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Evaluating Essential Oil Composition of *Morina persica* L. as an Endemic Ethnoveterinary Plant in Iran

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ABSTRACT

Aims: Ethnobotanical knowledge is widespread and vital among the tribespeople. However, this knowledge is based on experimental findings rather than scientific ones. Apart from this, there is a risk that this ethnobotanical knowledge may disappear and be neglected. One of the endemic plants in Iran is *Morina persica* L., known in ethno-veterinary and ethnobotany. In this study, the chemical compounds of *Morina persica* L., an endemic ethnoveterinary plant, were investigated for the first time in Iran using GC/MS.
Materials & Methods: All *M. persica* aerial parts were collected during the flowering stage. The extraction of essential oils was performed by a hydro-distillation method using a Clevenger device. Then, the gas chromatography-mass spectrometer device was employed to determine and analyze the essential oil composition. Kovats retention indices (KI=RI) were used to confirm the identification by the mass spectra**.**
Findings: Results showed that the plant has 31 types of compounds constituting 92.7% of the total volatiles identified via GC/MS. The most important compounds are as follows: germacrene d, *β*-gurjunene, bicyclo germacrene, acetophenone, (*2E,6E*)-farnesol, *δ*-cadinene, *α*-copaene, *n*-tricosane, *α*-cardinal, *para*-methyl-acetophenone, benzyl acetate, and *E*-caryophyllene.
Conclusion: Some important components of the essential oil were found germacrene d, bicyclo germacrene, acetophenone, and benzyl acetate. Germacrene d and bicyclo germacrene are used in industrial production and transformation. Acetophenone, α-cardinal, and especially benzyl acetate are also utilized in the pharmaceutical industry, perfumery, and the industry for producing plastics, rubber, cellulose acetate, nitrate, varnishes, glaciers, and printing inks. Based on the evidence, this plant can be recommended for medicinal purposes (human and livestock), the chemical and flavoring industries, and the perfume industry.

Keywords: Endemic Plants; Essential Oil; Ethnoveterinary; Herbal Plants.

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Introduction

Plants have been a rich source of producing medicine throughout history. It has been widely common to use plants for human health and animal treatment in all human societies and cultures, especially among rancher nomads [1]. Therefore, plant identification and application of medicinal plants are ordinary worldwide. Ethnoveterinary or traditional veterinary includes the study of folk beliefs and knowledge of ranchers and nomads based on their use of medicinal herbs and application of folk methods in prevention, health, and animal treatment. Generally, local diagnosis information on ethnoveterinary medicine is included a range of systems and knowledge. That information is based on accessible medicinal plants, metaphysics, skills, teachings, methods, technologies, and surgical procedures in treating livestock. On the whole, the main aim is to care for animal health.

The acceptance of ethnoveterinary medicine mainly refers to its power to enhance folk pharmacotherapy that is locally accessible, achievable, feasible regarding economic aspects, and culturally suitable [2]. In addition, the practice encloses some experimental data on self-management and environment [3]. In developing countries within their poor and low-income societies, ethnobotanical knowledge and ethnoveterinary-related approaches help as potent therapeutic practices to overcome and avoid livestock disease [2]. Within their rural economies and societies in developing countries, livestock production plays an important role, especially regarding food security, poverty relief, and different cultural activities [4,5]. Ranchers and shepherds in rural societies trust ethnoveterinary medicine as a sustainable practice to veterinary approaches [2].

Research and documents show that in many developed countries, traditional plants with medicinal applications are preferred by people to treat their livestock than modern medicine [6, 7, 8, 9, 10]. The indigenous knowledge of the people, especially medicinal appliable plants, is at high risk of various aspects, including deforestation, habitat loss, agriculture expansion, exploitation, and climate change. The inherited knowledge of people on herbal medicine is critical for the treatment of various diseases. Despite the attempt of a few researchers to document plants with medicinal veterinary applications in some cultural groups, these days, it is not sufficient to show how it is essential [4, 11, 12, 13, 14, 15, 16].

