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Full Length Research Paper

Comparison and evaluation of steroid alkaloid solasodine on *IN VIVO* and *IN VITRO* cultures of *SOLANUM SURATTENSE* Burm L.

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Steroid alkaloid solasodine is a nitrogen analogue of diosgenin that is used as a raw material for synthesis of steroid drugs. Plant medicinal *SOLANUM SURATTENSE* (*SOLANACEAE*) is a rich source of solasodine. The purpose of this study was production and maintenance of callus from leaves and comparison of solasodine steroid alkaloid production in outdoor plants and *IN VITRO* calli. The leaves were collected, sterilized and placed on MS medium supplemented with BAP (1 to 2 mg/l) and NAA (1 to 3 mg/l). The samples were kept in a growth chamber at 25°C with 16/8 (day/night) photoperiod and irradiance of 3000 to 2000 lux. The methanol extracts was performed to obtain solasodine from 2 g dry weight of each leaves and calluses and then injected into the high-performance liquid chromatography (HPLC) system. The results showed that solasodine produced by leaves was 0.3316 mg/g whereas, the solasodine content obtained from calluses were 0.3951 mg/g (in 1 mg / l BAP + 2 mg / l NAA) and 0.3908 mg/g (in 1 mg / l BAP + 3 mg / l NAA). Plant tissue cultures have been suggested as a potential tool for solasodine production.

Key words: Callus induction, *Solanum surattense* Burm, *in vitro*, *in vivo*, Solasodine, high-performance liquid chromatography (HPLC).

INTRODUCTION

The medicinal plant *Solanum surattense* Burm (syn. *Solanum xanthocarpum*) belong to family of *Solanaceae* which is known as a perennial, prostrate, ramose and prickly plant with green and white fruits but yellowish when mature. The fruits are known for several medicinal uses like anthelmintic, anti pyretic, laxative, anti-inflammatory and anti-asthmatic (Kiritikar et al., 1994). Vapor of burning seeds is used as an expectorant in asthma and cures toothache and crude plant extract caused hypotension, antitussive and is beneficial in bronchial asthma and non-specific cough. The plant is native in tropical areas of Asia, India, Australia, Pakistan and South-East of Iran. *S. surattense* is a rich source of solasodine (Kaul and Zutshi, 1982). Alkaloid steroid solasodine is an analog of the nitrogen from diosgenin which is used as a raw material in production of steroid drugs (Mann, 1987).

Solasodine is obtained by chemical or microbial hydrolysis of solamargine. It has potential as a substitute for diosgenin in the semi-synthetic production of steroidal hormones for use in pharmaceuticals (Figure 1). Therefore, steroidal glycoalkaloid from Solanaceae plants have become increasingly important as a starting material for the production of steroidal hormones. Solasodine synthesis *in vitro* callus *S. surattense* has been reported (Heble and Narayanswamy, 1968; Barnabas and David, 1988).

Production of secondary metabolite derived from medicinal plants is reliable in comparison with the compounds extracted from plants under natural conditions. The benefit of this method in the pharmaceutical industry is that it can be used in a short period by synthetic medium and away from climate

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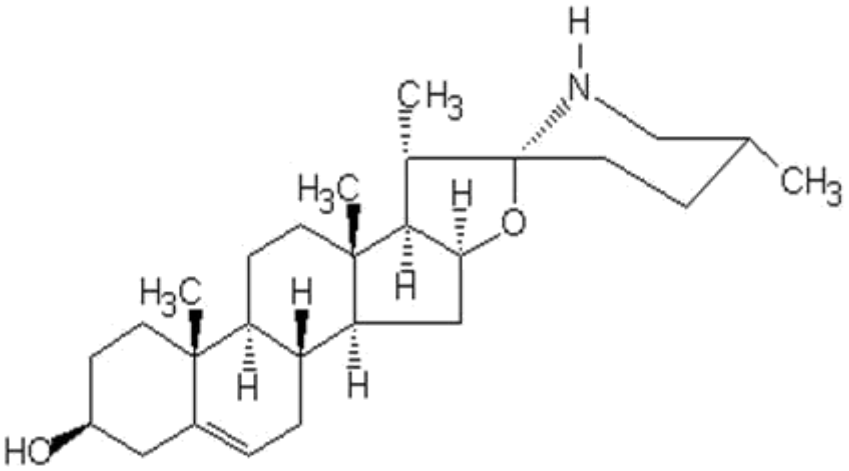

