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Research Article

Identification of ontogenetic and diurnal variability in oregano (*Origanum onites* L.)

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Abstract

Ontogenetic and diurnal variabilities in İzmir thyme (*Origanum onites* L.), an important essential oil and spice plant of Lamiaceae (Labiatae) family, were determined in this study. Experiments were conducted in randomized blocks - factorial experimental design with 3 replications. Two harvests were performed. Plants were harvested at different hours of a day (09:00, 13:00, 17:00) and in different growth periods (beginning of budding, beginning of flowering, 50% flowering and 100% flowering). Plant height, fresh herbage yield, drug herbage yield, fresh leaf yield, drug leaf yield, essential oil ratio and essential oil yield parameters were investigated. High essential oil ratios were obtained from the plants harvested at morning hours. Essential oil ratios varied between 2.11 - 4.41%. The greatest essential oil ratio was obtained from the plants harvested in morning hours of the beginning of budding period of the second harvests. Fresh herbage yields varied between 222.45 - 714.63 kg/da with the greatest value from the plants harvested in the beginning of flowering period of the second harvests. Drug herbage yields varied between 64.30 - 256.74 kg/da with the greatest value from the plants harvested in the beginning of flowering period of the second harvests.

Keywords: drug; essential oil; harvest hour; harvest period; oregano

Introduction

Thyme (*Origanum, Thymus, Satureja, Thymbra, Coridothymus*) is among the important medicinal and aromatic plants of Turkish flora. It belongs to Lamiaceae (Labiatae) family and is mostly used for essential oil and spice. There are several different species of thyme used as medicinal, aromatic and spice plant. Thyme essential oil is rich in carvacrol and thymol (Baydar, 2016). The oils sold under the brand of "thyme oil" are mostly obtained from *Origanum* species (Bağdat, 2011).

Mostly the leaves are used as spice, all above-ground parts of thymes are used as drug. Thyme leaves have essential oil contents of between 0.5-8.0%. The thyme species to be used in essential oil industry should have at least 2.5% essential oil content. Thyme is mostly used as spice, less used as thyme tea, thyme oil or thyme juice. Since thyme contains essential oils with quite strong antimicrobial and antioxidant effects, it is used as a significant additive in foods, perfumes, cosmetics, drugs, lotions, soaps and toothpastes (Baydar *et al.*, 2004; Özkan *et al.*, 2010; Kapluhan, 2013). Thyme oil is used as antioxidant to prevent spoilage of foodstuffs, as insecticide (carvacrol) to prevent storage pests, as herbicide to prevent some weeds and as fumigant to prevent

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some diseases (Baydar, 2016). Turkey is the leading thyme exporter country of the world. Among the thyme species both collected from the nature and exported, *Origanum onites* L. is the most valuable one both economically and culturally (Okkalıoğlu *et al*., 2014; Baydar, 2016). *Origanum onites* L. is especially widespread in Mediterranean region of Turkey. The spread zone also includes Greece, Islands and South-West Anatolia. It naturally grows at altitudes of between 0-1400 m. It is thermophilic plant and resistant to cold throughout the growing season, except for seedling stage and initial planting year. Thyme grows in almost every soil texture but grows better in clay-loam alluvial soils. Sandy soils are not good for thyme (Bayram, 2003).

Medicinal and aromatic plants have quite wide range of use and their significance and therapeutic characteristics are mostly designated by their active ingredients. Leaves, flowers, fruits and seeds of the plants are commonly used as drug and active ingredients of these sections of the plants generally vary with the growth seasons and changes in daily temperature and light intensity. Therefore, a drug producer should have a comprehensive knowledge about the changes in active ingredients and collect the plants from the proper places, at proper growth stages and dates when the plants were rich in drug-related active ingredients. By taking such changes into consideration, the most available organ, growth stage and harvest time for drug acquisition are decided. Previous studies revealed that the most appropriate harvest dates varied based on the plant species, climate and soil conditions of production sites (Aziz *et al*., 2013; Lakusic *et al*., 2013; Mammadov, 2014). In this study, effects of ontogenetic and diurnal variabilities on essential oil ratio and yield, drug and fresh herbage yield, drug and fresh leaf yield of oregano (*Origanum onites* L.) were investigated.

