



Publisher homepage: www.universepg.com, ISSN: 2663-6913 (Online) & 2663-6905 (Print)

<https://doi.org/10.34104/ajpab.024.018027>

American Journal of Pure and Applied Biosciences

Journal homepage: www.universepg.com/journal/ajpab



Isolation, Identification and Determination of Effective Substances of Three plants, Yarrow, False Chamomile and Fennel

Fatemeh Khezrian*

Faculty of Basic Sciences, Tehran University of Research Sciences, Tehran, Iran.

*Correspondence: anadanaalvand@gmail.com (Fatemeh Khezrian, PhD Student in Plant Biology (Systematics), Faculty of Basic Sciences, Tehran University of Research Sciences, Tehran, Iran).

ABSTRACT

This research was conducted in order to determine the amount of effective substances of yarrow, false chamomile and fennel in Hamadan region. First, the alcoholic extract of the three above-mentioned plants was obtained by maceration method. In this way, the body of the desired plant is dried and crushed and immersed in 70% alcohol and left in the same state for 14 days, and then the mixture is filtered and the dried extract is weighed and the ratio of the weight of the liquid extract to the weight of the dry plant was obtained. Also, the numerical amount of total ash and ash insoluble in acid was determined to determine the amount of solutes. Also, the amount of protein, calcium and phosphorus was obtained in three plants. By performing TLC chromatography (paper method), the type of active substances in three plants was determined and the pH of each of the manufactured products was also determined, and the results are as follows: The amount of insoluble ash in fennel acid was 1.6%, yarrow 2.8% and false chamomile 6.8%, and the amount of total ash was 12.9%, 13.7% and 7.4%, respectively. The percentage of calcium and phosphorus in fennel is 0.4% and 0.13%, in yarrow 0.98% and 0.5%, and in false chamomile is 0.74% and 0.39%. The amount of protein in fennel was 4.8%, in yarrow 24.5% and in false chamomile 19.2%. The pH of the products was as follows: the product prepared from fennel is 85/6, yarrow 80/4 and false chamomile 80/5. The results of paper chromatography of three plant samples are as follows: In fennel, in the vicinity of solvent 1 ($R_f = 0.6$ and 0.51), which is related to antole, and in the vicinity of solvent 2 ($R_f = 0.84$ and 0.76), which is related to fanchon. In yarrow plant, $R_f = 0.86$ is related to betapinene, $R_f = 0.72$ is related to sineon, and finally $R_f = 0.1$ is related to Borneon's special substance. In the false chamomile plant, $R_f = 0.21$ is related to gamma element, $R_f = 0.30$ to beta element, and $R_f = 0.39$ to the effective substance transmatricaria ester. By performing these steps and comparing the results obtained from previous researches on these plants, a step was taken towards the realization of the aforementioned goals, which is to determine the amount and type of effective substances of these three plants in Hamadan region.

Keywords: TLC active ingredients, False chamomile, Yarrow, Effective substances, and Fennel.

INTRODUCTION:

For many years, natural medicines, especially medicinal plants, were considered the basis and even in some cases the only way of treatment, and at the same time,

the raw materials contained in them were used in the pharmaceutical industry. At the beginning of this century, the progress of chemistry and the discovery of complex systems of organic synthesis led to the deve-

lopment of the pharmaceutical industry, which was able to cure many incurable and often fatal diseases. This is especially true for infectious diseases that have been treated with sulfamides, antibiotics, and other chemical compounds (Bedevian and Dip, 1994; Akha *et al.*, 2014; Sharif *et al.*, 2019).

Immunotherapy (severe stimulation of the body's defense system) also plays a big role in eliminating many infectious diseases. Despite this, medicinal plants and medicines prepared from them were never completely abandoned. The primary and effective materials that are stored in plants have been and will continue to be useful as irreplaceable materials. Also, folk medicine and perfumers have never stopped using these substances and have always passed traditional medicine in the form they inherited from their fathers and ancestors. With the passage of time, the number of known medicinal plants has increased and their fields of application have expanded. Discovering new plants among samples brought from distant lands, achieving new uses as auxiliary drugs in chemical or antibiotic treatments, realizing the health value of plants and finally, the discovery of new substances such as vitamins, hormones, anti-microbial, anti-viral, anti-tumor substances among known plants or newly discovered plants, once again helped in the progress of herbal medicine. Finally, in the new era of pharmaceutical industries, doctors and research groups of many countries again focused their attention on natural resources and medicinal plants, so that today we are witnessing vast experimental and production farms (Bruneton & Barton, 1987; Duke, 2002). Cultivation of medicinal plants is currently considered as an important branch of agriculture, which is used for the extraction and production of raw materials that are used in the manufacture and production of existing drugs. In this way, it can be seen that at no time has the attention to medicinal plants and the effects of their application and method of use been completely stopped.

