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Current Pharma Research 3 (4), 2013, 1010–1013.

Current Pharma Research

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Original Article

Phytochemical Evaluation of Total Glycoalkaloid of Dried Fruit of *Solanum Nigrum Linn.*

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Received 28 June 2013; received in revised form 1 Aug 2013; accepted 2 Aug 2013
Available online 15 September 2013

Abstract

Solanum nigum L. is one of the traditional medicinal plants with a current interest as a source natural medicine. There are steroidal glycoalkaloid and aglycone present in *solanum nigrum* that expected to be active phytoconstituents. Crude extract of dried fruit of *solanum nigrum* was analyzed for the presence of the phytoconstituents with the use of analytical methods like HPLC and GC-MS. From this study, Solasonine was the steroidal glycoalkaloid which had higher content (5.85 mg/g) than other relative constituents and aglycone solasodine was present in 75.94% which was significantly higher than solanidine.

Keywords: *solanum nigrum, glycoalkaloid.*

1. Introduction

The herbal drugs have been used very limited number of treatment because of several reasons like poor availability, traditional cultivation technology. Discovery of new therapeutic agent will helps to the pharma scientist to make effective product with minimum side effects [1]. The medicinal plants take up a place in modern medicine as a raw material for some important medicines. There is a continuous and urgent need to discover new phyto compounds with diverse chemical structures and novel mechanism of action for new and rising disease. Thus it is anticipated that phyto-constituents with adequate efficacy will be used for the treatment of various serious disease. Because of importance of Traditional medicine World Health Organization (WHO) has taken a part active in creating strategies, guidelines and standards for botanical medicines [2]. Modern physicochemical studies are helping the researcher to discover the research with more scientific approach. In certain part of Africa and Asia fruits of *solanum nigrum* being eaten frequently as a part of traditional medicinal plant. In Australia *solanum nigrum* utilized as the alternate host to prevent tobacco plant from the insects. The plant is more effective in doing this if it is infected with the bacterial parasite *Agrobacterium tumefaciens*. The fruits of *solanum nigrum* contain the solasodine. It is a glycoalkaloid which is used by pharmaceutical companies for the preparation of many important drugs such as nitrogen analogue of diosgenin.

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Sapogenin produced from the diosgenin is used as a base for preparation of cortisone and allied product. Finally Cortisone prepared from glycoalkaloid solasodine is found to be effective in treatment of leukemia and chronic cases of asthma [3]. *Solanum nigrum* express the phenotypic variation, particularly in vegetative features such as plant habit, leaf size and form, and stem winging. Black night shade known as *solanum nigrum,* causes belladonna poisoning in which active chemical constituents is the solanine group. Such findings indicate the importance of phytochemical studies of s*olanum nigrum L*. [4,5].

2. Material and Methods

The plant fruits of *solanum nigrum Linn.* was collected from Late Miss misha medicinal plant garden Dhanvantary pharmacy college, Kim Surat, Gujarat. Plant material was dried under shade until complete removal of water from it. Such dried fruits were powered by using pulveriser and passed through sieve no 80.

2.1. Extraction of Powdered Fruit

The dried fruit powder of each sample was dipped in *n*-hexane for 45 sec. The *n*-hexane was then filtered and analyzed. This process ensures the removal of surface fats and epicuticular wax so that they may not interfere in alkaloid and flavonoid analysis without disturbing the interior chemical make-up. Total glycoalkaloids for compositional analysis were extracted by a method based on a modification of the technique of Dao and Friedmanb [6]. 15 g of powdered sample plant material with 200 ml of 5% aqueous acetic acid was placed in conical flask and stirred for 30 min and then vacuum filtered through Whatman no. 42 filter paper and the residue re extracted thrice with the solution for 30 min. The

filtrates were combined in separating funnel and pH adjusted to 11 with ammonium hydroxide, and the alkaline extract was partitioned with 50 ml of saturated butanol. Then butanol extracts were evaporated to dryness and the residue was removed from the flash with three portions of methanol. The extract was evaporated to dryness and the powdered residue was weighed and dissolved in 10 ml of methanol and analyzed. Samples for HPLC and GC-MS analyses were filtered using polyamide filters.

3. Experimental Methods

3.1. Chromatogrhy

The solvent systems used for different glycoalkaloids of *solanum nigrum*.

a) Acetic acid- 1 ml: ethanol- 3 ml [7].

b) Chloroform- 2ml: methanol- 2ml: 1% aqueous ammonium hydroxide- 1ml [8].

b) Ethyl acetate-3 ml: pyridine- 3ml: water- 3ml [9].

