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Effect of plant ontogeny on the phytochemical composition and antioxidant activity of *Artemisia herba alba* essential oil

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ABSTRACT

Artemisia herba-alba is a medicinal and strongly aromatic plant widely used in traditional medicine by many cultures since ancient times. It is known that the quantitative chemical composition of plants and consequently their biological activities vary throughout the vegetative cycle. The main purpose of this study was to evaluate the phenophase effect on the chemical composition of essential oil and consequently on antioxidant capacity of A. herba-alba. Plant material has been harvested at three phenological stages (late vegetative. full-flowering and early vegetative stage). EO isolated by hydrodistillation were analyzed by GC/SM. The antioxidant capacity was determined by DPPH free radical scavenging ability. Results indicated the presence of 57 components. Thirty-three components accounting for 99.70% of the total composition were identified in the late vegetative stage. In the full-flowering stage. 36 compounds were identified amounting 99. 23% of total components. In the early vegetative stage. 38 compounds amounting 99. 09% of total components were identified. Chemotype corymbolone characterizing full-flowering stage is a new chemotype described for the first time in the essential oil of A. herba-alba.

Keywords: *Artemisia herba-alba,* chemotype. essential oil, phenological stages,

INTRODUCTION

Globally, research in herbal medicine has made great strides; consequentlyl, lthe potential for developing drugs from these plants is unlimited (Sood *et al.*, 2023). *Artemisia herba-alba* (Asteraceae) is a greenish-silver perennial dwarf shrub growing in arid and semi-arid climates. This plant is widely used in the traditional medicine to treat diabetes, bronchitis, diarrhea, hypertension and neuralgias (Tahraoui *et al.*, 2007). In a study conducted on certain Algerian medicinal plants, including *Artemisia herba-albal*, it was noted that these plants can be considered a good source of natural antioxidants for medicinal and commercial purposes (Goudjil, 2016). Numerous studies in the literature have reported the composition of *A. herba-alba* essential oil from different parts of the world and confirmed the many oil-dependent chemotypes assigned to the plant. Essential oils contain various secondary metabolites, the majority of which possess antibacterial, antifungal, antioxidant and bioregulatory activities (Kumar *et al.*, 2023). Seasonal variation has an effect on the active principles of medicinal plants (Gautam *et al*., 2025). The essential oil yield and the quality of plant material are the most important characteristics in commercial cultivation of spices and aromatic herbs. It is very important to determine the optimal harvesting period, which may differ depending on climatic conditions and plant properties.

According to Abad *et al*. (2012) the quality and yield of the essential oils from the Artemisia species is influenced by the harvesting season. Indeed, the climatic conditions and water availablity in the soil have an effect on the vegetal secondary metabolism and, consequently, alter the composition of essential oils, through the seasons of the year. Most users of medicinal plants believe that the harvest date has no effect on the biological activities of extracts from these plants. Through this research, we demonstrate the variation of chemical composition of *A. herba-alba* essential oils according to the harvest date, and we present, for the first time, a detailed study of the chemical composition of *A. herba-alba* essential oils during three growth stages.

MATERIALS AND METHODS

Aerial parts (leaves. stems. branche and flowers) of *Artemisia herba-alba* were collected from Ain Bel. south-west of Djelfa. Algeria. at three phenological stages, *viz.*, early vegetative (May, 2024); late vegetative (July, 2024); and full flowering (December 2024). For each stage, one sample was collected. resulting in a total of three essential oil samples. The samples were air - dried for 15 days in the laboratory of Plant Biology and Physiology. Faculty of Natural and Life Sciences. University Ziane Achour of Djelfa at room temperature till the weight stay stable. The aerial parts (leaves. stems. branche and flowers) of individual plants were subjected to hydrodistillation for 3hrs using a Clevenger-type apparatus. The EO (essential oil) obtained was separated from water and dried over anhydrous sodium sulphate and kept in amber vials at 4^0 C until chromatographic analysis. Essential oil yield percentage was calculated as weight (g) of EO per 100 g of plant dry matter.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC/MS is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Then gas phase chromatographic analyses were carried out with the aid of a Trace GC Ultra apparatus equipped with one injector in Split Play. a TRB-5 MS column (30 m x0. 25 mm. film thickness 0.25 µ.mL). The operating conditions are as follows: carrier gas: helium; solvent: ethyl acetate; injection volume: 50 µL; flow rate: 1 .mL/min; The operating condition of GC oven temperature was maintained as: initial temperature 40 °C for 1 min. programmed rate 5°C/min up to final temperature 220°C with isotherm for 1 min; injector temperature 280° C.The coupling with the mass spectrometer ITQ 900 was done with a temperature of 220 °C interface. The operating conditions are as follows: type electron impact ionization (70 eV); injector temperature was 200 °C. Mass spectra were recorded over 50–500 a. mL. u.range. The database used was NIST M Search. The identification of the constituents was assigned of the comparison of their retention indices and mass spectra with those given in the literature (Adams, 2001, Joulain and König, 1998).

