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RESEARCH ARTICLE

Chemical composition and insecticidal activity of *Origanum floribundum* Munby essential oil endemic plant from Algeria

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ABSTRACT

Among strategies for decreasing diseases, the use of larvicidal plant extracts against mosquito larvae gain an important place in vector control. The aim of the present study was to reveal the insecticidal effectiveness of an endemic medicinal plant on Mosquito-Borne Diseases. The chemical composition of the dried aerial part from *Origanum floribundum* Essential oil, obtained by hydrodistillation, was characterized by Fourier-transform infrared (FTIR) spectroscopy and gas chromatography-mass spectrometer (GC-MS) system. The potential larvicidal activity of this extract as bioinsecticide was assessed against *Culex pipiens*. Results showed many functional groups such as phenols and alcohols, besides, the chemical composition by GC-MS identified thymol (86.9%) and p-Cymene (5.1%) as major components. A significant larvicidal effect against the target mosquito species was noted, with values of LC_{50} at 21.19 mg/l and LC_{90} at 68.37 mg/l. The results obtained showed that the essential oil of *Origanum floribundum* with chemotype thymol possess a potent larvicidal activity and could be considered as an eco-friendly alternative for vector control.

Keywords: *Origanum floribundum* Munby; FTIR spectroscopy; GC-MS; *Culex pipiens*; Vector control.

INTRODUCTION

The genus *Origanum*, belonging to the Lamiaceae family, is known for its richness with medicinal properties. In addition, it is characterized by a large number of biological activities, including antimicrobial, antifungal, antioxidant and insecticidal, that have benefits on human health (Azizi *et al*., 2009). This genus includes about 42 species broadly spread around the Mediterranean (Kokkini, 1996). Our study was concerned with *Origanum floribundum* Munby, one of two species existing in Algeria (Quezel & Santa, 1963).

Based on the taxonomy of *Origanum* by Ietswaart (1980), *Origanum floribundum* Munby was classified into the section *Elongastipica.* This spontaneous species is rare and endemic to the north center of Algeria and systematically characterized by loose and tenuous spikes and tubular calyces with 5 equal teeth as well as by prostrate stems at the base (Quezel & Santa, 1963). In folk medicine, it is used as expectorant and against cough and also as culinary herb by the local population. Previous studies have shown that the oil of this species was rich in phenolic compounds and possess biological activities (Baser *et al*., 2000; Hazzit *et al*., 2006; Boulaghmen *et al*., 2019).

A large number of plant essential oils (EOs) may be potential sources of mosquito larvicides, because they constitute a rich source of bioactive components (Govindarajan, 2010). On the other hand, essential oils are considered as substances with a minimum risk for human health and environment (Isman, 2006). In addition, several studies have demonstrated the insecticidal effects of Essential oils against mosquitoes (Traboulsi *et al*., 2002; Bouguerra *et al*., 2018).

Mosquitoes are generally controlled by conventional insecticides which poses not only strong secondary effects on the environment and non target organisms but also another serious problem which is the development of resistant mosquito populations (Hodgson & Levi, 1996; WHO, 2012). *Culex pipiens* (Diptera: Culicidae) are the most widely distributed species in Algeria and many countries in the world (James, 1992; Rehimi & Soltani, 2002; Tine-Djebbar *et al*., 2016). Besides their severe morbidity and mortality to human and animals, it has been considered as major economic burden in disease endemic countries (Pavela, 2015). The treatment of the aquatic

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larval stage remains to be the most logical and easier approach for controlling the mosquito populations compared to adult (Cetin & Yanikoglu, 2006).

The development of new alternatives strategies to synthetic insecticides aimed at decreasing pest populations by developing pesticides based on plant extracts without adverse effects in non target organisms and environment (Dahchar *et al*., 2016). This is currently one of the most promising approaches for vector control (El Haddad *et al*., 2018; Rodzay & Zuharah, 2021).

To the author's knowledge, *Origanum floribundum* have been scarcely investigated to the insecticidal activity in vector control. In this context, the purpose of this paper was to explore the chemical composition of the essential oil extract using FTIR spectroscopy and GC-MS with assessing for the first time its insecticidal properties against fourth instar larvae of *C. pipiens*, the most abundant and interesting mosquito species in Algeria.