Materials and Methods

The seedlings produced from the *Origanum onites* L. seeds supplied from Aegean Agricultural Research Institute were used as the plant material of the present study.

Average temperatures of the experimental year (2018) were about 2 °C above the long-term seasonal averages. Total precipitation was greater than long-term averages especially in July, but almost half of long-term averages in June and August.

Origanum onites L. seeds were germinated in peat-perlite filled pots in 2017 and resultant seedlings were transplanted into the field when they reached to a height of 8-10 cm. The year 2017 was considered as the experimental set up year and harvests were performed in 2018 vegetation period. Experiments were conducted in randomized blocks - factorial experimental design with 3 replications. Seedlings were planted at 40 cm row spacing and 20 cm on-row plant spacing (40 x 20 cm). Harvests, observations and measurements were performed on two middle rows. Soil analyses were performed before the experiments and fertilization was practiced as to have 6 kg/da phosphorus (P_2O_5) and 6 kg/da nitrogen (N) to meet macro element needs of the plants. Harvests were performed at 4 different periods (beginning of budding, beginning of flowering, 50% flowering and 100% flowering) and at 3 different hours of the day (9:00, 13:00 and 17:00). Two harvests (the first and the second) were performed in this study. Harvests were performed at all periods and hours in the first harvest, but harvests at 100% flowering period were not able to be made in the second harvest. Harvested samples were dried in a drying cabin at 35 °C for 48 hours. Dried leaf samples (40 g) were placed into distillation flasks and supplemented with 1000 ml water. Then the flasks were heated in heating mantles for 3 hours as not to exceed 120 °C. Following heating, distillation apparatus was cooled down for 5-10 minutes and essential oil quantity (ml) collected over the water surface was measured from a graduated pipe (Telci *et al*., 2004; Yeşil, 2012). Experimental data were subjected to analysis of variance (ANOVA) with SAS-JMP. 13.0 statistical software. LSD multiple range test was used to compare significant means.

Results and Discussion

Plant height (cm)

Effects of harvest periods on plant heights of *Origanum onites* were found to be significant in both harvests ($p<0.01$). There were significant differences also between the first and second harvests. In the first harvests, the greatest plat height obtained from the beginning of flowering period (43.64 cm) and the shortest plant height was obtained from the beginning of budding period (36.33 cm). In the second harvests, the greatest plant height was obtained from the beginning of flowering period (29.43 cm) and the shortest plant height was obtained from the 50% flowering period (25.05 cm). On the other hand, effects of harvest hours on plant heights were not found to be significant in both harvests. In both harvests, the greatest plant heights were obtained from the beginning of flowering period. In the beginning of flowering period, average plant height of the first harvests was about 32% greater than the average plant height of the 2nd harvests (Table 1). Similar with the present findings for plant heights of oregano (*Origanum onites* L.) harvested at different periods, previous researchers also reported varying plant heights (25.0-58.0 cm) with harvest periods for different thyme species (Kızıl *et al*., 2010; Bahtiyarca Bağdat, 2011; Batıray and Kan, 2015, Özyazıcı and Kevseroğlu, 2019). Özyazıcı (2004) and Karık *et al*. (2007) reported significant effects of harvests on plant heights, but different from the present findings, they reported the greatest plants heights for 100% flowering period. Similar with the present findings, Kulan (2013) also indicated that harvest hours did not have any significant effects on plant heights.

Table 1. Effect of ontogenetics and diurnal variability on the plant length (cm) of *Origanum onites* L. at I. and II. harvest

First harvest						Second harvest				
Hour	Budding	First flowering	50% flowering	100% flowering	Mean	Budding	First flowering	50% flowering	100% flowering	Mean
9.00 am	35.83	45.07	43.33	39.23	40.86	27.80	28.40	23.46	-	26.55
1.00 pm	36.30	43.23	39.10	38.60	39.30	28.20	29.53	25.70	-	27.81
5.00 pm	36.86	42.63	42.00	42.93	41.11	27.40	30.36	26.00	-	27.92
Mean	36.33 C	43.64 A**	41.47 AB	40.25 B		27.80 A	29.43 A*	25.05 B	-	
LSD: 2.54						LSD: 2.22				
*:P<0.05 **: P<0.01										

Fresh herbage yield (kg/da)

