



The Effect of Oleander (*Nerium oleander* L.) Extracts on Seed Germination and Seedling Growth of Four *Pistacia* Species

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| ARTICLE INFO | ABSTRACT |
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| <p>Research Article</p> <p>Received : 17/10/2018 Accepted : 25/12/2018</p> <p>Keywords: Germination <i>Nerium oleander</i> <i>Pistacia</i> spp. Extract Gibberellic acid</p> | <p>In this study, the effects of the extracts obtained from flower, stem, leaf, branch and their mixture of <i>Nerium oleander</i> L. on the seed germination and seedling growth of four <i>Pistacia</i> species (<i>Pistacia terebinthus</i> L., <i>P. vera</i> L., <i>P. khinjuk</i> Stocks., <i>P. atlantica</i> Desf. and <i>P. terebinthus</i> L.) were investigated. Five hundred grams of <i>N. oleander</i> was taken from the plant parts and the extracts were mixed with distilled water (1.5 L). The extracts were applied to <i>Pistacia</i> species for 24 and 48 hours. The results showed that the extract had positive effects on their germination and growths of the species mentioned above. The highest germination rate for all the <i>Pistacia</i> species was obtained from the extracts of flower, while the lowest germination rate was recorded in the mixture of all parts of <i>N. oleander</i> mentioned above. Moreover, the treatment time was found to reduce the germination ratio. It was also found that the extracts from the stem of <i>N. oleander</i> were the most effective on the stem height of the <i>Pistacia</i> species studied. It was followed by the extracts from branches, leaves and flower, respectively. Furthermore, the findings indicated that the extracts from the stem of <i>N. oleander</i> had pronounced effect on the stem diameter of the <i>Pistacia</i> species studied. It was followed by the extracts from flower, leaves, and branches, respectively. In general, the effects of the extracts from several parts of <i>N. oleander</i> on the germination and other growing parameters were found to be almost comparable to those of synthetic promoter, Gibberellic Acid (GA₃).</p> |



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Introduction

Oleander (*Nerium oleander*) is an ever green shrub or small tree in the dogbane family Apocynaceae. It is the only species currently classified in the genus *Nerium*. It is native to a broad area from Morocco and Portugal eastward through the Mediterranean region and southern Asia to Yunnan in southern China. It occurs typically around dry stream beds (Pankhurst, 2005; İmalı and Koçer, 2016).

Oleander leaf extract is used to treat congestive heart disease and applied topically to treat skin disorders. It should only be taken under supervision and dosage of a licensed herbalist and physician. Moreover, because of the high amount of silicate in the root of oleander, oleander is known as a very well fire retardant (Pankhurst, 2005). The most significant of toxins in the oleander plant are oleandrin and nerverine, which are cardiac glycosides (Goetz, 1998; İmalı and Koçer, 2016; Khan, 2017). Ursolic and oleanolic acid are also found in *Nerium oleander* as one of the main components (Balkan et al., 2018; Cao et al., 2018).

The chemical secretion of one plant can inhibit the growth or germination of the same plant or different plants and microorganisms, which is called "allelopathy" (Rice, 1984; Latif et al., 2017). Allelochemicals are found everywhere in the nature for products and can be released from all plant tissues including leaves, stem, roots, flowers, seeds, rhizomes, pollen, bark and buds (Latif et al., 2017). So far, there have been many studies on the allelopathic properties of various plants (Alam, 1990; Alam et al., 1990; Ak et al., 1995; Alam et al., 1998; Friedman, 2017; Latif et al., 2017; Duke and Lydon, 2018; Einhellig, 2018; Shah et al., 2018). However, a few studies (Karaaltın et al., 2004) have been found on the effect of oleander extract on the germination of wheat plants. Allelopathic effects of oleander (*N. oleander*) flower extract on seed germination and seedling development of *Lolium multiflorum* were investigated (Uslu et al., 2018).

