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RESEARCH ARTICLE

Matrix solid phase dispersion extraction for Tropane alkaloid detection in cultured and collected *Hyoscyamus reticulatus* plants

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Abstract

Tropane alkaloids are important secondary metabolites with many medicinal uses. *Solanaceae* family and *Hyoscyamus* genus is an important source for this natural products. Hyoscyamine and scopolamine are important compounds that belong to tropane alkaloids class. For evaluation of scopolamine and hyoscyamine concentration, cultured and collected plants were analyzed by HPLC. For plant material extraction MSPD method was used. Results showed varied hyoscyamine and scopolamine concentrations in different organs. Maximum tropane alkaloid concentration found in the leaf of both cultured and collected plants. In cultured plant's leaf, hyoscyamine was more than 6.5 times as much as collected plants and this rate for scopolamine was 7.6.

INTRODUCTION

Global trade of medicinal plants is more than a hundred billion dollars per year (2013) and annual growth rate is about %8-15 (Tabriz and Kuchaki, 2014). Tropane alkaloids are natural compounds that have a structure named 8-azabicyclo [3.2.1] octane as a key structural element. Most of the alkaloids are in the form of an organic acid ester or hydroxyl compound (Christen, 2000).Scopolamine and Hyoscyamine belong to this class of natural compounds. Scopolamine has depressant effect on Central Nervous System and Hyoscyamine lowers activity of muscles and glands that is regulated by Parasympathetic Nervous System. Synthetic productions of these alkaloids are difficult and global demand for scopolamine is tenfold higher than hyoscyamine. (Sheludko, 2010). Solanaceae family has 90 genera and over 2,000 species, mainly in tropical and subtropical areas. *Hyoscyamus* genus find in Europe, North America, and large areas of North Africa and Asia, plants belonging to this genus contains tropane alkaloids (Aniszewski, 2007).

In Flora Iranica, 18 species of this genus have been reported but other researcher reported 13 species (Khatamsaz, 1998). This plant has been targeted for analysis in many research but all of these researches have been focused on collected plant (Dilmaghani *et al*, 2006; Bahmanzadeghan *et al*, 2008). In natural habitat plants are exposed to many environmental factors that may encourage or inhibit secondary metabolite synthesis, for finding real production potential of any species, we must test these plants in cultural condition.

Several analytical techniques have been used to identify Tropane alkaloids including enzyme-linked immune sorbent assay (ELISA), spectrometric method like atomic emission spectrometry (AES), atomic absorption spectrometry (AAS), uv spectroscopy ,Capillary electrophoresis (CE), Capillary Zone Electrophoresis (CZE) ,

micellar electrokinetic chromatography, mass spectrometry (MEKC-MS..) Thin layer chromatography (TLC), high performance thin-layer (HPTLC-densitometry,)gas chromatography–mass spectrometry (GC–MS),TLC(GC) and high performance liquid chromatography(HPLC)(Dehghan 2012). HPLC Analysis to identify various compounds has several stages, including the sampling, sample preparation, separation and measurement. The most important stage in sample preparation process is extraction. . Recently emphasis is on the preparation samples methods that cause reduction of the organic solvents use, inhibit the destruction of the sample content and reducing the need for purification and concentration of the samples before analysis. MSPD is a simple and inexpensive method that use for the extraction of solid and semi-solid compounds (Andrzej et al 2010).