Spray reagent:

Marquis reagent

Dragendorff's reagent

Antimony-3 chloride reagent

3.2. HPLC Analysis

HPLC instrument consisting of Shimadzu LC-10A system utilized for analysis of glycoalkaloid. The injector loop was 20 µl. Mobile phase was prepared fresh, sonicated and filtered through a 0.45 µm polyamide filter. Solasonine, α-Solamargine, β-Solamargine and α-Solanine were used as standards. Mobile phase- 0.05 M acetonitrile: ammonium di-hydrogen phosphate buffer (30:70 v/v) and solvent flow rate- 1.5 ml/min.

3.3. Hydrolysis by Acid

10 g of dried powdered sample and 20 µg standards were separately dissolved in 2 ml of 1 M HCl prepared in methanol and heated in water bath for 3 hour 70 °C. Aglycones were released from the hydrolysate by adding 2 ml of 25% ammonia to the cooled tube and extracted with 2 ml of dichloromethane with vigorous mixing and centrifuge to separate dichloromethane layer. This step was repeated twice with the help of dichloromethane. Finally Aglycon extracts were then evaporated to dryness.

3.4. Derivatization of Aglycone

20 µl of Tri methyl silylimidazole and 50 µl of dry acetonitrile were added via glass syringe to sample and standard. The mixtures were placed in an oven for 15 min at 60°C. Then they were cooled to room temperature and 1 µl of solution was injected into the chromatographic system.

3.5. GC-MS Analysis Aglycone Derivatives

The aglycone derivatives were determined by the method recommended by Laurila *et al.* (1999) for *solanum nigrum* using Shimadzu GC-MS system operating at an ionization voltage of 70 eV with ion source temperature of 180 °C [10]. Samples were analyzed with the help of fused-silica capillary column (15 m- 0.20 mm). 1 µl sample was taken for GC/MS analysis.

Injector and detector temperatures- 280 °C.

Flow rate of carrier gas Helium - 1 ml/min.

Identification of the aglycones in the dried fruits of was based on the GC-MS spectra of TMS derivatives [11,12].

Results and Discussion

The *Solanum* glycoalkaloids have been intensively studied during recent decades and as a result of substantial research efforts, thousands of articles concerning various aspects of glycoalkaloids have been published. *Solanum nigrum* is especially known for its toxicity because it contains solanine which is a neurotoxic glycoalkaloid [13]. The development of new glycoalkaloid analysis techniques is still continuing with various analytical methods like HPLC which is the most commonly used application and its advantage is that both entire glycosides and also aglycones can be analyzed. And the spectrophotometric techniques such as LCMS or GC-MS are available for the unknown glycoalkaloid. The most important advantage of GC analysis is its sensitivity and good separation of aglycone mixtures. As suggested by Tetenyi, alkaloids were helpful for the classification of the family Solanaceae on the basis of the metabolism [14]. GC-MS technique is cited for the easy determination and identification of alkaloids and the application of the technique to chemotaxonomic studies [15]. In this study phytochemical investigation was based on qualitative and quantitative determination of glycoalkaloid, aglycone contents in dried fruit powdered of *solanum nigrum*. There had been many reports on the compositional analysis of alkaloids of *solanum nigrum* by TLC. Various steroidal glycoalkaloids were detected by TLC of the crude alkaloids with some minor differences. The 5-alpha-solasodan3-beta-ol was found to be accumulated in *solanum nigrum*. The different components found in this method were A-Solanine, Solasodine, Solasonine, A-solamargine which having the R_f Value of 0.46, 0.24, 0.23, 0.40, 0.54 respectively. This method provides that the different phytoconstituents are present in the extract of Dried fruits of *solanum nigrum. Solanum nigrum* is well known for glycoalkaloid it is necessary to make quantitative analysis for a phytochemical investigation. The glycoalkaloids are difficult to separate due to structural similarity like α-Solamargine and β-Solamargine have specific sugar constituents, but aglycone attachment pattern is different, i.e. solasodine and solanidine. Similarly, solasonine and solanine contain the same sugar moieties, but solasodine and solanidine have aglycone backbones. There had been many reports on the SGA of different species of *solanum nigrum* but not a single one on *solanum nigrum.* With the help of Sotelo and Serrano method results were obtained [16]. In chromatograms standard analytes with greater signal intensities were selected for at 210 nm. Glycoalkaloid analyzed qualitatively and quantitatively by the HPLC of the alkaloids extracted using the standard compounds. The peaks were

determined by retention time and response when standards were added. (Figure 1)