According to some research, investigations in ethnoveterinary medicine are needed because plants depending on the situation, contain an extensive and varied range of phytochemicals. Herbal medicines can support the principal applicant for active product development and discovery of drugs. These issues can be helpful when trying to manage livestock's health and well-being [2, 17]. In addition, most of the local ethnobotanical knowledge and diagnostic methods employed in livestock healthcare are passed down orally within several generations or are in access due to apprenticeship [18, 19]. Since ethnoveterinary medicine is primarily local and based on culturally particular, these specific experiences, knowledge, and skills obtained over generations are susceptible to dying out. Thus, for many reasons such as immigration, urbanization, and technological development, if no effort is documented, this information will be soon forgotten [2, 20, 21, 22].

The Morina persica L. plant is from the Morinaceae family. This family has three genera, *Cryptothlsdia*, *Acanthocalyx* ,and *Morina*, and 13 species worldwide [23]. The name of the *Morina* species was taken from a French physicist and botanist, Louis Pierre Morin [24]. In Iran, this genus has only one species of herbaceous perennial plants with stunning and highly aromatic flowers, and a rich fragrance called the scientific name *Morina persica* L., also named Whorl-Flower in English, Khar Aros or Gol Charkhei in Persian in the western and central parts of Iran (Figure 1). In addition to Iran, this genus grows wild in southeastern Europe, Anatolia, Syria, Lebanon, Afghanistan, Pakistan, the Himalayas, and Kashmir Mountains [25, 26].

A few studies have been done on the effective ingredients of *M. persica* [27,28]. For this purpose, the fresh flowery parts of this plant were collected from Eskisehir of Turkey, and hydro-distillation was recruited to extract the essential oils, which were then subject to analysis using GC/MS device. The results of this study identified this plant as the richest source of (*2E,6E*)-farnesol composition in nature [27]. In another study, an examination of methanolic and ethanolic extracts of *M. persica* in Turkey showed the high antioxidant, enzyme inhibitor, anti-fungal and disinfectant effects of the plant, which indicated the importance of considering the plant as a high-quality natural resource for further use in pharmaceutical formulations [25]. In China, this plant is used as a traditional drug for curing migraines, tumors, backache, arthritis, and high heart rate, besides applying helminth in veterinary medicine [28, 29]. Subsequently, some research was done on the *M. longifolia* Wallich species. No (*2E,6E*)-farnesol combination was observed through the decomposition of chemical compounds of active ingredients [25,28,30,31]. Investigation of the effect of seasonal changes on the chemical composition of the active ingredient of *M. longifolia* species showed many changes in the Rudranath-Uttrakhand (India) region. The highest amount of essential oil produced from fresh aerial parts was obtained by the hydro-distillation method in the sum-

Figure 1) *Morina persica* in its naturally occurring habitat of the Dehsheykh area, the Maharloo Watershed, Iran. (a) the whole plant, (b) flowers, (c) sampling area landscape.

mer, and the end of the flowering period is the best harvest time for this plant. The most important chemical compounds identified by GC/MS were *α*-gurjunene, *β*-cadinene, bicyclogemacrene, dihydrofarnesol, germacrene d, methylcommate, and tetra methylcyclohexane [33]. In a study conducted in Deoband, Uttarakhand, India, the extraction of the essential oil of *M. longifolia* leaves by hydro-distillation method and performing chemical analysis via GC-FID and GC/MS resulted in the identification and separation of 47 chemical compositions. Most of the chemical compounds identified by the sesquiterpenes included germacrene d, *α*-cadinol, and germacrene d-4-ol [30]. In addition to its applications as an herbal remedy, this plant is used in veterinary medicines. Ethnobotanical plant studies of the Kavrepalanchowk district showed that the root-extracted juice of *M. longifolia* is employed to treat diarrhea and dysentery besides its usage as incense. Furthermore, the plant is used to treat worm-infected wounds [34]. Other studies reported that the plant had been used to treat gastroenteric disorders and maggot wounds [31,35,36].

