

## Breeding History, Phytochemical Properties and Distribution of *Paeonia* spp. in Iran

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### Abstract

*Paeonia* is the only genus of Paeoniaceae. In 1753, the *Paeonia* genus was described for the first time, and gradually more attention to *Paeonia* was paid. With the expansion of the *Paeonia* industry worldwide, the need to increase the number of suitable cultivars has urgently soared. Today, main methods of breeding techniques including selection and crossing are applied for breeding this plant. The horticultural utilization of *Paeonia* includes as ornamental plants, food, and medicinal applications. Different parts of *Paeonia* genus have various phytochemical compounds, although the root is of special interest to phytochemists because of its medicinal uses. The most important compounds in *Paeonia* are Paeonol from the phenols and paeoniflorin from the monoterpene glycosides. These compounds have various biological effects such as anti-inflammatory, anti-tumor, and antioxidant activity. There is little information about *Paeonia* genus in Iran. Nevertheless, until today, 5 species of the *Paeonia* genus have been reported in Iran that contain *P. mascula*, *P. tomentosa*, *P. wittmanniana*, *P. wendelboi*, and *P. archibaldii*. In *P. wittmanniana*, an extensive debate has taken place in the taxonomists. The first group considers *P. wittmanniana* and *P. tomentosa* identical, whereas the other group regards them as two separate species.

**Keywords:** Iranian *Paeonia*; Monoterpene Glycoside; Paeoniaceae; Paeoniflorin; Paeonol

## Introduction

The genus *Paeonia* was classified in the Ranunculaceae until the 20th. Recently, based on the characteristics of the stamen and specific chemical components, this genus was classified in separate family known as Paeoniaceae [1] and it's the only genus in Paeoniaceae family. The species and varieties of this genus include perennials and herbaceous plants with about 33 species and 26 subspecies distributed mostly over temperate regions of northern hemisphere. The wild woody *Paeonia* species are found only in China, while the herbaceous species are widely spread in central and East Asia, as well as Northwest America [2]. In latest classification, the *Paeonia* genus divided into three groups; sect. *Moutan*, sect. *Onaepia* and sect. *Paeonia* [3].

The peony cultivation and using it as ornamental plant started first in China, which was then released into other East Asian countries such as Japan and Korea. In the 18th century, varieties of Chinese trees and herbaceous peonies became very popular and were transferred to Europe and North America. In the 19th century, by establishing breeding programs as well as considerable crossings, many new cultivars of this genus were released as ornamental plants. Herbaceous peony varieties have been introduced as a new cut flower and several large collections of peony cut flowers have been established around the world, such as in China, Holland, North America, and New Zealand. In 2011, China's ministry of health approved seed oil extracted from *P. suffruticosa* and *P. ostii* as a new food source, consequently oil peony planting rapidly expanded [2]. In this review, breeding history, phytochemical properties, horticultural utilization and distribution of *Paeonia* spp. in Iran are discussed.

The application of peony cultivars as ornamental plants, is included three main aspects landscape architecture, potted plants and cut flowers. The special peony gardens are the most famous in China and Japan. In Luoyang, Heze and Beijing, China, and Shimane, Japan, there are over 30 large special peony gardens, with the largest number in Luoyang [4]. In the international flower market, herbaceous peony is becoming more and more popular as a new cut flower. More than 25 countries are engaged in the production of herbaceous peony cut flowers, with the

Netherlands being the largest global producer, annually producing more than 48 million herbaceous peony cut flowers [5]. The other utilization of *Paeonia* is the medicinal applications that will discuss this further.

Peony is usually propagated by division of a tuberous root with 3 to 5 vegetative and dormant buds [6]. Several studies have been carried out on the tissue culture of Peony. Although some progress has been made in this field, indirect shoot induction through callus is very difficult and requires a long time. One of the problems of callus production in peonies is excessive browning of the tissue. There are also few reports on peony somatic embryogenesis [7]. Tissue culture has been investigated in *Paeonia mascula* and the results showed that BAP, NAA and GA3 treatment increases branching [8].