Currently, patients are trying to gain more knowledge about the uses of effective substances in them and their use in the treatment of various diseases. At the same time, you should not lose sight of the fact that natural medicines are not always suitable for the treatment of any type of disease, and only a doctor can

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regulate the type of treatment and its schedule and during the treatment period, it is necessary for the patient to be under the supervision and care of a doctor. Due to the fact that the active substances in herbal medicines are in a state of biological balance due to their association with other substances. Therefore, they are not accumulated in the body and do not cause side effects, and therefore they have no significant superiority over chemical drugs. In this regard, only exceptions are poisonous plants that should never be consumed without a doctor's prescription (Loggia *et al.*, 1992; Ono *et al.*, 1996; Momtazi *et al.*, 2017).

The use of plants and their use in pharmaceuticals and replacing imported drugs with them in Europe has increased day by day and the results have been consistently positive. Doctors, pharmacists, and the general public are constantly discovering the beneficial effects of plants and the effective ingredients in them, so that they can heal patients by using the greatest treasure of health, nature. (Jean and Lag and Jiri Stodola, Medicinal plants) With the approach that has happened to medicinal plants and natural treatments, it is necessary to identify and classify the effective substances and healing compounds of plants so that pharmacists, doctors and consumers know their duties. By doing this research, information is obtained that was not available before or no special work has been done in this case, the work done in this research leads us to the amount and type of effective substances in the three plants of yarrow, chamomile and fennel that grow in Hamadan region and according to the studies done in other places, it is possible to compare the amount and type of compounds. Considering that each ecological region has its own weather, soil and climate, different effective substances and plant products can be expected in a plant that grows in different regions. This issue is very important to know, for example, the differences and similarities between the cultivated or wild yarrow in Hamedan region and the yarrow grown in the northern region of the country in terms of vital and medicinal substances, and which one is richer. (according to which material is more valuable for us).

Research background

The research done on these three plants has already been done by many researchers in different places and

the work done in this research is to compare the compounds obtained in different places with the desired plants in Hamedan. Extracting and determining the amount of dry extract and liquid extract and determining the amount of total ash and ash insoluble in acid, the amount of calcium, phosphorus, solutes and the amount of protein and TLC (thin layer) chromatography and determining the pH of the product made by Mr. Dr. Razaghi was done for this purpose and the results were compared with previous similar works.

Afaq Nazar Alipour and Fatemeh Sefidkan have conducted a quantitative and qualitative study of the medicinal and aromatic plant essence of false chamomile in the Tehran Forestry and Pasture Research Institute. In this research, the aerial parts of *Tripleurospermum disciforme* were collected in July 2010 in the full flowering stage from Taleghan region, in the north of Tehran and after drying in the shade at ambient temperature, the essential oil was extracted from the flowering branch of the plant three times by steam distillation. The essential oil was obtained as a light yellow oily layer with a yield of 45%. Zahedi Khorasani, Mehdi Taherian, Abbas Ali, Vafai, Abbas Ali Rajabi, Mohammad Reza Rashidipour have evaluated the effect of the hydroalcoholic extract of *Achillea millefolium* yarrow plant on modulating anxiety in small laboratory mice. Mohammad Bakarrezaei, Kamkarjaimand, Ahmad Majed, Mehdokht Madah have studied the effect of collection time on the amount of essential oil and chemical compounds of fennel plant *Foeniculum Vulgare* Mill in Tehran Forestry and Pasture Research Center. Fatemeh Sefidkan has also conducted a quantitative and qualitative study of fennel essential oil in different stages of growth at the Forest Research Institute in Tehran.

