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Original Article

Optimization of Essential Oil Extraction Conditions for *Rosmarinus officinalis* L. on a Laboratory and Semi-industrial Scale

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Abstract

Rosmarinus officinalis L.is of the most important species of medicine, which is used in various food, pharmaceutical and sanitary industries and for this reason, it is considered as one of the most important export figures in the world. In order to study the effect of extraction of essential oil by distillation with water, in a laboratory scale and semi-industrial scale, an experimental design was carried out at the headquarters of the Research Institute of Forest and Rangelands for two years (2018 and 2019).The samples were collected from Alborz research station located in Karaj city. Then the plant samples were dried in shade and essential oils were extracted by water distillation method in laboratory and semi-industrial scale. The compounds of essential oils were measured by GC and GC/MS. In this study, according to experimental and pilot studies on rosemary species, different results were obtained. At this time, the necessity of testing in the laboratory was evident in order to determine the method and the appropriate amount of powder of the plant. In this regard, the rosemary leaves were prepared in three methods: full leaf, semi-powder and complete powder. Then samples were extracted by water distillation method (Clevenger apparatus) for 4 hours. The essential oil yield was obtained from full leaf (0.44%), semi-powdered leaf (0.46%) and pure powder (0.70%).Therefore, the most important time for the extraction of essential oils from plant specimens was determined in pilot and laboratory methods for 4 hours of essential oil extraction and the sample of the semi-powdered plant was determined by mesh of 10. Finally, the results of the important combinations identified. The major combinations identified with plant leaf powder on a laboratory scale are: Camphene (27.49%), Octanol acetate (10.39%), Benzyl formate (9.64%), Dihydro- linalool acetate (8.64%), Verbenene (8.30%), Neo-iso-dihydrocarveol (7.46%) and majorcomponents in the pilot were :Camphene (31.53%), Verbenene (10.90%), Benzyl formate (8.18%), Octanol acetate (8.14%), and α-phellandrene (7.18%). Considering the importance of the experimental method for extraction of essential oil and the application of the laboratory method to the semi-industrial, it is suggested that in the pilot plant the specimen with the mesh 10 should be used.In this experiment, the essential oil content of the sample extracted in the pilot sample was 0.46%, which is equal to 0.46% in the laboratory sample. It shows the economic value of this method for the application of other species.

Keywords: *Rosmarinus officinalis* L., Essential oil, Chemical Composition, Extraction, Laboratory, Semi-industrial

Introduction

The genus *Rosmarinus* belonging to the Lamiaceae family, is a pleasant-smelling perennial shrub that grows in several regions of all over the world [1]. Rosemary is an aromatic, bushy, attractive evergreen shrub with pine needle-like leaves, native to the Mediterranean countries [2] southern Europe and in the littoral region through Minor Asia areas wildly. Plants, including herbs and spices with an intense pleasant smell reminiscent of pine wood. The herb is economically important that cultivated or imported and sold in many markets all

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over the world. It grows up to 2 m high, with densely-leafy erect branches. Flowers are white to pale blue, in small clusters in the leaf axils at the tops of stems and last throughout the year. Rosemary is used fresh or dried. It is a well-known valuable medicinal herb that is widely used in pharmaceutical products [3]. It is essential oil and herbs were widely used in folk medicine, cosmetics, phytocosmetics, flavouring and conservation of food products [4-6].

Multiple studies have been reported on the chemical composition of the essential oils of *Rosmarinus officinalis* belonging to different regions in the world [6-8]. The essential oil of *Rosmarinus officinalis* has been the object of several studies antioxidant activity [9-13], antibacterial [14- 18], toxicity insecticidal [19,20], anti-inflammatory and antinociceptive [21], antifungal [1,22] and pest control products [23].

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition [24]. Also the essential oils are used in traditional medicine for their antiseptic action, are constituted 1% of plant secondary metabolites and are mainly represented by terpenoids, phenypropanoids or benzenoids, fatty acid derivatives and amino-acid derivatives [25]. The oils also help increase the flow of digestive fluids, improve digestion and eliminate gas and stomach cramping [26]. The purpose of this study was to investigate the extraction of essential oils at laboratory and semi-industrial scale.