Breeding history, phytochemical properties and distribution of *Paeonia* spp. in Iran is discussed in this review.

## Genetic Breeding of *Paeonia*

With the expansion of the *Paeonia* industry around the world, the need to increase the number of suitable cultivars has urgently soared. However, *Paeonia* breeding is confronted with difficulties such as insufficient knowledge of the extent of germplasm resources, basic breeding techniques, fragmentary breeding knowledge, unclear breeding goals as well as low breeding efficiency [2].

Knowing the genetic characteristics of a plant can play an important role in its improvement. Weak self-compatibility, high chromosomal heterozygosity, and long juvenile period are the most important limiting factors in genetic studies of *Paeonia*. In recent years, some scientific institutions have paid more attention to *Paeonia* and some of its genetic mechanisms are gradually being investigated. Based on 33 species of *Paeonia*, more than 8000 varieties of this plant have been identified based on the color and type of flowers. Today, main methods of breeding techniques including selection and crossing are applied for breeding this plant [2].

Selection is still one of the most important methods of *Paeonia* breeding. This is a simple method in the

breeding of *Paeonia*, which mainly focuses on the selection of stem and bud mutation. Before 1960 in China, most of the new cultivars were modified and introduced by this method. Molecular breeding is a very efficient method to overcome the shortcomings of traditional breeding, which shortens the breeding cycle. Currently, *Paeonia* tissue culture mainly uses embryos or buds to produce secondary buds by direct tissue culture method. For this plant, it is dif-

icult to differentiate and produce callus and then produce stem. This limitation has caused the inability to edit genes in *Paeonia*. Although there are still many challenges in the success of molecular breeding of *Paeonia*, the mechanisms of several traits in this plant have been investigated, which have practical aspects in the production of this plant [2]. The history of *Paeonia* spp. identification and classification is showed in Table 1.

**Table 1:** The history of *Paeonia* spp. identification and classification

| Year | Events  | Reference |
|------|---|-----------|
| 1753 | The <i>Paeonia</i> genus was described for the first time and named one species <i>Paeonia officinalis</i> L., with two varieties, <i>feminea</i> and <i>mascula</i>  | [9]       |
| 1794 | The first tree peony plant was introduced and called <i>Paeonia suffruticosa</i> , from Guangzhou, China  | [10]      |
| 1818 | The first monograph on <i>Paeonia</i> was published, which included 13 species divided into two major species groups, <i>fruticosa</i> and <i>herbaceae</i>   | [11]      |
| 1829 | The first <i>Paeonia</i> species of the New World (North America) was recorded and named <i>Paeonia brownii</i>   | [12]      |
| 1890 | The <i>Paeonia</i> genus was reclassified into three subgenera, <i>Moutan</i> , <i>Onaepia</i> , and <i>Paeonia</i>   | [13]      |
| 1943 | The genus divided into three major groups: sect. <i>Moutan</i> , including all woody peonies; sect. <i>Onaepia</i> , including all herbaceous peonies in the New World; and sect. <i>Paeon</i> (= <i>Paeonia</i> ), including all herbaceous peonies in the old World | [3]       |
| 1963 | The intersectional hybrid peonies, so called Itoh hybrids, were bred using pollen of tree peony <i>P. suffruticosa</i> "Alice Harding" on the white, semi-double herbaceous peony "Kakoden"   | [14]      |
| 1988 | The chromosomal numbers of <i>Paeonia</i> spp. were calculated  | [15]      |
| 1991 | Propose to classify species and cultivars according to their morphological characteristics, flavone/flavanol patterns in the petals   | [16]      |
| 1997 | Propose to classify species and cultivars according to DNA analysis   | [17]      |

## Biochemical Studies and Medicinal Applications

The root or bark of many species of this genus is usually used as a traditional medicine to treat various diseases. For example, Cortex Moutan is the dried bark of the *P. suffruticosa* root, which is called mudanpi in Chinese and is used to remove heat, cool the blood, improve blood circulation, and reduce blood stasis. Also, the seeds of some species have high amounts of unsaturated fatty acids and are a suitable source for use as edible oil. In 2014, *P. suffruticosa* seed oil was approved as a new food source by the Chinese Ministry of Health [4].