The necessity and importance of the conducting research

Basically, in order to prepare medicinal products from plants, both industrially and modernly, as well as traditionally and domestically, it is necessary to be aware of the quantity and quality of the effective medicinal substances that exist in plants in complex form. The analysis factors mentioned in the previous few lines should be considered for all medicinal plants and prepare the ground for researchers who intend to make drugs and products. In addition to introducing

new and effective drugs to the medical community and patients, this work prevents intentional and unintentional frauds and mistakes, and the benefit of this process goes back to the consumers, because the lack of correct identification of the medicinal plant used can lead to poisoning and serious problems at least the problem that arises from the consumption of non-original plants will be the lack of favorable results. In addition to the mentioned materials, it is necessary that these materials help to complete the knowledge about the researched plants as basic information. Training and familiarization with the methods of measurement and analysis by device and manual is another goal of this research. Because during this research, experiences related to working with analytical devices and familiarity with reagents and detectors (chromatography) and methods of preparing solutions for pH measurement are obtained, which are useful and practical (WHO, 1999).

Conceptual framework

From plant to medicine

The fresh and live plant that has medicinal substances is known as the mother plant. In this case, it is not exactly a medicine and it will not become a medicine unless the actions that we explained to you are done, especially in relation to drying on it. Today, things like crushing, grinding, sieving, and mixing plants are done mechanically in pharmaceutical factories; And the only manual operation that needs a lot of practice and experience is peeling some parts of the plant, such as the roots of riwand and khatmi, etc. Dried medicinal plants or their used parts are called herbal medicines. According to the part of the plant from which these drugs are obtained, they are given Latin names as follows: Stem, stem (Single), seed, tuberous filaments, spore, root, rhizome (potato stem), tuber, bulb, fruit peel, peduncle, oung part of stem end, bud leaf, wood, bark, flower, stigma, fruit seed, vegetable, seta, along with these parts sap and extract (Succus), resins (Resinae), resin gum (Gummi-resinae), balsam (Balsamum) are also harvested. The name of the drugs also sometimes specifies way of preparation or their action:

- 1) Naturalis
- 2) Concissa
- 3) Mundata
- 4) Pulvis

The raw materials in the plants should be adapted to the specific nature of the desired plant and their therapeutic use by performing a series of chemical or mechanical actions. The methods of preparing the plant after collection and during its preparation are specified with precise instructions. The instructions that have been obtained as a result of long experiments and measurements of many effective substances of plants. These instructions include how to harvest or collect, dry, peel, chop, remove some parts, grind, sift, pound, roast (put on fire) and even the way plants are fermented. Each of these actions is to preserve and keep the effective substances in a type of plant. Drying of medicinal plants like other useful plants (such as spices and plants with industrial uses) should be done by their producers. The time to collect the plant in nature or in the field is chosen in connection with the presence of the maximum effective substance in the plant during its growth. Usually, the collected plant should be exposed to drying as soon as possible to prevent damage during wilting. In most cases, the method of drying the plant under direct sunlight is not used. Because sunlight destroys raw materials and the plant quickly turns yellow and brown. The plants that are collected for extracting essential oils lose one-third of their raw materials by drying them in sunlight, while the wheat and mint lose one-fifth of their raw materials.

In some cases, placing the plant in the sunlight for a short period of time before placing the plant in a covered place that has air flow and an aerator is recommended. Drying a plant means gradually taking moisture from the plant. Often, before drying, it is necessary to wash the plant for a short period of time in order to remove dust, dirt, and dirt.

Effective ingredients of medicinal plants

The effective substances of medicinal plants are of two types: First, substances resulting from primary metabolism (mainly saccharides) or necessary and vital substances, which are produced in all green plants by photosynthesis. The second type of substances resulting from secondary metabolism, which are produced as a result of nitrogen absorption by the plant. Apparently, these products are often useless for the plant, but on the contrary, their therapeutic effects are significant. These compounds mean oil essences (or natural

