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RESEARCH ARTICLE

Extraction, Isolation and Purification of Solanesol obtained from *Nicotiana tobaccum*

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ABSTRACT

Nicotiana Tobaccum, a polyprenol attracts the attention of bio-chemists because of their significant value as source of isoprene units for the synthesis of metabolically active quinones and Vitamin - K analogues. The present investigative study aims at the isolation of solanesol - a polyprenol from tobacco. The principle of isolation includes the extraction at 50ºC with n-hexane resulting 15-20 percent yield of solanesol. Elution of solanesol with hexane and ethyl acetate (95:5 v/v) has also been identified. The solanesol was further separated from elution by various Chromatographic techniques such as Column Chromatography, TLC, HPLC, produced good yield of solanesol with appreciable percentage of purity. Modified heat reflux extraction method for extraction and separation of solanesol from tobacco was established and showed better yield than the traditional methods.

KEYWORDS

Solanesol, Extraction, Separation, Silica Gel Column Chromatography, TLC, HPLC

INTRODUCTION

In present years, it becomes important to know that even in commonly cultivated crop species; there are significant levels of medicinal compounds that would form the basis of therapies. The best example is isoprenoid family, known as terpenoids representing the largest and oldest class of natural products known, consisting of more than 40,000 different molecules. Solanesol a naturally occurring trisesquiterpenoid (C45) alcohol, all trans-nonaprenol, of tobacco is one of the important precursor of the tumorigenic polynuclear aromatic hydrocarbons (PAHs) of tobacco smoke.[1] Solanesol, a long-chain terpenoid alcohol mainly existing in tobacco leaves, is the starting material for many high-Value biochemicals, including Vitamin K analogues and co-enzyme Q10 which is useful in the treatment of heart diseases, cancers and ulcers. Solanesol itself can be used as cardiac stimulant, lipid antioxidant and antibiotics, and clinical trials are also developing the use of solanesol as an anti-cancer drug.[2] There is a great demand for solanesol for production of Coenzyme Q10 and other uses. Its isolation not only reduces the risks of PAH from tobacco smoke but also makes use of it as a starting material in synthesis of several value added products such as Q10 and other analogues. Therefore, isolation of solanesol from tobacco is gaining a lot of importance in recent years.[13] Western medical researchers found that highly purified Solanesol itself can be directly used as a clinical drug, main clinical uses are as follows: anti-heart failure, treatment of liver injury, as well as adjuvant therapy for cancer.

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The most conventional method for extraction of solanesol from tobacco leaves is heat-reflux extraction.

The major problem in extraction of solanesol from tobacco is the selection of a suitable solvent for maximum yield and also purification pose several problems because of the presence of closely related fatty acids, alcohols, alkaloids, tobacco pigments, tar and other organic impurities. Especially the food and pharmaceutical grade of solanesol has to be of highest purity. Therefore, it is quite important to develop processes that can selectively separate the pure solanesol from the crude extracts of tobacco leaves. Most of the methods involve multiple step procedures, which are non-specific, quite tedious and time consuming. A number of chromatographic methods for determination of solanesol in tobacco were reported including thin layer chromatography, column chromatography, HPLC, UV were used to determine solanesol. Most of the methods reported before 2006 were in normal phase mode with UV detection. However, reversed phase HPLC with UV detection is often preferred not only because of its higher sensitivity but also wide availability and suitability.[6]

In the present investigation, a protocol involving the use of Heat reflux extraction with n-hexane, followed by saponification with ethanolic KOH, column chromatography for isolation of solanesol from waste of *Nicotiana tobaccum L.* was developed. Further, a simple non-aqueous RP-HPLC with UV method for determination of purity of solanesol in tobacco waste (crude extract), saponified extract, and isolated/purified solanesol of *Nicotiana tobacum L*. was described and compared to the standard solanesol.

MATERIAL AND METHODS

Chemicals and Reagents

All the reagents were of analytical-grade unless stated otherwise. HPLC-grade isopropanol (IPA) and methanol (MeOH) (Ranbaxy, SAS Nagar, India) were used. Dried *Nicotiana Tobaccum* leaves were received from local farmers from Rajasthan, India. Solanesol as reference standard was purchased from Naturite Agro Products LTD Hyderabad, India. Analytical standards from Sigma Aldrich (>90%) are considered for solanesol and solvents such as Hexane, Ethyl acetate, Ethyl alcohol, Isopropanol of HPLC grade chemicals like Potassium Hydroxide , Silica gel and Acetonitrile have been employed for extraction (by chromatography), identification (by TLC) and for evaluation of its purity (by HPLC).