Due to the outstanding characteristics of plants of the Paeoniaceae family, complete phytochemical and medicinal research has been conducted on these plants for food and medicinal applications during the past few decades. Among the different parts of the *Paeonia* plant, the

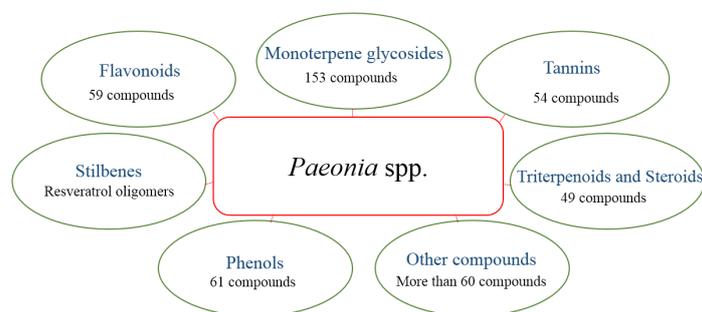
root is of special interest to phytochemists because of its medicinal uses. Although the leaves, flowers, and stems of the plant have been studied to investigate chemical compounds [4].

Plants of the Paeoniaceae family are known for producing monoterpene glycosidic compounds and other metabolic compounds such as flavonoids, tannins, stilbenes, triterpenoids, steroids, and phenolic compounds. New pharmacological research has shown that compounds and extracts obtained from plants of this genus have wide biological activities such as antioxidant, anti-inflammatory, anti-tumor effects, liver protection, cardiovascular protection, and neuron protection [4].

According to the latest findings, up to now 153 monoterpene glycosides, 59 flavonoids, 53 tannins, 15 stilbene, 49 triterpenoid and steroids, 61 phenolics, and more

than 60 compounds, including alkyl hydrocarbons, fatty acids, fatty alcohols, L-amino acids, nucleotides, coumarin, anthraquinone, sugars, amygdalin, benzamide, and (Z)-

hex-3-enal, have been isolated in the *Paeonia* genus [4] (Figure 1).



**Figure 1:** Phytochemical compounds in *Paeonia* genus

In particular, there are 3 key bioactive compounds in the plants of this genus that has been used therapeutically or have been clinically tested, which include total glucosides of peony (TGP), paeoniflorin, and paeonol. TGP includes paeoniflorin, paeonin, albiflorin, lactiflorin, and benzoyl-paeoniflorin compounds, that paeoniflorin being the most important active component of this compound [18]. The two most important bioactive, paeoniflorin and paeonol are in the monoterpene glycosides and phenols categories, which will be discussed further.

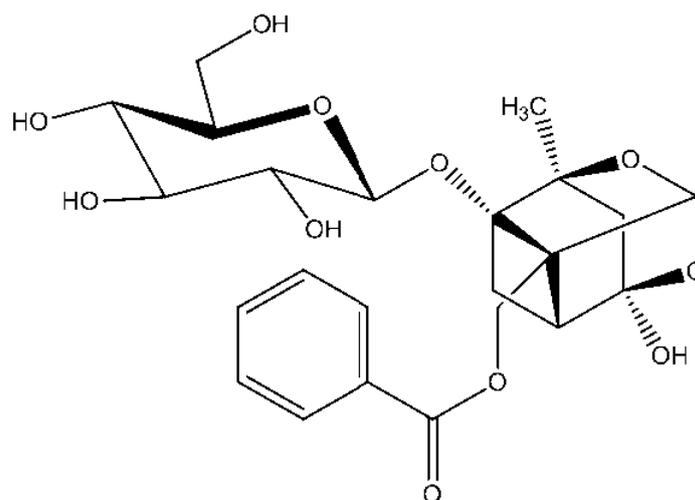
### Monoterpene Glycosides

To date, 153 monoterpene glycoside compounds have been reported in the genus *Paeonia*. This group of compounds is the dominant compound in all *Paeonia* plants. Most of these compounds are well known due to their cage-like pinane skeleton, for example, paeoniflorin. While other monoterpene compounds are p-menthane, such as paeonilactone (A to C). Pinane and p-menthane compounds are types of monoterpenes and their glycosidic compounds are the characteristics of *Paeonia* plants. It should be explained that a small amount of osmane and myrceane types of monoterpenes have also been observed in this genus [4].

