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The Effectiveness of Whole Concentration of Homemade Herbal Distillates on the Result of Qualitative Methanol Detection by the Chromotropic Acid Method

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Abstract

The occurrence of blindness after drinking some herbaceous distillates is created some anxieties in Iran. In this study, the effectiveness of concentration of Khalvash distillate was studied on the function of the new modified suggested chromotropic acid method for qualitative detection of methanol. For this purpose, three samples of Khalvash distillate were purchased from vendors of Rasht. According to the obtained results, due to special chemicals and their high concentrations in the studied homemade samples, using of 1:5 dilution ratio in the distillate water is necessary for test performance. Also, it was confirmed that methanol was present in all Khalvash samples like other herbal ones.

Keywords: Chromotropic acid, Herbaceous distillates, Methanol, Qualitative detection.

INTRODUCTION

In spite of spirits, existence of methanol in herbaceous distillates is due to biologic activities of plant in its life time (Niinemets and Reichstein, 2003a; Niinemets and Reichstein, 2003b; Niinemets *et al.,* 2004). Also, kinds of stresses like hypoxia, frost and high ozone concentrations, senescing, injuring (e.g. herbivore attacks, cutting), drying of leaves and even aging of plant can cause to increase methanol production in plant (Von Dahl *et al*., 2006; De Gouw *et al*., 1999; Wu, *et al*., 2007; Karl *et al*., 2005; Loreto *et al.*, 2006; Holzinger *et al.*, 1999; Nemecek-Marshall *et al.*, 1995). Furthermore, methanol plays several roles in plant physiology and signaling, in plant-herbivore relationships and in defense against micro-organisms (Arora *et al.,* 2007). Therefore, presence of methanol in juices, herbal distillates and the other plant products is fully logical. According to American standard, existence of 120-460 mg/L (with mean 140 mg/L) methanol in fresh and canned juices (such as orange and grape fruit) is permitted (Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, Volume 5). In Iran, herbal distillates are prepared by industrial and non-industrial or homemade ways. Guilan Province doesn't have especial place for production of herbaceous distillates among the other ones, but, usually, a herbal distillate that is named khalvash (a: *Salvia virgate* and b: *Calamintha umbrosa*) is traditionally prepared by local people which its concentration is more than industrial ones. khalvash as a self-propelled plant is one member of pennyroyal family and its distillate is prepared from green leaves and stems (with tiny white to pink flowers) that have large relative amount of methanol, therefore, there always are some nearly huge amounts of methanol in its distillate.

Methanol poisoning is an uncommon but extremely harmful for central nervous system (Von Dahl, 2006; Birnbaumer and Bessen, 1998). Generally, methanol is a CNS depressant, but, blindness is the most common symptom of its' poisoning in sever conditions (Kruse, 1992; Belson and Morgan, 2004; Younger, 1995). Recently, some reported cases of blurred vision leading to blindness (chronic methanol poisoning) after drinking of some herbal distillates that has not relationship to fermentation process of wooden or cellulose parts of consumed plant tissues and it has motivated the contra's health officials (Karimi *et al.*, 2007; Solhi *et al.*, 2009).

At present, methods based on high performance liquid chromatography (HPLC), gas chromatography (GC), selective flow-injection, enzymatic method, GC–MS, fourier transform infrared spectroscopy (FT-IR) and etc. are used to qualitative detection or quantitative determination of methanol content in various samples directly (Quanmin and Huanhuan, 2008; Garcia de Maria *et al*., 1995; Savary and Nu˜nez, 2003). Analysis of the samples with HPLC or GC needs very expensive apparatus that make them inapplicable in common laboratories (Von Dahl *et al*., 2006). On the other hand, some different methods based on spectrophotometric, spectrofluorimetric and chemilumenscence techniques have been utilized for quantitative determination of formaldehyde in various samples. Therefore, it can use of one of these methods for detection of methanol indirectly after oxidation of methanol to formaldehyde (Von Dahl *et al.*, 2006; Ashraf *et al.*, 2008). One of these procedures that was recommended by the National Institute for Occupational Safety and Health (NIOSH) and is adopted as a standard spectrophotometric method for the determination of 0.02–4.00 μg mL^{-1} HCHO is the chromotropic acid (CA) spectrophotometric method. However, this old chromotropic acid colorimetric method is very sensitive, easy, simple, inexpensive and selective, but, it has been recommended as official methods by AOAC and ISO for determination of methanol only in spirits (Ashraf *et al.*, 2008; Fagnani *et al*., 2003; Garcia de Maria *et al.*, 1995). Also, it has some application problems that the major drawback of it is the consumption of large amount of hot concentrated sulfuric acid, which is potentially hazardous and corrosive (Savary and Nu˜nez, 2003; Ashraf, *et al.,* 2008). On the other hand, this method is not recommended to use in none alcoholic drinks, therefore, it was modified for qualitative detection of methanol in herbal distillates, but, the first obtained results were different in industrial and homemade distillates and this is caused, studies were continued to find the reason. This paper is a portion of results of

a prolonged study about a modified CA method (which in it is using 1:2 dilution of herbal distillate sample prepared by industrial method for doing of test) about the reasons of application of higher dilution of samples (1:5) for doing of test in homemade herbaceous distillates.