Extraction of Solanesol

An important method has been introduced for the extraction of solanesol from the Flue-cured tobacco leaves. In this method the tobacco leaves were dried at 70^{o}C for 3 hours, grounded and passed through a 40-mesh sieve the grounded leaf powder (500gm) was extracted by employing n-hexane (3litres) on the water bath at 50^{o}C under reflux for 2 hours and filtered. The sample solution was stirred constantly at 75rpm and a distillation column was used to prevent the solvent loss due to evaporation. The residue was re-extracted successively with n-hexane. The amount of hexane extract was a semi-solid material with a dark brown colour and a pungent smell. The hexane extracts were mixed and concentrated by rotary vacuum evaporator at 40^{o}C. The pasty residue was saponified with 10% ethanolic potassium hydroxide (50ml) and then extracted with hexane, washed free of alkali, concentrated and again dried by rotary vacuum evaporator. The concentrated n-hexane extract was subjected to TLC, using silica gel.

Silica Gel Low-Pressure Column Chromatography

Silica gel low-pressure column chromatography technique has been developed for the purification of the solanesol. In this method, the crude solanesol dissolved in hexane at a ratio of 10:1 (v/w) of hexane-to-crude solanesol has been run on a silica gel column (30 cm×2.0 cm i.d.), which was preconditioned with n-hexane. The column was eluted with n- hexane: ethyl acetate (95:5, v/v). The eluent was collected in fraction of 5ml and tentative identification has been carried out using TLC. The fractions containing solanesol were dried by the rotary evaporation.

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Figure 1: Silica gel column Chromatography

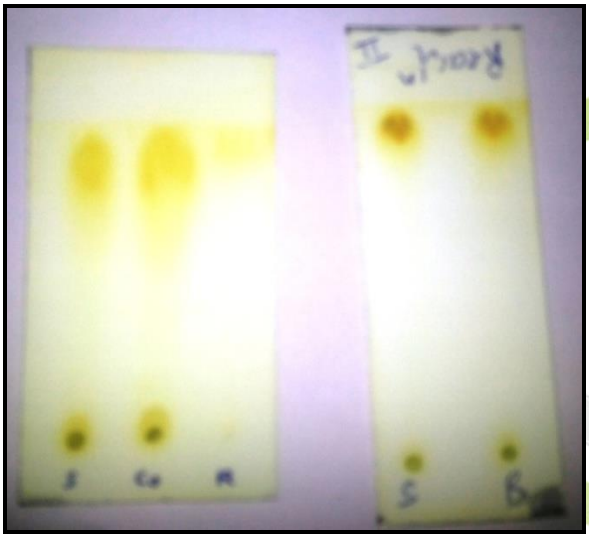

Figure 2: TLC of the Standard Solanesol and the Extract

TLC Detection

Silica gel plates were activated at 120°C for 1h before used. 10µ l of each fraction collected from column chromatography along with solanesol standard solution was loaded to the marked points about 10mm from the bottom of silica plate. The plates were developed in hexane: ethyl acetate (90:10, v/v) at room temperature and the separated spots were visualized by iodine fume. Ingredients of each fraction were compared with standard for identification.[17] The solanesol has been identified by TLC and the TLC chromatogram is presented in Figure below (Figure 2).

HPLC Method Development

Solanesol is the lipid soluble fraction of tobacco constituted with C45 terpenoid and is soluble in polar solvents but insoluble in water. The preferred stationary phase for analyses separation is silica. Low polar solvents must be used to achieve ample retention in normal phase silica. Except the trace amounts of water in the solvents are carefully controlled it becomes absolutely difficult to maintain reproducibility in such systems. By using reverse-phase chromatography, the problems encountered in the separation of the hydrophobic compounds by normal phase chromatography are generally conquered.