In the *Paeonia*, glycosidic monoterpene compounds exist due to various structural lies. First, the cage-

like pinane skeleton is the most abundant type of monoterpene glycoside in the genus *Paeonia*. Most of these compounds are mono-cargo dibenzoate monoterpene glycosides that differ in the substitution of their aromatic rings, such as galloyl, vanilloyl, hemisuccinyl, and hemiglutaryl. Paeoniflorin compound is the most important active compound present in almost all species of the *Paeonia* genus. Second, the pinane skeleton is highly unstable and highly variable. It has been reported that other monoterpene glycoside compounds such as lactiflorin and paeobrin have the same biosynthetic pathway as paeoniflorin [4].

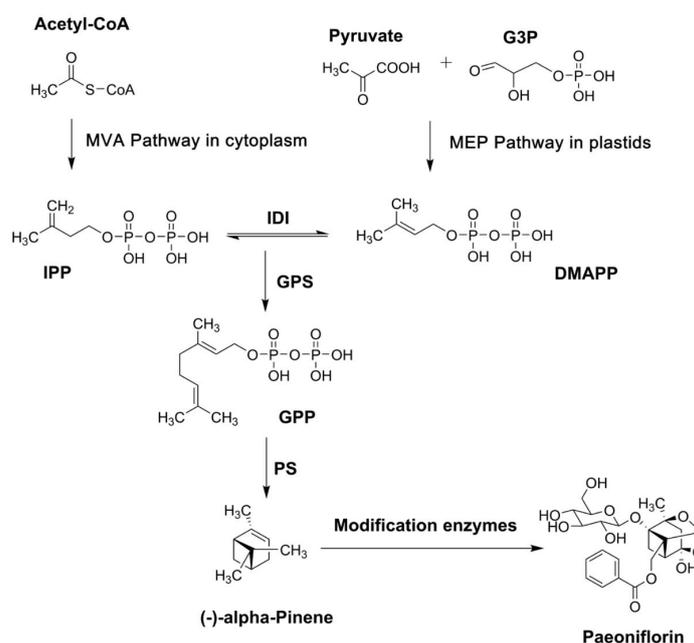
Paeoniflorin ( $C_{23}H_{28}O_{11}$ ) belonged to the monoterpene glycoside compounds. The most important active substance in *Paeonia* is paeoniflorin (almost in all species) which has broad and powerful biological effects. There are found often in fresh unripe fruits and roots in the cortex. Paeoniflorin has some biological effects such as anti-inflammatory, immune system regulating, anti-tumor activity, cardiovascular protective, therapeutic effects on central nervous diseases, antioxidant, diabetes improvement, prevention of arterial thrombosis, liver oxidative protection, and liver cholesterol regulation [4]. Paeoniflorin is insoluble in water but soluble in ethanol and other organic compounds. However, due to its size, it cannot be separated by steam distillation [19]. Structure of paeoniflorin is shown in Figure 2.



**Figure 2:** Structure of Paeoniflorin compound

Based on reported research, paeoniflorin biosynthesis pathway could be branched into three stages apart. During first step, isopentenyl pyrophosphate (IPP), and dimethylallyl diphosphate (DMAPP), as common precursors, are synthesized. Then the pathway continues into two

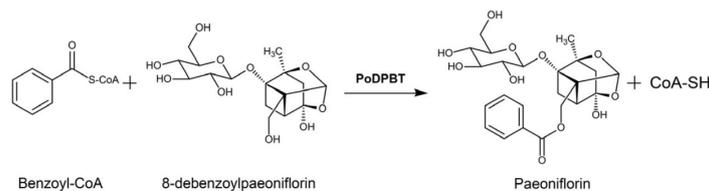
routes for producing IPP and DMAPP. The first is the mevalonate (MVA) pathway located in the cytoplasm, and the second one is the 2-C-methyl-D-erythritol4-phosphate (MEP) pathway located in plastids [20]. Biosynthesis pathway of paeoniflorin is shown in Figure 3.