Effects of different harvest periods and hours on fresh herbage yields of *Origanum onites* were found to be significant in both harvests. Significant differences were also observed between the 1st and 2nd harvests. Considering the average fresh herbage yields of harvest periods, the greatest fresh herbage yield was obtained from 100% flowering period (436.54 kg/da) in the first harvests and from the beginning of flowering period (520.61 kg/da) in the second harvests. In the second harvests, the greatest fresh herbage yield was obtained from the plants harvested at 17:00. Considering the effects of ontogenetic and diurnal variabilities together, it was observed that the greatest fresh herbage yield was obtained from the second harvests made at 17:00 in the beginning of flowering period (714.63 kg/da). The greatest fresh herbage yield of the first harvests was about 30% less than the greatest fresh herbage yield of the second harvests (Table 2). Similar with the present findings for the first harvests, Karık *et al*. (2007), Kızıl (2009) and Özyazıcı (2004) reported the greatest fresh herbage yields for 100% flowering period. Güngör *et al*. (2005) reported greater fresh herbage yields of oregano for 50% flowering of the first harvests. Air temperature was high and dry conditions were dominant in the first harvests of the present study. Such high temperatures and dry conditions might have slowed down vegetative development and accelerated transition into flowering period. Therefore, fresh herbage yields of the first harvests were lower than the second harvests.

Table 2. Effect of ontogenetics and diurnal variability on fresh herbage yield (kg/da) of *Origanum onites* L. at I. and II. harvest

First harvest						Second harvest				
Hour	Budding	First flowering	50% flowering	100% flowering	**Mean**	Budding	First flowering	50% flowering	100% flowering	**Mean**
9.00 am	255.88 e	254.42 e	341.96 cd	496.65 a**	337.23 B	355.39 cd	327.04 d	398.41 cd	-	360.28 C
1.00 pm	222.45 e	378.78 bc	246.94 e	487.58 a	333.94 B	349.15 cd	520.17 b	418.09 cd	-	429.14 B
5.00 pm	251.25 e	482.66 a	385.58 b	325.38 d	361.22 A*	373.56 cd	714.63a*	436.36 bc	-	508.18 A*
Mean	243.19 D	371.95 B	324.82 C	436.54A**		359.37 C	520.61A*	417.62 B	-	
LSD (Period): 23.34; LSD (Hour): 20.21; LSD (PXH): 40.43						LSD (Period): 54.03; LSD (Hour): 54.03; LSD (PXH): 93.58				
*:P<0.05 **: P<0.01										

Drug herbage yield (kg/da)

In the first harvests, different harvest periods alone and together with harvest hours and significant effects on drug herbage yields of *Origanum onites*. In the second harvests, harvest periods and hours alone and together had significant effects on drug herbage yields. There were significant differences also between the first and the second harvests. With regard to average drug herbage yields of different periods, the greatest drug herbage yield was obtained from the beginning of flowering period of the second harvests (175.92 kg/da). Such a value was 6% greater than the greatest value of the first harvests. Harvest hours were found to be significant in the second harvests and the greatest drug herbage yield was obtained from the plants harvested at 17:00 (186.32 kg/da). Considering the together effects of harvest periods and hours, the greatest drug herbage yield was obtained from 9:00 of 100% flowering period in the first harvests (186.49 kg/da) and from 17:00 of the beginning of flowering period in the second harvests (256.74 kg/da) (Table 3). The average drug herbage yield of the second harvests was about 37% greater than the average drug herbage yield of the second harvest. Güngör (2002) conducted a study on İzmir thymes with harvests and reported greater drug herbage yields than the present values.

Table 3. Effect of ontogenetics and diurnal variability on drug herbage yield (kg/da) of *Origanum onites* L. at I. and II. harvest

First harvest						Second harvest				
Hour	Budding	First flowering	50% flowering	100% flowering	Mean	Budding	First flowering	50% flowering	100% flowering	Mean
9.00 am	87.41 d	64.30 e	110.92 c	186.49 a**	112.28	112.39 bc	107.43 c	122.84 bc	-	114.22 B
1.00 pm	74.23de	108.64 c	80.49 de	185.21 a	112.14	111.15 c	163.61 b	139.81 bc	-	138.19 B
5.00 pm	82.62 d	134.58 b	124.34 bc	123.10 dc	116.16	147.06 bc	256.74 a*	155.16 bc	-	186.32 A*
Mean	81.42 C	102.50 B	105.25 B	164.93 A**		123.53 B	175.92 A*	139.27 B	-	
LSD (Period): 10.36 LSD (PXH): 17.95						LSD (Period): 29.63; LSD (Hour): 29.63; LSD (PXH): 51.32				
*:P<0.05, **: P<0.01										