Some researchers have studied on the allelopathic effect of oleander on the 9 plant species: *Abutilon theophrastii* Medik., *Amaranthus retroflexus* L., *Avena sterilis* L., *Conium maculatum* L., *Descurania sophia* (L.) Webb. Ex Prant, *Lepidium sativum* L., *Lolium perenne* L., *Rumex crispus* L., *Trifolium repens* L. (Kadioglu and Yanar, 2004). It was found that oleander had positive effects on the germination of the plants mentioned above. Also, Karaaltin et al. (2004) reported that the oleander extracts had positive effects on the germination of wheat.

So far, no study has been done on the effect of oleander extracts on *Pistacia* species. Therefore, with this study, we aimed to find out the allelopathic effect of oleander extract on the germination and growth of the seed of four *Pistacia* species.

Material and Methods

Material

Oleander plants (*Nerium oleander* L.; Apocynaceae) were collected in Kahramanmaraş province which is located in the East Mediterranean of Turkey. For germination and growth test, seed and seedlings of four *Pistacia* species (*Pistacia vera*, *Pistacia terebinthus*, *Pistacia atlantica* and *Pistacia khinjuk*) in the greenhouse were used.

Preparation of the Extract

Firstly, different parts of the oleander plant such as leaf, stem, flower, branch as well as their mixture, were washed under tap water and then cut into small pieces by scissors. Five hundred grams of each part were placed into glass beaker (2.5 L) and then mixed with distilled water (1.5 L). Finally, the resultant mixture was filtered by filter paper. The filtrate was used as an extract solution.

Determination of Seed Germination and Growth

For each *Pistacia* species studied herein and the extract of each part, at least 5 seeds (having 3 replications) were put into a glass jar and treated with the extracts for the treatment periods of 24 and 48 h. For each *Pistacia* species and treatment period, seven treatments were conducted. Therefore, for each species and treatment time, 105 seeds were used. All the extract-treated seeds were compared with untreated ones and seeds treated with conventional GA₃ (Gibberellic Acid) (250 ppm) used as reference promoter. For seed germination, stem diameter and height, the average number of the three replications were reported in this study.

The treated and untreated control seeds located in each box were transferred to greenhouse medium consisting of sand, soil and peat mixture having a weight ratio of 1:1:1 in boxes (9×5 cm). Finally, the germination and development of the *Pistacia* species were recorded by following conventional methods. At least, the average of 10 measurements was reported as mean value in this study. The whole data were statistically analyzed by using Duncan's mean separation test (P<0.05).

Results and Discussion

Table 1 shows the effect of the extracts obtained from various parts of *N. oleander* on the germination of the seeds of *Pistacia* species for 24 h in view of mean values of the germinated seed number, standard deviations and Duncan's Mean Separation Test. As can be seen from Table 1, the extracts of *N. oleander* play an important role on the germination of the seeds of all the *Pistacia* species studied here. On the basis of the extracts of all the parts of *N. oleander*, the extracts are found to be mostly effective on the seed germination of *P. atlantica*. It was followed by *P. vera*, *P. terebinthus* and *P. khinjuk*, respectively.

Table 1. Duncan's mean separation test results of the average number of the germinated seed of *Pistacia* species depending upon the extracts obtained from various parts of *N. oleander* for 24 h as well as GA₃ and control