Measurement of antioxidant capacity of essential oil of *A. herba-alb*

The DPPH radical scavenging is a commonly used method to evaluate the ability of plant extracts to scavenge free radicals generated from DPPH reagent. The most commonly used DPPH assay is simple and highly sensitive. In this part of our work. the *in vitro* antioxidant activities of the leaves essential oils extracts stored at 4°C have been evaluated based on the DPPH test. Antioxidant scavenging activity was studied using 1. 1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Adedapo *et al*. (2009) with some modifications: 2 mL of various dilutions of the plant extracts were mixed with an equal volume of 0. 135 mM methanolic DPPH solution. After an incubation period of 30 min in the dark at room temperature. the absorbance at 517 nm and the wavelength of maximum absorbance of DPPH. were recorded as A (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). Ascorbic acid is used as reference. Measurements were performed in triplicate.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Differences between phenological stages were analyzed using one-way analysis of variance (ANOVA). followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism 9. Additionally. Pearson correlation analysis was performed to evaluate the relationship between the relative abundance of major oxygenated sesquiterpenes and antioxidant activity (IC_{50} values).

RESULTS AND DISCUSSION

Essential oil yield and chemical composition

As shown in Table 1. variable essential oil concentration as calculated on the basis of dry plant weight (v/w) was observed for *A. herba-alba* depending on the different growth stages. The essential oil yield showed a decreasing trend from the early vegetative stage (0.54%) to the flowering stage (0.16%). The higher yield recorded during the vegetative period may be attributed to intense metabolic activity associated with plant growth and the accumulation of volatile compounds in secretory structures prior to reproductive development. The decline during flowering could reflect metabolic reallocation toward reproductive processes and the synthesis of non-volatile secondary metabolites. Such seasonal fluctuations in oil yield have been widely reported in aromatic and medicinal plants and are often linked to environmental factors such as temperature. photoperiod. and water availability (Mansinhos *et al.*, 2024). Little has been published in connection with this matter, although there is some controversy concerning the EO yielded by different Asteraceae species at different phenological stages Farhadia *et al*. (2020) indicated that the harvested aerial parts at the flowering stage gave the highest essential oil content (0.24 % v/w) (*Achillea millefolium L*.). which agrees with the results obtained by Verdian-Rizi (2008). the yields of EO at different growth stages of *Artemisia annua* were in the order of pre-flowering < post-flowering < full-flowering. These results clearly indicate that harvesting time should be carefully selected to ensure maximum yield of EO. In the case of *A.herba-alba*.the spring season (vegetative stage in young leaves) could be favoured.

The composition of the EO is affected by many factors. such as the genetic factorsl. the developmental stage. the extraction method and the conditions of analysis (Kim and Lee, 2004). The chemical composition of the various extracted EOs is presented in Table 1. In all. 57 components of the oils have been identified in different growth stages of *A. herba-alba*; 33 components accounting for 99.70% of the total composition were identified in the late vegetative stage (L. V.S.). The major constituents of this oil were davana ether (65.51%), spathulenol (13.59%), 2-Propenal.3-(2.6. 6-trimethyl-1-cyclohexen-1-yl)- (7.22%). In the full-flowering stage (F.F.S.), 36 compounds were identified amounting 99.23% of total components with corymbolone(40.99%), davana ether (29.44%), Benzene,1.3-bis (1.1-dimethylethyl)- 2-methoxy-5-methyl- (6.63 %), 2-Propenal. 3-(2. 6. 6-trimethyl-1-cyclohexen-1-yl)- (4.1 %) were identified. Finally, in the early vegetative stage (E.V.S.), 38 compounds amounting 99.09% of total components were identified which included davanone C (67.65%). davana ether (8.69%). α-campholenal(5.11 %) as main components. The observed discrepancies between oil samples may be attributed to the fluctuation of some climatic factors. essentially rainfall and temperature. since they grow under the same conditions. It should be borne in mind that EO production and composition depends on plant genetics and the ecological factors of the growing area (Dutta *et al*, 2005). which explains much of the controversy between the results obtained by different researchers. According to our results. it seems that chemical composition of *A. herba-alba* EO varied significantly with physiological stage of the plant.

