

Determination of Methanol Content in Herbal Distillates Produced in Urmia Using Spectrophotometry

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ABSTRACT

Background: Herbal distillates have been used for many centuries as drinks, flavors, and herbal medicine in Iran, especially in the city of Urmia. Recently, some studies claimed the presence of methanol in different types of herbal distillates. Methanol is a highly toxic compound which can cause acute or chronic toxicity in humans. Acute poisoning with methanol can cause different complications and even death while chronic methanol exposure has a wide range of nonspecific and misleading findings. The main purpose of this study was to determine methanol content in the commonly-used industrial herbal distillates produced in Urmia.

Methods: Five samples of six types of most commonly used herbal distillates (peppermint, musk willow, lemon balm, pennyroyal, dill, and rose water) were purchased from five active herbal distillates manufacturers in Urmia. All samples were transferred to the laboratory and methanol content of each sample was measured two times according to the standard method of analysis using spectrophotometer.

Results: The lowest and highest concentration of methanol were found in rose water (mean=72.4±32.1 ppm) and musk willow (mean=278.3±106 ppm) samples, respectively. One-way ANOVA showed statistically significant differences among methanol concentrations in the studied herbal distillates (F=60.9, P <0.001).

Discussion: Different amounts of methanol were found in herbal distillates and it seems that there are statistically significant differences in methanol concentrations of various types of herbal distillates. Therefore, considering the harmful effects of methanol on human health, further studies are required for determining permitted levels of methanol in herbal distillates.

Keywords: Herbal distillate, Iran, Methanol, Spectrophotometry, Urmia.

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INTRODUCTION

Methanol, also named methyl alcohol, wood spirit, carbinol, wood alcohol, or wood naphtha, is a colorless

and volatile liquid with a characteristic odor, primarily used for manufacturing other chemicals and as a solvent, glass cleaner, windshield washer fluid, carburetor cleaner, antifreeze, de-icing

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solution, paint remover, varnish, photocopying fluid, canned solid (picnic) fuel, and small engine fuel (1,2). Methanol may also be found as an adulterant of alcoholic drinks (3).

Methanol is highly toxic following ingestion, inhalation or dermal exposure (4). Ingestion is the most common route of exposure (2,4). Susceptibility to methanol poisoning varies greatly and ingestion of 0.25 mL/kg of 100% methanol (pure methanol) would theoretically, assuming complete absorption, results in severe toxicity. Death has also been reported after ingestion of about 15 mL of 40% methanol (5).

Methanol is metabolized in the liver by the alcohol dehydrogenase enzyme to formaldehyde and then by aldehyde dehydrogenase enzyme to formic acid; the latter is responsible for the most adverse effects observed in methanol toxicity (2, 4).

Acute methanol ingestion commonly produces gastrointestinal complaints due to mucosal irritation, including nausea, vomiting, and abdominal pain. Most patients note visual disturbances as one of the first symptoms. Visual impairment, secondary to optic nerve necrosis or demyelination, ranging from blurred vision to visual field deficits and even total blindness may be seen. Severe high anion gap metabolic acidosis, hypotension, central nervous system depression, confusion, and ataxia are other common observable signs. Large amounts of methanol ingestion can result in convulsion, stupor, coma, and sometimes death (2,5).

The majority of the existing information about methanol toxicity is related to acute rather than chronic exposure. The toxic effects of repeated or prolonged exposures to methanol are supposed to be similar but less severe than those induced by acute exposure. These effects may include gastrointestinal (abdominal pain, nausea, and vomiting), ophthalmic (irritation, blurred vision),

central nervous system (headaches, unsteadiness, dizziness, tinnitus, hearing loss, seizures, amnesia, anxiety, phobias) and other nonspecific symptoms (weakness, malaise, fatigue, palpitation). This wide range of symptoms may falsely mimic some chronic diseases such as fibromyalgia, myasthenia gravis (MS), systemic lupus erythematosus (SLE), diabetes mellitus, chronic fatigue syndrome, Alzheimer, and attention disorder (5,6). Hence diagnosis of chronic methanol toxicity is highly important.

Production of herbal distillates is an ancient practice in Iran. Great Iranian scientists, such as Avicenna, Rhazes and Attar, have been familiar with herbal medicines and used them for treating patients. Nowadays, there are still many Iranian people who tend to take herbal distillates and other herbal remedies to regain their own well-being.

