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Isolation of polyprenols from tobacco and their separation by adopting new chromatographic techniques*

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ABSTRACT

In recent times polyprenols attract 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 studies further include Chromatographic techniques employed for separation which produced good yield of solanesol with appreciable percentage of purity.

Keywords : Solanesol; Extraction; Separation; Silica gel column chromatography.

INTRODUCTION

Solanesol is primarily found in plants from the solanaceae family particularly tobacco. Solanesol, a polyisoprenoid alcohol (Figure-1) isolated for the first time by from flue cured tobacco (Rowland et al) [1] and its existence as free and esterified forms were recognized (Stedman et al) [2]. Gas chromatographic method for the determination of free and total solanesol in tobacco has been carried out by (Severson et al) [3]. Studies on variation in

solanesol levels among tobacco stalk positons, growth stages and air curing has been carried out by (Narsimha Rao et al) [4]. Studies with quantitative gas chromatographic method for the analysis of aliphatic hydrocarbons, terpenes, fatty alcohols, fatty acids and sterols in tobacco have been carried out by (Severson et al) [5].

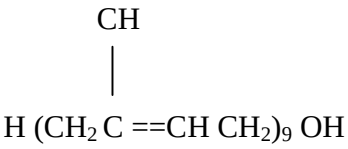

Fig.1: Molecular Structure of Solanesol

Polyisoprenoid alcohols are found in diverse life forms including higher plants, mammalian tissues and microorganisms. Many other polyprenols were found to contain both *cis* and *trans* units, but solanesol is recognized to be compound of all trans isoprene units. Numerous classical methods have been reported on the extraction of solanesol from tobacco leaves [6-8] and many solvents were employed such as n-hexane, toluene, propanol and methanol. (Scholtzhauer et al) [9] isolated solanesyl esters fractions. Saponification of the solanesyl esters fractions in 10% KOH / Ethanol yielded a series of fatty acids and solanesol. Silica gel chromatography had been investigated for separating the hexane extract of flue-cured tobacco [10]. Thin Layer Chromatography (TLC) has been used for quality identification of solanesol (Li et al) & (Woollen et al) [11], [12].

Literature survey revealed the methods of synthesis of solanesol (RR Uegg, RR Uegg, et al) [13a], [13b] in which *cis* and *trans* forms are isolated. Presently solanesol has drawn much attention as a potential source of isoprene units for the synthesis of metabolically active quinones such as Coenzyme Q_{10} (Lipshutz et al) [14a], [14b]. The aim and objective of this present investigative study is to develop a new method for isolation of solanesol from tobacco and its separation by a novel chromatographic technique.

MATERIALS AND METHODS

Experimental

i. Chemicals and materials

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).

ii. Extraction of solanesol

In the present research study, 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°C for 3 hrs, grounded and passed through a 40-mesh sieve the grounded leaf powder (500 g) were extracted by employing n-hexane (3 liters) on a water bath at 50°C under refluxed for 2 hrs and filtered. The residue was re-extracted successively with n-hexane. The hexane extracts were mixed and concentrated by rotary vacuum evaporator at 40°C. The pasty residue was saponified

with 10% ethanolic potassium hydroxide (50 ml) and then extracted with hexane, washed free of alkali, concentrated and again dried by rotary evaporation.

iii. Silica gel low-pressure column chromatography

Silica gel low-pressure column chromatography technique has been developed for the purification of the solanesol. In the Present developed 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 dried by rotary evaporation.