The chemical composition of *Artemisia herba-alba* essential oil showed marked qualitative variation across the different phenological stages. reflecting dynamic regulation of terpenoid biosynthesis during plant development. At the early vegetative stage. the oil was strongly dominated by Davanone C (67.65%). indicating a relatively simple sesquiterpene profile characterized mainly by ketonic structures. This stage corresponds to active vegetative growth. where metabolic resources are primarily directed toward biomass production. and secondary metabolism remains less diversified. During the late vegetative stage. a transitional chemical pattern was observed. marked by a decrease in Davanone C and the emergence of Corymbolone (40.99%) and Davanaether (29.44%). suggesting progressive enzymatic oxidation and structural rearrangement of sesquiterpene precursors. This shift reflects activation of terpene-modifying enzymes and increasing metabolic plasticity as the plant prepares for reproductive development. At the full flowering stage. the oil became predominantly rich in Davanaether (65.51%) and Spathulenol (13.59%). indicating a higher degree of oxidation and structural complexity. Flowering represents a physiologically demanding phase during which plants enhance production of oxygenated terpenoids involved in ecological defense. protection of reproductive organs and adaptation to environmental stress. From a biosynthetic perspective these results suggest a sequential transformation of sesquiterpene skeletons through oxidation–reduction reactions regulated by developmental gene expression and environmental cues. Overall. the observed compositional changes confirm that phenological stage is a key determinant of chemotypic expression in *A. herba-alba*. driven by metabolic reprogramming throughout plant growth.

Davanon was reported as constituting major in the essential oil of some chemotypes of Spain and Algerian (Djelfa) species (Salido *et al*., 2001). In a Tunisian oil, camphor, α/β-thujones, 1,8-cineole and chrysanthenyl derivatives were the major components (Haouari and Ferchichi, 2009). Moreover Zaim *et al*. (2012) showed that the essential oil of *Artemisia herba-alba* South of Morocco (Ouarzazate) contains chrysanthenone as major compound. Generally, the oil was largely reported to be composed of monoterpenoids, mainly oxygenated, such as 1, 8-cineole, chrysanthenone, chrysanthenol (and its acetate), α/β-thujones, and camphor as the major components. Corymbolone has been reported as major components of the oil of *Artemisia alba* Tura (Đorđević *et al*., 2013) and as so far we known, it was tentatively determined in this species for the first time. The effect of different phenological stages on oil and its components may be due to its effect on enzyme activity and metabolism of essential oil production (Sellami *et al*., 2009). Plant ontogeny or growth stage has very close relation to secondary metabolite accumulation in plants, and thus, has a significant influence on oil yield and its composition, which varies with place to place and plant-to-plant (Ozcan and Chalchat, 2006).