**Figure 3:** The biosynthetic route to the formation of paeoniflorin. G3P, glyceraldehyde-3-phosphate; MVA, mevalonate; MEP, 2-C-methyl-D-erythritol4-phosphate; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl diphosphate; IDI, isopentenyl diphosphate isomerase; GPS, geranylgeranyl pyrophosphate synthase; GPP, geranyl diphosphate; PS, pinene synthase [20]

Also, it was reported that PoDPBT, an O-benzoyl-transferase belonging to the BAHD family, can catalyze the benzylation of 8-debenzoylpaeoniflorin to paeoniflorin in *Paeonia ostii*. The PoDPBT enzyme was identified as the

first enzyme involved in the modification stage of paeoniflorin biosynthesis. These results suggested PoDPBT could utilize benzoyl-CoA and 8-debenzoylpaeoniflorin to synthesize paeoniflorin [21] (Figure 4).



**Figure 4:** PoDPBT catalyses the benzoylation in the paeoniflorin biosynthesis [21]

## Phenols

There are about 61 phenolic compounds and their analogs in all plants of the *Paeonia* genus, which are mostly present in the roots and root cortex. Some of these compounds such as phenethyl alcohol, gallic acid, and benzoic acid are widely found in plants of this genus [4]. One of the most important phenolic compounds in this genus is paeonol, which is often found in the root cortex and has wide medicinal uses.

Paeonol ( $C_9H_{10}O_3$ ) is a simple phenolic compound that has a phenolic hydroxyl group and methoxy group [19], and is a white crystalline powder, that has molecular weight 166.17 g/mol and melting point 52.5 °C [22]. Paeonol easily dissolves in hot water and polar organic solvents such as ethanol and methanol, but its solubility in cold water is very low. This is a volatile compound that can be isolated by steam distillation. Paeonol has wide pharmacological and physiological effects, including anti-inflammatory, anti-tumor, antioxidant activity, anti-allergic, anti-diabetic, platelet aggregation inhibition, anti-atherosclerosis, and cardiovascular protective activities [19]. Paeonol extracted from plants like *Paeonia* genus, *Dioscorea japonica*, and *Arisaema erubescens* [23]. *Dioscorea japonica* belongs to the Dioscoreaceae and is a folk remedy used in Korea to treat syndromes related to metabolic disorders such as diabetes. Plants of the genus *Dioscorea* have been reported to possess various pharmacological activities such as anti-inflammatory, anti-tumor and immunomodulatory effects [24]. *Arisaema erubescens* in traditional Chinese medicine, several biological diseases have been treated with this plant and it used in traditional Chinese medicine to clear wet mucus, prevent spasms, and help reduce hardening and swelling [25].

In *paeonia*, it is often found in the root cortex [22]. Structure of *paeonol* is shown in Figure 5.

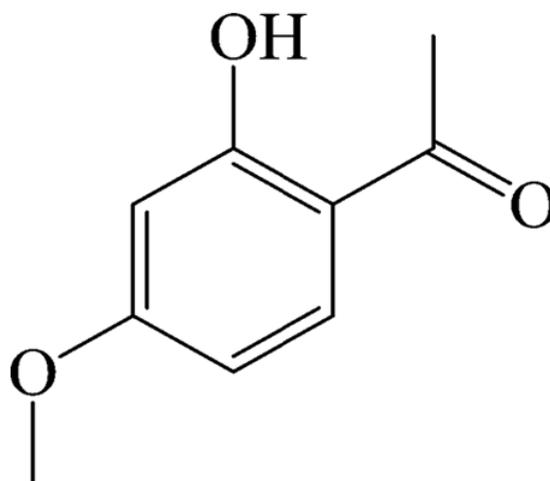
There are many problems associated with paeonol's solubility and bioavailability that have hindered the development of the drug as a pharmacological agent. A variety of derivatives of paeonol were synthesized and studied to enhance its stability. For example, drug delivery one of the best methods to enhance paeonol bioavailability [22].