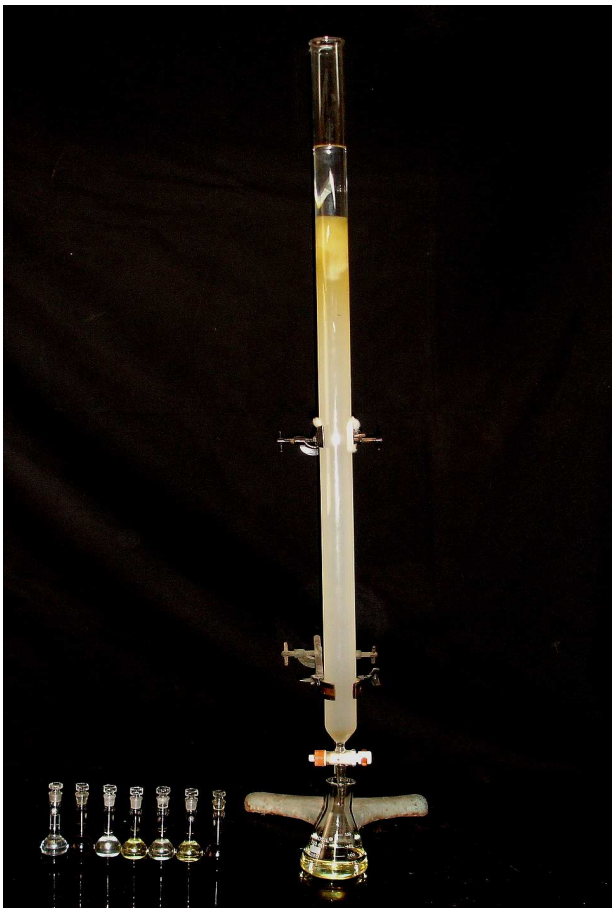

Fig.2: Silica gel low pressure column chromatography

iv. TLC detection

Silica gel plates were activated at 120°C for 1h before used. 10µl 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. The solanesol has been identified by TLC and the TLC chromatogram is presented in
figure-3

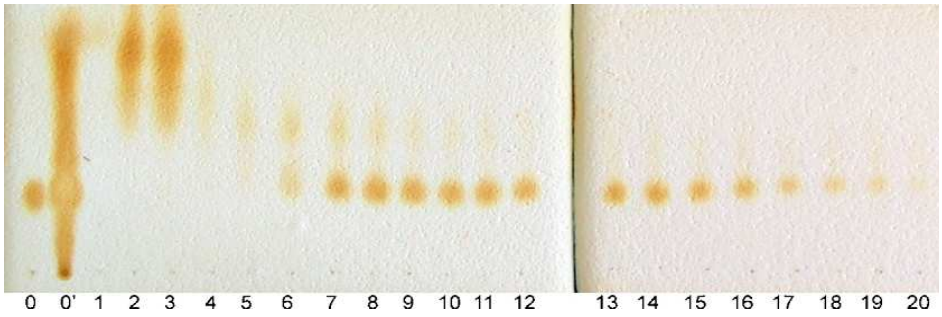

Fig.3: Thin-layer co-chromatogram of standard (0), original sample (0') and eluted fractions from silica gel column chromatography (from 1 to 20).

v. HPLC analysis

The HPLC system (Shimadzu, LC8A) auto sampler with employed for the research study and a C_{18} column (250mm x 4.6mm ID) used at 25ºC. Mobile phase comprising isopropanol-methanol (60:40 v/v) used and calibrations made 1.0ml/min with UV absorbance (210 nm) (Narasimha Rao et al) [15].

The extract of tobacco was analyzed before and after saponification of free and total solanesol were (Fig. 4A), (Fig. 4B) and solanesol isolated using silica gel column chromatography (Fig.4C) respectively.

Fig.4(A)

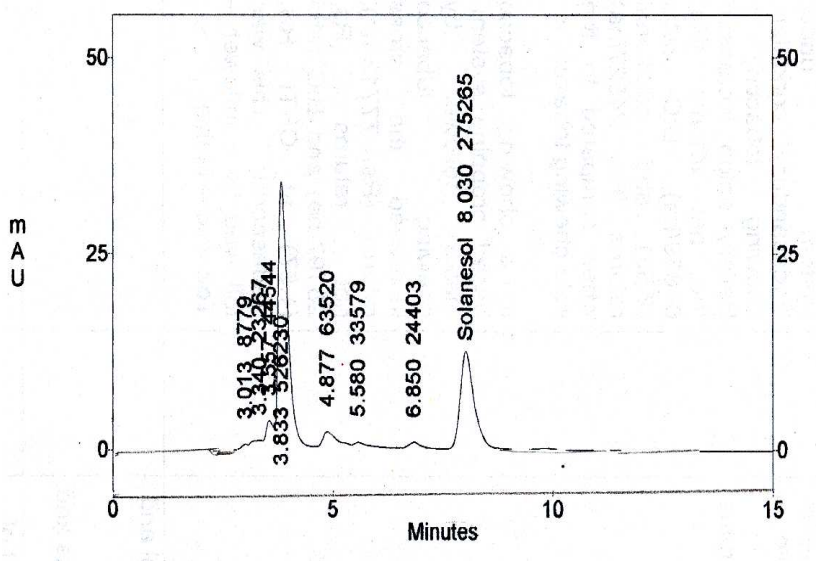

Fig.4(B)

