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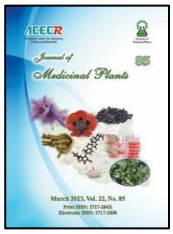

Research Article

Some physicochemical and phytochemical characteristics of Iranian *Ferula assa-foetida* L. oleo-gum resin

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ABSTRACT

Background: *Ferula assa-foetida* is an endemic medicinal plant of Iran. Its oleo-gum resin or assafoetida gum (AG) is an important herbal product whose standardization is important to ensure effectiveness and safety. **Objective:** Due to the lack of accurate and complete data about Iranian AG, the objective of this study is to extend the available knowledge by reporting some physicochemical and phytochemical properties of an Iranian AG sample. **Methods:** Organoleptic properties were determined by sensory evaluation. Total ash, acid-insoluble ash, dry residue as well as total phenolic and flavonoid content were measured by accepted methods to determine some physicochemical and phytochemical properties of AG. Analysis of its essential oil by GC/MS as well as microbial load assessment were performed. **Results:** The AG was a yellowish brown mass with a sulfur-like pungent odor similar to garlic, with total ash and acid-insoluble ash of 2.83 % and 2.06 %, respectively, and a dry residue of 3.43 %. Total phenol content was 4.15 ± 0.26 mg gallic acid equivalent/g in dry AG. (*E*)-1-Propenyl sec-butyl disulphide, (*Z*)-1-propenyl sec-butyl disulphide, and 2,4-thiazelidinedione were the major essential oil component of this oleo-gum resin. Microbial load was in acceptable range according to Pharmacopeia. **Conclusion:** The current findings provide data on some of the physicochemical and phytochemical properties of Iranian AG which is applicable for completing current knowledge. Although more information is needed for establishing Iranian AG standardization criteria, which will be obtained from more extensive researches.

1. Introduction

The genus *Ferula* belongs to the Apiaceae family includes a number of important medicinal plants that are distributed in the flora of Iran. With more than 170 species of this genus identified worldwide, there are approximately 30 species in Iran, of which about 16 species as *Ferula. assa-foetida* L. are endemic [1, 2].

Abbreviations: PM, Persian Medicine; GC-MS, Gas chromatography-mass spectrophotometer; WHO, World Health Organization; TSB, Tryptic Soy Broth; SDA, Saburated Dextrose Agar; CFU, Colony Forming Unit

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F. assa-foetida L. is growing in the eastern regions of Iran and has two types, bitter and sweet [3]. Oleo-gum resin exuded from the rhizome and root of this plant is called Angūzeh (in Persian), which is one of the important export items of Iran. According to the Chamber of Commerce, in 1399 alone, the value of exports of this product to more than ten countries exceeded $ 4 million [4, 5].

Many therapeutic effects in various fields such as gastro-intestinology, neurology, dermatology and urology have been mentioned in Persian medicine (PM) resources for AG. Interestingly, many of these effects have also been shown in recent studies. For example, its use as a treatment for gastrointestinal diseases, inflammation and swelling, as a pain reliever, anticonvulsant and antidote are just some of the things that the PM scholars have stated. New evidences also suggest anti-inflammatory, analgesic, antiparasitic, antifungal, and antimicrobial effects in animal and clinical studies [6-8].

The effectiveness of any herbal medicine depends on the amount of its active ingredients. The influence of environmental factors over time reduces these compounds. Contamination, impurities and fraud are also factors that reduce the quality of medicinal herbs. As quality control of herbal medicines plays an important role in their effectiveness and safety, determining the tests and the acceptable range for them is very important in evaluating the quality of medicinal plants in each region, which is done by preparing herbal Pharmacopoeias in each country.

In addition to long history of use of AG as a spice as well as herbal medicine in folk medicine, it has also been offered in the form of some new formulations. Among them is a drug that is known to be useful for the symptomatic treatment of COVID-19 and also applicable as an antivirus [9, 10]. These novel therapeutic uses of AG, highlight the importance of its standardization and quality control.

Although AG is mentioned in the Iranian Herbal Pharmacopoeia as the first monograph with few tests suggested for its initial identification and quality control, but the presented ranges are obtained from studies of foreign AG [3]. A recent study has reported essential oil components of thirteen Iranian AG. It concluded that there are high levels of variation in their essential oil yield and components [11]. In general, it can be mentioned that accurate and comprehensive data for evaluating the quality of Iranian AG is not available. Due to lack of sufficient data on the physicochemical and phytochemical properties of various types of Iranian AG, this study intends to measure some of these properties to help provide the required data for standardization of this valuable herbal medicine.

