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Quantitative Analysis of Ethanol and Methanol in Herbal Distillates Distributed in Ilam City in Western Iran Using Gas Chromatography

Ali Zargoush-Nasab[1], Elahe Karimi[2], Ahahita Jalilian[3], Mahmoud Bahmani[4]

[1]Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran. E-mail: a.zargoosh74@gmail.com

[2]Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran. E-mail: elahehkarimi3913@gmail.com

[3]Department of Microbiology, Faculty of veterinary Medicine, Urmia University, Urmia, Iran. E-mail: a.jalilian@ymail.com

[4]Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran. E-mail: mahmood.bahmani@gmail.com

Corresponding Author, [1]Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran. E-mail: mahmood.bahmani@gmail.com

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Abstract

Background: In this study, five types of popular herbal distillates including licorice, fennel, orange blossom, lavender, and cinnamon were purchased from the supply level stores in the city of Ilam by cluster sampling method and analyzed for the amount of ethanol and methanol.

Materials and Methods: In the first step, the samples of herbal distillates were taken for testing. In addition, analytical grade ethanol and methanol were purchased from Merck Company and used as controls. Dates of production and expiry of each sample were separately checked and recorded. The samples of every herb distillate were kept in their original containers and used for testing. The names and brands of the manufacturing plants were mentioned as A, B, C, etc., in view of the ethical aspects of the research. The concentration of methanol and ethanol in the products of each company was analyzed by gas chromatography (GC) and each sample was measured five times and the mean value was reported.

Results: On the basis of the obtained results, the mean concentrations of ethanol and methanol in lavender distillate were 7.153 and 17.002 ppm, respectively. The mean ethanol and methanol levels in orange blossom distillate were 164.2551 and 64.2472 ppm, respectively. The average ethanol and methanol content in cinnamon distillate were 11.9482 and 12.769 ppm, respectively. The mean ethanol and methanol contents in fennel distillate were 30.8897 and 7.5103 ppm, respectively. The mean ethanol and methanol contents in licorice distillate were 4.1524 and 38.3075 ppm, respectively. The results of this study showed that herbal distillates have ethanols and particularly methanols.

Conclusion: Herbal distillates that contain methanol and ethanol can be toxic, especially to pregnant women, if taken over a long period of time.

Introduction

Different cultures and nations use herbal medicines and medicinal plants to meet their treatment needs. The global demand for herbal medicines is increasing despite the medical and technological advances of modern times [1]. Various parts of each plant such as roots, leaves, fruits, flowers, seeds, gum, resin, seed mucilage, sap, manna, and plant oil are used in various forms such as poultices,

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infusions, syrups, and herbal distillates in the pharmaceutical industry and in traditional medicine [2-7]. Since ancient times, herbal distillates have been prepared from the medicinal plants. These herbal distillates have been used in Iran to treat various diseases. Sometimes, these herbal distillates are added as flavoring agents and aromatic compounds to a variety of foods and edible products. There has been a long history of using the properties of herbal distillates to treat some diseases and maintain the health of various organs of the body [8]. Herbal distillates are mentioned as a supplement to chemical drugs, as a protector of the body's health, and also as an effective substance for the prevention of various diseases in traditional and Islamic medicine [9]. One of the most widely used forms of medicinal plants is herbal distillates. Today, there are more than 50 types of herbal distillates in Iran that have medicinal uses. In general, herbal distillates are the attenuated type of essential oils. In the preparation of herbal distillates, more water is used in order to dilute them for domestic and medicinal purposes. As a result, herbal distillates should be consumed in greater amounts than essential oils. However, excessive consumption may cause side effects, particularly when they contain large amounts of alcohol, such as methanol and ethanol [10]. The importance of measuring methanol in such products is demonstrated by published and anecdotal reports by some physicians of the presence of methanol poisoning symptoms, including blindness, in chronic users of herbal distillates [11]. Methanol belongs to the category of aliphatic alcohols and is a highly toxic compound. Methanol is used in industry as a solvent. It is also used in the production of methylated compounds and formaldehyde [12]. It has been reported that the toxic and poisonous amount of methanol is in the range of 6 to 100 ml [13]. A lethal dose of 30 to 240 ml of methanol has been reported in other reports [14, 15]. Methanol has also been found in herbal distillates. It has been reported that consumption of some of these herbal distillates causes blurred vision and blindness [16].