To achieve the elution in acceptable time should be used non-aqueous solvents such as methanol, acetonitrile and tetrahydrofuran because hydrophobic compounds exhibited large retention time on chemically bonded C_{18} phases. For example fats, carotenoids and sterols are usually separated by non-aqueous reverse phase chromatography. An additional advantage of reverse phase separation is enhanced resolution of homologues/ isomers under such conditions. Hence Solanesol was separated effectively from the other components of tobacco by using non-aqueous reverse phase HPLC.[4]

Phenomenex Luna C18 column (250nm x4.6mm i.d., particle size 5µm) with the mixture of MeOH: IPA (60:40v/v) used for better separation and resolution. The total run time was 30 minutes and the solanesol has been eluted with the retention time was found to be 6.29 min. Solanesol was identified on co-injection and comparison of retention time with that of the reference standard .[15] The HPLC Chromatograms of the Solanesol standard and isolated /purified solanesol are shown below (Figure 3, 4).

Preparation of Standard Stock Solution

10mg of the reference standard solanesol was dissolved in methanol(1mg/mL). Aliquots of the standard solutions(0.1 to 0.5mL) were diluted with methanol to get working standard solutions at concentrations 10 -50 µg/mL range. Readings were taken at 210 nm (Figure 5).

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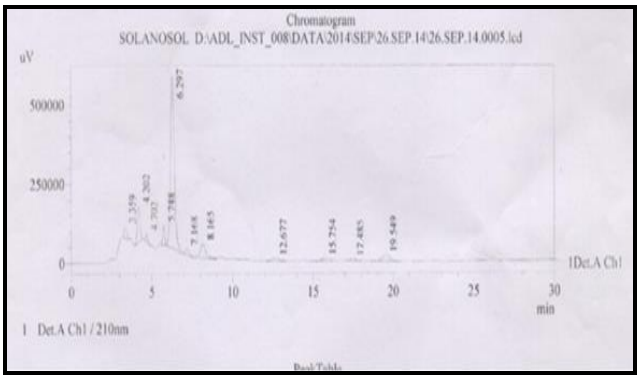