essences), resins, various alkaloids such as opium. Generally, these materials are not found in their natural state and are combined with other elements that strengthen their effects. In this case, even if the medicinal plant has only one active substance, its effect on the human body is more useful than the same substance obtained from chemical synthesis. This feature proves the preference of herbal therapy or the use of drugs that have herbal roots. Here, the effective substance is not only a chemical compound, but it has a physiological balance that the body tolerates better and does not leave side effects, which is a good reason for the preference of natural medicine. In this case, we can mention opium, which is the dried sap of poppy mace, which contains a significant number of important alkaloids in addition to a large number of different substances (Pharmacopoeia, 2000; Wagner & Bladt, 1996; Uddin *et al.*, 2023). Each alkaloid that we separate from other elements has a completely different effect from the total opium and shows its own specific effects on the human body (pharmacological effects). The digital plant (thimble flower) is also the same. Currently, there are modern methods that allow elements in plants to be discovered. In the first stage, microscopic studies are carried out on the anatomical structure and appearance of the plant (microscopic atlas of medicinal plants).

Then physical methods such as microsublimation, which includes heating small amounts of medicine and collecting the emitted vapors on special glasses, which are then separated by chemical analysis. Some materials are identified by the fluorescent light they emit under the radiation of a mercury lamp. Finally, the presence of some elements in the plant is investigated through quantitative and qualitative chemical techniques. These methods are presented in specialized articles that respond to the existing standards at the national level and the expectations of the quality of medicinal plants.

From the point of view of chemistry, the type and type of drug by the amount of substances in the main groups such as: alkaloids, legosides, saponins, bitter agents, tannins, aromatic substances, essential oils and terpenes, fatty oils, glucokinin, plant mucilages, hormones and plant disinfectants, which are among the most important substances, are studied.

Extracting constituents of medicinal plants

The extraction of effective substances in the plant is done by different solvents. It is worth mentioning that the practice of extraction has been common since ancient times, especially during the time of Galen. In general, the method of extracting effective substances in plants depends on the type of plant tissues and plant compounds (Shahen *et al.*, 2019).

MATERIALS AND METHODS:

In the present study, three plant samples of yarrow, false chamomile and fennel were collected from Shahid Madani Agricultural Research Center. The samples were dried separately, then weighed and crushed to perform the extraction process and poured into jars containing 70% alcohol and dated on the containers and remained in this state for 14 days. During this time, the contents of the jars were mixed and stirred with a spoon, and if the volume of the solvent decreased, some 70% alcohol was poured on the plants so that the plants remained immersed. After the elapsed time, we filtered the contents of the jars with a special mesh and weighed the obtained solution as well as the plant pulp and put the obtained solution (extract) in a suitable tray and dried it in a shady place away from light and the dried extract of plants (yarrow, false chamomile, fennel) was recorded by weight and numbers obtained to obtain the amount of extract obtained from a certain weight of the plant. Ointments and products were made from the dried extracts according to the special formula of Mr. Dr. Razaghi. Ointment against nosebleeds was made from yarrow plant, cream against wrinkles and skin inflammation

was made from false chamomile, and anti-hair growth cream was made from fennel extract. In order to obtain total ash and ash insoluble in acid and chromatography and the amount of calcium, phosphorus and protein of powdered plants and determine the pH of the products, the following steps were performed:

Total ash determination method:

Required equipment:

- 1) Accurate scale with a sensitivity of 0.1 mg
- 2) Electric furnace with the ability to adjust the temperature at 550 ± 20 degrees Celsius
- 3) China plant suitable for ashing made of silica, nickel or china plant
- 4) Desiccator

RESULTS AND DISCUSSION:

In this chapter, in brief, the results obtained from the tests to determine calcium, phosphorus and protein, the percentage of solutes (ash insoluble in acid), the percentage of total ash, the percentage of moisture and pH of the products, and the results of chromatography and Rf obtained with the standards recorded in Iranian herbal pharmacopoeia was compared.

Moisture percentage

The amount of 5 grams of the desired plant has been turned into powder and poured into the Chinese plant and weighed. (Weight of empty Chinese bush and bush with plant) is recorded. The desired plant is incinerated in an electric furnace, and with the obtained information, the moisture percentage was calculated as follows.

Table 1: Moisture percentage.