2. Methods

2.1. Authentication of herbal medicine

In this research that is an experimental and laboratory study, AG was purchased from a reputable seller of medicinal plants (supplied from eastern Iran, near the Afghan border). In order to authentication, it was sent to the herbarium of the Faculty of Pharmacy, University of Tehran.

2.2. Investigation of organoleptic characteristics

Properties such as color, odor and physical appearance are examined by sensory evaluation of AG powder.

2.3. Determination of some physicochemical properties

Properties such as the percentage of total ash and acid-insoluble ash as well as the dry residue

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of AG powder are measured according to Pharmacopoeia method [12]. For instance, oleo-gum resin (2 g) was precisely weighed and burned in a weighted crucible. The temperature gradually raised to 675 ± 25°, until no carbon remained in the sample. Then the ash was accurately weighted. For measuring the acid-insoluble ash, the total ash which was previously obtained, was boiled in 25 mL of 3 N hydrochloric acid for 5 min. The insoluble matter was collected on a tared filtering crucible or ash less filter. Afterward it was washed with hot water, then it was burned and weighed. Finally, the percentage of acid-insoluble ash was calculated from the weight of the initial sample.

Dry residue was measured based on Pharmacopoeia method [13]. By crushing the mass of AG, 2 g sample with reduced the particle size to about 2 mm, was prepared. A glass container with a lid, was dried at room temperature and in a desiccator for 30 minutes, then it was weighed exactly. The weighted sample was poured into glass container and the lid was closed, then it was weighted. The sample was spread gently into the bottle so that the thickness is about 5 mm and not more than 10 mm, before placing the bottle in the drying chamber and removing the stopper. After drying, upon opening the chamber, the bottle was closed and wait until coming to room temperature in a desiccator, then it was weighted.

2.4. Essential oil isolation and analysis procedure

The essential oil was isolated from the AG by hydro-distillation method according to European Pharmacopeia [14]. Gas chromatography coupled with mass spectrometer (GC-MS) was used to analyze the essential oil. Various parameters such as inhibition time, inhibition index, comparison of mass spectra with standard compounds and available information in the computer libraries of GC-MS (NIST05a.L. and wiley7n.l) were used to identify the constituents of AG essence.

The gas chromatography device used was Agilent 6890 with a column length of 30 m, an inner diameter of 0.25 mm and a layer thickness of 0.25 μm of BPX5 type. To identify the constituents, a 1 μl- derived sample was injected into the GC / MS. The temperature program of the column was set as follows: The initial temperature of the oven was 50 °C and stopping at this temperature for 1 minute, with a temperature gradient of 3 °C per minute, increasing the temperature to 133 °C and stopping at this temperature for 0.2 minutes. Again with a temperature gradient of 2 °C per minute, increase the temperature to 199 °C and stop at this temperature for 0.2 minutes and finally with a temperature gradient of 1.5 °C per minute, increase the temperature to 295 °C and stop at this The temperature was 0.2 minutes. The response time was 127 minutes. The temperature of the injection chamber was 290 °C as SPLILESS and helium gas was used as the carrier gas with flow rate (flow) of 1 ml per minute. The mass spectrometer used was Agilent 5973 with ionization voltage of 70 electron volts, EI ionization method and ionization source temperature of 220 °C. Copper scan range was set from 40 to 700. The software used was Chemstation. The spectra were identified using their inhibition index and compared with the indices in reference books and articles, using the mass spectra of standard compounds and the information available in the computer library (NIST05a.L. and wiley7n.l).

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2.4.1. Determination of the total phenol and flavonoid contents

Total phenolic content of AG was analyzed based on Gallic acid substance by Folin Ciocalteu reagent according to strategy depicted by Zargaran et al. [15]. The Gallic acid (Sigma) was utilized as standard. The instance of AG was extracted by methanol. Then 5 ml of Folin Ciocalteu reagent (diluted with distilled water 1:10) and 4 ml of Na2CO3 1M, were added to both sample and standard solutions. The total phenol content was specified by colorimetric trial at 765 nm after 15 minutes. Standard curve was collected by measuring the absorption of Gallic acid solution in 0, 50, 100, 200 and 250 mg/ml concentration in methanol: water (50:50 v/v). The tests were repeated three times and their mean was presented as Gallic acid equivalent (mg/ml of AG oil).