Figure 1. Structure alkaloid steroid solasodine (Mann, 1987).

restrictions. Commercially, Secondary metabolites have been reported through tissue and cell culture for anti-drug cancer from *Taxus brevifolia* and solasodine extracted from cell suspension culture from *Solanum eleganifolium*. Although, the species of *S. surattense* has important medicine, little work has been done on *in vitro* culture (Prasad and Chaturvedi, 1978; Prasad et al., 1988; Pawar et al., 2002). Due to poor information about the Iranian *S. surattense*, the objective of this study was optimizing the callus induction from the leaves explants and comparison of solasodine product from field grown plants and *in vitro* callus.

MATERIALS AND METHODS

The seeds of *S. surattense* which were collected from south east of Iran, Sistan and Balouchstan province were cultured on April 2010 in the Botanic Garden of Zabol University, Iran. The young leaves were divided into pieces of 1 to 2 cm. The explants were surface sterilized with 70% ethyl alcohol for 30 s to 1 min and rinsed with distilled water for 3 to 4 times. After that, the explants were transferred to a laminar air flow chamber and disinfected with 3% hypochlorite sodium for 10 min and washed with sterile distilled water for 3 to 4 times. Three explants were transferred into the 25 ml MS basal medium supplemented with different concentration of BAP (1 to 2 mg / l) and NAA (1 to 3 mg / L) with 20 g sucrose for callus induction. The samples were kept in the growth chamber at 25°C with 16/8 h (day/night) photoperiod and irradiance of 2000 to 32000 luxe. The leaves were either grown in field conditions or in laboratory at room temperature and calli developed *in vitro* were dried at 60°C in the oven for 24 h (Figure 2).

High-performance liquid chromatography (HPLC)

The model used for HPLC analysis was Cecil. All samples were filtered through 0.45 µm membranes. The main column was C18 with length of 25 cm and flow rate of 0.5 per minute. The solasodine detected by UV absorption at 210 nm and the volume of each injection was 20 ∝l. The mobile phase consisted of 70% methanol and 30% water (20 mM phosphate buffered) and pH = 3.5.

Solasodine extraction

Two gram dried callus and leaves acquired from field plants were ground in 20 ml ethanol 96% until a uniform solution was obtained. The samples were placed on the shaker for 10 min and tubes were left for 24 h at 4°C. The solution was filtered and evaporated under vacuum and then the riffraff was dissolved in 20 ml of sulfuric acid (5%) and 20 ml of the diethyl ether and transferred into the separatory funnel. The top phase containing fats and pigments was discarded and the beneath aqueous layer (acidic phase) containing alkaloids was collected. The pH of the acidic phase was increased by ammonia 25% to almost 11 under cold condition (water and ice) to prevent heat reaction caused by ammonia and acid. The heat will cause decomposition of alkaloids.

The top aqueous layer was transferred into the separatory funnel and 50 ml of chloroform was added in three stages. The underneath phase (acidic phase) include alkaloids was collected and evaporated by Rotary evaporator under vacuum. The pellets were dissolved in 1 ml methanol and transferred into a clean vial. The crude alkaloid extract was used to measure the content and type of alkaloid by HPLC. The pure Solasodine was prepared from Latoksan, France and 1 mg/ml methanol was used for injection to HPLC system content of solasodine obtained according to Yoganth et al. (2009).

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard weight}}{\text{Dilution}} \times \frac{\text{Dilution}}{\text{Sample weight}} \times \text{Standard purity}$$

RESULTS

The results showed that the highest percentage of induced callus (100%) was observed on all MS medium containing 1 mg/l BAP + 1 mg / l NAA, 1 mg / l BAP + 3 mg / l NAA, 2 mg/l BAP +1 mg / l NAA (Table 1). The highest callus fresh weight was observed (2038 mg) in the MS medium contained 1 mg / l BAP + 1 mg / l NAA