Materials & Methods

Habitat Description

The study area is located in the southern parts of the Zagros Mountains next to the west of Shiraz, the capital City of Fars Province, Iran, between 29° 37′ to 46° 00′ north longitudes and 52° 19′ 24″ east latitude with the average altitude of 1920 m above sea level. It is a sub-area of the western part of the Maharloo Watershed. It is in a dry climate with an average rainfall of 390 mm/year and an average temperature of 18°C. (Figure 2). The study area has a Mediterranean climate with a dry season from May to September, followed by a rainy season from October to April. The dominant plant species are shrubs such as *Astragalus sp., Convolvulus leiocalycinus* Boiss, *Ebenus stellate* Boiss., and *Phlomis elliptica* Benth., followed by perennial forbs and grasses including *Bromus tomentellus* Boiss., *Gundelia tournefotii* L., *Hordeum bulbosum* L., and *Stipa barbata* Desf. The soils in this area are mainly silty and marl limestone with shale and weathered marl, and in some parts, silty shale, conglomerate, and sandstone.

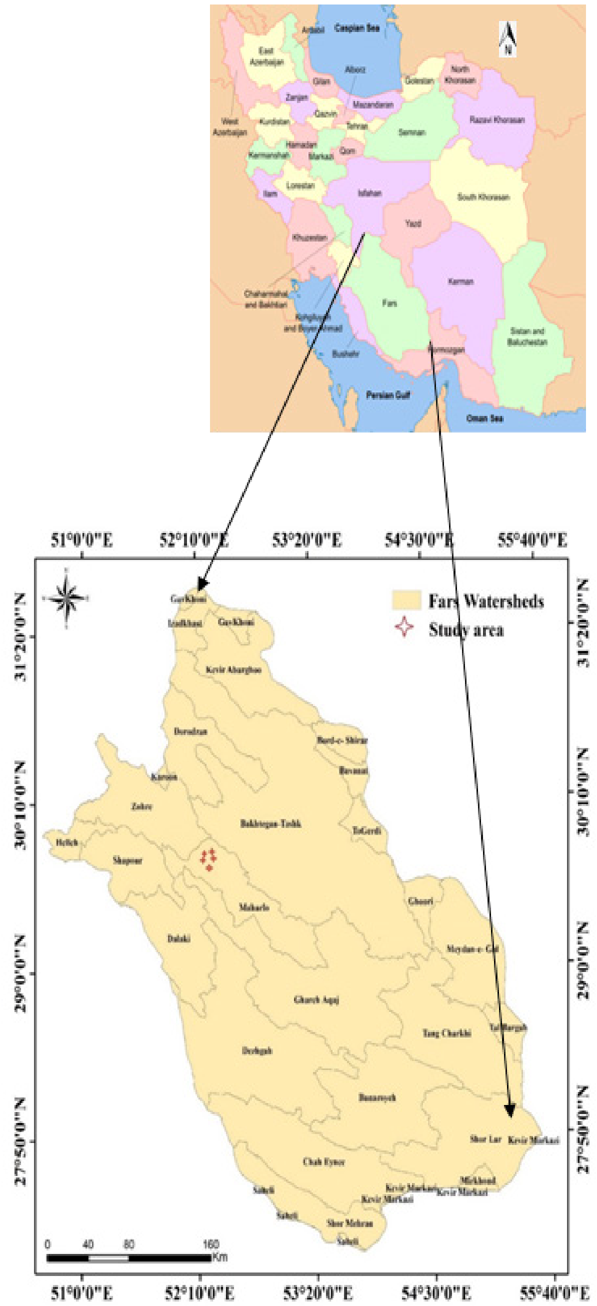

Figure 2) Location of the study area in Iran, Fars Province, and the Maharloo Watershed.

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Collection and Preparation of Plant Samples for Phytochemical Studies

The study target was first presented to the indigenous participants in the current research. Then the ethnobotanical information was collected through face-to-face interviews employing a semi-structured questionnaire. To obtain the most and the best information, the questionnaire of data collection was classified into two parts, interviews and a field walk to the collection of plants. Before collecting the interviews mentioned plants, the field observations were carried out in allocated areas of study. Over the field walks, indigenous ranchers contributed to identifying and collecting the plants. Plants applied for livestock diseases treatment identified. In this way, *Morina persica* was selected as an endemic ethnoveterinary plant based on folk knowledge of one of the famous Iranian nomads, namely the Fars nomads. This plant was identified and approved by the Herbarium of Research Center of Agriculture and Natural Resources of Fars Province, Iran (Voucher No. 12821). *M. persica* is spread and found as a small colony (community). For studying the essential oils, five colonies of this plant were found, and the geographic coordinates were recorded (Table 1).