Quantification of the total flavonoid content of AG carried out pursuant to method described before [16]. A blend of 5 ml of 2% aluminum trichloride in methanol and 5 ml of AG was centrifuged at 4000 rpm for 8 minutes. The clear phase was separated and the total flavonoid content was determined through its calorimetric test at 415 nm with a blank sample of AG and methanol. The standard curve by Rutin (Sigma, Aldrich) solution was used and related concentrations were: 0, 5, 20, 50 and 80 mg/ml). Measurement repeated three times and the mean of them stated as the total flavonoid content as mg of Rutin equivalent per milliliter of AG.

2.5. Microbiological control

The total number of live aerobic bacteria and yeasts and molds was evaluated in the laboratory.

Microbial control was performed according to the method mentioned by WHO, with two objectives, determine the total number of living aerobic microbes and investigate the presence of specific microorganisms [17].

For primary preparation, 10 g or 10 ml of the AG was blended with 5 g of 20 R polysorbate and, warmed to 40 °C and mixed whereas warming in a water bath. 85 ml of broth lactose was added and warmed to about 40 °C to create an emulsion whereas pH was adjusted to about 7.

In arrange to determine the total viable aerobic count, 1 ml of the sample was added to a test tube containing 5 ml of sterile Tryptic Soy Broth (TSB) medium. Also, 1 ml of it was included into the primary test tube of the dilution tube series. For dilution, 6 tubes, each containing 9 ml of TSB, were pre-prepared and sterilized. Within the same way, one ml was transferred from the primary pipe to the second pipe. After stirring, one ml was transferred from the second pipe to the third pipe and so on until the end. In arrange to determine the colony count, four solid culture medium were prepared for every dilution, two containing Caso Agar for bacteria and others containing Saburated Dextrose Agar (SDA) for fungi. 1 ml of every dilution was poured in each plate and mixed by sterile melted medium. TSA plates were incubated at 36 °C and SDA plates at 25 °C. The resulting colonies were counted in TSA plates after 24 hours and in SDA plates after 72 hour.

3. Results

3.1. Authentication

The oleo-gum resin sample was authenticated with the scientific name *F. assa-foetida* L. (Family: Apiaceae) in the herbarium of the Faculty of Pharmacy, University of Tehran and PMP-888 as voucher code was assigned to it.

3.2. Organoleptic characteristics

The studied oleo-gum resin was a viscous, brown mass with a pungent, strong sulfur odor.

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3.3. Physicochemical properties

Physicochemical properties including total ash, acid insoluble ash and loss on drying are shown in Table 1.

3.4. Phytochemical characteristics

3.4.1. Essential oil analysis

The findings of GC-MS analysis of AG essensial oil are presented in Table 2, Table 3 and Fig. 1.

3.4.2. Total phenol and flavonoid Content

The mean of total phenolic content measured in AG was 4.15 ± 0.26 mg Gallic acid/g. The total flavonoid content equivalent as Rutin was reported to be low quantity.

3.5. Microbial control

The results are shown in Table 4.

Table 1. Physicochemical properties of AG

Test Name	Test reference	Test results	Unit
Total ash	561/USP 40	2.83*	w/w %
Acid insoluble ash	561/USP 40	2.06*	w/w %
Loss on drying	Moisture Analyzer	3.43*	w/w %

*Repeated three time

Table 2. AG essential oil analysis by GC-MS

No	RT	%	Components	RI	Type
1	23.40	0.68	Disulfide, ethyl hexyl	1173	Other
2	23.66	23.30	(*Z*)-1-Propenyl sec-butyl disulfide	1178	Other
3	23.97	43.90	(*E*)-1-Propenyl sec-butyl disulfide	1182	Other
4	25.73	1.30	Bis (1-methyl propyl) disulfide	1220	Other
5	35.69	5.13	4-Chloro-2-nitrotoluene	1325	Other
6	35.81	8.19	2,4-Thiazolidinedione	1445	Other
7	37.3	2.92	Tetrahydro thiazole	1460	Other
8	38.1	0.15	*β*-Selinene	1494	SH
9	38.49	0.20	*α*-Farnesene	1507	SH
10	38.80	1.09	*β*-Dihydroagarofuran	1517	SO
11	42.41	0.37	Guaiol	1609	SO
12	42.74	0.24	Carotol	1618	SO
13	42.88	0.21	unknown	-	
14	43.5	1.92	*B*-Maaliene	1622	SH
15	44.00	0.35	Hinesol	1651	SO
16	47.48	1.52	1,1-Dimethyl germacyclobutane	1684	Other
17	52.28	0.62	1,3-Xylyl-15-crown-4,2,3-pinanedioxyboryl	1690	Other
		91.6	**Total Identified**		
		2.5	***Sesuiterpenes Hydrocarbons SH***		
		2.23	***Oxygenated Sesquiterpenes SO***		