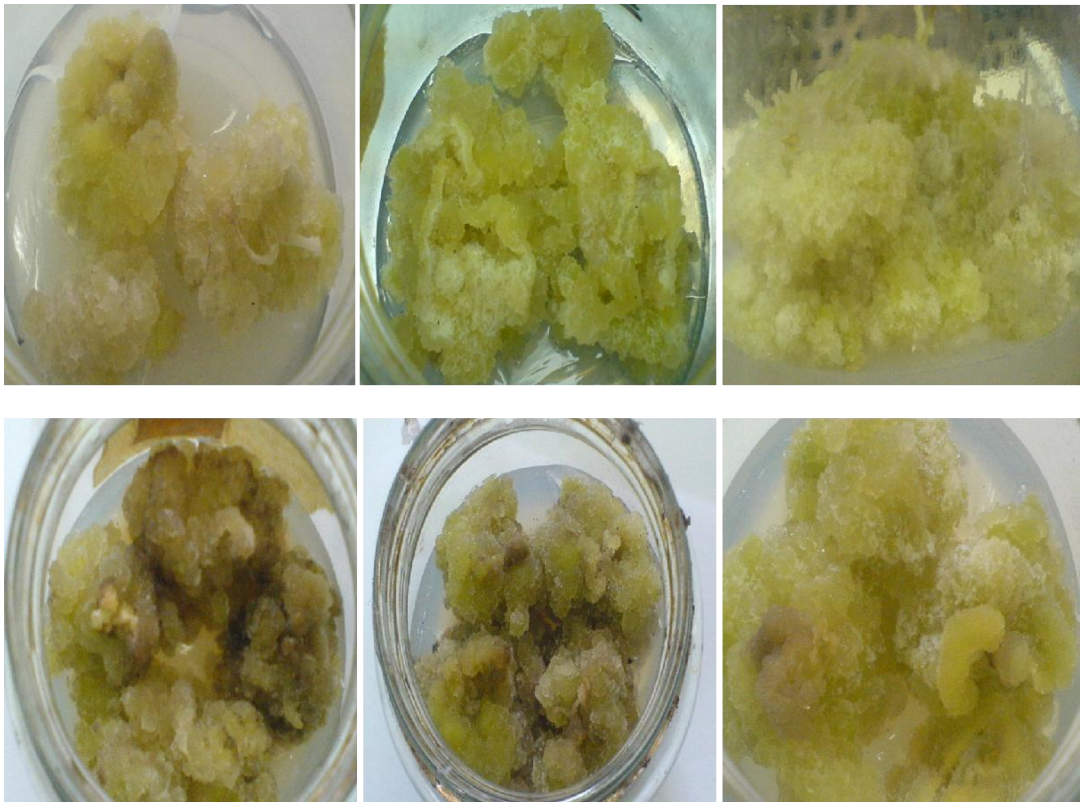

Figure 2. Calli production from young leaves explants of *Solanum surattense* with the various color and form treated with BAP and NAA.

Table 1. Effect of BAP and NAA on calli of young leaves explants of *Solanum surattense.*

Hormones mg/l BAP + NAA	Percentage callus induction	Fresh weight (mg)	Dry weight (mg)
1.0 + 1.0	100.00	2038.00	4677.00
1.0 + 2.0	91.67	1417.00	3194.00
1.0 + 3.0	100.00	1898.00	4278.00
2.0 + 1.0	100.00	1722.00	3882.00
2.0 + 2.0	75.00	1332.00	3001.00
2.0 + 3.0	91.67	1624.00	3401.00

and the highest callus dry weight (4666 mg) was observed in the MS medium supplemented with 1 mg / l BAP and 1 mg / l NAA (Table 1).

The results obtained from analysis of chromatography (HPLC) showed that solasodine content extracted from field grown leaves was 0.3316 mg/gwd where as, the solasodine content *in vitro* callus was 0.3951 mg/gwd in the MS medium containing 1 mg / l BAP + 2 mg / l NAA and 0.3908 mg/gwd in the MS supplemented with 1 mg / l BAP + 3 mg / l NAA. The results indicated that the *in vitro* culture leads to improvement of solasodine synthesis (Figure 3).