MATERIALS AND METHODS

Plant material

The aerial part of *O. floribundum* plant was collected in July 2015 at the flowering stage from Lakhdaria (80 Km East of Algiers, altitude: 1100 m). The taxonomic identity of the plant was confirmed by Dr S. Benhouhou with the herbarium voucher specimen from the department of botany at the National High School of Agronomy (ENSA), Algiers.

Mosquito rearing

The larvae of *C. pipiens* used in the assays originated from Réghaïa Lake, Algiers and reared in VALCOR Laboratory. Each 20 larvae were kept in pyrex storage jar containing 150 ml of stored tap water and placed in screened cages (20cm x 20cm x 20cm) and maintained at temperature between 25-27°C. Larvae were daily fed with fresh food consisting of a mixture of Biscuit-dried yeast (75:25 by weight) (Rehimi & Soltani, 1999). The 4th instar larvae were used in the bioassays. Taxonomic identity of *Culex pipiens* was confirmed with Pr. Baba Aissa from ENSA, Algiers.

Extraction of essential oil

Entire dried aerial part (about 100g) was subjected to hydrodistillation for 3 hours using a modified Clevenger type apparatus according to the European Pharmacopeia (2012). The obtained essential oil was dried of water traces over anhydrous sodium sulfate (Na_2SO_4) and conserved at 4°C in a sealed amber flask until use.

Infrared spectroscopy analysis

The FTIR spectra of *O. floribundum* EO was measured for qualitative determination of characteristic bands and various functional groups. The chemical structures of the essential oil were characterized by FTIR spectroscopy using JASCO-1400 type spectrophotometer, Deutschland. The frequency range was between 4000 and 600 cm^{-1}. The KBr (100 mg) pellet was prepared, pressed then analyzed after adding and spreading a drop of oil on this pellet. The results are tentatively identified by Coates (2006), Lambert *et al*. (1998) and Agatonovic-Kustrin *et al*. (2020).

Gas chromatography-Mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analysis were carried out by an Agilent 7890B-5799A system USA using the apolar column HP 5MS (30 m × 0.25 mm × 0.25 µm film thickness). Conditions for GC-MS spectra were: The column temperature program was 60°C for 8 min. increasing at 2°C/min towards 280°C and held at 280°C during 15 min. Sample (0.2 µl) without dilution was injected by splitting and the split ratio was 1:20. Injection was performed at 250°C. A flow rate of 0.5 ml/min carrier gas used was Helium and an ionization mode with electronic ionization is used at 70 eV over a scan range of 30-550 atomic mass units. (Kerbouche *et al*., 2015).

Identification of EO constituents was tentatively done by comparing their mass spectral fragmentation patterns with those stored in the MS database (NIST 2014 and Wiley 7N libraries) and with mass spectra literature data and those of ADAMS spectral database (Adams, 2007; Babushok *et al*., 2011).

Treatment and larvicidal activity

Biological tests were realized according to World Health Organization (1996) protocol, with minor modification (Pavela & Sedlák, 2018). *Origanum floribundum* EO was diluted in ethanol (solvent) in order to make a serial dilution ranging from 5 to 60 mg/l of the larvicidal test. In order to produce a homogeneous test solution for experimental treatment, we added 1 ml of serial dilution to 99 ml of lodging water which we shook gently.

The test for each concentration is carried out using 5 replicates with 20 larvae each for the treated and control series. The positive controls were exposed to ethanol (1 ml of ethanol + 99 ml of lodging water) while the negative controls were exposed to lodging water only. After the exposure time, larvae were removed in clean water and scored dead when they failed to move after probing with a needle in the siphon or cervical region. No mortality detected in negative and positive control tests.

Statistical analysis

Results are presented as mean ± standard deviation (SD). The significance between different series was tested using Tukey's test at 5% level. Data were subjected to one-way analysis of variance (ANOVA). Probit analysis was conducted to estimate lethal concentrations (LC_{50} and LC_{90}) and their respective 95% confidence limit (CL) were determined. All statistical analyses were performed using SPSS Statistics 26.0 Software. The significance level was set at a probability value lower than 0.05.