Table 3. Mass fragmentation ions observed for major compounds from the AG essential oil

No.	Compound name	Mass fragmentation ions (percentage)
2	(Z)-1-Propenyl sec-butyl disulfide	162 (56.15), 106 (100), 57 (28.92), 72 (12.75), 59 (11.54)
3	(E)-1- Propenyl sec-butyl disulfide	162 (52.67), 106 (100), 73 (11.62), 72 (11.34), 57 (25.29)
6	4-Chloro-2-nitrotoluene	90 (5.13), 89 (100), 79 (5.7), 73 (11.08), 61(14.40)
7	2,4-Thiazolidinedione	90 (5.04), 89 (100), 79 (5.64), 73(11.19), 61(15.17)
8	Tetrahydro thiazole	91(4.73), 90 (5.17), 89 (100), 73(6.6), 61(11.76)
15	*β*-Maaliene	204 (96), 189 (100), 133 (50), 161 (75), 59(40.9)

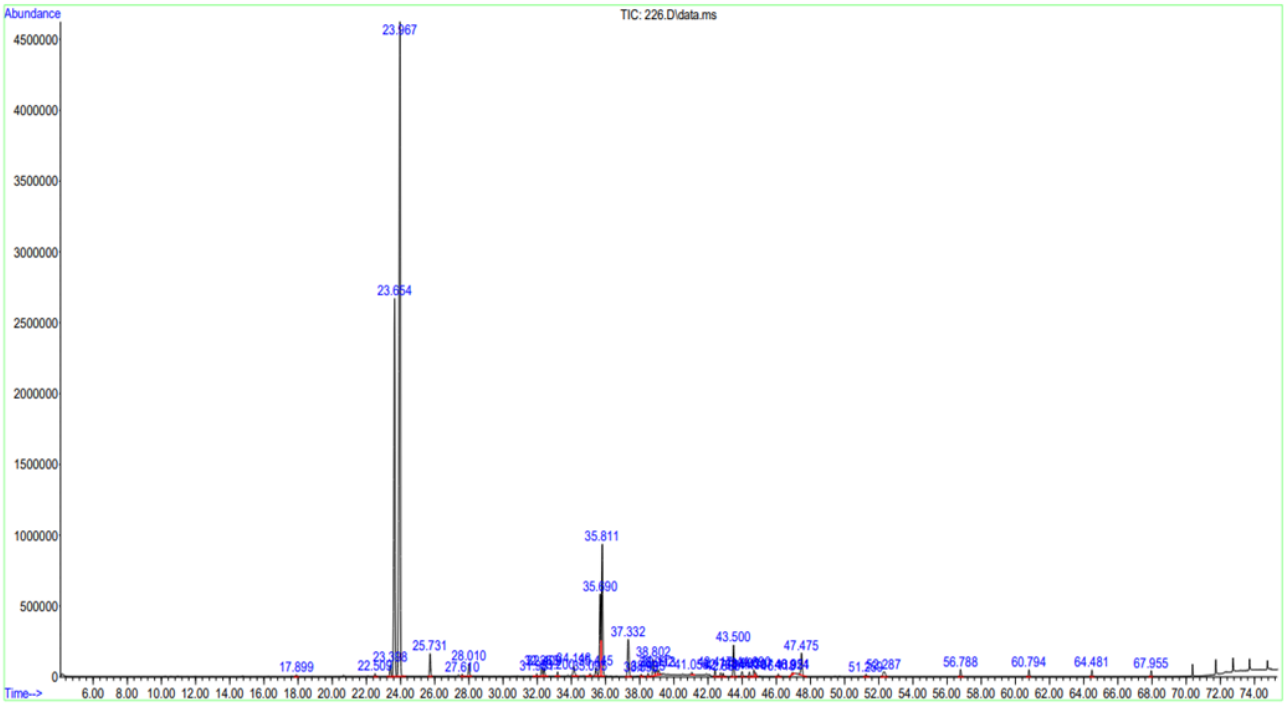

Fig. 1. GC chromatogram of AG essential oil

Table 4. Microbial load of AG

Method & Test name	Test Reference	Specifications	Test Results	Unit
Total Plate Count	USP 40	Max 10^5	< 10	cfu/gr
Total yeast & Mold Count	USP 40	Max 10^3	< 10	cfu/gr
E. coli	USP 40	Negative	Negative	cfu/gr
Salmonella spp.	USP 40	Negative	Negative	cfu/gr