DISCUSSION

The results showed that the highest percentage of induced callus (100%) was observed on all MS medium containing 1 mg/l BAP + 1 mg/l NAA, 1 mg/l BAP + 3 mg/l NAA, 2 mg/l BAP +1 mg/l NAA. The NAA and BAP have been reported as the best hormones for callus induction (Biswas et al., 2007). Generally, the callus induction is promoted by combination of auxin and cytokenin (Albarello et al., 2006). The callus growth is based on different type and concentration of growth regulators (Khan et al., 2006). In this study, all treatments leaded to

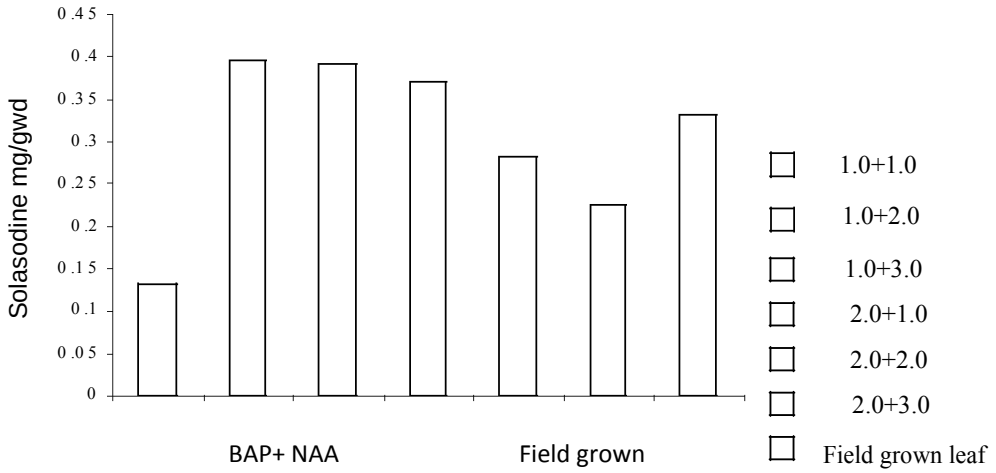

Figure 3. Solasodine production in field grown leaf and callus of *Solanum surattense* Burm L.

production of calluses with the various color as green, yellow and brown. The synthesis of pigments in some samples was observed which is due to the absorption of carbohydrates and elements such as calcium, phosphate, nitrate and ammonium (Zhang et al., 1998). Various PGR and different concentrations of them are often an important factor in the production of secondary metabolites (Albarello et al., 2006).

Plant regenerations *in vitro* are a very useful method for quality producing of herbal medicines plants. Productions of plant secondary metabolites with medicinal properties *in vitro* are more reliable than those obtain from field grown plants. Precise control of various parameters increase the quality of the material and no change is observed during the treatments. While in normal conditions frequently effect of climate and pest influence the secondary products. The natural production of solasodine is restricted. Hence, the pharmaceutical industry is exploring alternative methods such as *in vitro* production of natural compounds. Manipulating of the PGR, quality and quantity of irradiance, carbon source, size of explants, temperature and medium ingredient affect the rate of secondary metabolite production (Nigra et al., 1989; Yu et al., 1996; Rao and Ravishankar, 2002; Bhatnagar et al., 2004; Xu et al., 2008).

In our experiment, the best results for synthesis of solasodine were obtained on MS medium supplemented with 2 mg/l NAA and 1 mg/l BAP, 3 mg/l NAA and 1 mg/l BAP. The same result has been reported for optimizing of solasodine production in combination of NAA and BAP on medicinal plant of *S. nigrum* (Yoganth et al., 2009). More evidences have been observed in tissue culture method on some other plants such as *S. jasminoides* and *S. verbascifolium* (Jain et al., 1995), *S. platfolium* (Jaggi and Singh, 2001) and *S. Khasianum* (Bhalsing, 2000). Also, in suspension cultures of *S. auritianum*, synthesis of solasodine was raised when NAA and BAP were used (Biswas, 2007). Another report stated that the use of the hormonal treatments of NAA and BAP on *solanum laciniatum* led to high production of solasodine (Chandler, 1984). By using tissue culture, larger content of compounds of Visnagin and Diosgenin were obtained in *Solanum xanthcarpum* and *Ammi visnagal* (Heble et al., 1968). Therefore, plant tissue cultures have been suggested as a potential tool for the high production of secondary metabolites.

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