Plant sample	Weight of empty plant	The weight of the ash plant and plant	Plant weight with fresh plant	Moisture percentage
Fennel root	29/139	33/885	34/139	%5/08
Yarrow leaves	27/579	32/302	32/579	%5/54
False chamomile flower	22/610	27/361	27/610	%4/98

$$MD = \frac{\text{The weight of the ashed container and sample} - \text{Weight of empty plant}}{\text{Sample wet weight (gram)}} \times 100$$

Ash insoluble in acid

The amount of two grams of the powdered plant was weighed in a china bush and the ash was filtered with hydrochloric acid and distilled water in filter paper, UniversePG | www.universepg.com

and the remaining solutes were weighed in the filter paper and the percentage of ash insoluble in acid was obtained.

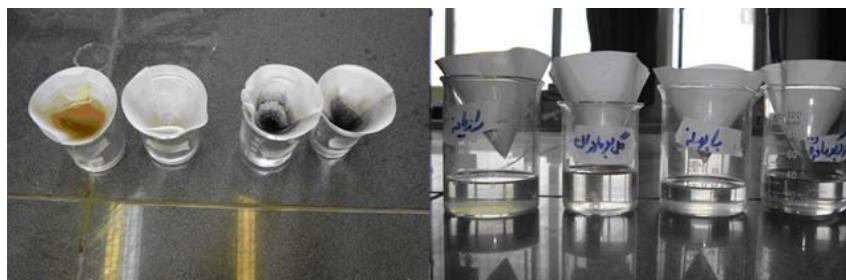


Fig. 1: Ash insoluble in acid.

Table 2: The percentage of insoluble ash.

Plant sample	Weight of empty plant	Bush with ashes	Weight of empty filter paper	Smooth paper with insoluble ash	The percentage of insoluble ash
Fennel root	26/533	26/774	0/900	0/933	% 1/6
yarrow root	25/609	25/877	0/903	0/960	% 2/8
False chamomile flower	26/805	26/949	0/914	1/05	% 6/8

$$\text{The percentage of insoluble ash} = \frac{\text{Weight of ash and filter paper} - \text{empty filter paper}}{\text{Sample weight (gram)}} \times 100$$

Total ash

The amount of 1 gram of the powdered plant is weighed in a Chinese pot and then placed in the

furnace and finally the percentage of total ash is calculated.

plant sample	Weight of empty plant	Bush with ashes	Total ash percentage
Fennel root	22/691	22/820	12/9%
yarrow root	21/529	21/666	13/75%
False chamomile flower	25/314	25/388	7/4%

$$\text{Total ash} = \frac{\text{The weight of the container and ash} - \text{the weight of the empty container}}{\text{Sample weight (gram)}} \times 100$$

Determining the percentage of calcium and phosphorus

During the steps described in the materials and

methods chapter, the percentage of calcium and phosphorus was obtained, and the results are as follows:

Table 3: Percentage of calcium and phosphorus.

Plant sample	Reagent number	Percentage of calcium	Percentage of phosphorus	Spectrophotometer number
Fennel root	0/96	0/4	0/13	0/006
Yarrow root	1/54	0/98	0/5	0/023
False chamomile flower	1/30	0/74	0/39	0/18



Fig. 2: Image of spectrophotometer.



Fig. 3: Final calcium titration step.

$$\text{Phosphorus percentage} = \frac{100}{20} \times \frac{100}{1000} \times \frac{100}{1} \times \text{amount read} \times (0.4365) \text{ constant number}$$

$$\text{Calcium percentage}$$

$$= ((0.02 \times \text{Normality of potassium permanganate} \\ \times (\text{The volume of potassium permanganate used in the sample} \\ - \text{The amount of potassium permanganate consumed by the control})) \\ / (\text{Volume taken from filtered solution} \times \text{sample weight in grams}) \times 100$$

Determination of protein percentage

During the steps mentioned in the materials and methods section, the percentage of protein was obtained:

Plant sample	Reagent number	Weight of dry samples	Protein percentage
Fennel root	3/77	0/625	4/80
yarrow root	10/69	0/375	24/50
False chamomile flower	14/01	0/631	19/02

$$\text{Protein percentage} = \%N$$

$$= \frac{\text{Weight of ash (useful acid of control} - \text{consumed acid of plant sample}) \times 0.014N \times 100}{\text{sample weight}} \times 100$$

$$\%CP = \%N \times \text{Protein coefficient (the protein coefficient differs in different cases)}$$

$$\%N = \text{Nitrogen percentage}$$

$$\%CP = \text{Percentage of crude protein}$$

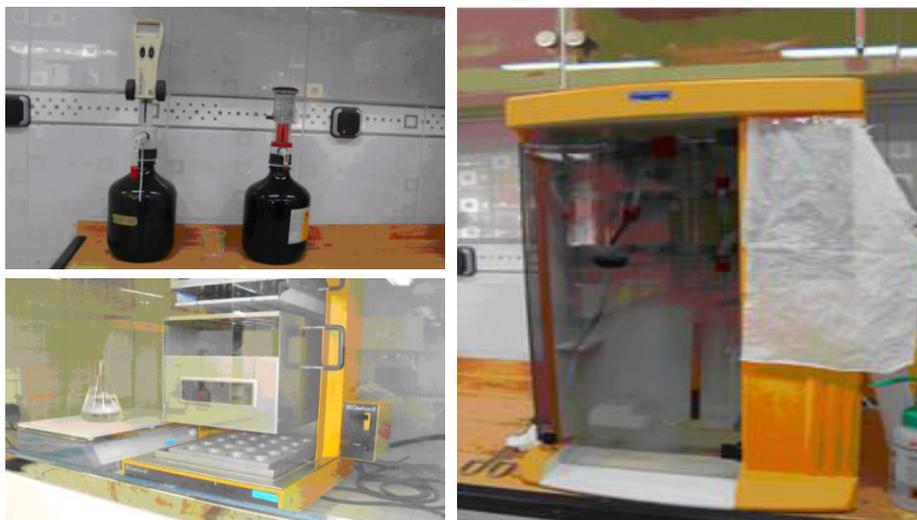


Fig. 4: Image of the protein percentage determination device.

Determination of pH of products

The pH of the products made by Dr. Razaghi was measured with a pH meter.



Fig. 5: Image of pH meter device.



Fig. 6: Chromatographic image.

Table 4: Determination of pH of products.

Sample name	pH
Fennel anti-bleeding product	6/85
Yarrow anti-bleeding product	4/80
Chamomile anti-wrinkle and anti-inflammatory product	5/80

The results of paper chromatography to identify and determine the type of effective substances in plant extracts

The paper chromatography of plant extracts (three samples) was performed with 3 repetitions and the following results were obtained:

Chromatography of fennel

Two solvent series of fennel extract were used in chromatography. In the solvent system number 1, which included: toluene - normal hexane, the obtained Rf's were: $R_f = 0.6$ and $R_f = 0.51$, which are related to Entol. In the solvent system number 2, which used toluene-ethyl acetate solvents, the following Rf's were obtained:

$R_f = 0.72$ and $R_f = 0.84$. Finally, because concentrated sulfuric acid reagent was used to make the spots more visible, the tulle immediately turned red to violet, which corresponds to $R_f = 0.51$. In the picture below, you can see the cut fennel root.

Solvent 1	$R_f=0/51, 0/6$	Anne Tull
Solvent 2	$R_f=0/72, 0/84$	Funchon

Yarrow chromatography

In the chromatography of Yarrow alcoholic extract, toluene-ethyl acetate solvent was used, during which the following Rf's were obtained:

Table 6: Solvents used in yarrow chromatography.

R_f	0/86	0/72	0/51	0/1
The desired material	Batapi nan	betatogon	Cineol	Borneo

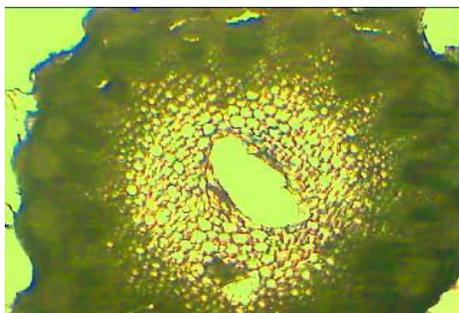


Fig. 8: Yarrow stem cut.

$R_f = 0/86, 0/72, 0/51, 0/1$ Vanillin-sulfuric reagent was also used to make the spots appear. In the figure below, a view of the yarrow stem section is shown.

Chamomile chromatography

The following Rf's were obtained in false chamomile chromatography, where dichloromethane and toluene were used:



Fig. 7: Image of cut fennel root.