## *Paeonia* Genus in Iran

There is little information about *Paeonia* genus in Iran. Until now, according to the latest classification all the species of the *Paeonia* genus that have been reported in Iran belonged to the *Paeonia* section. The number of reported species in Iran varies among different sources and different names have been used for them [26]. In some references it was reported that *P. corallina* Retz, with two varieties, var. *tridentate* (Pall.) Boiss and var. *corallina*, and *P. wittmanniana* recorded in Iran [27]. One reference reported that there is only one species of *P. wittmanniana* with two varieties, var. *wittmanniana* with hairy follicles, and var. *nudicarpa* Schipcz with glabrous follicles in Iran [28]. Other reference reported that *P. cf. mlokosewitschi* Lomark has been observed in Northwestern Iran [29]. Later, this species was considered as a new species which was named as *P. wendelboi*. Moreover, it was reported *P. mascula* L. subspecies *mascula* in western Iran [30].

During research on the diversity of the genus *Paeonia* in Iran until 2018, it has been determined that there were 4 species of *Paeonia*, including *P. wendelboi*, *P. wittmanniana*, *P. tomentosa*, and *P. mascula* subsp. *Mascula* [26]. However, the most recent survey identified a new species in Iran which was named *P. archibaldii* [31]. Therefore, until today, 5 species of the *Paeonia* genus have been reported in Iran, of which two are endemic to Iran; *P. archibaldii* and *P. wendelboi*. Pictures and descriptions of locations of *Paeonia* genus in Iran are presented in Figure 6 and Table 2,

respectively.



**Figure 5:** Structure of paeonol compound



**Figure 6a:** *Paeonia mascula*, subsp. *Mascula* [26], b: *P. tomentosa* [31], c: *P. wittmanniana* in some sources [26], d: *P. wendelboi* [26], e: *P. archibaldii* [31]

In the case of *P. wittmanniana*, there has been a long debate among plant taxonomists. The first group, consider *P. wittmanniana* and *P. tomentosa* to be same species [32], while the other group consider them as two independent species [33]. These studies are often based on morphological traits that must be confirmed in detail by molecular investigations.

Important morphological traits in the *Paeonia* plant include floral traits such as petal color, petal and sepal size, filament and anther traits and carpel number as well as branching habits and leaf related traits [34]. Some morphological traits of Iranian *Paeonia* genus mentioned in Table 3.

**Table 2:** Locations of *Paeonia* genus in Iran

| species             | Locations   | References |
|---------------------|---|------------|
| <i>P. mascula</i>   | Quercus forest, in Kurdistan province   | [32]       |
| <i>P. tomentosa</i> | Drazno Mountain and Golestan forest, in Golestan province                             | [32]       |
|                     | Sangdeh forest, Chamestan, Siah Bisheh, Kelardasht and Ramsar, in Mazandaran province |            |
|                     | Manjil, Damash, Chaboksar and rodsar, in Gilan province                               |            |
|                     | Asalem to Khalkhal Road, in Ardabil province  |            |

|                        |   |      |
|------------------------|---|------|
| <i>P. wittmanniana</i> | For identification, there has been a debate between plant taxonomists | [26] |
| <i>P. wendelboi</i>    | Khalkhal, Ardabil province  | [32] |
| <i>P. archibaldii</i>  | Firouzkoh, Mazandaran province  | [31] |