Material and Methods

Vegetal Material

In order to study the effect of extraction of essential oil by water distillation, in a laboratory scale and semi-industrial scale, an experimental design was carried out at the headquarters of the Research Institute of Forest and Rangelands for two years (2018 and 2019). The samples were collected from Alborz research station located in Karaj city. Then the plant samples were dried in shade and essential oils were extracted by water distillation method in laboratory and semi-industrial scale. Three specimens of rosemary leaf were prepared as whole leaf and half powder leaf (with mesh 10) and whole powder leaf (with mesh 40),(Since we were not able to fully powder the noodle, we tested it as a half-powder),and the next issue was the sample size in the distillation container, which we decided to reduce the sample size. In vitro, essential oils from three rosemary leaf samples were completely and partially powdered and whole powdered.

Essential Oil Extraction by Water Distillation

In this study, according to experimental and pilot studies on rosemary species, different results were obtained. At this time, the necessity of testing in the laboratory was evident in order to determine the method and the appropriate amount of powder of the plant. In this regard, the rosemary leaves were prepared in three methods: full leaf, semi-powder and complete powder. Then samples were extracted by water distillation method (Clevenger appratuss) [27,28], for 4 hours. The essential oil yield was obtained from full leaf (0.44%), semi-powdered leaf (0.46%) and pure powder (0.70%).Therefore, the most important time for the extraction of essential oils from plant specimens was determined in pilot and laboratory methods for 4 hours of essential oil extraction and the sample of the semisweet plant was determined by mesh of 10.

The extraction of essential oils from the leaves of rosemary was performed by water distillation in a Clevenger-type extraction took 3.5 hours for mixing 100g of plants in 1600 ml of distilled water. The essential oil obtained was dried by anhydrous sodium sulfate and then stored at low temperature (below 4°C) and dark before use.

Specifications of Semi-industrial Essential Oil Extraction Machine

In 2012, the Institute of Forests and Rangelands Research in Iran decided to install, design, build, and operate a semi-industrial machine for extracting essential oils. For this purpose, a double wall distillation boiler with 500 kg heat insulation (width of 75 cm and length of 125 cm), first stainless steel wall 316L and second wall stainless steel 304 were constructed. The distillation boiler door is opened and closed with the help of a piston, which is the entire pneumatic system and works with a process control Programmable Logic Controller (PLC). There are also door open and door close buttons for emptying the distillation pot. The amount of water entering the double-walled tank is measured by a liquid turbine flow meter.

Steam Boiler

Steam boiler from J.A.M. Nazarians Factory, a 15-horsepower, Model BN.15.AG Colombian - American design, all-steel, all-automatic body, 225 k steam per hour, five types of protection system, McDonnell America design control level, Fantini Italy pressure switch, purchased and installed.

Cooling Tower

To cool the distillation system of the suction cooling tower (fan above), a 10 ton cross-flow cubic fiber glass with 1 kw motor power of a 1.20 m height axial fan with a diameter of 73 cm, splash type drops the German 2h design polymer clamp has been used.

Condenser Device

Also for cooling distilled water from cooling a steel distillation converter 2/316 L pass with a total length of 900 mm with a 10 x 1 mm steel tube with 82 bushings, flanges and steel 316 L bushings was used.

Process Control by Programmable Logic Controller (PLC)

A Programmable Logic Controller (PLC) is an industrial computer control system that continuously monitors the state of input devices and makes decisions based upon a custom program to control the state of output devices.

Touch Panel Computer System

Standard computer, printer, control board, sensor board, touch screen**,** wireless pane, for connection monitoring purposes, including PT100 temperature sensor (four sensors at the bottom of the distillation boiler, above the distillation boiler, at the bottom of the cooling and at the top of the cooling condenser device),pH measurement sensor, indicator, stability, Siemens Programmable Logic Controller **(**PLC) monitoring system includes the necessary hardware and software modules: turbine flow meter, glob control valve, ball valve, Control valves: on/ off valve, proportional valve, connect to computer

Semi-industrial Scale Essential Oil Extraction

In this experiment, essential oil of 15 kg of semi-powdered leaf (with mesh 10) of rosemary was taken for 4 hours. Percentage of oil by laboratory scale was 0.46% with compare semi-industrial scale 0.46% were equals.

Gas Chromatography

GC analyses were performed using a gas chromatography, Ultra-Fast Module –GC, made in Italia. Profile column machine brand Ph-5 capillary column, manufactured by Shimadzu with Length of 30 mm and an inner diameter of 1.0 mm with 0.25 μm film thickness, the inner surface of the stationary phase material is covered by 5% Phenyl Dimethyl Siloxane. Column temperature program: initial temperature 60 °C to start the final temperature of 210 °C. The initial temperature 3 °C per minute to be added and then injected sample into the chamber to a temperature of 280 °C. The carrier gas inlet pressure to the column: helium with a purity of 99/999% of the inlet pressure to the column equal to 5/1 kilogram per square centimeter is set.

