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Study of Ethanol Presence in Some Ornamental and Aromatic Plants Using Gas Chromatography

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Abstract

Plants re-emit a substantial fraction of their assimilated carbon into the atmosphere as biogenic volatile organic compound. According to the presence of relatively high amount of methanol in herbal distillates, finding different concentrations of ethanol in such drinks is expectable, as several studies appeared that produced ethanol by plants emits into the atmosphere as do methanol. In this study, ethanol content of 30 industrial herbal distillates made by three different companies was measured. For this purpose, some solutions with concentration of 10000 mg L^{-1} were prepared from ethanol and methanol separately as original and internal standard sources that in continue, different concentrations of standards were prepared from ethanol solution and after it, 50 µL methanol solution was added to all of the standards and samples as internal standard and then, all of them were examined with GC. The obtained results indicated that some of the herbal distillates contain ethanol. Maximum and minimum amount of ethanol existed in *Rosa damascena* L., (rose water) with mean value 96.4 mg L^{-1} and *Salix aegyptiaca* L., distillate (only one sample had 15.5 mg L^{-1} and in two other samples were not seen) respectively, while most of the examined samples had no ethanol. Presence of ethanol in some of the examined samples showed that further study is necessary to achieve correct information about this subject.

Keywords: Ethanol, Gas chromatography, Herbaceous distillates, Volatile compounds.

INTRODUCTION

A biochemical basis for the different sensitivity of plants to anoxia is the accumulation of fermentation products (mainly ethanol) (Perata *et al.*, 1986). It seems ethanol play only a minor or secondary role in anoxia injury to roots (Jackson *et al.,* 1982; Barata, 1984; Alpi *et al*., 1985). Also, cytoplasmic acidosis are the major adverse effect of anoxia that has been recently proposed (Roberts *et al.*, 1984).

Alcohols have been shown to have effects on the photosynthetic pathway of plant tissues C3 (Andres *et al.,* 1990; Hemming *et al.,* 1995), and they cause to retard senescence of these tissues (Rajala *et al*., 1998). Plants re-emit a substantial fraction of their assimilated carbon into the atmosphere as biogenic volatile organic compounds (BVOCs) that affect the chemical and physical properties of the atmosphere. BVOCs are produced in many different physiological processes of plant tissues. These are diverse and including isoprene, terpenes, alkanes, alkenes, alcohols, esters, carbonyls and acids (Peñuelas and Llusià, 2003).

One of the biochemical basis of the different behavior of plants about anoxia is their sensitivity to accumulation of fermentation products, particularly ethanol (Perata and Alpi, 1990). Ethanol with biologic origin is produced by plants and fermentative microorganisms. Perata and Alpi indicated that ethanol is likely a non-toxic compound for plant and presumably its toxicity may be related to a product of ethanol metabolism (probably the acetaldehyde). Several researchers believe that ethanol is accumulated on the plants under anaerobic conditions. Therefore, the damages resulted from the anaerobic environment can't just be attributed to ethanol (Jackson *et al.,* 1982; Alpi *et al*., 1985). Although, it had been generally believed that ethanol is produced in plant tissues during anaerobic situations that in conditions, it can have an important role in causing tissue injuries (Jackson *et al.*, 1982).

The first step of ethanol metabolism in plant tissues is the oxidation of ethanol to acetaldehyde; this oxidative step is considered to be mediated exclusively by alcohol dehydrogenase enzyme. The fate of acetaldehyde depends on the nature of the metabolizing tissue. But, the oxidation of this aldehyde in different tissues is lead to the acetic acid formation. The Ethanol boiling point is near to water and it can easily be solved in water. On the other hand, its chemical and physical properties are very similar to methanol that is easily found in all herbal distillates (Rafizadeh *et al.,* 2010). Therefore, presence of ethanol in such products is possible. Thus, in this study, we tried to investigate the presence of ethanol in some high consumed of herbal distillates. Of course, herbal distillates are frequently used in Iran and some neighboring countries and in this regard, there is no research about it in developed countries. Also, there is no study about ethanol contents in herbal distillates at this time; so, the results of this study can be new and unique.

MATERIALS AND METHODS

Materials

Ten samples of each high consumed herbal distillates (*Citrus aurantium* L., *Salix aegyptiaca* L., *Rosa damascena* L., *Glycyrrhiza glabra* L., *Mentha spicata* L., *Origanum vulgare* L., *Cichorium intybus* L., *Carum carvi* L., *Anethum graveolens* L. and *Trigonella foenum-graecum* L.) made by three different factories (shown by A, B and C) were purchased from Rasht stores cluster- random sampling (30 samples).