| Parts of <i>N. oleander</i> | Average number of the germinated seed | | | | |
|-----------------------------|---------------------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| | <i>P. vera</i> | <i>P. khinjuk</i> | <i>P. atlantica</i> | <i>P. terebinthus</i> | General means |
| Control | 3.00±0.00 ^{bcd*} | 2.00±0.00 ^{ef} | 2.67±0.58 ^{cde} | 2.00±0.00 ^{ef} | 2.42±0.14 ^c |
| Leaf | 3.67±0.58 ^{ab} | 3.00±0.00 ^{bcd} | 3.00±0.00 ^{bcd} | 2.33±0.58 ^{def} | 3.00±0.29 ^b |
| Branch | 3.33±0.58 ^{abc} | 2.33±0.58 ^{def} | 3.33±0.58 ^{abc} | 3.00±0.00 ^{bcd} | 3.00±0.43 ^b |
| Stem | 3.67±0.58 ^{ab} | 2.33±0.58 ^{def} | 3.67±0.58 ^{ab} | 3.00±0.00 ^{bcd} | 3.17±0.43 ^{ab} |
| Flower | 3.33±0.58 ^{abc} | 2.67±0.58 ^{cde} | 4.00±0.00 ^a | 4.00±0.00 ^a | 3.50±0.29 ^a |
| MAP | 1.67±0.58 ^f | 3.00±0.00 ^{bcd} | 3.33±1.15 ^{abc} | 1.67±0.58 ^f | 2.42±0.58 ^c |
| GA ₃ | 4.00±0.00 ^a | 2.67±0.58 ^{cde} | 3.67±0.58 ^{ab} | 2.67±0.58 ^{cde} | 3.25±0.43 ^{ab} |
| General mean | 3.24±0.41 ^a | 2.57±0.33 ^b | 3.38±0.49 ^a | 2.67±0.25 ^b | |

*Values with the same letters are not significantly different (Duncan's Mean Separation Test), MAP: Mixtures of all the parts

Table 2 Duncan's mean separation test results of the average number of the germinated seed of *Pistacia* species depending upon the extracts taken from various parts of *N. oleander* as well as GA₃ and control for 48 h.

| Parts of <i>N. oleander</i> | Average number of the germinated seed | | | | |
|-----------------------------|---------------------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| | <i>P. vera</i> | <i>P. khinjuk</i> | <i>P. atlantica</i> | <i>P. terebinthus</i> | General means |
| Control | 3.33±0.58 ^{abc} | 2.33±0.58 ^{def} | 1.67±0.58 ^{fg} | 3.00±0.00 ^{bcd} | 2.58±0.43 ^c |
| Leaf | 2.00±0.00 ^{efg} | 1.33±0.58 ^g | 1.67±0.58 ^{fg} | 1.67±0.58 ^{fg} | 1.67±0.43 ^d |
| Branch | 3.33±0.58 ^{abc} | 2.00±0.00 ^{efg} | 3.00±0.00 ^{bcd} | 3.00±0.00 ^{bcd} | 2.83±0.14 ^{bc} |
| Stem | 3.33±0.58 ^{abc} | 2.00±0.00 ^{efg} | 3.67±0.58 ^{ab} | 3.33±0.58 ^{abc} | 3.08±0.43 ^b |
| Flower | 3.67±0.58 ^{ab} | 1.67±0.58 ^{fg} | 3.00±0.00 ^{bcd} | 2.33±0.58 ^{def} | 2.67±0.43 ^c |
| MAP | 3.00±0.00 ^{bcd} | 1.67±0.58 ^{fg} | 2.67±0.58 ^{cde} | 2.67±0.58 ^{cde} | 2.50±0.43 ^c |
| GA ₃ | 4.00±0.00 ^a | 2.67±0.58 ^{cde} | 4.00±0.00 ^a | 3.33±0.58 ^{abc} | 3.50±0.29 ^a |
| General mean | 3.24±0.33 ^a | 1.95±0.41 ^c | 2.81±0.33 ^b | 2.76±0.41 ^b | |

*Values with the same letters are not significantly different (Duncan's Mean Separation Test), MAP: Mixtures of all the parts