This method was introduced in 1989 and is used for identification of drugs, pesticides, natural compounds and other compounds in a variety of plant and animal samples , such as pesticides residues in apples, beans, corn, garlic, nuts, olive oil, rice and secondary metabolites such as caffeine in tea, carotenoids in spinach, isoflavonoids in herbs, phenolic substances in green tea . Results of analysis showed that the efficiency of MSPD extraction method was better than or equal to traditional extraction methods. In this technique, the amount of solvent requirement and consumed time for extraction reduced 5% and 10%, respectively (Barker, 2007). This study aimed to compare tropane alkaloid concentration in both collected and cultured plants of *Hyoscyamus reticulatus*

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Materials and methods:

Plant material:

H.reticulatus plants collected from ″33° 38′ 22 .45 North latitude and 48 ° 24. 52 ″ east longitude in Khorramabad, Lorestan province. Plant completely removed from the surface at maturing stage. Seeds of this plant sowed in 15 cm pots containing 50% Perlite and 50% sterilized garden soil. Pots placed in a greenhouse with 16h light and 8 h dark and 26±4 temperature. Capsule, stem and leaf from collected plants and leaf and root from cultured plants after 10 weeks were detached and air dried in room temperature so kept in plastic bags.

Propane alkaloids identification:

Sample preparation was carried out by matrix solid phase extraction (MSPD) method.50 mg from air dried plant sample and 50 mg from C18 sorbent blended in glass mortar for 5 minutes. Then the mixture was homogenized and transferred to a cartridge by a spatula and compressed. Then analyte eluted by 600 micro liter of methanol and filtered before HPLC system injection. The eluent was a gradient of di-Sodium hydrogen phosphate dehydrate(50mM,PH=3)and methanol(Methanol ratio at 0.01 minutes50%, at 10 minutes 50%, at 15 minutes 20%, at 20 minutes 50%, and 30 minutes was 50%) .Flow rate was 1 ml $min^{-1.}$

Equipment

HPLC analysis of the samples was conducted using a Shimadzu (Model L-10AD) instrument consisting of two reciprocating pumps, a DGU-14A in-line degasser, a Model CT10-10AC oven, a high-pressure manual injection valve (20 µL injection loop) and a UV/VIS (Model SPD-10A) detector. The software used for the data acquirement and processing was Class-vp v.R 6.1. The analytical column was a 25 cm □ □ 4.6 mm i.d. RP18 column (Shim-Pack CLC-C18) packed with 5 µm particles and equipped with a 1-cm guard column (C18-B197) packed with 10 µm particles of the same type. A 25-µL HPLC microsyringe (F-LC, SGE, Australia) was used for sample withdrawal and injection. A 2-ml polyethylene (PE) syringe was used. . Calibration was made by Scopolamine Hydro bromide (SIGMA-ALDRICH'S) (Rt =4.7) and Atropine (SIGMA-ALDRICH'S) (Rt =3.62).

Results and D Discussion:

In collected plants (Table 1), maximum hyoscyamine concentration was found in the leaf, and minimum concentration belongs to stem, scopolamine also had the same concentration of the same. In leaf, stem and capsule of collected plants, hyoscyamine was dominant alkaloid. Alkaloid concentration difference between cultured and collected plants is very much. In cultured plant, hyoscyamine concentration in leaf was more than 6.5 times of collected plant and this rate for scopolamine was 7.6 (Table 1).

TABLE 1: Tropane alkaloid analytical results in *Hyoscyamus reticulatus*

Plant organ	Hyoscyamine(mg.gr^{-1})	Scopolamine(mg.gr $^{-1}$)	Total alkaloid	**Hy/Sco**	Scopolamine (%)	Hyoscyamine (%)
Collected						
leaf	0.3515	0.3611	0.7126	0.9734	49.32	50.68
stem	0.0788	0.1311	0.2099	0.601	7.55	62.45
capsule	0.3192	0.0494	0.3686	6.4615	13.41	86.59
Cultured						
leaf	2.3377	2.7467	5.0844	0.8511	50.02	45.98
root	0.1683	0.6873	0.8556	0.2448	80.32	19.68

Total alkaloid content in both leaf and root organs in cultured plant were higher in comparison to collected plant (Figure 1). The data showed both scopolamines percent and concentrations were higher in cultured plants.(Figure 2, 3).