Iran has 11 of 13 existing climate zones of the world and, as a result, it has rich plant species (7). The flora of the country comprises more than 8000 species of seed plants, including 2400 native and 1727 endemic species (8).

The land covered by Iran's natural flora is four times more than that of Europe. More than 1000 herbal medicines are sold in Iranian traditional markets and around 100 plants are used for producing herbal medicines in national pharmaceutical companies (9).

Over 800 plant species grow in West Azerbaijan province of Iran and around 70 plants are used for extracting herbal distillates through traditional or industrial methods. Urmia is the central city of the West Azerbaijan province and each year more than 10 million liters of herbal distillates are produced in this city both by traditional or industrial methods (10).

Determination of the composition of herbal distillates is a difficult task, but due to the nature of distillation process, methanol is likely to be produced in distillates. With this assumption and because of the harmful effects of methanol

on human health, this article was done to study the concentration of methanol in six types of herbal distillates produced commercially in Urmia, West Azerbaijan, Iran.

MATERIALS AND METHODS

Samples collection

Six types of the most commonly used herbal distillates [peppermint (*Mentha piperita*), musk willow (*Salix aegyptiaca*), lemon balm (*Melissa officinalis*), pennyroyal (*Mentha pulegium*), dill (*Anethum graveolens*), and rose water (*Rosa damascene*)] were purchased from 5 main manufactories, producing and supplying these distillates in Urmia. Five samples of each distillate were purchased from each manufactory. Production date of all samples was less than 2 months. All 150 samples were collected in 200 ml sterile polyethylene containers and sealed with paraffin.

Then they were transferred to the laboratory in cold conditions. Regarding the ethical aspects of research, manufacturers were defined with code and the examiners did not know anything about the producers.

Methods

AOAC standard method proposed to measure "methanol in alcoholic beverages" was used to measure the methanol content of the samples (11) as follows:

- 1 ml of sample is added to a 50 mL balloon that contained 2 mL potassium permanganate 3% solution (containing 15% acid orthophosphoric).
- Balloon was put in cold water bath for 30 minutes.
- Sodium metabisulfites 10% aqueous solution was added drop by drop with a pastor pipette into the balloon to be completely colorless.
- 1 ml of chromotropic acid aqueous 5% solution was added to the balloon.

- 15 ml of concentrated sulfuric acid (95-97%) is gradually added to the balloon.
- Balloon was put in 60 °C water bath for 15 minutes and then cooled to 25°C.
- It was volumed by distilled water to 50 ml.
- Light absorption of the balloon's content was measured using spectrophotometer at 575 nm wavelength.

Methanol content of the samples were determined using prepared standard curves. Different concentrations of methanol (50, 100, 150, 200, 250, 300, 350, 450, 500 parts per million) was used in the 575 nm wavelength for preparation of the standard curves. All trials were examined twice by 2 separate laboratory expert technicians.

Chemicals and equipment

Solvents (methanol) and chemicals, such as potassium permanganate, chromotropic acid and orthophosphoric acid, were obtained from Merck (Darmstadt, Germany). Absorbance was measured by a spectrophotometer (Shimadzu UV-1700, Japan).

Statistical analysis

Data obtained from methanol measurements of the samples were entered into SPSS software version 15. There were 6 cases with missing value (4%) in data processing. Analysis was performed on the remaining 144 specimens.

RESULTS

Intercorrelation among twice sample tests was high (Cronbach's alpha=0.96). Frequency distribution of data is shown in Table 1.

Table 1. Frequency distribution for tested samples

| Manufacturer code | Rose water (Rosa damascene) | Dill (Anethum graveolens) | Peppermint (Mentha piperita) | Pennyroyal (Mentha pulegium) | Lemon balm (Melissa officinalis) | Musk willow (Salix aegyptiaca) | Total |
|-------------------|--------------------------------|------------------------------|---------------------------------|---------------------------------|-------------------------------------|-----------------------------------|---------------|
| 1 | 5 (22.7%) | 5 (20.8%) | 5 (20%) | 5 (21.7%) | 5 (20%) | 5 (20%) | 30 (20.8%) |
| 2 | 5 (22.7%) | 5 (20.8%) | 5 (20%) | 5 (21.7%) | 5 (20%) | 5 (20%) | 30 (20.8%) |
| 3 | 2 (9.2%) | 5 (20.8%) | 5 (20%) | 3 (13.2) | 5 (20%) | 5 (20%) | 25 (17.4%) |
| 4 | 5 (22.7%) | 4 (16.8%) | 5 (20%) | 5 (21.7%) | 5 (20%) | 5 (20%) | 29 (20.2%) |
| 5 | 5 (22.7%) | 5 (20.8%) | 5 (20%) | 5 (21.7%) | 5 (20%) | 5 (20%) | 30 (20.8%) |
| 6 | 22 (15.2%) | 24 (16.7%) | 25 (17.4%) | 23 (15.9%) | 25 (17.4%) | 25 (17.4%) | 144 (100%) |