Antioxidant capacity of essential oil of *A. herba-alb*

The free radical scavenging activity is usually expressed as percentage of DPPH•inhibition but also by the antioxidant concentration required fora 50% DPPH•reduction (IC_{50}). The estimate of the antioxidant capacity of EO during different growth stages from *A. herba-alba* was carried out by DPPH assay (Table 3). The antioxidant activity evaluated by the DPPH radical scavenging assay also varied significantly among stages. The strongest activity was observed during the full flowering stage (IC_{50} = 13.65 µg/mL) followed by the late vegetative stage (14.30 µg/mL). whereas the early vegetative stage showed comparatively weaker activity (18.45 µg/mL). Although all oils exhibited notable antioxidant capacity. their activity remained slightly lower than that of ascorbic acid (IC50 = 10.5 µg/mL). The enhanced antioxidant activity observed in the flowering-stage essential oil may be mechanistically explained by the predominance of oxygenated sesquiterpenes. particularly Davanaether and Spathulenol. Oxygenated terpenoids possess functional groups such as hydroxyl ether and carbonyl moieties that facilitate hydrogen atom transfer (HAT) and/or single electron transfer (SET) mechanisms during free radical neutralization. These functional groups increase electron density and promote resonance stabilization of the resulting radical intermediates thereby improving radical-scavenging efficiency. Interestingly the stage with the highest oil yield (early vegetative stage) did not correspond to the strongest antioxidant activity. This finding emphasizes that biological activity depends more on qualitative composition than on quantitative yield. The predominance of specific oxygenated compounds during flowering appears to enhance antioxidant performance. supporting the hypothesis that compositional profile is a determining factor in bioactivity. These results are similar to those reported by Bouzidi (2016) and Goudjil (2016). The study conducted on *A. herba-alba* essential oil harvested in Bouzidi *et al*., (2016), in which the maximum IC50 reached 20.64 µg/mL. These values appear less effective compared to ours. Goudjil (2016) tested the antioxidant activity of *A.herba alba* essential oil from Djelfa. and observed an IC_{50} of 17.73 µg/mL. Indeed. our oils appear to be consistently more active. Mighri *et al*. (2010) studied the antioxidant activity of four types of essential oils of *A. herba-alba* harvested in southern Tunisia. they found that Type I oil which is characterized by a high content of β-thujone the IC50 = 8.552 µg/mL. Type II oil which is dominated by α-thujone the IC_{50} = 17.961 µg/mL. Type III oil whose major compounds are α-thujone. β-thujone the IC50 = 8.236 µg/mL. Type IV oil where the main elements are 1.8-cineole / camphor / a-thujone / b-thujone the IC50 = 18.036 µg/mL. Based on our results. we can see that our tested *A. herba-alba* oils are more potent than type II and IV oils but less effective than type I and III oils.

Correlation between chemical composition and antioxidant activity

A negative correlation was observed between the total percentage of oxygenated sesquiterpenes and IC_{50} values. indicating that higher concentrations of oxygenated compounds were associated with stronger radical scavenging activity (lower IC_{50}) (Table 2). In particular, Davanaether content showed an inverse relationship with IC_{50} values, suggesting it significantly contributes to antioxidant potential. Conversely, positive correlation was found between total essential oil yield and antioxidant activity. Confirming that biological efficacy depends primarily on qualitative composition rather than quantitative production. These results statistically support the hypothesis that phenological-stage–dependent chemical variation directly influences the antioxidant performance of *Artemisia herba-alba* essential oils.

The ANOVA analysis revealed a significant effect of phenological stage on essential oil yield ($p < 0.05$). with the early vegetative stage producing higher yields compared to the late vegetative and full flowering stages. Similarly. phenological stage significantly influenced antioxidant activity (IC_{50} values. $p < 0.05$) (Table 2). Post hoc analysis indicated that the full flowering stage exhibited the lowest IC_{50} values. reflecting stronger antioxidant activity. whereas differences between the late vegetative and flowering stages were less pronounced (Table 3). These findings demonstrate that seasonal variation significantly affects both the quantity and biological activity of *Artemisia herba-alba* essential oil. and that qualitative chemical composition. especially the content of oxygenated sesquiterpenes such as Davana ether. plays a key role in determining antioxidant potential.

CONCLUSION

Overall. the results confirm that phenological stage plays a crucial role in determining both the quantitative and qualitative characteristics of *A. herba-alba* essential oils. The observed variation in essential oil composition across phenological stages reflects dynamic modulation of terpenoid biosynthesis in *Artemisia herba-alba*. Early vegetative stages favor accumulation of precursor ketonic sesquiterpenes (Davanone C). whereas flowering stages promote oxidative diversification and accumulation of oxygenated derivatives (Davana ether. Spathulenol). These findings confirm that plant developmental stage is a key determinant of chemotypic expression. driven by metabolic reprogramming and ecological adaptation mechanisms. These findings provide valuable insight for the rational exploitation of this species in pharmaceutical. Cosmetic.and nutraceutical applications.