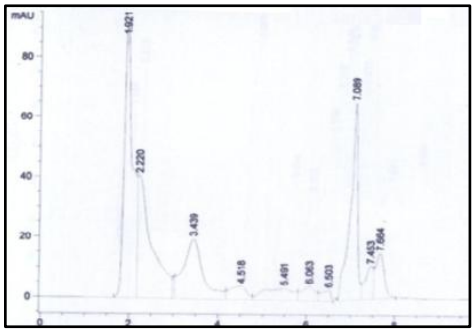

Fig. 1. Chromatogram of glycoalkaloid of *Solanum nigrum* for HPLC.

The separation was carried out by following linearity gradient in the terms of peak area response of the standard sample. Finally in present study, α-Solamargine, β-Solamargine, Solasonine, and α-Solanine contents of *solanum nigrum* was 5.03 mg/g, 1.69 mg/g, 5.85 mg/g, 4.75 mg/g respectively. The chemical profile for *solanum nigrum*, containing secondary metabolites is remarkably distinctive [17]. The glycoalkaloid determined from *solanum nigrum* α-Solamargine, Solasonine, and α-Solanine relatively higher concentration than of the β-Solamargine. Gas chromatographic technique used to determine the qualitative and quantitative analysis on the basis of thermal stability and volatility of the compounds. Using Gas chromatograpy aglycones can be separated and quantified in a single run with a nitrogen-specific detector [18]. Derivatization is also major factor for the fragmentation pattern which elucidates clear structure. Before analyze the compound derivatization is necessary in the terms of the modification of functional group of a molecule by derivatizing with reagents. The aglycones can be analyzed without derivatization below the temperature less than 280°C because above this temperature the aglycone moiety may be decomposed [19]. Various reagents are used to derivatives, but derivatization reactions belong to one of three categories: silylation, acylation or alkylation. Among all these reactions, silylation is the most widely used. In silylation derivatives are produced by proton displacement in –OH, -SH or –NH groups. Gas chromatography has been applied for the determination of the aglycones of steroidal glycoalkaloids from potato. The need of GC-MS reports on the alkaloids of *solanum nigrum* prompted to quantify the aglycones in the terms of solanidine and solasodine by this technique. Solanidine produced peaks at *m/z* 469 for mono-trimethylsilylation derivatives. Di- trimethylsilylation derivative of solasodine showed the base peak of *m/z* 125 and at *m/z* 559. It is accounted that derivatives made up by attaching tetrahydrofuran ring and hydroxyl group to the trimethylsilylation group due to presence of the nitrogen ring in silylation reaction of diosgenin containing oxygen [11]. Quantification of aglycones was carried out using an external standard calibration method. From graphical representation and calibration method glycoalkaloid present in *solanum nigrum* was solasodine with a percentage of 75.94% and Solanidine having 16.00%. (Figure 2) Calibration was performed by injecting standard mixtures of solasodine and solanidine at levels ranging from 4 to 200 mg/l. Good linearity of response was found for solanidine and solasodine in this concentration range with correlation coefficient value greater than 0.99.

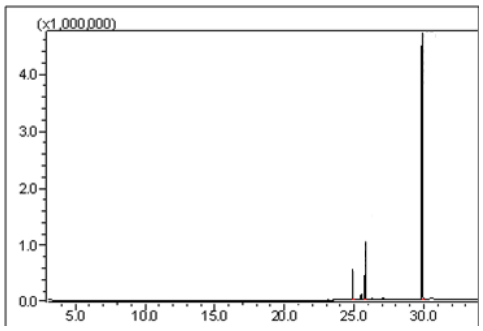

Fig. 2. Chromatogram of aglycon of *solanum nigrum* for GC-MS.

Conclusion

Phytochemical investigation is important tool to determine the active phytoconstituents in different part of the medicinal plants. We know the importance of the medicinal plants traditionally and many of the plants or parts of the plants are utilized worldwide. So there are techniques like HPLC and GC-MS to analyze the phytoconstituents present in the *solanum nigrum.* From the present study, it can be concluded that this analytical technique is indicating the presence of glycoalkaloid Solasonine which was in higher concentration than other glycoalkaloid α-Solamargine, β-Solamargine, α-Solanine and for aglycone solasodine was significantly present with higher percentage. As outcome, the study will help to identify the different phytoconstituents which can be applied for pharmacological screening.

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