In traditional medicine, orange blossom distillate is used to strengthen the heart and the nerves. It is an anticonvulsant, a sedative and an anti-hysteric. It is used to cure depression, insomnia and hiccups. It breaks down fat and increases the body's metabolism through norepinephrine in beta-3 receptors. Lavender distillate is useful as a stimulant, for headaches, migraines, and anxiety. It also has a calming effect and is a sleep aid. The mixture of lavender distillate with honey and vinegar is a remedy for dizziness, myoclonus, epilepsy, obsession and forgetfulness. Cinnamon distillate strengthens the stomach and the nerves, it is anti-anxiety, obsession, wind breaker, sedative and astringent. Moreover, the nature of cinnamon distillate is dry and warm. Fennel distillate also has a warm nature. It is carminative. It is a cure for colic. It removes phlegm. Because it contains female hormones, it increases breast milk. It is also stimulating, antispasmodic, diuretic, and tonifying. It should be noted that to treat the above cases, one cup should be taken before each meal. Licorice distillate has a warm nature, cures gastric and duodenal ulcers and strengthens the digestive system. It is antitussive and expectorant. It removes constipation and hemorrhoids and cures lung and kidney infections.

In traditional and complementary medicine of Iran, different herbal distillates are used as medical beverages. Licorice, fennel, orange blossom, lavender and cinnamon are among the most widely used medicinal plants and herbal distillates. Since methanol cannot be separated from ethanol, methanol is always found as an unwanted byproduct along with ethanol. Therefore, the present study was carried out to quantitatively analyze ethanol and methanol in herbal distillates distributed in Ilam City, Western Iran using gas chromatography.

Materials and Methods

Sample Collection

Common herbal distillates including licorice, fennel, orange blossom, lavender, and cinnamon were collected and sampled from Ilam City's supply level. Table-1 shows the botanical information and characteristics of the herbal distillates.

Table-1. *Botanical information and characteristics of herbal distillates.*

Herbal Distillate Name	Scientific Name	Family	Country
Licorice	*Glycyrrhiza glabra*	Fabaceae	Iran
Fennel	*Foeniculum vulgare*	Apiaceae	Iran
Orange blossom	*Citrus aurantium*	Rutaceae	Iran

Lavender	*Lavandula angustifolia*	Lamiaceae	Iran
Cinnamon	*Cinnamomum verum*	Lauraceae	Iran

Sample Size Calculation Method

Five samples of each type of herbal distillates were selected at random from the stores of the supply levels of Ilam City. The herbal distillates that were tested included licorice, fennel, spring orange, lavender and cinnamon.

Conditions and Method of Chromatography

The concentrations of ethanol and methanol were determined using a model Agilent 7890B GC instrument with an FID detector. The column used was RTX-5MS, 30 m in length, 0.25 mm internal diameter, and 0.25 µm thickness of stationary phase. The initial temperature of the device was 40 °C, which was maintained for 3 minutes, and then it reached the temperature of 160 °C with a ramp of 20 °C per minute and was maintained at this temperature for 1 minute. Injections were made in splits with a ratio of 1:25. The injection volume was 1 microliter. The temperature of the injector was 200 °C and the temperature of the detector was 250 °C. Due to the transparency of the samples, the 1 microliter sample volume was diluted in splits with a ratio of 1:25. This means that the apparatus divided the sample into 25 parts and 1 part of the sample was analyzed and 24 parts were taken out of the apparatus. Nitrogen was also used as the carrier gas for the make-up.

Results

The results obtained showed that all the plant distillate samples were found to contain ethanol and methanol. Our results revealed that the mean concentrations of ethanol and methanol in lavender distillate were 7.153 and 17.002 ppm, respectively. In addition, we found that the mean ethanol and methanol levels in orange blossom distillate were 164.2551 and 64.2472 ppm, respectively. The average ethanol and methanol content in cinnamon distillate were 11.9482 and 12.769 ppm, respectively. Furthermore, the results indicated that the mean ethanol and methanol contents in fennel distillate were 30.8897 and 7.5103 ppm, respectively. The mean ethanol and methanol contents in licorice distillate were 4.1524 and 38.3075 ppm, respectively (Table-2).

