INTRODUCTION
An alkaloid is any organic chemical compound that has at least one nitrogen (nitrogen) atom in a heterocyclic ring. Alkaloids are characterized by nitrogenous compounds. So far, more than 10,000 alkaloids have been identified in nature or in chemical experiments (ASADOLAHI, 2014). Alkaloids are biologically and chemically nitrogenous and are mostly found in plants; they are also seen in a number of animals. Alkaloids contain the amine factor, which is why they are often alkaline. Especially alkaloids that are used in pharmacy and medicine and have physiological effects. Plant-derived alkaloids are basic compounds that contain at least one nitrogen atom (usually in a heterocyclic ring). These compounds usually have prominent physiological effects on humans and animals (BALAZOVA et al., 2008; MUSHTAQ et al., 2016). The name proto-alkaloid or amino-alkaloid is sometimes used for compounds such as hordenine, ephedrine, and colchicine that lack one or more of the properties of common alkaloids. Other alkaloids whose properties do not correspond to the properties given in the general definition are those obtained by synthesis but not found in plants.

These compounds are very similar to natural alkaloids (for example: hematropin). In practice, those compounds found in plants that respond positively to qualitative tests for alkaloids are called alkaloids, and if plants respond positively to these tests, they are usually considered to contain alkaloid-containing plants. Alkaloids are often insoluble in water and show the production of water-soluble salts with acid. Free alkaloids are soluble in atrochloroform, but their solutes are insoluble in these solvents. Most alkaloids are crystalline and solid. Some are amorphous and some lack in oxygen; Like nicotine. Alkaloid salts are usually crystalline. The microscopic properties and shape of these crystals are often a means of rapid detection. Alkaloids are often bitter. Some of the functions of alkaloids in plants generally include the following:

- Toxins that protect plants from insects and animals
- Have a similar effect to hormones and have a regulating effect on plant growth
- Storage materials that later serve as a source of nitrogen or nutrients

The cause of alkaloid production in plants can be considered as a result of the evolution of plant metabolism; because the production of alkaloids in plants is controlled by genes, in plants the material of alkaloids is supplemented by the phenomenon of mutation of additional metabolic reactions. The genus Thalictrum comprises 120 species of perennial herbaceous plants (BEHZADIRAD et al., 2016). Over 200 alkaloids, almost all of isoquinoline group have been reported from this genus and a large number have biological activity (mainly hypotensive, antimicrobial and antitumor properties, (BADAMJAV et al., 2021; CHEN et al., 2014). So far, the only alkaloids reported to occur in the minus of Iranian origin were berberine and magno florine (BHAGWATH et al., 2000). The aim of the present work was to continue studies on the alkaloid content of the minus and identifying major alkaloids of the above ground parts of Th. minus of Iranian origin.
**METHOD**

In the present study, in order to select a suitable eliminator to increase the production of the valuable medicinal alkaloids Thebaine and sanguinarine, the stimulants of fluoroglucinol and methyl jasmonate, alone and in combination, were used on P. bracteatum root explant cell culture in vitro and 40 hours after in vitro treatment. The amount of desired metabolites produced was measured. Benzyl isoquinolines are a large group of alkaloids that are mainly identified in the plant families Papaveraceae, Fumariaceae, Berberidaceae and Ranunculaceae. Many alkaloids in this group have important drug effects such as analgesics (morphine and anti-cancer), anti-morphine and stone, codeine. They are bacteria (berberine) and muscle relaxants (papaverine).

The biosynthesis of Benzylisoquinoline Alkaloids begins with the conversion of tyrosine to dopamine and 4-hydroxyphenyl acetaldehyde (with a network of decarboxylation, orthohydroxylation and deamination). Tyrosine converts decarboxylase, tyrosine and dehydroxyphenylalanine to similar amines. Dopamine and 4-hydroxyphenyl acetaldehyde are combined by norcoclarine synthase (NCS) to produce S-norcoclarine (a central precursor for all Benzylisoquinoline Alkaloids in plants). All 2133 central Benzylisoquinoline Alkaloids are concentrated.