Table 1) Geographical coordinate of samples.

Site (Point)	Longitude	Latitude
1	52⁰ 19′ 12.36″	29⁰ 38′ 6.00″
2	52⁰ 19′ 16.75″	29⁰ 38′ 10.36″
3	52⁰ 19′ 16.61″	29⁰ 38′ 10.93″
4	52⁰ 19′ 13.62″	29⁰ 38′ 9.74″
5	52⁰ 19′ 15.74″	29⁰ 38′ 8.05″

All aerial parts of *M. persica* were collected in June 2018 during the flowering stage. The drying process was conducted in the laboratory, with no exposure to direct light, and at an ambient temperature, which took more than a month. After cutting, they were mixed, and the extraction operation was performed by hydro-distillation method using a Clevenger device. This way, three samples of that mixture were selected for three test repetitions. Four hours after boiling water, the essence extraction was finished. The obtained essential oil was collected in special glasses, and sodium sulfate was added to purify the essential oil and dehydrate it. It is then stored in a sealed glass amber vial and kept in the refrigerator until it is time to be injected into the gas chromatography-mass spectrometer device.

Identification of Essential Oil Components

To detect and analyze the essential oil composition, a gas chromatography-mass spectrometer device was used and recognized by reliable references [37]. The device features are Column: HP-5MS, 30m× 0.25mm (ID) × 0.25micron (FT), GC Condition: The temperature program of the oven was set up as follows: from 60 to 210 ˚C with Rate: 3˚C min -1 then increased to 240˚C with Rate 20 ˚C min -1 and the final temperature held for 8.5min. Running Time was 60min.

The electron ionization energy was 70eV in the electronic ionization (EI) mode, ion-source: 230 ˚C, Detector: MS, Interface line temperature: 280 ˚C, Injector: 280 ˚C, Split ratio: 1:50, Carrier Gas: He, 1ml.min^{-1}, mass range: 50-480. For this purpose, extracted essential oil sample was delivered to the Medicinal Plants Laboratory of the Agricultural and Natural Resources Research Center of Fars Province. Molecular structure determination of the essential oil components was detected by precise studying of the retention time (RT), the Kovats retention indices (KI=RI), mass spectrometry, and comparing these parameters with standard compounds and comparing with mass spectra and Library information and use of data analysis software. In other words, the numbers in the

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vertical column of the chromatogram represent the amplitude amount of the essential oil components, and the horizontal column expresses the separation time and detection of each component of the essential oil in the column. Regarding the exit pattern of the normal alkanol and comparing the mass spectra obtained by the GC/MS with the standard mass spectra (found in the sources), it was determined that each mass spectra is precisely related to which compound [38]. In order to verify the detection by the mass spectra, the Kovats retention indices (KI=RI) were employed, and the results are given in Table 2.

Findings

The yield of extracted oil from a dried form of *M. persica* in its flowering stage was 0.35% on a moisture-free basis (w/v). The essential oil compositions and the percentage of each one are shown in Table 2.

As shown in Table 2, 31 constituents were detected, in total, in the essential oil of *M. persica* at the flowering stage. Moreover, the shape of the chromatogram is shown in Figure 3. At the

Table 2) The most significant hydro-distillation extracted chemical constituents of *M. persica* essential oils at its flowering stage*.

NO	Compound	RT	%GC$_c$	NO	Compound	RT	%GC$_c$
1	*n*-decane	7.24	1.54	17	geranyl acetone	25.31	0.63
2	acetophenone	9.54	7.12	18	γ-muurolene	26.27	0.63
3	benzyl formate	9.93	1.42	19	germacrene d	26.53	18.78
4	*n*-Undecane	10.74	1.61	20	Bicyclogermacrene	27.04	9.62
5	benzyl acetate	13.33	2.30	21	α-muurolene	27.18	0.72
6	*para*-methyl-acetophenone	13.96	2.30	22	δ-cadinene	28.01	6.20
7	1-phenyl ethyl acetate	14.59	1.08	23	*n*-hexyl benzoate	30.08	1.71
8	thymol	18.70	1.52	24	Viridiflorol	30.70	0.63
9	carvacrol	19.00	1.66	25	*Epi*-α-muurolol	32.59	0.96
10	δ-elemene	20.53	1.60	26	α-cadinol	33.06	2.51
11	α-copaene	22.14	3.34	27	(*2E,6E*)-farnesol	35.53	5.40
12	β-bourbonene	22.50	1.10	28	*n*-hexadecanoic acid	43.57	1.16
13	β-elemene	22.82	0.82	29	*n*-tricosane	52.23	3.02
14	*E*-caryophyllene	23.88	2.05	30	*n*-tetracosane	53.86	0.29
15	β-gurjunene	24.43	11.63	31	*n*-pentacosane	55.79	0.80
16	aromadendrene	24.69	0.88				