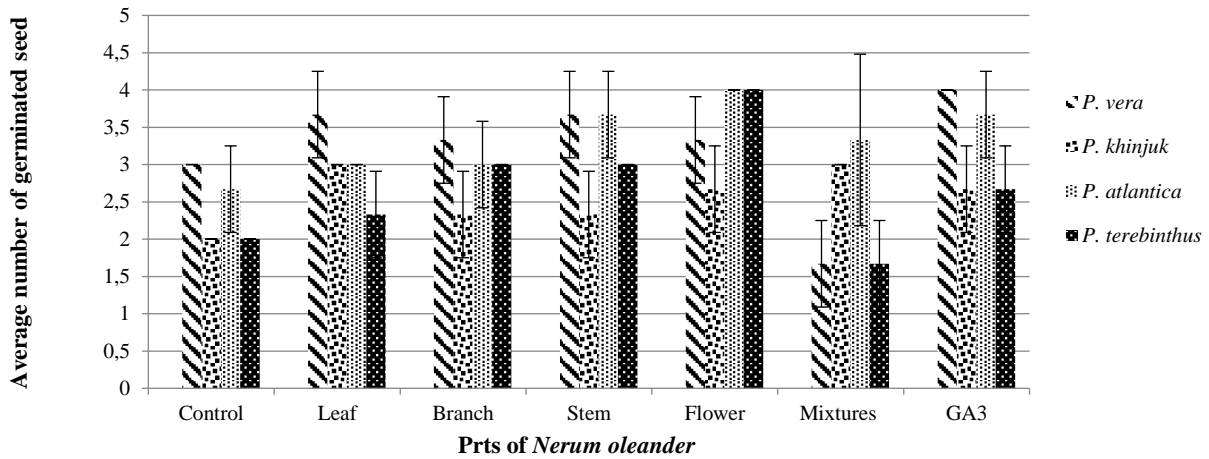


Figure 1 The effect of the extracts taken from various parts of *N. oleander* on the average germinated seed number of *Pistacia* species for 24 h.

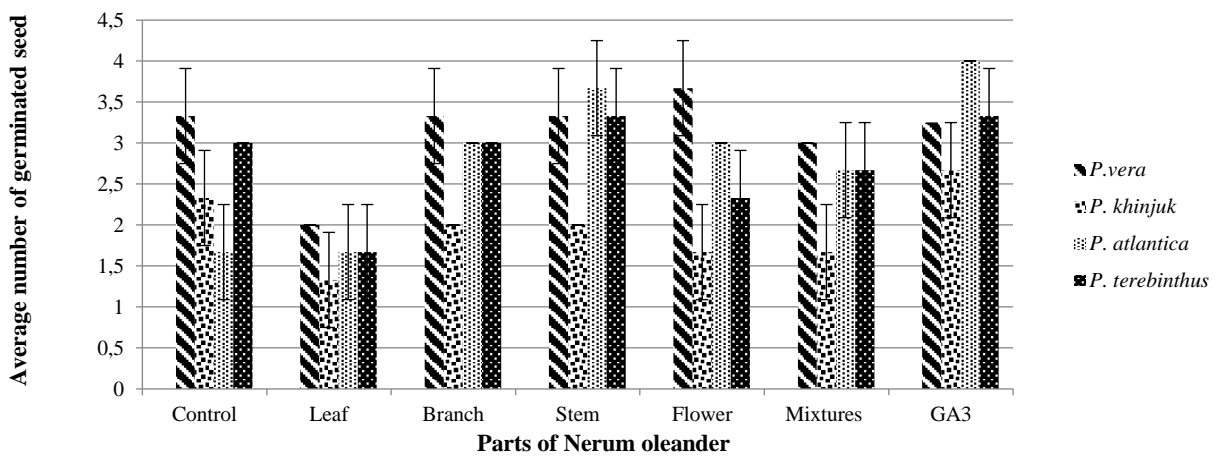


Figure 2 The effect of the extracts obtained from various parts of *N. oleander* on the average germinated seed number of *Pistacia* species for 48 h.

As shown in Fig. 1, the extracts obtained from the flower of the oleander plant were the most effective part of *N. oleander* on the seed germination of the *Pistacia* species, which is followed by stem, leaves or branches and mixtures of all the parts studied, respectively. However, the GA₃ (Gibberellic Acid) promoter resulted in the greatest seed germination.

Table 2 indicates the effect of the extracts taken from various parts of *N. oleander* on the germination of the seeds of *Pistacia* species for 48 h in view of mean values of seed, standard deviations and Duncan's Mean Separation Test. As can be seen from this table, all the extracts obtained from various parts of *N. oleander* have an important role on the germination of the seeds of all the *Pistacia* species studied here in comparison to control and somewhat to GA₃ promoter.