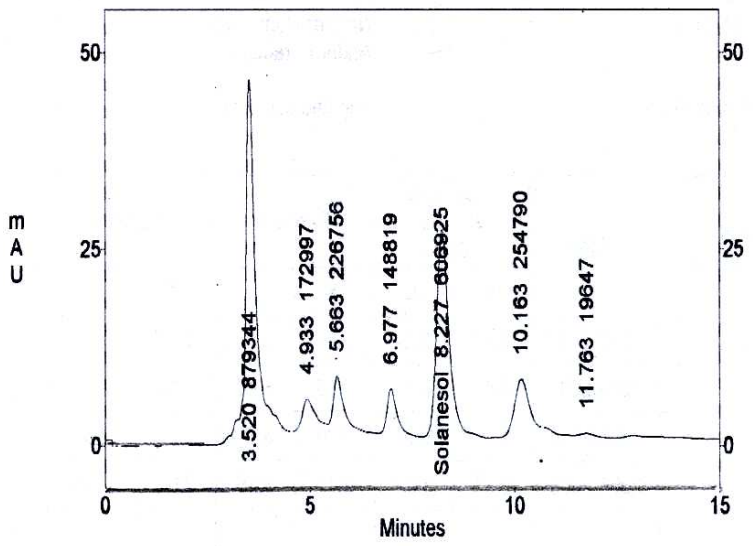

Fig.4(C)

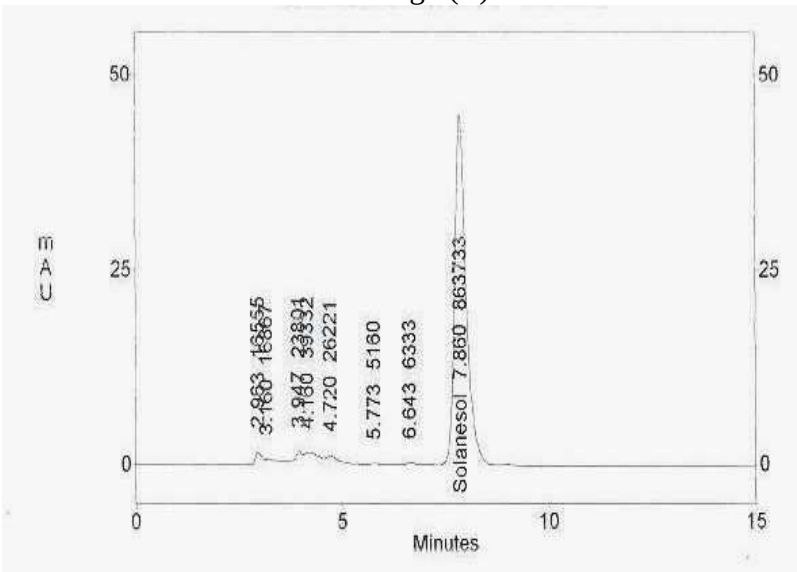

Fig. 4: HPLC chromatograms of: (A) before and (B) after saponification of hexane Extract of tobacco and (C) solanesol isolated using silica gel column chromatography.

vi. 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: extract rate (%) = (weight of extracted solanesol / weight of tobacco leaf samples) x 100 %. (%)

= (weight of The solanesol content in extract was counted as: solanesol content in extract extracted solanesol / total weight of tobacco extract) x 100%. Results indicated that n-hexane have 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.

The extraction abilities of various solvents have been presented in table1.