Distribution of methanol content in the samples, using Kolmogorov-Smirnov test, represents normal distribution (KSZ=0.99, Pv=0.27, Mean=172±103.3). Mean values of methanol content in samples are summarized in Table 2, based on herbal distillates types.

Table 2. Mean values of samples' methanol content based on types of herbal distillates

| Herbal distillate type | Mean values of methanol content |
|------------------------|---------------------------------|
| Rose water | 72.40 ± 32.1 |
| Dill | 195.4 ± 109.8 |
| Peppermint | 151.3 ± 80 |
| Pennyroyal | 121.2 ± 74.2 |
| Lemon balm | 198.3 ± 58 |
| Musk willow | 278.3 ± 106 |

Comparison of mean values of methanol content in herbal distillates was performed using one way ANOVA. Results indicated significant differences among herbal distillates ($\alpha < 0.001$, F=60.9). Duncan test was used for classification of herbal distillates according to methanol content. Results are given in Table 3.

Table 3. Herbal distillates classification according to methanol content

| Herbal distillate | Ascending sort (From the lowest to the highest methanol content) |
|--|---|
| Rose water | 1 |
| Peppermint, Pennyroyal Dill, Lemon balm | 2 |
| Musk willow | 3 |
| | 4 |

DISCUSSION

Due to the nature of distillation process used in production of herbal distillates, methanol (wood alcohol) is likely to be formed in the final product (12). Karimi et al. (2007) measured methanol contents in 10 types of herbal distillates including musk willow, camel thorn, ajowan, cumin, caraway, fenugreek, dill, succory, rose water, and peppermint produced by 6 manufactories in Mashad, Iran. They found maximum methanol content in a dill distillate sample (1477.7±23.8 ppm) and minimum methanol concentration in a musk willow distillate sample (79.4±3 ppm). In their study, the minimum detectable and minimum measurable amounts were 50 and 75 ppm, respectively, and methanol

content was lower than the minimum measurable amount in 18 cases. It seems that their sample size was so small that they could not find significant relationships between methanol content and the type of herbal distillates (13).

Solhi et al. (2009) also investigated methanol content in 6 types of herbal distillates (peppermint, musk willow, camel thorn, fenugreek, dill and succory) produced by traditional and industrial methods (from each type 3 traditional and 3 industrial samples) in Arak, Iran. In a recent study, maximum and minimum methanol content in traditional products was in musk willow (266.02 ppm) and fenugreek (60.26 ppm) distillate samples, respectively. The highest and the least methanol content in industrial products were reported in a peppermint (415.04) and a musk willow (88.08 ppm) samples, respectively. In their study, Solhi et al. did not find statistically significant differences between methanol content in traditional and industrial distillates (14).

In the present study, 150 samples were investigated. All trials were repeated twice, a total of 300 experiments were done. Regardless of the 6 missing values, a significant relationship was found between methanol concentration and herbal distillates types so that in rose water, which is simply made of rose flowers, methanol content was significantly lower than other herbal distillates and, in musk willow distillate, methanol content was significantly higher than the other herbal distillates.

CONCLUSION

It seems that the less the medicinal plant has branches, stems and leaves and the more only flowers are used for preparation of herbal distillates, the less methanol content will appear in the final product. However, this conclusion may not be completely correct, and other factors may also affect methanol concentration in the final product. In our study and the other two similar studies, dilutions of herbal

distillates samples were not determined before methanol content measurements. This can be a confounding factor because there is the possibility of adding water to the final products to increase volume due to jobbery of some herbal distillate manufacturers.

In conclusion, due to the adverse effects of methanol on human health, it is suggested that similar studies should be done with sufficient samples and considering all influencing and confounding factors on more types of herbal distillates produced by traditional and industrial methods to allow determination of standard range of methanol in herbal distillates.

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