**Table 3:** Some morphological traits of *Paeonia* genus characterized in Iran

|               | <i>P. muscala</i>                         | <i>P. tomentosa</i>                               | <i>P. wittmanniana</i>                               | <i>P. wendelboi</i>                                    | <i>P. archibaldii</i>  |
|---------------|---|---|--|--|--|
| Inflorescence | solitary and terminal                     | solitary and terminal                             | solitary and terminal                                | solitary and terminal                                  | solitary and terminal  |
| Sepals        | - Elliptic                                | - Oblong and obtuse                               | - Orbicular or ovate orbicular                       | - Broad  | - Orbicular  |
|               | - Pale green to purplish                  | - Pale green                                      | - Green but with purple periphery or entirely purple | - Pale green   | - Brownish green with red innervation and a wide reddish purple margin |
|               | - Hairy or                                | - Hairy on the outside                            |  | - More or less hairy on outer side                     |  |
|               | glabrous on the outside                   |   |  |  |  |
| Petals        | - Elliptic                                | - Elliptic  | - Elliptic   | - Elliptic to broadly ovate                            | - Elliptic   |
|               | - Pink, red, or white                     | - White   | - Pale Yellow to white                               | - Pure yellow  | - Pure white   |
| Stamen        | - Purple filament                         | - Yellow or violet filament                       | - Yellowish white to purple filament                 | - Yellow filament                                      | - White filaments and staining pinkish at the base                     |
|               | - Anthers are approximately 4.5 mm long   | - Anthers are approximately 4.5 mm long           | - Anthers are approximately 4.5 mm long              | - Anthers are approximately 4.5 mm long                | - Anthers are approximately 4.5 mm long                                |
| Carpels       | - 2 carpels                               | - 3-5 carpels                                     | - 1-5 carpels  | - 3-4 carpels  | - Glabrous   |
|               | - Densely hairy                           | - Densely hairy and very rarely are glabrous      | - Glabrous   | - Densely hairy carpels                                |  |
|               |   |   |  |  |  |
| Stems         | - 40-70 cm height                         | - 20-50 cm height                                 | - 50-70 cm height                                    | - 20-40 cm height                                      | - Dwarf plant  |
|               | - Glabrous                                | - Simple stems                                    | - Simple stems                                       | - Simple stems   | - Glabrous   |
|               | - Simple stems                            |   |  | - Brownish violet to purple.                           | - Simple stems   |
|               | - Oblong and purple                       |   |  |  | - Green, but at the base with some reddish staining                    |
| Leaves        | - Lower leaves biternate                  | - Lower leaves biternate                          | - Lower leaves biternate                             | - Lower leaves biternate                               | - At first purplish red gradually be dark green at end of vegetation   |
|               | - Upper leaves smaller and simple ternate | - Upper leaves smaller, simply ternate or pinnate | - Upper leaves smaller, simply ternate or pinnate    | - Upper leaves smaller and simply ternate              | - Lower leaves biternate   |
|               | - Hairy in lower surface of leaves        | - Ovate to elliptic shapes                        | - Broad—obovate, oblong                              | - More or less hairy only on the lower surface of leaf | - Distinctly glabrous throughout                                       |
|               |   |   |  |  |  |

|            |      |      |      |      |      |
|------------|------|------|------|------|------|
| References | [32] | [32] | [35] | [32] | [31] |
|------------|------|------|------|------|------|

## Conclusion

To assess the distribution range of *Paeonia* spp in Iran and to survey their therapeutic potential, this study was conducted by collecting information via library and electronic search. Based on our knowledge, there is not much information about the *Paeonia* genus in Iran. So far, the most research work had been focused on the identification of species of *Paeonia*, without considering the genetic diversity and bio compound properties of this plant, especially the endemic species. Considering that so far there is not much information about the *Paeonia* genus in Iran, its genetic and phytochemical properties can improve the *Paeonia* breeding program and phytochemical studies. As stated, the selection is still one of the most important methods of *Paeonia* breeding. So, for selecting peony, it is necessary to study and evaluate plant genetic diversity in the world. It is possible to improve breeding resources using Iranian species due to the limited *Paeonia* germplasm available. For example, due to the lack of yellow color in the *Paeonia* genus, *P.*

*wendllboi* can be used in breeding program.

No study was reported on the identification and extraction of important compounds such as Paeonol and paeoniflorin. These two compounds are the main bioactive compounds that have wide biological effect. They exist in almost all species. So, Iranian *Paeonia* genus can have significant amounts of these two valuable compounds. Due to the gaps in research, particular attention will be paid to *Paeonia* breeding studies and bio compounds characteristics as future research needs.

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## Conflict of Interest

The authors declare no conflict of interest.

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