For *in vitro* seed germination in the *Pistacia* species, different concentrations of GA₃ were tested to provide the best rootstock production and percentages of seed germination of *P. vera* var. Siirt, *P. khinjuk* Stocks, *P. atlantica* Desf., *P. terebinthus* L. were detected as 80%, 92%, 60% and 48%, respectively (Onay et al., 2016). At the same time, seed germination of *Lolium multiflorum* was found to be 93% in *N. oleander* flower extracts by Uslu et al., (2018). The extracts of the flower are the most effective part of *N. oleander* on the seed germination of the *Pistacia* species, which is followed by GA₃, stem, branches, leaves and the mixtures of all the parts studied, respectively.

When Table 1 is compared to Table 2, it can be said that the general mean values of the whole seed numbers of all the *Pistacia* species obtained from 48 h application are found to be less than those of the seed numbers for 24 h. It is also clear from Fig. 2 that the number of seed germinated decreases when increasing the germination time. This phenomenon might be attributed to the side effect.

As seen from Fig. 2, on the basis of the extracts of all the parts of *N. oleander*, the extracts were most effective on the seeds germinated of *P. vera*. It was followed by *P. atlantica*, *P. terebinthus* and *P. khinjuk*, respectively. On the other hand, the highest number of seeds germinated were obtained from the flower extract and it was, followed by stem, branch and leaf extracts in *P. vera* for 48 h. The mixture of all the extracts studied here gave average seed numbers as is expected. It is also important to notice that the leaf extract has a negative effect on the seed germination for all the *Pistacia* species for 48 h in comparison to control.

Table 3 demonstrates the effects of the extracts taken from various parts of *N. oleander* on the stem height of *Pistacia* species in view of mean values of seed, standard deviations and Duncan's Mean Separation Test. As can be seen from Table 3 and Fig 3, the whole extracts obtained from a various part of *N. oleander* show important role on the stem height of all the *Pistacia* species studied here in comparison to control.

Table 3 Duncan's mean separation test results of the stem height of *Pistacia* species as functions of the extracts obtained from various parts of *N. oleander* as well as GA₃ and control

| Parts of <i>N. oleander</i> | Stem Height (cm) | | | | General means |
|-----------------------------|--------------------------|--------------------------|---------------------------|--------------------------|---------------------------|
| | <i>P. vera</i> | <i>P. khinjuk</i> | <i>P. atlantica</i> | <i>P. terebinthus</i> | |
| Control | 46.49±3.35 ^{ab} | 31.19±1.86 ^d | 32.11±1.99 ^d | 24.43±3.05 ^d | 33.55±2.56 ^d |
| Leaf | 57.78±2.04 ^a | 35.23±4.56 ^{bc} | 40.25±1.03 ^{bc} | 29.14±0.96 ^d | 40.6±2.15 ^{bc} |
| Branch | 58.46±1.53 ^a | 36.31±4.78 ^{cd} | 39.59±8.05 ^{abc} | 35.13±4.61 ^{bc} | 42.37±4.74 ^{abc} |
| Stem | 59.16±0.84 ^a | 39.92±5.91 ^{bc} | 41.24±10.2 ^{abc} | 35.3±9.26 ^{bc} | 43.91±6.55 ^{ab} |
| Flower | 52.81±3.95 ^a | 34.84±4.92 ^c | 35.39±4.21 ^c | 32.44±2.14 ^d | 38.87±3.81 ^c |
| MAP | 48.84±1.42 ^{ab} | 30.54±0.55 ^d | 35.43±4.45 ^d | 25.07±4.56 ^d | 34.97±2.75 ^d |
| GA ₃ | 61.43±1.54 ^a | 42.24±4.27 ^{ab} | 44.56±6.75 ^{ab} | 35.81±7.49 ^{bc} | 46.01±5.01 ^a |
| General mean | 55±2.1 ^a | 35.75±3.83 ^b | 38.37±5.24 ^b | 31.05±4.58 ^c | |

*Values with the same letters are not significantly different (Duncan's Mean Separation Test), MAP: Mixtures of all the parts