*The unidentified peaks > 0.5% are not to be included in the table.

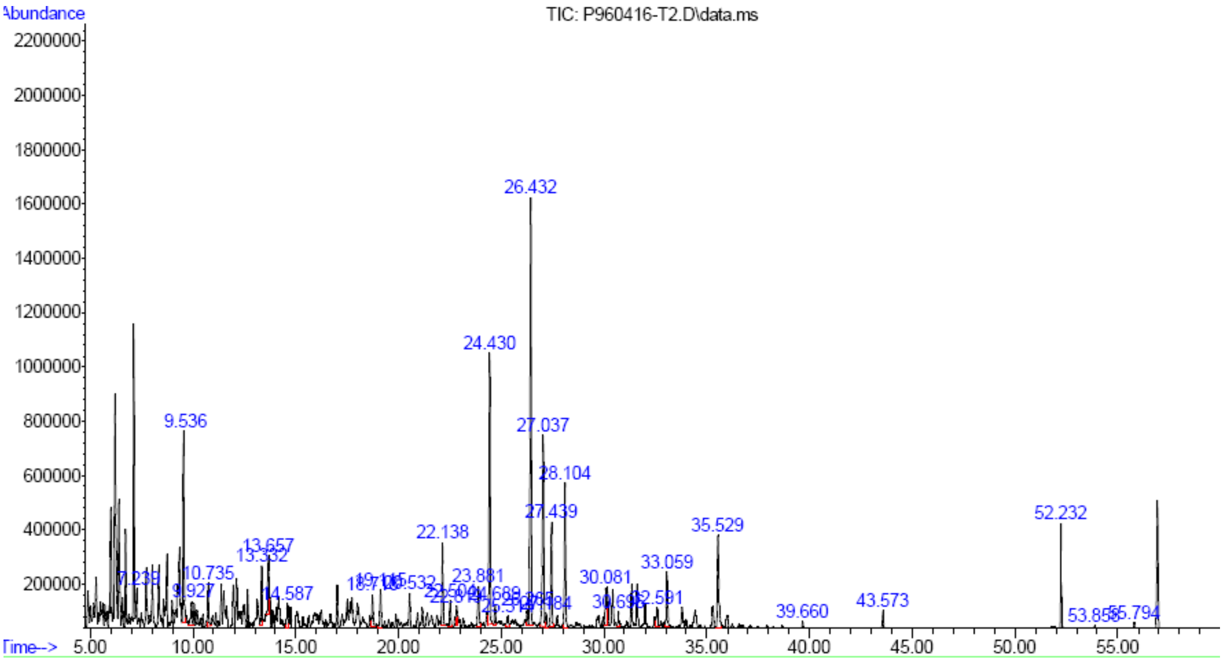

Figure 3) *Morina persica* chromatogram by Gc/MS

flowering stage, *M. persica*, the essential oil, was rich in terpenes, representing about 70% of the total volatile. The oil was dominated by sesquiterpenes (44.7%). The major components of the essential oil turned out to be germacrene d (18.78%), *β*-gurjunene (11.63%), bicyclo germacrene (9.62%), acetophenone (7.12%), *δ*-cadinene (6.20%), (*2E,6E*)-farnesol (5.40%), *α*-copaene (3.34%), *n*-tricosane (3.02%), *α*-cardinal (2.51%), *para*-methyl-acetophenone (2.30%), benzyl acetate (2.30%) and (*E*)-caryophyllene (2.05%), respectively.