Fresh leaf yield (kg/da)

Effects of ontogenetic and diurnal variabilities on fresh leaf yields of *Origanum onites* L. were found to be significant in both harvests. Considering the fresh leaf yields of different harvest period, the greatest fresh leaf yield was obtained from 100% flowering period (297.87 kg/da) in the first harvests. Considering the different harvest hours, the greatest fresh leaf yield was obtained from the harvests made at 17:00 (255.83 kg/da). Considering the effects of harvest periods and hours together, the greatest fresh herbage yield of the first harvests was obtained from harvests made at 13:00 of 100% flowering period (347.82 kg/da) and the lowest fresh leaf yield was obtained from the harvests made at 13:00 of the beginning of budding period (124.61 kg/da). In the second harvests, the greatest fresh leaf yield was obtained from the beginning of flowering period

(327.23 kg/da) and from the harvests made at 17:00 (351.42 kg/da). Considering the together effects of ontogenetic and diurnal variability, the greatest fresh leaf yield was obtained from the harvests made at 17:00 of the beginning of flowering period (482.05 kg/da) and the lowest fresh leaf yield was obtained from the harvests made at 13:00 of the beginning of budding period (213.16 kg/da) (Table 4). Kırman (1993) (in flowering period) and Bahtiyarca Bağdat (2011) reported greater fresh leaf yields in the second harvests than in the first harvests. Similar with the findings of previous studies, the greatest fresh leaf yields in both harvests were obtained from the harvests made in flowering period.

Table 4. Effect of ontogenetics and diurnal variability on fresh leaf yield (kg/da) of *Origanum onites* L. at I. and II. harvest

First harvest						Second harvest				
Hour	Budding	First flowering	50% flowering	100% flowering	**Mean**	Budding	First flowering	50% flowering	100% flowering	**Mean**
9.00 am	143.90 hı	173.09 fg	239.44 e	324.27 b	220.18 B	230.40 b	222.65 b	266.06 b	-	239.70 B
1.00 pm	124.61 ı	263.92 d	176.49 f	347.82 a**	228.21 B	213.16 b	276.99 b	283.16 b	-	257.77 B
5.00 pm	152.16 gh	358.69 a	290.96 c	221.52 e	255.83 A**	267.16 b	482.05 a*	305.06 b	-	351.42 A*
Mean	140.22 D	265.24 B	235.63 C	297.87 A**		236.90 B	327.23 A*	284.76 AB	-	
LSD (Period): 12.59; LSD (Hour): 10.91; LSD (PXH): 21.82						LSD (Period): 56.56; LSD (Hour): 56.56; LSD (PXH): 97.96				
*:P<0.05 **: P<0.01										

Drug leaf yield (kg/da)

Effects of ontogenetic and diurnal variability of drug leaf yield of *Origanum onites* L. were found to be significant in both harvests. In the first harvest, the greatest drug leaf yield was obtained from 1005 flowering period (162.84 kg/da) and the lowest drug leaf yield was obtained from the beginning of budding period (89.74 kg/da). Considering the together effects of harvest periods and hours in the first harvests, the greatest drug leaf yield was obtained from the harvests made at 9:00 of 100% flowering period (207.78 kg/da). In the second harvests, the greatest drug leaf yield was obtained from the beginning of flowering period (209.25 kg/da) and the lowest drug leaf yield was obtained from the beginning of budding period (124.49 kg/da). Effects of diurnal variability alone was not found to be significant in the first harvests, but significant in the second harvests. Drug leaf yields of the second harvest varied between 180.15 kg/da (17:00) - 119.02 kg/da (9:00). Considering the effects of variabilities together in the second harvests, the greatest drug herbage yield was obtained from the harvests made at 13:00 of the beginning of flowering period (260.00 kg/da) and the lowest from the harvests made at 9:00 of the beginning of flowering period (110.70 kg/da) (Table 5). Yalçıntaş Özyazıcı (2004) reported the greatest drug leaf yield of *Origanum onites* L. in the first and second harvests for the harvests made in 100% flowering period. Those findings comply with the present findings for the first harvest, but not with the findings for the second harvests.