R_f	0.21	0.30	0.39
The desired material	Gamaal man	Betaalman	trans trans matrix ester

$R_f = 0/21, 0/30, 0/39$ Of course, the anisaldehyde-sulfuric acid detector should have been used, but it was not possible to prepare this substance. Finally, an ultraviolet lamp was used to better see the stain (creating a fluorescence state).

CONCLUSION:

The obtained results of total ash for yarrow is 13.7%, which is maximum 10% in pharmacopoeia. This difference can be due to different test conditions or differences in the habitats and climate of different regions where this species is grown. The percentage of total ash for false chamomile was 7.4% and for fennel was 12.9%, which was not reported in the pharmacopoeia about the total ash of these two plants. Among these three plants, yarrow had the highest percentage of total ash. The percentage of ash insoluble in acid is also measured in such a way that first, 2 grams of the desired plant is weighed and poured into a bush that is

weighed separately and placed in an electric oven for 5 to 6 hours at a temperature of 105 to 110 degrees Celsius and then put it inside the desiccator to cool down. In the next step, mix 15 milliliters of water and 10 milliliters of 36% hydrochloric acid and pour some of this solution inside each of the plants and then boil the desired mixture slowly and after cooling, a relatively smooth solution has appeared, which is poured into a small beaker, in the mouth of which a filter paper was placed in the form of a funnel, and they wait for it to become smooth and from time to time, they wash the inside of the funnel with distilled water, and finally, they burn the dried filter paper that contains insoluble ash and weigh the residue according to the formula, the percentage of insoluble ash in yarrow leaves was 2.8%, in false chamomile was 6.8% and fennel root was 1.6%. which were slightly different from the numbers obtained in the pharmacopoeia, in that 2.5% for yarrow and 1.5% for fennel in the pharmacopoeia, and similar work had not been done for false chamomile. Basically, the plants that are interested in siliceous soils have salts and a higher percentage of ash insoluble in acid. Among the mentioned plants, Chamomile grows mostly in limestone and siliceous soils, and the ratio of solutes in it is almost high compared to the other two plants. The obtained results showed that the percentage of calcium in fennel is 4%, yarrow is 98%, and false chamomile is 74%. In the book of herbal knowledge of Mirhaydar, volume 6, the percentage of calcium in fennel leaves is reported as 0.2% which has a lower percentage compared to fennel root. Fennel root contains higher amounts of calcium. Which is probably due to the accumulation of minerals and salts in the plant roots that are in the vicinity of the soil and fertilizers. Phosphorus percentage of false chamomile in the conducted research is 0.39 and no other information was obtained in this case. The results obtained in the present research are relatively close to the results of the pharmacopoeia and it seems that the difference is due to the different working conditions in the laboratory. Another thing is that the studied species in Hamedan has different climatic conditions from Tabriz, where the information about fennel from that city is recorded in the pharmacopoeia. In the case of yarrow, the obtained results have a partial difference with the Rf recorded in the herbal pharmacopoeia of Iran, which could be due to

the difference in the location and climate of the study which has an effect on the concentration of effective substances and as a result Rf (the distance traveled by the object to the distance traveled by the solvent) and also the test error. The second major component of false chamomile essential oil is cis-calamenene, which constitutes about 23% of the weight of the essential oil. Another important compound is beta-farnesin, which is insoluble in water and is a component of many aromatic essential oils. Farnesin is present in the amount of 12.5% in false chamomile essential oil, which gives the essential oil a good aroma and smell. Another major compound identified in false chamomile essential oil is maalin, which is a tricyclic terpene sescoside. In general, most of the components of false chamomile essential oil are terpene, among which matricaria ester gives it medicinal properties and farzene gives it a special aroma.

ACKNOWLEDGEMENT:

We are grateful to all the dear professors for providing their information regarding this research.

CONFLICT OF INTERESTS:

The authors declare conflicts of interest obviously in the manuscript and have no conflict of interest.

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Citation: Khezrian F. (2024). Isolation, identification and determination of effective substances of three plants, yarrow, false chamomile and fennel. *Am. J. Pure Appl. Sci.*, **6**(1), 18-27.

<https://doi.org/10.34104/ajpab.024.018027>