RESULTS

Extraction yield and organoleptic properties of essential oil

The EO isolated by hydrodistillation from the aerial part of *O. floribundum* was obtained in yield of 4.06% (w/w). The extraction afforded a pale yellow oil with a very strong and persistent *Origanum* fragrance.

Identification of functional groups: Infra Red spectroscopy

The FTIR spectroscopy analysis of the *O. floribundum* EO provided a spectrum presented in Figure 1. Prominent spectral bands were found at a wavelength of 3407, 3025, 2958, 2925, 2869, 1619-1457, 1421-1363, 1290, 1253, 1230, 1176-1060, 995 and 944-809 cm^{-1}.

The FTIR spectrum of the studied sample illustrates the chemical composition of different compounds present in the EO. Table 1 presents the spectral ranges and related band assignments of the EO. Further, these results of FTIR spectroscopy analysis was confirmed by GC-MS.

Gas Chromatography-Mass Spectrometer

The GC-MS analysis of the studied sample has led to the identification of 19 compounds, representing 97% of total identified components. Oxygenated monoterpens (89.1%) and monoterpens hydrocarbons (5.3%) classes were the main groups of the constituents in *O. floribundum* EO. This oil was rich in thymol (86.9%) followed by p-Cymene (5.1%) and linalool (1.3%) with lower percentages. Chemical composition and percentages of this oil are given in Table 2.

Larvicidal effect

Different concentrations of *O. floribundum* EO: 5, 15, 30, 45, 60 ppm were applied on 4th instar larvae of *C. pipiens*. The mortality