Extractant	Hexane	Toluene	Isoproponal	Methanol
Extract rate (%)	0.75	0.72	0.71	0.69
Solanesol content in extract	5.76	5.75	2.58	0.98

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

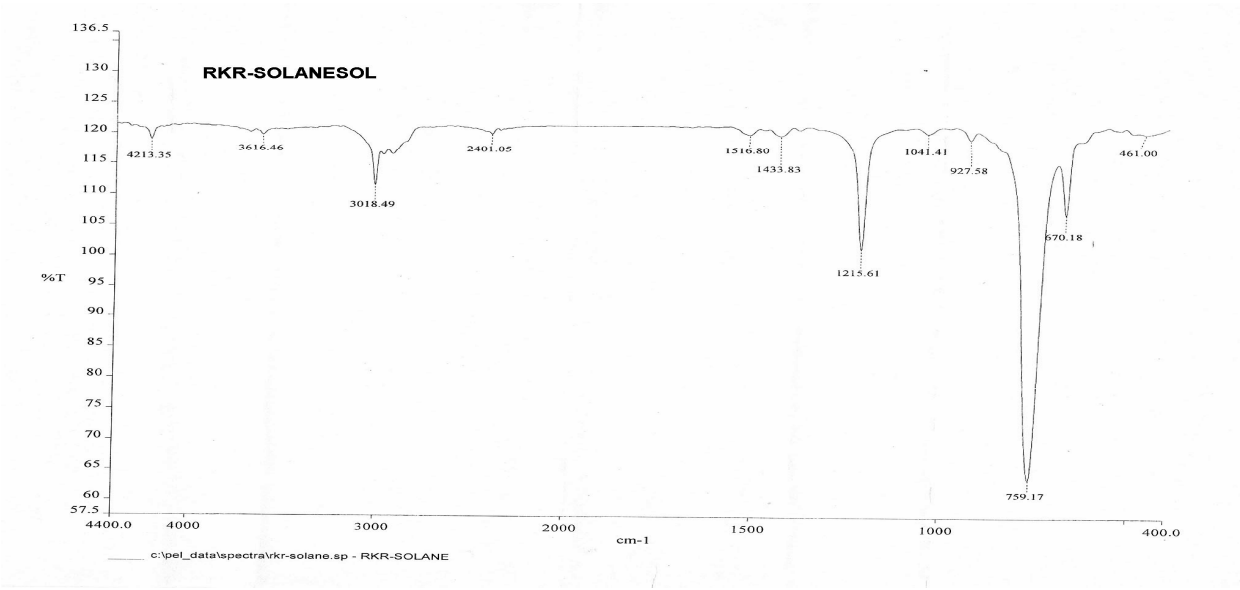

Fig. 5: FT-IR spectrum of solanesol

In this method, an appreciable yield with a purity (98%) has been recorded and the product isolated has been spectral characters of the samples were established by FT-IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectrometry (Figs.5-8).