4. Discussion

The amount of ash and moisture in Iranian AG have been measured and reported in a few studies. Also, these data are not mentioned in the Iranian herbal Pharmacopoeia [3]. In a recent study on Iranian AG, the total ash content and moisture content were 3.85 ± 0.11 and 10.54 ± 0.75 %, respectively [18]. Given that pure type of AG (namely tear-shape) are very rare and out of reach; also, the mass-shape AG contain many impurities, this difference in the amount of total ash may be due to external contamination, which is very variable in different samples. Also, the difference in humidity can depend on the freshness of the sample. Thus, the samples evaluated in the current study seem to be cleaner but older than the previous study. Considering the maximum total ash as 15 % mentioned in the Iranian herbal Pharmacopeia; the sample evaluated in the current study with 2.83 % total ash, is of sufficient quality.

The results of GC-MS analysis of AG essential oil in the current study indicated that compounds including (*E*)-1-propenyl sec-butyl disulphide, (*Z*)-1-propenyl sec-butyl disulphide, 2,4-thiazelidinedione, 4-chloro-2-nitrotoluene, tetrahydro thiazole and *β*-maaliene had the highest values, respectively. While

(*Z*)-*β*-ocimene and (*E*)-1-propenyl sec-butyl disulphide are mentioned in Iranian herbal Pharmacopoeia as the most important components of AG essential oil [3]. Also, in a study evaluating the composition of AG essential oil from 13 regions of Iran and one sample of Afghanistan, major constituents were reported as follows: (*E*)-1-propenyl sec-butyl disulfide, *β*-pinene, (*Z*)-1-propenyl sec-butyl disulfide, *α*-pinene, thiophene and thiourea [11].

In the current study, two of the major constituents of the AG essential oil were (*E*)-1-propenyl sec-butyl disulfide (43.90 %) and (*Z*)-1-propenyl sec-butyl disulfide (23.30 %). These compounds have also been identified as the most abundant constituents in several studies conducted on Iranian *F. assa-foetida* essential oil. For example, Hassanabadi et al. reported the values of (*E*)-1-propenyl sec-butyl disulfide and (*Z*)-1-propenyl sec-butyl disulfide, as (13.66-49.35%) and (2.02-15.29 %) respectively [11]. Also, in a recent study by Karimi et al., the presence of these compounds in the essential oil has been shown (0.18-49.25 %) and (0.62-15.86 %) in ten Iranian *F. assa-foetida* samples, respectively [19]. Two other studies that were conducted on some Iranian *F. assa-foetida* samples, reported the amounts of these compounds as the main components of the essential oil as (27.7 %) and (40.15 %, 44.36 %) for (E)-1- Propenyl sec-butyl disulfide as well as (27.7 %) and (23.93 %, 27.98 %) for (Z)-1-Propenyl sec-butyl disulfide respectively [20, 21].

Other major constituents of essential oil in the current study were 2,4-thiazolidinedione (8.19 %), 4-chloro-2-nitrotoluene (5.13 %), tetrahydro thiazole (2.92 %) and *β*-maaliene (1.92 %). Among them, only *β*-maaliene had been reported as one of the major constituent of Iranian *F. assafoetida* essential oil in a recent study [22].

According to current findings and previous studies, a high level of variation is observed in the composition of essential oils extracted from Iranian AG [11]. These variations primarily due to genetic variation, different geographic origin and climatic situations; however, extraction and storage methods also may affect it. On the other hand, another study showed that different types of AG, including tear, mass and paste, are different in terms of chemical composition [23]. Given that in the Iranian herbal Pharmacopoeia, there is no quantitative standard for the permissible content of essential oil components obtained from AG of different regions of Iran; so, it is not possible to judge the quality of the examined gum based on the available data.

The total phenol content of AG was determined in this study as 4.15 ± 0.26 mg Gallic acid/g. Although, assay of flavonoids by thin layer chromatography using Rutin solution as a control solution, has been suggested in Iranian herbal Pharmacopoeia for evaluating the purity of AG, but in the current study, the amount of total flavonoids measured by HPLC method, resulted to small amounts and no report was obtained.