The results provided by other researchers also show difference in the amounts of hyoscyamine and scopolamine in different organs and different species of Hyoscyamus genus. Dilmaghani *et al* (2006) studied two species of *Hyoscyamus reticulates* and *Hyoscyamus Arachnoideus* Pojark and observed that the hyoscyamine and scopolamine concentration varied in different organs and different stages of development.

Fig .1: Total alkaloid concentration in *Hyoscyamus reticulatus* different organs

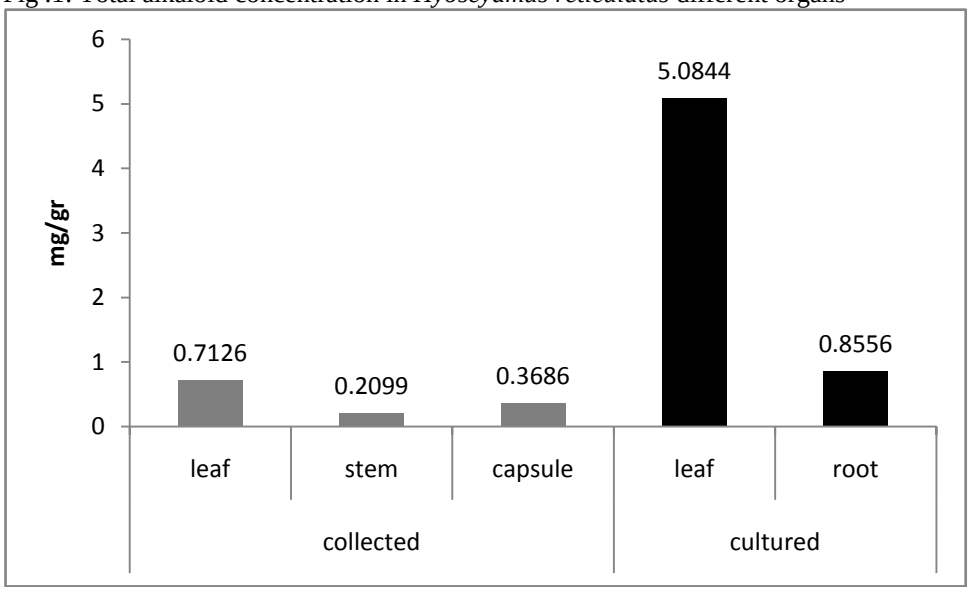


Fig .2: Scopolamine concentration in *Hyoscyamus reticulatus* different organs

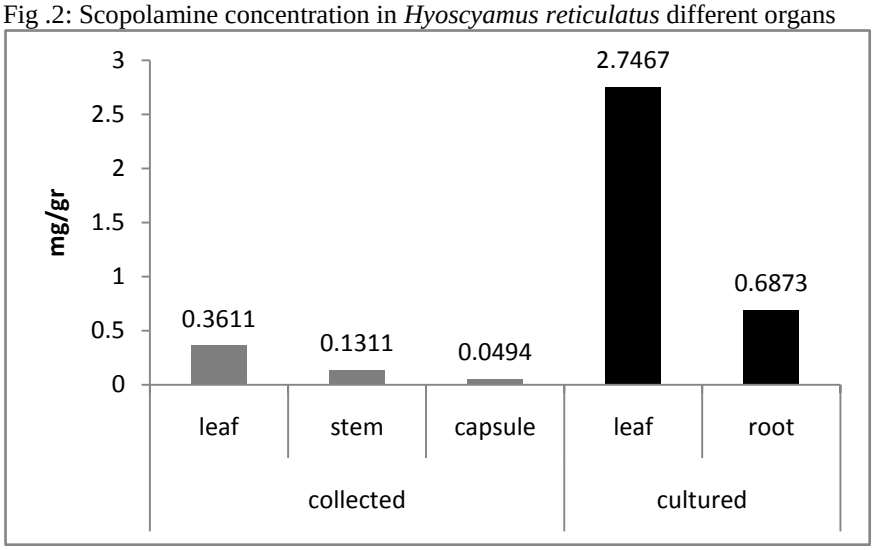
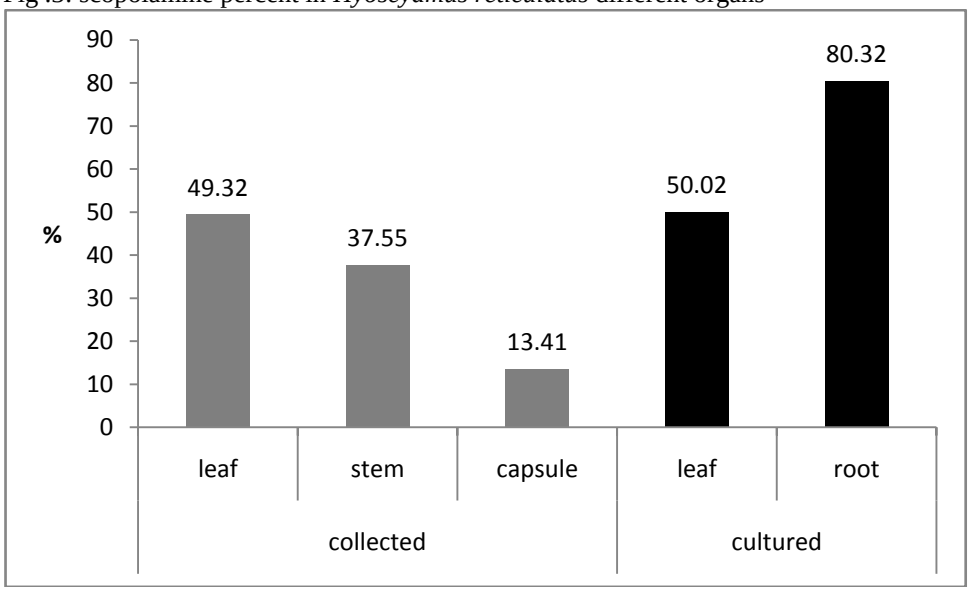


Fig .3: scopolamine percent in *Hyoscyamus reticulatus* different organs



Bahmanzadeghan *et al* (2008) observed hyoscyamine and scopolamine concentration in different parts of two different species *Hyoscyamus pusillus. L* and *Hyoscyamus reticulatus* varied. Scopolamine was dominant alkaloid in *Hyoscyamus pusillus*, *Hyoscyamus niger* and *Hyoscyamus kurdicus* species, but in *Hyoscyamus reticulatus*, hyoscyamine was dominant tropane alkaloid. Maximum concentration of scopolamine and hyoscyamine

varied between species and different organs in one species. In *Hyoscyamus Pusillus*.L and *Hyoscyamus kurdicus* leaves and *Hyoscyamus niger* seeds scopolamine had maximum concentration and in *Hyoscyamus reticulatus* seeds hyoscyamine maximum concentration was observed. Hyoscyamine to scopolamine ratio in roots and flowers of the species *Hyoscyamus pusilus* in *Hyoscyamus reticulatus* was at the highest rank.

A study by Christian *et al* (2000) on *Hyoscyamus albus* showed hyoscamine was dominant alkaloid in this plant and obtained hairy roots, hyoscyamine concentration in young leaves was higher than older leaves. Par *et al* (1990) observed wide range of tropane alkaloid concentration in 7 species of *hyoscyamus* genus.

Conclusion:

Genetically traits control quantity and quality of secondary metabolites, but environmental factors play a major role in this area. Tropane alkaloid content difference among cultured and collected plants show alkaloid production capacity in this plant is high and for reach to proper concentration, cultural conditions must be optimized. The amount and composition of these materials is not constant due to the effects of environmental change and natural selection. Plants in their natural habitats provide certain values of secondary metabolites. Cultural condition cause change in assimilation rate and this change affect secondary metabolite synthesis pathway.

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