Figure 3: HPLC Chromatogram of the extract

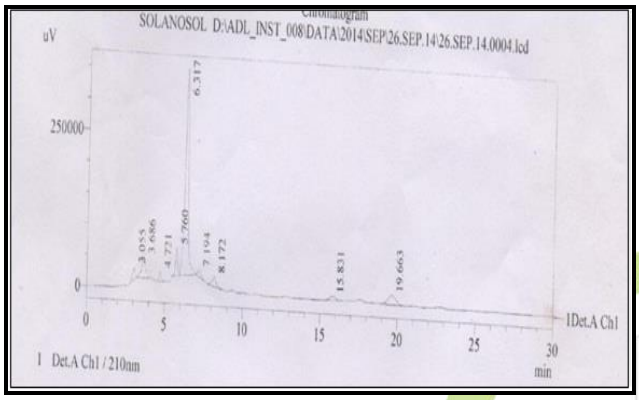

Figure 4: HPLC Chromatogram of the Std Solanesol

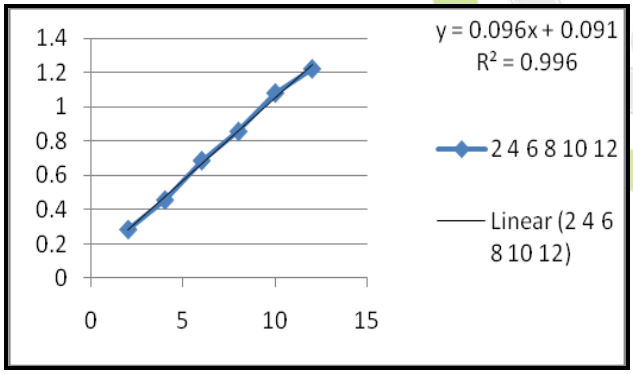

Figure 5: Calibration of Solanesol with methanol

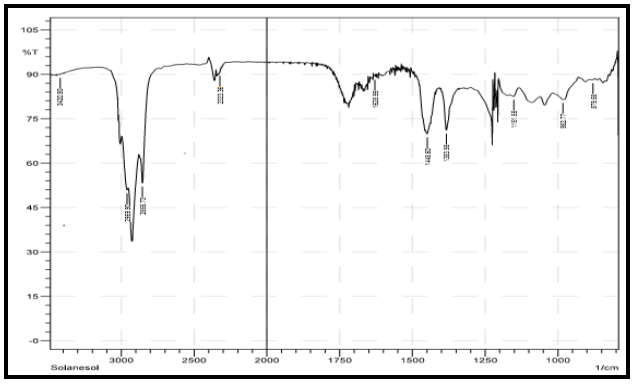

Figure 6: FT-IR spectrum of solanesol

Selection of Extractant

Extraction of solanesol from tobacco leaves; many solvents were employed such as n-hexane, toluene, propanol and methanol. In order to evaluate the solvents extract ability, 1 g of ground leaf powder were extracted using 80 ml solvent in a water bath at 50°C under reflux for 2 hrs, and filtered. The residue was washed. The extract and washings were combined and dried by rotary evaporation. The dried extract was carefully weighed and the solanesol content was determined by HPLC analysis. The percentage extraction of solanesol (w/w) was counted as:

$$\text{Extract rate (\%)} = \frac{\text{(Weight of extracted solanesol)}}{\text{(Weight of tobacco leaf samples)}} \times 100\%$$

Results indicated that n-hexane has good extract ability for solanesol and the content of solanesol in the crude tobacco extract was higher than those obtained using other solvents (Table: 1). It suggested that solanesol could be extracted by n-hexane with much less impurities than extracted using other solvents, which could make the subsequent purification easier. Hexane is preferred solvent for extraction.

Selection of Eluent

The crude solanesol solution was loaded on to the column; hexane was added when the sample solution layer went down almost to the surface of the column bed. The objective is to keep the solanesol in as small a volume as possible to diminish band broadening and to prevent the separation from initiating before the entire sample solution has reached the adsorbent top. After the hexane reached the surface of the column bed, develop the silica gel column successively with hexane and then 2, 5, 8, 10 and 15% ethyl acetate in hexane. Solanesol could not be eluted by hexane, and with lower concentration of ethyl acetate in hexane, it could be eluted but large amount of eluent was needed. In this investigation, 5% ethyl acetate in hexane was good at separation of solanesol on the silica gel column, while the ethyl acetate concentration in hexane higher than 10%, solanesol moved

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downed to quickly and could not be separated from other components.

RESULTS AND DISCUSSION

Preparing the solanesol crude extract is always economical in heat reflux extraction by n-hexane followed by saponification with methanolic KOH at 50-55^{0}C and the solvent was concentrated under vacuum evaporation. The results (Table: 1) showed that N-hexane has good extracting ability of solanesol as compared to other solvents like toluene, isopropanol and methanol. The quantity of solanesol in extract with n-hexane is higher compared to the other solvents. The percentages of impurities are also low with n-hexane extraction. The yield besides the percentage purity is also significant. Elution of solanesol with hexane and ethyl acetate (95:5 v/v) has also been identified by the Column Chromatography method.

The samples upto 5 mL were collected and the TLC was compared with that of the standard using n-hexane: ethyl acetate (7:3, v/v) as the mobile phase and Iodine chamber as the visualising reagent. Thus Thin Layer Chromatography has been used for quality identification of solanesol. Silica gel chromatography had been investigated for separating the hexane extract of flue-cured tobacco. A simple and rapid method for the separation and determination of solanesol from tobacco using non-aqueous RP-HPLC in an isocratic elution mode and using UV detector at 210nm was developed.

The total run time was 20 min and the solanesol standard and the solanesol extract has been eluted with the retention time of 6.29 and 6.31 min. Solanesol was identified on co-injection and comparison of retention time with that of reference standard. The HPLC chromatograms of solanesol standard, crude extract of solanesol are shown in (Figure 3, 4). The regression obtained was 0.996. The IR of Solanesol extracted from tobacco leaves was taken in (Figure 6). Therefore it is the convenient efficient method for the extraction of solanesol from tobacco leaves and shows greater potential for the large scale industrial application in the near future.

CONCLUSION

One method of extraction and separation of solanesol from tobacco was established. The extraction of solanesol with n-hexane from flue cured tobacco leaves and its separation by silica gel column chromatography by elution with a binary solvent mixture of n-hexane, ethyl acetate (95:5) is a significant method for the separation of solanesol with a purity of 95 to 98%. However further purification is necessary before employing for the synthesis of ubiquinones and vitamin k - analogues.

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