Discussion

As is seen from the investigation of the chemical composition of the *M. persica* essential oil in the present study, germacrene d has the highest content among other chemical compounds (18.78%). This volume of germacrene d is higher than the reported amount in the research of Baser and Kurkcuoglu (1998) (10.99%). Furthermore, the amount of (*2E,6E*)-farnesol compound was 5.40% in this study, while it was reported to be 58.79% in Baser and Kurkcuoglu's research (1998). Given that the ecological characteristics of each area can affect the quantity and quality of essential oils of plants, and since the plant species, exploitation time, and essence extracting methods of these two studies are the same, the existing difference is due to the ecological changes such as climate, soil and physiographic characteristics of the sample collection areas.

In this study, most compounds are related to germacrene d, *β*-gurjunene, and bicyclo germacrene. Seven joint combinations were reported in the study conducted in Turkey, and the research carried out in Iran, including *α*-copaene, *β*-bourbonene, germacrene d, bicyclo germacrene, *δ*-cadinene, *α*-cardinal and (*2E,6E*)-farnesol. According to the results, the amount of all similar compounds in these two studies was higher in the present study, except (*2E,6E*)-farnesol [24,30,33]. This issue emphasizes the role of habitat and ecological vegetative areas on the active ingredients of a plant species. Furthermore, (*2E,6E*)-farnesol was not observed in the analysis of the chemical compounds of the active ingredients on the *M. longifolia* due to the difference between the species

[30,32,33]. As perceived in research, the most essential chemical compounds obtained from *M. longifolia* were germacrene d, bicyclogemacrene ,and α-cadinol, underscoring the possibility of using this plant in herbal supplements and perfumery [25,30,32,33]. It should be noted that the findings of this research indicate that the presence of germacrene d and bicyclogemacrene in *M. persica* is approximately two times more than in *the M. longifolia* plant. Also, the analysis of the essential oil of *M. longifolia* demonstrated 47 types of chemical composition caused by different devices used (GC-FID), in addition to the differences in species type and habitat [35]. In general, the differences in the compositions derived from the essential oil depend on the environmental conditions (such as ecological and geographical), the phonological stage, and the harvesting time [39, 40].
Some of the compounds in the essential oil of this plant, such as germacrene d, α-copaene, bicyclo germacrene ,and *E*-caryophyllene, can be used to manufacture pesticides and insecticides. Germacrene d and bicyclo germacrene are also applied in industrial productions and transformations. Some compounds, such as acetophenone and benzyl acetate, can be used in soap-making, perfumery, and other cosmetics. It is worth noting that the composition of benzyl acetate (which is also found in most flowers) is also utilized in industries to make plastics, gum, cellulose acetate, nitrate, lacquers, glaciers, and inks. Compounds like α-cardinal and acetophenone are used in the pharmaceutical industry owing to their anti-fungal properties [40].

Conclusion

Human communities have shown a deep interest in herbs and herbal medicines in recent decades. Of course, the ultimate purpose is not to collect herbs in nature. Due to the current and future demand for herbal medicines, new opportunities should be created in agriculture by producing medicinal plants as alternative crops [40]. Considering the amount of essential oil and valuable existing compounds in this plant, agricultural experts have begun domesticating *M. persica* to take practical steps toward using its natural compounds in the pharmaceutical and cosmetic industries through proper cultivation and exploitation. Iranian nomads have a great traditional background in ethnobotany and ethnoveterinary medicine and practices. The current research is also carried out on rich biodiversity and endemic species. On the other hand, the individuals enjoy tremendous local knowledge about ethnoveterinary plants with medicinal applications, which is vital for treating different kinds of livestock illnesses. Generally, the result of this study indicated that the region's medicinal plants and this knowledge are both at high risk of extinction due to various aspects. Some of the most important reasons are included deforestation, habitat loss, agriculture expansion, exploitation, and climate change. Therefore, great attention must be presented to the government, researchers, and, more importantly, local people and ranchers.
To modernize this knowledge, there needs to be a proper analysis of medicinal plants to verify the authenticity of new drugs. Thus, it is critical to perform science-based cultivation and conservation to preserve the endangered and rare plant species along with this priceless local knowledge for the next generations. At the same time, the correct, sustainable, and respectful use of plant species by ethnic communities would also benefit local socio-economic development. Therefore, the plant seems to have high economic and pharmaceutical values that are still unknown and open to further research.

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