Gas Chromatography-Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used. The column was same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V. The identity of the oil components was established from their GC retention indices, relative to C7- C25 n-alkanes standards mixture, and by comparison of their mass spectra and retention indices with those reported in the literature [29, 30, 31], and by computer matching with the Wiley 5 and NIST mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Result and Discussion

Many studies have been reported on the chemical composition of *Rosmarinus officinalis* essential oils in different regions of the world [6, 7, 8]. The major combinations identified with plant leaf powder on a laboratory scale are: Camphene (27.49%), Octanol acetate (10.39%), Benzyl formate (9.64%), Dihydro- linalool acetate (8.64%), Verbenene (8.30%), Neo-iso-dihydrocarveol (7.46%) and majorcomponentsin the pilot were: Camphene (31.53%), Verbenene (10.90%), Benzyl formate (8.18%), Octanol acetate (8.14%), and α- phellandrene (7.18%). According to the results obtained and the comparison with the results of others is quite different, only with the example of Angioni *et al*., 2004. In rosemary oil, major compounds such as α-pinene, borneol, camphene, camphor, verbonone, and bornyl acetate have been reported [32]. Rosemary has good antibacterial and antioxidant properties due to its tropical and cyclic compounds in its structure.

Table 1 Chemical composition of essential oil of *Rosmarinus officinalis* L. on laboratory and semi-industrial scale

Compounds name	R.I.	Laboratory scale	semi-industrial scale
α- pinene	942	0.33	0.39
Camphene	951	27.49	31.53
Verbenene	968	8.30	10.90
Cis- pinene	980	0.23	2.38
α- phellandrene	1005	6.88	7.18
α- terpinene	1014	0.82	1.00
(E)- β- ocimene	1046	0.27	0.20
γ- terpinene	1056	1.81	2.63
n- octanol	1065	4.98	6.16
Benzyl formate	1077	9.64	8.18
Terpinolene	1086	0.17	0.27
(2E)-heptenyl acetate	1113	0.28	0.35
Trans – thujone	1117	2.48	2.45
Trans-dihydro- β- terpineol	1134	0.49	0.69
Camphor	1155	0.51	0.66
Octanol acetate	1205	10.39	8.14
Trans-carveol	1214	1.34	1.28
Cis- sabinene hydrate acetate	1219	0.29	0.33
Neo-iso-dihydrocarveol	1228	7.46	5.07
Isobornyl acetate	1236	1.35	1.26
Methyl ether carvacrol	1241	1.60	1.01
Isoamylhexanoate	1247	0.64	0.57
Dihydro- linalool acetate	1271	8.64	3.25
n-tridecane	1302	0.27	0.49
n-nonanol acetate	1313	0.44	0.31
Myrtenyl acetate	1325	2.09	3.16
Geranylpropanoate	1482	0.68	0.68
Oil percentage	-	0.46 %	0.46%

The most important compounds in the rosemary structure were alpha-pinene, 1, 8-cineol and camphene, all of which are terpenes. Another advantage of rosemary is the high amount of biologically active compounds and its volatile oils, which increases the extraction efficiency of its essential oil.

This study has confirmed that most of the identified components and the composition of essential oils of *R. officinalis* from Iran are different from other countries. This could be markedly affected by geographical environment, physical and chemical characteristics of soil, time of harvest, method of extraction, relative humidity, distillation equipment, condition of the twigs and leaves, plant age, plant cultivation techniques, plant population density, climate and management [33]. Therefore, for different use of essential oils of rosemary different geographic origins may be considered for growing rosemary.

Conclusion

Based on our experience in the essential oil extraction scale in the lab, always sample powder kept in a 2-liter balloon water. However, on a semi-industrial scale, due to the large volume of the sample, the ratio between the volume of water and the sample is small, so the essential oil does not have the opportunity to bring itself to the surface of the distillation pot.

This means that when the sample size is large, the essential oil cannot bring itself to the surface through the sample and some of the essential oil remains in the pot. But when the sample is low, that is, the water-to-sample ratio is high, so the sample is immersed in water, making it easier to extract the essential oil. As you can see on a lab scale. Different results were obtained from three samples of whole rosemary leaf and half powder and whole powder, yield of whole leaf sample (0.44%), half powder sample (0.46%) and whole powder sample (0.70%).In this study, the semi-powder sample in the laboratory was compared with the semi-powder sample in the semi-industrial scale with low volume (0.46%).

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