Essential oil ratio (%)

Effects of harvest periods on essential oil ratios were found to be significant in both harvests and effects of harvest hours were found to be significant only in the second harvests. The greatest essential oil ratio was obtained from the beginning of budding period of the second harvest (4.41%). Such a value was 27% greater than the greatest value of the second harvests in 100% flowering period. Essential oil ratios at different hours of the second harvests varied between 4.36% (9:00) - 3.71% (13:00). Considering the effects of harvest period x harvest hour interactions, the greatest essential oil ratio was obtained from the harvests made at 9:00 of the beginning of budding period (4.82%) and the lowest from the harvests made at 13:00 of 50% flowering period (3.22%) (Table 6). Plant essential oil ratios generally vary based on climate, environmental and topographic conditions and morphogenetic, ontogenetic and diurnal variability (Arabacı *et al*., 2012). In present study, high essential oil ratios were achieved in harvests made at morning hours of flowering periods. It was reported in

previous studies conducted in Turkey with *Origanum onites* and the other thyme species that essential oil ratios varied with the harvest periods and hours. Similar with the present findings, greater essential oil ratios were generally observed in the second harvests (Yalçıntaş Özyazıcı, 2004; Kaçar *et al*., 2006; Avcı and Bayram, 2006). Sağlam (2005) reported the greatest essential oil ratio (1.58%) for morning hours of 50% flowering period; Yaldız *et al*. (2005) reported the greatest essential oil ratio (1.62%) for post-flowering period; Kızıl *et al*. (2009) reported the greatest essential ratio (3.65%) for afternoon hours of full flowering period. Arabacı *et al*. (2012) investigated the effects of diurnal variability on *Coridothymus capitatus* (L.) genotypes and reported the greatest essential oil ratio for full flowering period. Such findings support the differences observed in this study with ontogenetic and diurnal variability. Kaya *et al*. (2013) harvested *Thymus vulgaris* L. plants in one-hour intervals between the hours 06:00 - 17:00 and reported the greatest essential ratio (2.20%) for the harvests made at 06:00 and indicated that essential oil ratios decreased within the day. Similar with the findings of those studies, the greatest essential oil ratio of the present study was also obtained from the harvests made at morning hours.

Table 5. Effect of ontogenetics and diurnal variability on drug leaf yield (kg/da) of *Origanum onites* L. at I. and II. harvest

First harvest						Second harvest				
Hour	Budding	First flowering	50% flowering	100% flowering	**Mean**	Budding	First flowering	50% flowering	100% flowering	**Mean**
9.00 am	93.71 cde	72.90 e	117.14 bc	207.78 a**	122.88	119.02 b	110.70 b	127.35 b	-	119.02 B
1.00 pm	83.76 de	115.18 bcd	137.94 b	188.15 a	131.26	126.81 b	260.00 a*	122.96 b	-	169.93 A
5.00 pm	91.76 cde	140.82 b	137.64 b	92.61 cde	115.71	127.63 b	257.04 a	155.77 b	-	180.15A*
Mean	89.74 D	109.63 C	130.90 B	162.84 A**		124.49 B	209.25 A**	135.36 B	-	
LSD (Period): 18.83; LSD (PXH): 32.62						LSD (Period): 30.98; LSD (Hour): 30.98; LSD (PXH): 53.65				
*:P<0.05, **: P<0.01										

Table 6. Effect of ontogenetics and diurnal variability on essential oil ratio (%) (kg/da) of *Origanum onites* L. at I. and II. harvest

First harvest						Second harvest				
Hour	Budding	First flowering	50% flowering	100% flowering	**Mean**	Budding	First flowering	50% flowering	100% flowering	**Mean**
9.00 am	2.33	3.37	2.90	3.50	3.03	4.82	4.25	4.00	-	4.36 A**
1.00 pm	2.16	3.40	2.56	3.55	2.92	4.19	3.72	3.22	-	3.71 C
5.00 pm	1.83	3.26	2.77	3.37	2.81	4.22	3.85	3.83	-	3.97 B
Mean	2.11 C	3.34 A	2.75 B	3.47 A**		4.41 A**	3.94 B	3.68 C	-	
LSD (Period): 0.27						LSD (Period): 0.22; LSD (Hour): 0.22				
*:P<0.05, **: P<0.01										