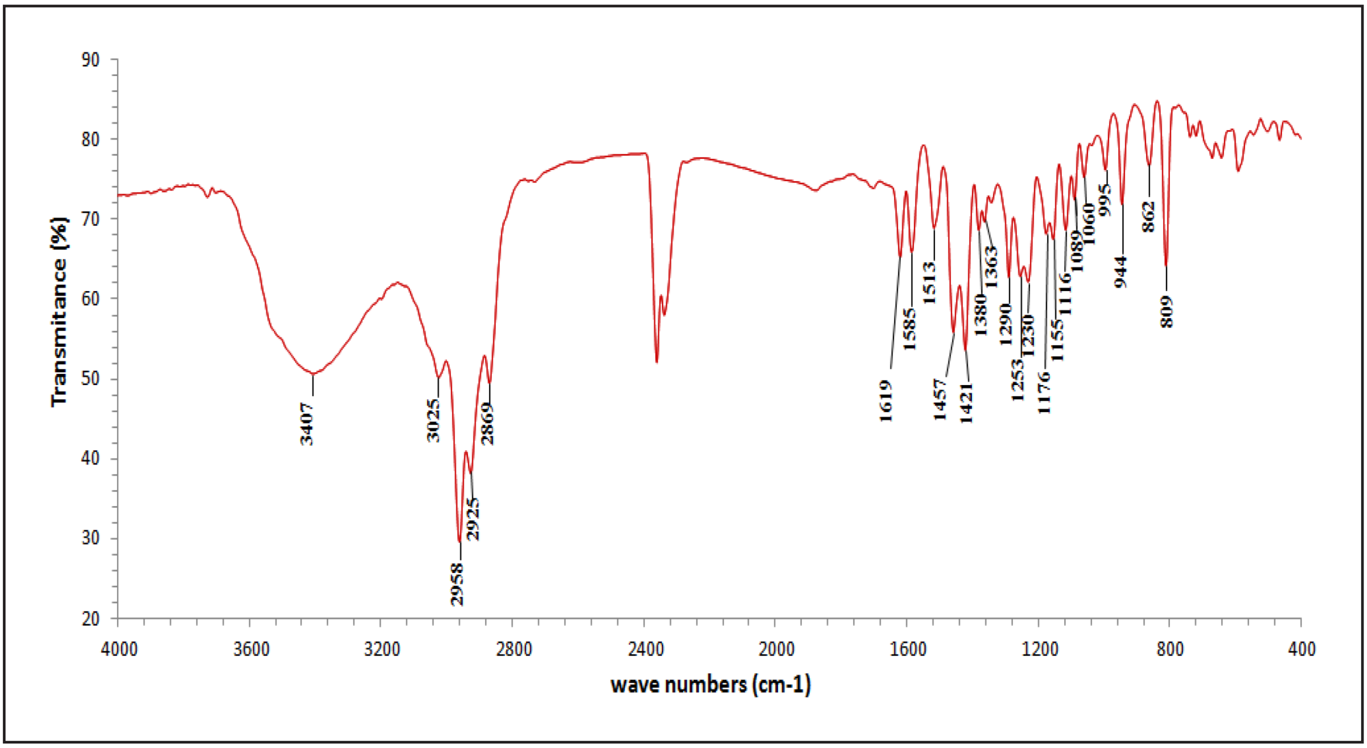

Figure 1. FTIR bands observed in spectra from *O. floribundum* essential oil.

Table 1. Functional groups of the components of *Origanum floribundum* essential oil (Referred to Coates, 2006; Lambert *et al*., 1998 and Agatonovic-Kustrin *et al*., 2020)

Bands (cm^{-1})	Band assignments
3407	O-H stretching
3025	C-H stretching
2958	C-H asymmetric stretching CH_3
2925	C-H symmetric stretching CH_2
2869	C-H symmetric stretching CH_3
1619, 1585, 1513, 1457	C=C stretching
1421, 1380, 1363	C-H symmetrical and asymmetrical bending
1290, 1253	C-C-O stretching
1230	C-O stretching
1176, 1155, 1116, 1089, 1060	C-O stretching
995	C-H bending
944, 862, 809	C-H bending

was noted at 24 hours after treatment while variations in mortality was reported in Table 3 with rates ranging from 11.6% (5 mg/l) to 96.66% (60 mg/l) (x^2=18.239, df=3, F=525.9). The results of this investigation clearly indicate that by increasing concentrations, the rate of mortality increases. Also, the lethal concentrations LC_{50} and LC_{90} and their confidence limits at 95% was showed in Table 4.

DISCUSSION

In this study, the obtained yield of *O. floribundum* essential oil is in range of those mentioned in literature (2.9-5.8%) (Hazzit & Baaliouamer, 2009; Hadjadj & Hazzit, 2020).

FTIR spectroscopy provides very interesting information about the structure of a molecule. It is considered an excellent technique for qualitative analysis. Many functional groups can be identified by their characteristic vibration frequencies (Belboukhari *et al*., 2013).

Previous studies has reported these spectral ranges (Agatonovic-Kustrin *et al*., 2020; Berechet *et al*., 2020; Taylan *et al*., 2021). The broad band located at 3407 cm^{-1} may be due to alcohols and phenols. The bands from 3025 cm^{-1} to 2869 cm^{-1} could be assigned to alkanes. The band 1421 cm^{-1} is due to saturated aliphatic groups. The band 1290 cm^{-1} may corresponds to phenols. The bands from 1176 to 1060 cm^{-1} may be attributed to alcohols. The band at 995 cm^{-1} could be assigned to alkenes. The peak at 809 cm^{-1} may be due to aromatic.

The FTIR spectrum of the *Origanum floribundum* essential oil was quite similar, by a high percentage of identical peaks with different intensities, to the Results obtained in previous studies from Agatonovic-Kustrin *et al*. (2020) and Berechet *et al*. (2020) who worked on the essential oil from Oregano and other Lamiaceae family with FTIR and ATR-FTIR spectroscopy. Also, the main essential oil components (thymol and p-cymene) from *Origanum vulgare* and *Thymus vulgaris* determined in the study of Valderrama & De (2017) have showed some similar spectral characteristics with our sample.

The major components of *Origanum floribundum* EO, identified by the GC-MS, have shown carvacrol (Baser *et al*., 2000; Houmani *et al*., 2002; Djebir *et al*., 2019) and thymol (Sahraoui *et al*., 2007; Kerbouche *et al*., 2015; Ksouri *et al*., 2017; Kerbouche *et al*., 2021; Zahi *et al*., 2021). In contrast, other studies of the same oil have mentioned p-Cymene (Hadjadj & Hazzit, 2020) and γ-Terpinene (Brada *et al*., 2012) as major compounds. Although variations are often found in the same site (Hadjadj & Hazzit, 2020).