Table 4 Duncan's mean separation test results of on the stem diameter of *Pistacia* species as functions of the extracts taken from various parts of *N. oleander* as well as GA₃ and control

| Parts of <i>N. oleander</i> | Stem Diameter (mm) | | | | General means |
|-----------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | <i>P. vera</i> | <i>P. khinjuk</i> | <i>P. atlantica</i> | <i>P. terebinthus</i> | |
| Control | 5.64±0.67 ^b | 4.26±0.07 ^c | 4.55±0.66 ^c | 3.57±0.58 ^c | 4.5±0.49 ^c |
| Leaf | 6.82±0.43 ^a | 5.98±0.17 ^b | 5.7±0.51 ^b | 4.16±0.68 ^c | 5.67±0.45 ^b |
| Branch | 6.75±0.63 ^a | 5.63±0.55 ^b | 5.52±0.44 ^b | 4.03±0.04 ^c | 5.48±0.41 ^b |
| Stem | 7.58±0.49 ^a | 6.7±0.61 ^a | 6.69±0.6 ^a | 5.01±1 ^b | 6.49±0.67 ^a |
| Flower | 7.61±0.59 ^a | 6.91±0.28 ^a | 6.79±0.42 ^a | 4.38±0.65 ^c | 6.42±0.48 ^a |
| MAP | 5.53±0.41 ^b | 4.58±0.58 ^c | 4.57±0.66 ^c | 3.74±0.59 ^c | 4.6±0.56 ^c |
| GA ₃ | 8.61±0.53 ^a | 7.2±0.85 ^a | 7.0±0.18 ^a | 4.37±0.57 ^c | 6.8±0.53 ^a |
| General mean | 6.93±0.53 ^a | 5.89±0.44 ^b | 5.83±0.49 ^b | 4.18±0.59 ^c | |

*Values with the same letters are not significantly different (Duncan's Mean Separation Test), MAP: Mixtures of all the parts

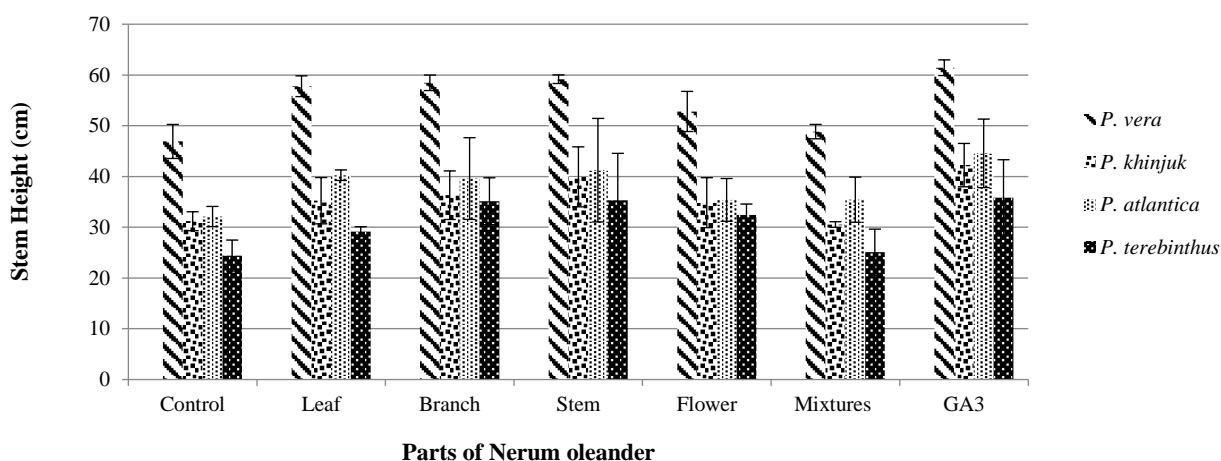


Figure 3 The effects of the extracts taken from various parts of *N. oleander* on the stem height of *Pistacia* species