MATERIALS AND METHODS

All the chemicals with purity analysis degree in this study such as potassium permanganate, sulfuric acid, sodium hydrogen sulfite and chromotropic acid were purchased from the Merck Company and used without any more purification. We also used three khalvash distillates that had been purchased from local vendors. According to them, the date of production didn't exceed two weeks. It is necessary to mention that lack of access to more samples was one of limits of this study. Then, aqueous solutions of 2.5% W/V potassium permanganate, 2.5% V/V sulfuric acid, 5% W/V sodium hydrogen sulfite, 1% W/V chromotropic acid and three series of dilutions (1:2 , 1:3 , 1:4 , 1:5, 1:6 , 1:7 , 1:8 , 1:9 , and 1:10) from samples were prepared.

After it, 0.5 mL of each prepared dilution was added in clean test tubes with 50 µl potassium permanganate and 50 µl sulfuric acid solutions. Then, after one minute waiting, 50 µl of sodium hydrogen sulfite solution was added to them and shake well to decolorize. At last, 50 µl chromotropic acid solution and 1 mL concentrated sulfuric acid were added to the tubes and after shaking, the result deduced as follows:

Negative: the mixture in the test tube would be remained colorless.

Positive: the color of mixture in the test tube would become violet.

RESULTS AND DISCUSSION

The results were shown, all of 1:2 dilutions were negative, while, in the 1:3 dilutions, light purple color were appeared that it was increased and completed in the 1:4 and 1:5 dilutions respectively and again, they were light and completely disappeared in upper dilutions. So, the maximum color was visible in 1:5 dilution ratios that it decreased in next one, so that, after 1:7 dilutions, any purple color was recognizable. Therefore, all of samples contain various amounts of methanol. Generally, with due attention to large amount of methanol content in green leaves and stems of plants and also, high sensitivity and low limit of detection (LOD) of suggested method , appearance of strong positive results (full purple) in all samples are expected but like observed differences between obtained outcomes in previously studies about industrial and homemade herbal distillates, comparison of obtained results from different dilutions in this study were indicated, all of them follow relatively similar pattern. As, a negative result with low opacity was observed in all of 1:2 dilutions, while, with increasing of dilution to 1:3 ratio, the light purple was appeared and at 1:4 and 1:5 dilutions the intensity of color was increased and opacity completely disappeared, but, at upper dilutions (1:6 and its upper), the intensity of color was gradually decreased to colorless. So, it was conclude, like the other distillates, there are various essences and effective medical compounds in khalvash distillate (as a homemade sample) that their concentrations are more higher than industrial ones and it could be accompanied with more interference effects of these chemical compounds and as a result, apparition of uncertain weakly positive answer (pale purple), unexplainable and false negative outcome or light visible opacity in lower dilutions (1:2). So, unlike industrial samples, application of 1:5 dilution instead 1:2 is necessary for doing of test. On the other hand, as it is observed in this study, in the upper dilutions (1:6 ratio and more than of it), the factor of dilution has more effect on the methanol concentration and it is lead to negative outcome. Therefore, it can to conclude, like the other industrial herbal distillate, the methanol content of homemade distillates are similar industrial ones, but, the high whole concentration of them are prevented to react of methanol with chemical reactants in 1:2 dilutions. Therefore, using of 1:5 dilutions is necessary to detecting of methanol in homemade samples by suggested method.

CONCLUSION

Generally, obtained qualitative outcomes in this study were indicated, negative result in 1:2 dilutions is not due to presence of less amount of methanol in homemade distillates than industrial ones, but, it is due to existence of whole concentration of homemade khalvash distillate. So, in different such homemade products, it is necessary to do this test by suggested method on the upper dilution (1:5) of samples to obtain completely clear results. However, In this section of study, correct determination of methanol concentration or proportional appointment content of it in used samples with together was not possible, but, the methanol existence were confirmed in them by an easy qualitative method.

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