**RESULTS AND DISCUSSION**

From the non-phenolic alkaloid fraction of Th. minus two alkaloid were separated. The structure of the known alkaloid, thaliglucine (Jin et al., 2020), was confirmed by comparison of its spectroscopic data (H-NMR, UV, IR, Mass) with the references (ZHAO et al., 2005)

It was previously reported to occur in Th. polygamum and Th. Rugasum

The phenanthrene nature of the new alkaloid, was deduced from its HNMR spectral data that exhibited four aromatic protons a two singlets at 7.21 and 7.10 and two as ABq system centered at 7.62 with a coupling constant of 9.4 Hz (H9 and H10 of the phenanthrene skeleton);

A methoxyl group at 4.01 and one singlet for a methylen dioxy group at 6.11 and one singlet for a methylenoxy bridge at 5.59.

It was similar to that of 1 with the exception that the Me-N-Me signal was shifted down field to 3.41 [(2) - (1) = 0.094].

The positive nature of N may cause this deshielding.

Consequently, two structural formulas 2.3, were proposed in N-methyl thaliglucine (Mushtaq et al., 2016), the ABq system appears at 7.47 and N trimethyl signal is present as singlet at 3.27 that’s different from our data and therefore this structure was rejected.

Consequently, this alkaloid may be N-oxide as structure 2.

The N-oxide structure of 2 was supported by the presence of the (M+) at m/z: 367 in a low intensity, as well as the apparent Fragment ion

\[(M-16)^+ \text{ at m/z:351 in the mass spectrum that are typical for N-oxides. Another intensive ion is} \ (M-61)^+ \text{ at m/z: 306 that’s typical for some of Phenanthrene N-oxides.} \]

Therefore, Alkaloid 2 is Thaliglucine N-oxide (2), isolated for the first time.

**Experimental apparatus**

UV Spectra were measured on Shimadzu UV-160A in CDCl3 and IR spectra were obtained by using FTIR Nicolet 550.

The 400 MHz H-NMR spectra were recorded on Varian FT- 400 Unity plus in CDCl3. Mass
spectra were measured with Finnigan Mat TSQ-70.

**Plant material**
The aerial parts of plant were collected from Karaj (the north slopes of mountain) in May 2000 and was identified at the herbarium of Tehran University, Faculty of Pharmacy.

**Extraction and Isolation**
The air-dried above ground parts (737g) were extracted by percolation to exhaustion with about 4 liters of ethanol. The dark syrup (90g) remaining after removal of the solvent, at reduced pressure at 40 was dissolved in 900 ml of chloroform and shaken with an equal volume of 2% aqueous citric acid solution. The citric acid extract was made alkaline to pH 9-10 with ammonium hydroxide solution and extracted with equal volumes of chloroform. The tertiary alkaloids in chloroform extract was divided into the phenolic and non-phenolic fractions by partitioning between chloroform and 5% Sodium hydroxide solution. The chloroform layer dried and (sodium sulfate) and after evaporation 0.22 g of the crude tertiary non-phenolic alkaloids left. This residue (0.22g) was dissolved in 5% Acetic acid solution and was made alkaline to pH 9-10 with ammonium hydroxide solution, extracted with equal volumes of ether and this work was repeated twice more. The ether layer I was washed with water, dried and after evaporation left 210 mg of non-phenolic alkaloids (BHAGWATH and HJORTSO, 2000).

These residue was further purified with the aid of Preparative TLC. The following TLC system were used for the plates coated with silica gel 60HF (CHCL₃, 40 cc- etherdepetrol 40 cc, acetone 10 cc, methanol10cc). This system yields two alkaloids:

1) Traliglucine (20mg) with the Rf value of 0.3
2) Thaliglucine N-oxide (23mg) with the Rf value of 0.1.