The production date of each factory product was maximum two months before their purchase and this date between products of different factories were four months. Also, the production and expiration dates, range of all distillates were 2013-2015. Also, both ethanol and methanol with purity analysis degree were purchased from the Merck Company and used without any other purification. In this study, all of the standards and Ostwald- Folin pipet were prepared from valid Germany trade mark sand GC with Yanglin brand (model YL6100 GC) made by South Korea were used.

Table 1. Instrumental conditioning of GC.

The primary temperature at injection time for 1 minute	Thermal slope	Column temperature	Detector	Column	Column length	Column diameter	Carrier gas	The injected sample volume
50 °C	10 °C min^{-1}	80 °C	FID	Tr25 (glass)	30 m	0.53 mm	Helium	0.3 µl

Methods

First, 50 ml of each herbal distillates sample was poured into labeled test tubes. Also, the solutions with concentration of 10000 mg L^{-1} were prepared from ethanol and methanol separately as original and internal standards. Then, different concentrations of standards were prepared from 10000 mg L^{-1} methanol solution. Then, 50 µl of 10000 mg L^{-1} ethanol solution is poured into all of the standards and samples as internal standard. Then all of them were injected into GC (operation conditions shown in Table 1) and results were analyzed with SPSS software.

RESULTS AND DISCUSSION

According to the obtained results, *Menta spicata* L., *Carum carvi* L., *Cichorium intybus* L., *Citrus aurantium* L., *Origanum vulgare* L., and *Glycyrrhiza glabra* L., distillates had no ethanol. Therefore, in spite of methanol (Rafizadeh *et al.,* 2011; Rafizadeh *et al.*, 2013.), the amount of produced ethanol in such plants is too low, that in the final product is not detectable. So, it can be concluded that ethanol has no important role in their biology. But there is some ethanol in all *Rosa damascena* L. samples (with mean value 96.4 mg L^{-1}), as, their amounts were higher than the other examined products and since, the concentration of ethanol is similar to methanol level in flowers (*Rosa damascena* L.) (Rafizadeh *et al*., 2011), it seems, ethanol is an important chemical compound in Rosa damascena L., flower biology. However, it is possible, like methanol, technical differences, quality and method of distillation can be effective on the presence, absence or amount of ethanol in herbal distillates. But, the minimum amount of ethanol existed in *Salix aegyptiaca* L., distillate (15.5 mg $L^{-1)}$ that is just observed in one sample.

This result indicates that, the ethanol existence is not essential in all flowers and its amount can be variable in kinds of flowers and its related distillates, because, in spite of rose water, there were not ethanol in the two samples of *Salix aegyptiaca* L., distillates in this study. Although, it seems, more investigations are necessary to attain correct conclusion. According to the obtained results, the significant level (0.02) is smaller than 5% that confirmed the test accuracy (the results are significant). So, 95% of the produced herbal distillates by three different factories were evaluated. As a result, ethanol was determined only in some kinds of herbal distillates (not all of them)

Table 2. The amount of ethanol (based on mg L^{-1}) in examined herbal distillates.

Name distillates	A	B	C
Mentha spicata L.	ND	ND	ND
Trigonella foenum-graecum L.	ND	ND	20.1
Carum carvi L.	ND	ND	ND
Anethum graveolens L.	34.4	ND	67.3
Cichorium intybus L.	ND	ND	ND
Salix aegyptiaca L.	ND	15.5	ND
Citrus aurantium L.	ND	ND	ND
Origanum vulgare L.	ND	ND	ND
Rosa damascena L.	86.7	77.7	124.8
Glycyrrhiza glabra L.	ND	ND	ND

ND: Non-Detectable
Notice: The names of companies are shown by alphabet letters (A, B, C).

Table 3. t test statistics.

Test	N	Mean (mg L^{-1})	Standard Deviation (mg L^{-1})	Standard Error Mean (mg L^{-1})
Paired comparison	30	14.2157	31.85054	5.81509

+Table 3 shows the number of data, mean, standard deviation and standard error mean, respectively.

Table 4. t test of the hypothesis.

	Test Value = 0					
Test	t (mg L^{-1})	df (mg L^{-1})	Sig. (2-tailed) (mg L^{-1})	Mean Difference (mg L^{-1})	95% Confidence Interval of the Difference	
One-Sample Test	2.445	29	0.21	14.21567	2.3225	26.1089

and its amount is not sufficient to effecting after drinking it with these products. The obtained results are shown in Table 2.

CONCLUSION

The obtained results were shown some of herbal distillates have ethanol that apparently, the most amount of it was measured in flower distillates with maximum and minimum amounts in *Rosa damascena* L., (with mean 96.4 mg L^{-1}) and *Salix aegyptiaca* L., distillate made by factory B (15.5 mg L^{-1}), respectively.

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