Table-2. *The mean concentrations of ethanol and methanol in herbal distillates including licorice, fennel, orange blossom, lavender, cinnamon.*

Herbal Distillate Name	Ethanol (ppm)	Methanol (ppm)
Lavender	7.1953	17.002
Orange blossom	164.2551	64.2472
Cinnamon	11.9482	12.769
Fennel	30.8897	7.5103
Licorice	4.1524	38.3075

Discussion

Herbal distillates are one of the traditional medicines in Iran and widely consumed by people. The chemical and microbial health and safety of traditional and industrial herbal distillates is very important due to the importance of their various medicinal uses. Recently, health authorities and the Iranian health officials have become concerned and sensitive to reports of blurred vision leading to blindness after unknowingly drinking methanol along with the consumption of some herbal distillates. Our results showed that all herbal distillates, regardless of manufacturer, contained varying amounts of methanol and ethanol. We found that the mean concentrations of ethanol and methanol in lavender distillate were 7.153 and 17.002 ppm, respectively. Moreover, the results showed that the mean ethanol and methanol levels in orange blossom distillate were 164.2551 and 64.2472 ppm, respectively. The average ethanol and methanol content in cinnamon distillate were 11.9482 and 12.769 ppm, respectively. Furthermore, the results indicated that the mean ethanol and methanol contents in fennel distillate were 30.8897 and 7.5103 ppm, respectively. The mean ethanol and methanol contents in licorice distillate were 4.1524 and 38.3075 ppm, respectively. In contrast to alcoholic beverages, the production of methanol in herbal distillates is caused by the metabolic processes of the plant during its growth and even after the harvest of the plant up to the preparation of the distillate [17]. Thus, the presence of methanol in the distillates we examined may be attributed to two sources: the metabolic

processes of the plant and the addition of methanol to extract the distillate.

In many processed plant foods, including herbal distillates, methanol is present as an impurity. Methanol causes numerous deadly poisonings. Significant poisoning can also result from prolonged consumption of small amounts of methanol. There are unpublished and anecdotal reports from some physicians about the presence of methanol poisoning symptoms, including blindness, in chronic users of herbal distillates. There is a possibility of methanol production in the production process of herbal distillates, depending on the type of herbal distillate preparation and the symptoms caused by its chronic use [18-20]. There is a lack of pasteurization and a possibility of ethanol and methanol contamination in the sample due to non-observance of optimal and standard conditions for production of herbal distillates. The process of growing plants is considered the main source of methanol production in nature and releasing it into the atmosphere.

The results of a study by Rafizadeh A et al. showed that the lowest amount of methanol in the rose water sample from factory "A" was equal to 65 mg/l, and the highest amount of methanol in the distillate sample of Alhagi from factory "C" was equal to 310 mg/l. Meanwhile, the average amount of methanol of the three factories studied for rose water and Alhagi distillate was equal to 79 mg/l and 300 mg/l, respectively [21]. The results of this study are in agreement with the results of our study. Growing conditions and environmental stress on plants, such as hypoxia caused by snow, increasing ozone concentration in the air, damage caused by herbivore attack and branch cutting, dry leaves, etc., cause an increase in the production of methanol in plant tissues, which leads to the degradation of lignin enzymes, DNA demethylation, protein repair pathways and pectin demethylation occur in their matrix, and the last factor plays an important role in the expansion of the cell wall of plant cells during growth [22, 23]. In a study by Madani et al., methanol concentrations in dill distillate ranged from a high of 4.25 ppm to a low of 0.00 ppm, with 7 samples tested within the standard range. The results of this study are in almost complete agreement with the results of the present study. It can be concluded that the amount of methanol in these samples may be reduced by timely packaging, as well as the type of plant and ambient temperature of these distillates [24]. There have been numerous reports of contamination with methanol and other harmful compounds such as heavy metals in alcoholic beverages and even fruit and vegetable juices, which clearly shows the importance of this area for further research [25, 26]. Methanol appears to be induced by the action of enzymes on cell wall pectins during the production or storage of such herbal distillates. One of these important enzymes is pectin methylesterase-1 (PME). PME can methylate pectin and release methanol [27, 28]. Therefore, it can be concluded that having more woody organs in the plant may make it susceptible to producing methanol. One study has shown that soaking time increases methanol production [29]. Industrial pressing has also been shown to increase the amount of cell wall enzymes released compared to the manual method. Pasteurized herbal distillates had a lower concentration of methanol compared to fresh herbal distillates. This is because the pasteurization process reduces enzyme activity [30]. The contribution of PME is related to its function during the growth and maturation of the plant, and therefore the amount of methanol is higher in plants with a more wood vessel such as mint than in plants such as fennel [31].

Conclusion

From this we may conclude that in the production of herbal distillates, by removing the wood structure of the plant and using direct steam distillation during the production, the activity of the enzyme decreases and, consequently, the concentration of methanol becomes much lower. Herbal distillates such as lavender, orange blossom, fennel, cinnamon and licorice are widely used by people. However, the presence of alcohols such as ethanol and methanol in these products requires food and beverage control through regular testing of these products.

Conflicts of Interest

The authors hereby declare no conflicts of interest.

Authors` Contribution

All authors contributed in the experiments, analysis and preparation of this manuscript.

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