CONFLICT OF INTEREST STATEMENT

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

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Table 1: Chemical composition of the *Artemisia herba-alba* essential oils at three phenological stages

tr (min)	Percentage composition			
Compound		LVS	FS	EVS
Santolinatriene	7.34	0.09	0.63	0.50
Cyclohexanol. 1-acetyl-2-ethylidene-	7.38	0.02	0.02	0.04
Ketone. 1.5-dimethyl bicyclo [2.1.0]pent-5-yl methyl	7.53	0.02	0.02	0.10
Bicyclo[3.1. 1]hept-2-ene. 2.6.6-trimethyl-. (±)-	7.72	0.01	0.00	0.06
3.3-Dimethyl-hepta-4.5-dien-2-ol	8.36	0.28	0.31	0.58
3-Carene	8.46	0.41	0.11	1.55
τ-terpinen	9.19	0.03	0.02	0.21
1S-à-Pinene	9.72	0.18	0.63	1.37
2-Propenal. 2-methyl-. diethylhydrazone	9.97	0.01	0.00	0.03
o-cymene	10.71	0.07	0.44	0.23
2-Cyclohexen-1-ol. 1-methyl-4-(1-methylethyl)-. trans-	10.87	0.56	0.06	2.44
2.3-dimethyl-1.3-Pentadiene	11.01	0.00	0.00	0.06
2(3H)- Furanone. 5-ethenyldihydro-5-methyl-	11.05	0.00	0.03	0.00
Bicyclo[l3l.1. l1l] lheptan-lendo-l6-lolll. lsyn-7-bromo-	12.00	0.00	0.02	0.00
6-Hepten-2-ol. 4-methylene	l12.02	0.00	0.00	0.07
Bicyclo[3.1.1]hept-2-ene-2-methanol. 6.6-dimethyl-	12.97	0.00	0.00	0.25
1.5-Cyclooctadiene. 1.3-dimethyl-	13.02	0.06	0.09	0.00
2.4-Hexadiene. 2.3-dimethyl-	13.37	0.01	0.00	1.66
Bicyclo[3. 1. 0]hexan-2-ol. 2-methyl-5-(1-methylethyl)-. (1à.2à.5à)-	13.52	0.04	0.00	0.16
α-Campholenal	14.15	1.38	0.25	5.11
Thujol	14.55	0.00	0.09	0.20
3-Caren-10-al	14.69	0.09	0.00	0.00
α-Santoline alcohol	14.79	0.19	0.00	1.18
Cyclopentane. 2-methyl-1-methylene-3-(1-methylethenyl)-	14.95	0.05	0.00	0.00
Bicyclo [3. 1. 1] hept-2-ene-2-carboxaldehyde. 6. 6-dimethyl-	15.66	0.06	0.00	0.18
Isothujol	16.61	0.15	0.11	0.11
Bicyclo[3.1.1]hept-2-en-6-ol. 2.7.7-trimethyl-. acetate. [1S-(1à.5à.6á)]-	17.61	0.04	0.00	0.04
3-Ethenyl-1.2-dimethyl-1.4-cyclohexadiene	18.05	0.19	0.00	0.07
2-Cyclopenten-1-one. 3-methyl-2-(2.4-pentadienyl)-. (Z)-	18.20	0.00	0.00	0.07
Acetic acid. 1. 7. 7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	18.41	0.38	0.00	0.41
à-Cubebene	21.73	0.63	0.86	0.23
2-Cyclopenten-1one. 3-methyl-2-(2-pentenyl)-. (Z)-	22.54	0.00	1.77	0.13
(1.2.3-Trimethyl-cyclopent-2-enyl)-methanol	23.24	2.15	0.89	0.00
Caryophyllene	23.46	0.16	0.15	0.17
7-Octylidenebicyclo[4.1.0]heptanes	25.61	0.89	0.00	0.42
Spiro-1-(cyclohex-2-ene)- 2'-(5'-oxabicyclo [2.1.0]pentane). 1'.4'	25.69	0.00	1.28	0.00
ç-Muurolene	26.07	0.00	0.70	0.00
Isoledene	26.1	0.00	0.00	2.65
1-[3-(2. 6.6-Trimethyl-cyclohex-2-enyl)-4.5-dihydro-3H-pyrazol-4-yl]-ethanone	26.19	0.00	1.28	0.00
Naphthalene. 1. 2. 3. 4. 4a.5. 6. 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-. (1à.4aà.8aà)-	26.76	0.00	0.54	0.95
Davanaether	27.51	65.51	29.44	8.69
2.3. 4.5-Tetramethylcyclopent-2-en-1-ol	27.89	0.00	0.00	0.38
2(1H)-Naphthalenone. 4a. 5. 6. 7.8.8a-hexahydro-6-[1-(hydroxymethyl) ethenyl]- 4. 8a-dimethyl-. [4ar-(4aà.6à.8aá)]-	28.98	0.00	0.51	0.19