**H-NMR**

*Thaliglucine N-oxide (2): C₂₁H₂₁N₀₅*

UV CHCl₃: 234, 265, 308, 320, 362, 371.
IR: 763,960, 1045,1209, 1270, 1454, 1608,2852,2924
Mass: M⁺ 367 (2%), 351 (M⁻16,54%), 306 (M⁻61,59%), 293 (M⁻16-58, 16%), 58 (100%)
H-NMR: 3.41(S, Me-N-me), 3.65 (t, CH₂-N), 3.69 (m, Ar-CH₂-) 4.011 (s, OCH₃), 5.59 (s, Ar-CH₂-O) ,6. 11 (s, O-CH₂O) 7. 107(S, Hα) 7.21(S, H₂), 7.49 (d, J= 9.4, H-9), 7.76(d, H-11).

Various species from genus Thalictrum have been used for centuries as herbal medicines especially in Russia, Japan and China (Chen et al., 2014). The genus is well known for its diverse pharmacological activities, including antitumor, antimicrobial, anti-tuberculosis, antimalarial and anti-inflammatory activities. It belongs to the family Ranunculaceae (Buttercup)and consists of about 110 species of herbaceous perennials. Thalictrum minus L; (Lesser meadow rue) is an important species of the genus Thalictrum distributed in many parts of the world.

In India, it is found in the states of Himachal Pradesh, Uttar Pradesh and Jammu & Kashmir (GBIF Secretariat: GBIF Backbone Taxonomy, 2013-07-01; available at: http://www.gbif.org/species/3927432 Accessed on 2016-02-05). The roots of this plant species are of medicinal importance and traditionally used as anti-inflammatory and anti-infective agents as well as in veterinary medicine.
CONCLUSION
The objective of the research was to continue studies on the alkaloid content of the minus and identifying major alkaloids of the above ground parts of Th. minus of Iranian origin. What was observed in this study was the high excitability of root cells against the accumulation of both alkaloids due to the treatment with elicitor. The stimuli used on the biosynthetic cycle of production of secondary metabolites of P. bracteatum were also very effective. Therefore, by using eliminators, especially the combined approach of eliminators with hormones and benefiting from their synergistic effect, it is possible to stimulate the biosynthetic cycle of these metabolites in a callus that lacks the active gene producing tebaine and sanguinarine.

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Isolation and characterization of alkaloids from thalictrum minus

Resumo
O objetivo do presente trabalho foi continuar os estudos sobre o conteúdo de alcalóides do menos e identificar os principais alcalóides das partes acima do solo de Th. menos de origem iraniana. O Thalictrum menos a população do Irã, continha dois principais alcalóides fenantrenos cuja estrutura foi estabelecida por métodos espectrais. A Thaliglucina foi detectada pela primeira vez no estado negativo. Presume-se que o N-óxido de taliglucina seja um novo N-óxido de fenantreno.

Palavras-chave: Thalictrum minus. Isolamento. Alcaloides. Atividade antibacteriana.

Abstract
The aim of the present work was to continue studies on the alkaloid content of the minus and identifying major alkaloids of the above ground parts of Th. minus of Iranian origin. Thalictrum minus population of Iran, contained two major phenanthrene alkaloids that their structure were established by spectral methods. Thaliglucine has been detected for the first time in the minus. Thaliglucine N-oxide is assumed to be a new phenanthrene N-oxide.

Keywords: Thalictrum minus. Isolation. Alkaloids. Antibacterial activity.

Resumen
El objetivo del presente trabajo fue continuar con los estudios sobre el contenido de alcaloides del signo negativo e identificar los principales alcaloides de las partes aéreas de Th. menos de origen iraní. Thalictrum menos la población de Irán, contenía dos alcaloides fenantrenos principales cuya estructura se estableció mediante métodos espectrales. La taliglucina se ha detectado por primera vez en el signo menos. Se supone que el N-óxido de taliglucina es un nuevo N-óxido de fenantereno.

Palabras-clave: Thalictrum minus. Aislamiento. Alcaloides. Actividad antibacteriana.