In another study on Iranian AG, the amount of total phenol and flavonoids measured based on quercetin was reported to be 9.67 ± 0.45 mg Gallic acid/g dried extract and 0.11 ± 0.02 mg QE/g dried extract, respectively [18]. Of course, these values have been reported for the alcoholic extract of AG, which will usually contain a higher concentration of active ingredients compared to crude sample. There is no standard range for the total phenol and flavonoid content of AG in Iranian herbal Pharmacopoeia as well, to judge about the quality of the current sample.

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Finally, the results of microbial control of AG sample showed that the microbial load according to US Pharmacopoeia criteria is acceptable. Extensive antibacterial and antifungal effects that have been reported in various studies on *F. assa-foetida* can be a reason for low contamination of AG [6, 23].

5. Conclusion

Although the Iranian herbal Pharmacopoeia has not provided the standardization criteria derived from Iranian AG samples, but compared to the limited available data, the sample examined in this study was of relatively good quality. The findings of this study provided data on some physico-chemical properties of an Iranian AG sample. However, more information is needed to achieve accurate standardization criteria, which will be obtained from extensive research.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

FA: Concept, Design, Manuscript preparation and editing. FE: Manuscript review. MN: Supervising. ZB: Manuscript review. ND: Manuscript review.

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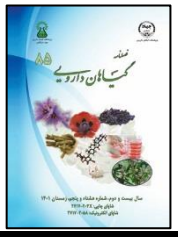

مقاله تحقیقاتی

برخی خواص فیزیکوشیمیایی و فیتوشیمیایی اولئو گام رزین آنقوزه ایرانی

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اولئو گام رزین

چکیده

مقدمه: آنقوزه یک گیاه دارویی انحصاری ایران است. اولئو گام رزین آن یا صمغ آنقوزه، فرآورده گیاهی مهمی است که استانداردسازی آن برای اطمینان از موثر بودن و ایمنی آن اهمیت دارد. **هدف:** به دلیل فقدان داده‌های دقیق و کامل در مورد صمغ آنقوزه ایرانی، هدف از این مطالعه این است که با گزارش بعضی خواص فیزیکوشیمیایی و فیتوشیمیایی یک نمونه صمغ آنقوزه ایرانی، دانش موجود توسعه داده شود. **روش بررسی:** خوص ارگانولپتیک با ارزیابی حسی تعیین شدند. همچنین خاکستر تام، خاکستر نامحلول در اسید، باقیمانده خشک و نیز محتوای تام فنولی و فلاونوئیدی با روش‌های پذیرفته شده اندازه‌گیری شد تا بعضی از خواص فیزیکوشیمیایی و فیتوشیمیایی این اولئو گام رزین تعیین گردد. آنالیز اسانس آن بوسیله GC/MS و نیز ارزیابی بار میکربی انجام گردید. **نتایج:** صمغ آنقوزه توده‌ای قهوه‌ای مایل به زرد با بوی تند گوگرد- مانند شبیه به بوی سیر، با خاکستر تام و خاکستر نامحلول در اسید به ترتیب برابر با ۲/۸۳ درصد و ۲/۰۶ درصد و باقیمانده خشک ۳/۴۳ درصد بود. محتوای تام فنلی برابر با ۰/۲۶ ± ۴/۱۵میلی‌گرم گالیک اسید در هر گرم صمغ خشک بود. ترکیبات عمده در آنالیز اسانس آن شامل (*E*)-۱- پروپنیل سکو بوتیل دی سولفید، (*Z*)-۱- پروپنیل سکو بوتیل دی سولفید و ۴،۲- تیازلیدین دیون بودند. بار میکربی در محدوده قابل قبول فارماکوپه بود. **نتیجه‌گیری:** یافته‌های حاضر اطلاعاتی را در مورد پاره‌ای خواص فیزیکوشیمیایی و فیتوشیمیایی صمغ آنقوزه ایرانی فراهم می‌سازد که برای کامل کردن دانش موجود کاربردی است. اگرچه اطلاعات بیشتری برای استانداردسازی مورد نیازاست که از مطالعات وسیع‌تر حاصل خواهد شد.

مخففها: PM، طب ایرانی؛ GC-MS، کروماتوگرافی گازی متصل به طیف سنجی جرمی؛ WHO، سازمان بهداشت جهانی؛ TSB، تریپتیک سوی براث؛ SDA، سابوریتد دکستروز آگار؛ CFU، واحد تشکیل کلنی

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