1-Oxetan-2-one. 4.4-diethyl-3-methylene-	29.11	0.00	0.47	0.00
Desconegut	29.82	0.00	0.00	0.47
Spathulenol	30.42	13.59	0.00	0.00
Caryophyllene oxide	30.69	0.00	1.41	0.00
Perhydrocyclopropa[e]azulene-4.5.6-triol. 1.1.4.6-tetramethyl	30.91	3.66	0.00	0.00
Davanone C	31.46	0.00	0.00	67.65
Benzene. 1. 3-bis (1.1-dimethylethyl)- 2-methoxy-5-methyl-	31.83	0.00	6.63	0.00
4.6. 6-Trimethyl-2-(3-methylbuta-1. 3-dienyl)-3-oxatricyclo[5.1.0.0(2.4)]octane	32.89	0.00	1.05	0.00
3-(2.6.6-Trimethyl-1-cyclohexen-1-yl)-2-propenal	35.04	7.22	4.10	0.00
2.6-dimethyl-3. 5 Heptadien-2-ol	13.58	0.15	0.14	0.48
1H-Cycloprop[e] azulen-7-ol. decahydro-1.1.7-trimethyl-4-methylene-. [1ar-(1aà.4aà.7á.7aá.7bà)]-	30.49	0.00	2.18	0.00
Corymbolone	31.37	0.00	40.99	0.00
Cyclobutanecarboxylic acid. 2-methyloct-5-yn-4-yl ester	32.29	1.42	0.98	0.00
3-Chloropropionic acid. tridec-2-ynyl ester	36.01	0.00	1.03	0.00
Monoterpene hydrocarbons		0.69	1.20	3.36
Oxygenated monoterpenes		1.81	0.45	6.49
Sesquiterpene hydrocarbons		1.68	1.71	3.05
Oxygenated sesquiterpenes		89.98	82.57	76.34
Other compounds		5.54	13.30	10.76
Total oil		99.70	99.23	99.09
Yield (%. v/w)		0.16	0.23	0.54

E.V.S; L.V.S; F.F.S; represent the early vegetative stage; the late vegetative stage; the full-flowering stage respectively

Table 2: Statistical correlation between chemical parameters, essential oil yield and antioxidant activity

Parameter	LVS	FS	EVS	Pearson r	R²	p-value	Interpretation
Oxygenated sesquiterpenes (%)	76.34	82.57	89.98	−0.73	0.53	0.445	Moderate negative correlation
Davanaether (%)	65.51	29.44	8.69	-0.694	0.482	0.511	Moderate negative correlation
Total essential oil yield (%)	0.16	0.23	0.54	0.956	0.913	0.190	Strong positive correlation
IC_{50} (mg/mL)	18.45 ± 4.88	18.45 ± 4.88	18.45 ± 4.88				

Table 3: Effect of phenological stage on essential oil yield and antioxidant activity (IC_{50}) with Tukey's HSD multiple comparison test

Phenological stage	Oil yield (%)	IC_{50} (mg/mL)
EVS	0.54 ^{a}	18.45 ± 4.88 ^{a}
FS	0.23 ^{b}	13.65 ± 0.61 ^{b}
LVS	0.16 ^{b}	14.30 ± 0.92 ^{b}

Note: Means followed by different letters are significantly different according to Tukey's HSD test ($p < 0.05$).