Essential oil yield (l/da)

Different harvest periods and hours had significant effects on essential oil yields of *Origanum onites L.* in both harvests. In the first harvests of *Origanum onites* L., the greatest essential oil yield was obtained from the 100% flowering period (5.67 l/da) and the lowest from the beginning of budding period (1.88 l/da). Essential oil yields of different harvest hours varied between 4.16 l/da (13:00) - 3.29 l/da (17:00). Considering the together effects of ontogenetic and diurnal variability on essential oil yields of the first harvests, the greatest value was obtained from the harvests made at 9:00 of 100% flowering period (7.26 l/da) and the lowest from the harvests made at 17:00 of the beginning of budding period (1.67 l/da). In the second harvests of *Origanum onites* L., the greatest essential oil yield was obtained from the beginning of flowering period (8.08 l/da) and the lowest from the 50% flowering period (4.99 l/da). Considering the effects of diurnal variability, the greatest essential oil yield was obtained from the harvests made at 17:00 (7.08 l/da) and the lowest from the harvests made at 9:00 (5.19 l/da). Considering the effects of variabilities together, the greatest essential oil yield was

obtained from the harvests made at 17:00 of the beginning of flowering period (9.93 l/da) and the lowest from the plants harvested at 9:00 of the beginning of flowering period (4.72 l/da) (Table 7). Essential oil ratio and essential oil yield based on drug leaf yield vary with the plant genetics, ecological conditions of growing sites (Özyazıcı and Kevseroğlu, 2019). In previous studies on oregano (*Origanum onites* L.), Güngör (2002) and Baydar (2002) reported decreasing essential oil yields after the first harvest. Contrarily, greater essential oil yields were obtained from the second harvests in present study.

Table 7. Effect of ontogenetics and diurnal variability on essential oil yield (%) (kg/da) of *Origanum onites* L. at I. and II. harvest

First harvest						Second harvest				
Hour	Budding	First flowering	50% flowering	100% flowering	**Mean**	Budding	First flowering	50% flowering	100% flowering	**Mean**
9.00 am	2.16 def	2.43 def	3.41bcd	7.26 a**	3.81 AB	5.76 b	4.72 b	5.08 b	-	5.19 B
1.00 pm	1.80 ef	3.92 bc	4.22 bc	6.69 a	4.16 A*	5.34 b	9.58 a	3.95 b	-	6.29 AB
5.00 pm	1.67 f	4.60 b	3.82 bc	3.07 cde	3.29 B	5.36 b	9.93 a*	5.94 b	-	7.08 A*
Mean	1.88 C	3.65 B	3.81 B	5.67 A**		5.49 B	8.08 A**	4.99 B	-	
LSD (Period): 0.78; LSD (Hour): 0.67; LSD (PXH): 1.34						LSD (Period): 1.16; LSD (Hour): 1.16; LSD (PXH): 2.01				
*:P<0.05, **: P<0.01										

Conclusions

Active substances in the parts of plants used as drog vary depending on the development periods of the plant and the changes in temperature and light during the day.When these changes are taken into account, it is important to decide which part of the plant, which stage of development and when it is best to obtain the drog. Significant ecological factors (soil, water, climate, etc.) influence the yield of drog and the ratio of essential oil. Essential oils of oregano are used for various purposes. For this reason, Herba yield and essential oil ratio in thyme cultivation are among the most important parameters to focus on. Finally, greater fresh herbage yields, drug herbage yields, fresh leaf yields, drug leaf yields and essential oil ratios, which are important parameters for oregano production, were obtained from the plants harvested in the beginning of flowering and 100% flowering periods. With regard to harvest hours, the greatest fresh herbage yield, drug herbage yield, fresh leaf yield and drug herbage yield were obtained from the plants harvested at late afternoon hours and the greatest essential oil ratios were obtained from the plants harvested at morning hours.

Authors' Contributions

Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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