Kerbouche *et al*. (2015), Kerbouche (2016) and Zahi *et al*. (2021) results are in agreement with those of our sample that contains a higher amount of thymol, lower amount of p-Cymene and absence of γ-terpinene than from Lakhdaria (33.6-15.5 and 19.9%) (same site), Maala (45-16.8 and 21.6%) and Kadiria (31.7-16 and 21.2%) respectively. However, the same species from Chrea and Hammam Melouane was rich in p-Cymene (73.4-60.7%) and poor in thymol (2.5-6.1%) (Hadjadj & Hazzit, 2020). The essential oil composition of *O. floribundum* differs qualitatively and quantitatively in major and minor compounds. This observed variability in yield, diversity in the essential oil chemical composition and chemotypes of the same

Table 2. Percentage composition of *Origanum floribundum* essential oil

No.	Compounds	RI	*Origanum floribundum*	Identification
1	α-Pinene	929	0.1	RI, MS
2	Camphene	952	t	RI, MS
3	β-pinene	979	t	RI, MS
4	1-octen-3-ol	980	0.4	RI, MS
5	3-Octanone	986	0.1	RI, MS
6	β-Myrcene	991	t	RI, MS
7	p-Cymene	1025	5.1	RI, MS
8	Limonene	1030	0.1	RI, MS
9	Cis Sabinene hydrat'	1070	t	RI, MS
10	Linalool	1099	1.3	RI, MS
11	Terpinen-4-ol	1177	0.8	RI, MS
12	L-α-Terpineol	1190	0.1	RI, MS
13	Carvacrol methyl ether	1244	0.7	RI, MS
14	Thymol	1291	86.9	RI, MS
15	Caryophyllene	1419	0.2	RI, MS
16	β-Bisabolene	1509	0.1	RI, MS
17	β-sesquiphellandrene	1524	t	RI, MS
18	Caryophyllene oxide	1581	1.1	RI, MS
19	Humulene epoxide 2	1606	t	RI, MS
	Total identified (%)		97	
	Monoterpene hydrocarbons		5.3	
	Oxygenated monoterpenes		89.1	
	Sesquiterpens hydrocarbons		0.3	
	Oxygenated sesquiterpens		1.1	
	Others		1.2	
	Yield % (w/w)		4.06	

Components listed in the order of elution from HP 5MS column
MS. comparison of mass spectra libraries and mass spectra of literature data
RI. comparison of retention indices with library
t= trace (< 0.1%)

Table 3. Mortality (%) of *Origanum floribundum* EO applied on fourth instar larvae of C. pipiens with mortality and standard deviation (m ± SD)

Time (hours)	5 mg/l	15 mg/l	30 mg/l	45 mg/l	60 mg/l	p
24	11.66±3.71	31.66±3.76	55.00±3.75	80.00±3.75	96.66±3.76	< 0.001

Table 4. Lethal concentrations LC_{50} and LC_{90} with confidence interval of *Origanum floribundum* EO against fourth larvae instar of *C. pipiens*

Species	Regression equation	mg/l	LC_{50} 95 % CL	mg/l	LC_{90} 95 % CL
C. pipiens	y= 2.54x-3.29		21.19 (15.58-27.62)		68.37 (48.15-128.68)

species is probably related to several factors such as: environmental conditions of collection site (seasonal variation, climatic and soil variation, geographic location), genetically determined properties, stage of vegetative cycle, harvest time, plant part used, extraction method, extraction time, method of essential oil isolation or even the operator conditions of the GC-MS analysis (Azizi *et al*., 2009; Babushok *et al*., 2011; Brada *et al*., 2012; Daoudi-Merbah *et al*., 2016; Pavela & Sedlák, 2018; Djebir *et al*., 2019; Agatonovic-Kustrin *et al*., 2020; Kerbouche *et al*., 2021).