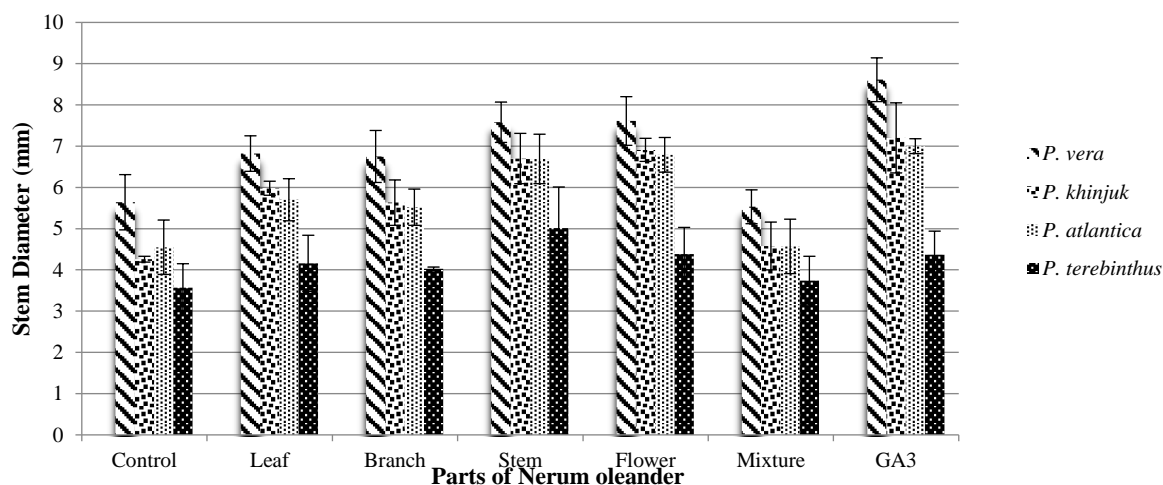


Figure 4 The effects of the extracts obtained from various parts of *N. oleander* on the stem diameter of *Pistacia* species

The 10% and 7.5% *Nerium oleander* leaf extracts used for germination of *Cyperus rotundus* seeds were reported to be 90% more efficient in germination of *Cyperus rotundus* seeds (Al-Samarai et al., 2018). The extracts taken from the stem extract of *N. oleander* is determined to be the most efficient promoter on the stem height of the *Pistacia* species studied, and it was followed by the extracts obtained from branches, leaves and flower, respectively. In general, for all the extracts used herein, *P. vera* has the highest stem height. It was followed by *P. atlantica*, *P. khinjuk* and *P. terebinthus*, respectively. It is also interesting to report that there is no significant difference in stem height values between all the extracts and GA₃ for *P. vera* (Table 3).

Table 4 depicts the effects of the extracts obtained from various parts of *N. oleander* on the stem diameter of *Pistacia* species in view of mean values of seed, standard deviations and Duncan's Mean Separation Test. As can be seen from Table 4 and Fig. 4, the whole extracts taken from a various part of *N. oleander* are determined to be effective on the stem diameter of all the *Pistacia* species studied here in comparison to control. It is also clear from this table that the extracts from the stem extracts of *N. oleander* are the most effective promoter on the stem diameter of the *Pistacia* species studied and it was followed by the extracts obtained from flower, leaves and branches, respectively. Moreover, *P. vera* has the highest stem height and it is followed by *P. khinjuk*, *P. atlantica* and *P. terebinthus*, respectively. However, the effects of the extracts taken from various parts of *N. oleander* on the stem diameter are lower than GA₃ promoter (see Fig. 4). It has been reported that different applications of gibberellic acid give successful results in seed germination and growth studies of *P. khinjuk* (Acar et al., 2017).

In this study, 250 ppm GA₃ was used in addition to oleander extracts. Seeds were grown in a greenhouse environment. In a study conducted by Nikpeyma, (2016), germination of different *Pistacia* species applied GA₃ was investigated in different environmental conditions.

As a result of the application on *Pistacia* species of *N. oleander* extract was obtained statistically significant results. In this study, it was determined that *N. oleander* extracts of different species of *Pistacia* made positive contributions to seed and seedling development compared to 250 ppm GA₃ application.

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