvae, using a strategy based on synthetic insecticides. Although they are effective, they have generated many problems such as insecticide resistance, environmental pollution, and adverse effects on human beings and livestock (Liu *et al.* 2005; Lixin *et al.* 2006).

The present study revealed that the EO of O. basilicum has a potent larvicidal activity against C. pipiens larvae. In line with our results, the larvicidal activity of O. basilicum EO was reported against four other mosquito species Culex tritaeniorhynchus, Aedes albopictus, Anopheles subpictus, and Aedes aegypti with LC<sub>50</sub> values of 14.01, 11.97, 9.75, and 75.35 ppm, respectively (Govindarajan et al. 2013; Manzoor et al. 2013). Furthermore, the EO of O. basilicum displayed the highest larvicidal activity against the larvae of the lymphatic filariasis vector (Culex quinquefasciatus), in comparison to O. sanctum and O. gratissimum (Rajamma et al. 2011). Diverse species of Ocimum from different countries displayed a potent larvicidal activity against mosquito larvae such as O. sanctum L. from India and Nigeria (Pathak et al. 2000; Gbolade and Lockwood 2008), O. americanum L. and O. gratissimum L. from Brazil (Cavalcanti et al. 2004), and O. lamiifolium Hochst./O. suave Willd from Ethiopia (Massebo et al. 2009). This activity could be related to the presence of important chemical compounds in the EOs. The main compounds found in the EO of O. basilicum were stragole and eucalyptol. Previously, the major EO constituents of O. basilicum plants included methyl chavicol (estragole), linalool, eugenol, and 1,8-cineole (eucalyptol) (Sajjadi 2006; Telci et al. 2006; Chalchat and Özcan 2008; Pripdeevech et al. 2010).

The *Eucalyptus* genus (Family: Mitraceae) includes more than 700 species and is one of the world's most important widely planted genera (Menut *et al.* 1995). In the present study, *E. gomphocephala* EO showed a remarkable larvicidal activity ( $LC_{50} = 30.07 \text{ mg/L}$ ) against *C. pipiens*. Confirming the present results, similar larvicidal activity of *E. camaldulensis* EO was reported with an  $LC_{50}$  value of 31 µg/mL against *A. aegypti* (Cheng *et al.* 2009). *S. aromaticum* is an evergreen tree in the family Myrtaceae. Its aromatic flower buds are widely used in traditional medicine (Alqareer *et al.* 2012). The methanolic extract of *S. aromaticum* displayed higher larvicidal activity ( $LC_{50} = 58.73 \text{ mg/L}$ ) against *C.* 

*pipiens* compared with its EO (LC<sub>50</sub>= 128.92 mg/L). A very similar result of the larvicidal activity of the EO from *S. aromaticum* was reported against *A. aegypti* (LC<sub>50</sub> = 124.69 ppm) and *C. quinquefasciatus* (LC<sub>50</sub> = 124.42 mg/L) (Sutthanont *et al.* 2010; Fayemiwo *et al.* 2014).

### CONCLUSIONS

- 1. The essential oils extracted from *T. grandis* (acetone extract), *S. aromaticum* (methanolic extract), and *E. gomphocephala* (branch oil) had remarkable antifungal activities against the three tested pathogenic fungi, *A. solani*, *F. oxysporum*, and *R. solani*.
- 2. The essential oils from *O. basilicum*, *E. gomphocephala*, and *S. aromaticum* as well as the methanolic extract of *S. aromaticum* have remarkable larvicidal effects.
- 3. By GC/MS analysis, the most abundant compounds identified in S. aromaticum methanolic extract were 3-allylguaiacol and eugenol acetate; in S. aromaticum essential oil were eugenol acetate, isoeugenol, *trans*-caryophyllene and  $\alpha$ -humulene; in *Tectona* grandis wood acetone extract were cyclohexylpentyl oxalate, 1,2-bis(TMS-oxy)-3-(1,3-benzodioxol-5-yl)-5-hydroxy-4-nitrocyclotechtoquinone, cyclooctene, hexanone, and 2-thiobarbituric acid, TMS; in T. grandis water extract were (E)-4,4dimethyl-2-pentene, vinylather, and divinyl carbinol; in E. gomphocephala branch oil were *p*-cymene, (+)spathulenol,  $\Delta$ 3-carene, 2-methyl-3-phenylpropanal, and 1,8cineole; in *E. paralias* leaf extract were  $1\beta$ H-romneine,  $\beta$ -amyrin, TMS derivative, and 8-bromo-neoisolongifolene; in *E. paralias* seed extract were  $\alpha$ -linolenic acid, TMS, 2-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-N-(4-fluorophenyl)acetamide, 8bromo-neoisolongifolene, gallic acid, 4TMS,  $\alpha$ -D-glucopyranose, 5TMS derivative, 24-methylene-cycloartenol, acetylated, palmitic acid, TMS, cyclohexyl pentyl oxalate, Shikimic acid (4TMS), and  $\beta$ -amyrin-TMS, and in *P. granatum* peel extract were furfural, orotyl amide, D-allose, n-capric acid, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, 2,5-dimethylfurane, ethyl  $\alpha$ -D-glucopyranoside, and *cis*-isoeugenol; in O. basilicum essential oil were estragole, eucalyptol, linalool, trans-4methoxycinnamaldehyde and fenchyl acetate.
- 4. These results support the idea of using natural plant extracts or essential oils as alternative antifungal compounds to control phytopathogenic fungi and the mosquito population.
- 5. This application could reduce the use of synthetic fungicides and insecticides.

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