Our results of larvicidal activity were compared with those of genus *Origanum* and some plant families against *Culex* sp. and other *Culicidae* insects. Similar studies have been done on different species of *Origanum* against *C. pipiens* larvae revealed a variation in lethal concentrations such as *O. majorana* (LC_{50} and LC_{90} : 258.71 and 580.49 µg/ml) (EL-Akhal *et al*., 2014), *O. minutiflorum* (LC_{50} : 73.8 and LC_{90}: 118.9 ppm) (Cetin & Yanikoglu, 2006), *O. syriacum* (LC_{50}: 36 mg/l) (Traboulsi *et al*., 2002), *O. onites* (LC_{50} : 24.8 and LC_{90} : 61.3 ppm) (Cetin & Yanikoglu, 2006), and *O. compactum* (LC_{50} : 7.50 ppm and LC_{90} : 18.60 ppm) (Lahlou *et al*., 2001). Larvicidal activity of *O. vulgare* essential oil against the same species indicated different toxicities level basing on treatment periods: 24, 48 and 72h (LC_{50}: 13.69, 13.70 and 12.41ppm and LC_{90}: 36.98, 38.37 and 37.93 ppm) (Bouguerra *et al*., 2019).

Among these results mentioned above, it is concluded that the lethal concentrations LC_{50} and LC_{90} in our study are in good accordance with those of *O. onites* followed by *O. syriacum* and *O. Minutiflorum*. Our results are better than those of *O. majorana* and less effective compared to *O. vulgare* or even *O. compactum*.

Otherwise, several studies on EOs have reported their effectiveness against mosquito, Dris *et al*. (2017a, b) demonstrated that *Ocimum basilicum* and *Lavandula dentata* essential oils have insecticidal activity against *C. pipiens* larvae. In addition, Guenez *et al*. (2018) found that the *Mentha pulegium* essential oil exhibits a larvicidal activity against the same mosquito and *Aedes caspius*. Kharoubi *et al*. (2020, 2021) also reported that *Mentha rotundifolia* has a larvicidal activity against *C.pipiens* with LC_{50} and LC_{90} 62.08 and 178.64 ppm respectively and revealed that *Mentha piperita* against *C. pipiens* had a potent larvicidal activity with LC_{50} 448.74, 181,97 and 152.05 ppm at 24, 48 and 72 h respectively.

In the other study of Bouguerra *et al*. (2018), the larvicidal activity of *Thymus vulgaris* against *C. pipiens* have been examined and the lethal concentrations showed a variations according to treatment periods: 24, 48 and 72h (LC_{50}: 72.04; 68.61 and 62.12 ppm and LC_{90}: 207.01; 190.54 and 169.82 ppm). Traboulsi *et al*. (2002) reported the insecticidal activity of three medicinal plants harvested in Lebanon (*Myrtus communis*, *Lavandula stoechas* and *Mentha microphylla*) against *C. pipiens molestus*; the LC_{50} values were between 16 and 89 mg/L.

In contrast, Vatandoost *et al*. (2012) have examined the larvicidal activity of *Kelussia odoratissma Mozaffarian* against *C. pipiens* and *A. stephensi*, the LC_{50} values of this oil were 2.69 and 7.90 ppm respectively. Also, volatile oils from *Thymus broussonetti* and *Thymus maroccanus* species showed an effective toxicity against *C. pipiens* larvae with LC_{50} and LC_{90} values (0.23 ppm - 0.76 ppm) and (0.31 ppm - 1.53 ppm) respectively (Belaqziz *et al*., 2010).

The contents of major constituents in *Origanum* EO also significantly influence their insecticidal efficacy, while in general, phenol thymol increases EO efficacy considerably (Traboulsi *et al*., 2002). Moreover, concerning the insecticidal activity of essential oils, mosquito larvicide with an LC_{50} below 100 ppm may be considered promising (Pavela, 2015).

On the other hand, insecticidal efficacy of EO is often varied because it depends not only on the mosquito species but, particularly, on the chemical composition of essential oils and on the synergic and antagonistic relationship between substances contained therein (Pavela, 2015). Although the relationship between temperature and EO efficacy could be very important for practical recommendations regarding application of plant insecticides developed based on EO (Pavela & Sedlák, 2018).

From this study it can be concluded that the essential oil of *Origanum floribundum* with many functional groups and thymol as major component was revealed to possess a potent larvicidal activity against *C. pipiens*. This plant essential oil could be considered as an important promising substance for development of environmentally insecticides from plants and moreover, for the prevention of Mosquito-Borne Diseases and vector control.

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Conflict of Interest

The author declares that they have no conflict of interests.

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