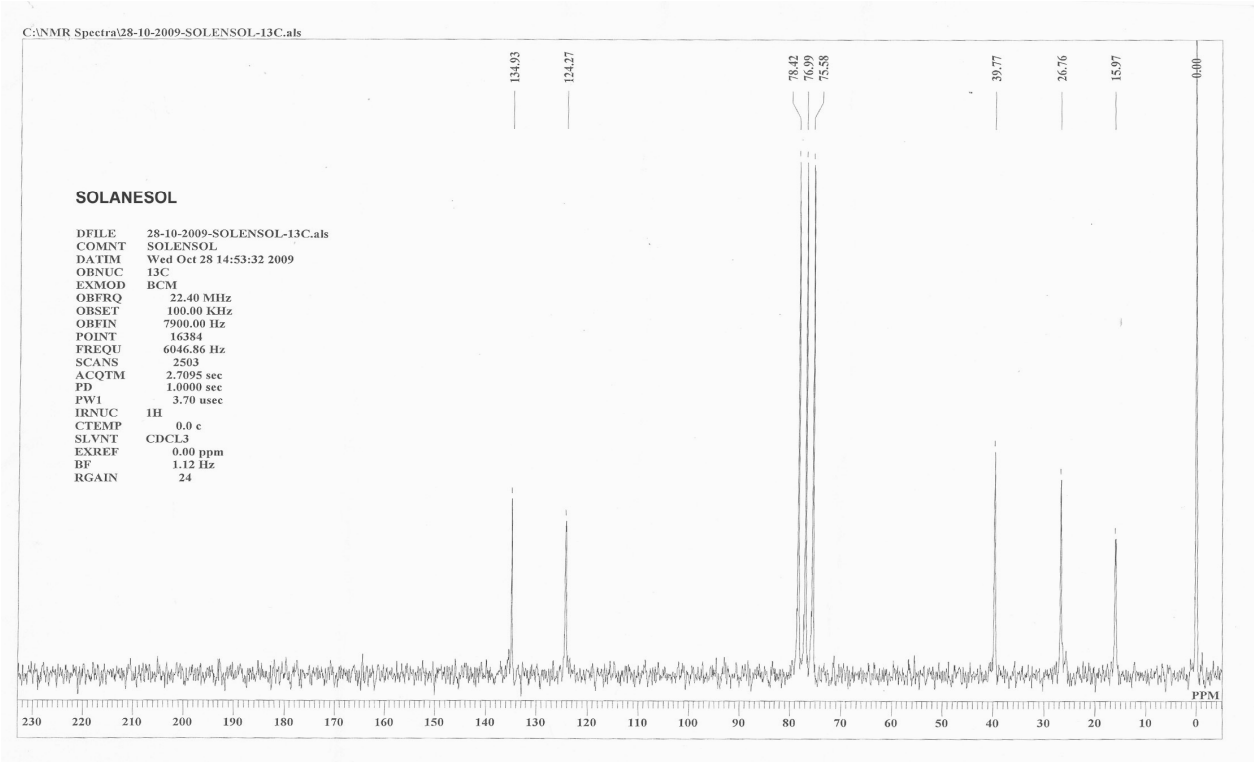

Fig. 6: ^{13}C NMR (75MHz, $CDCl_3$) spectrum of solanesol

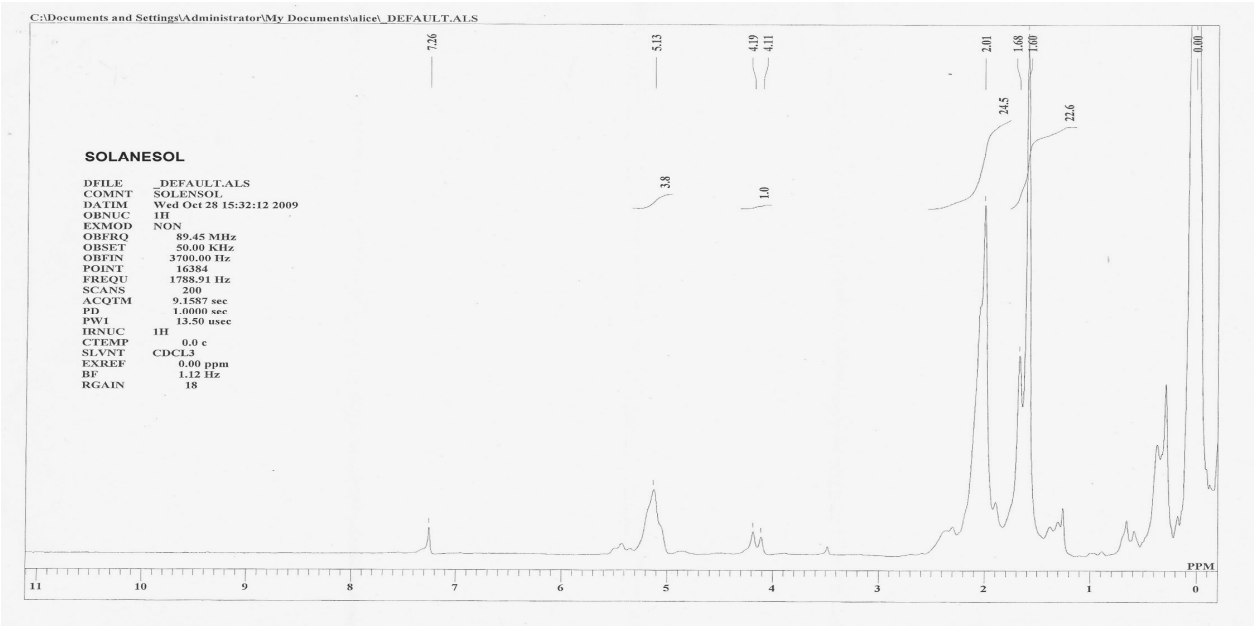

Fig. 7: ^{1}H NMR (300MHz, $CDCl_3$) spectrum of solanesol

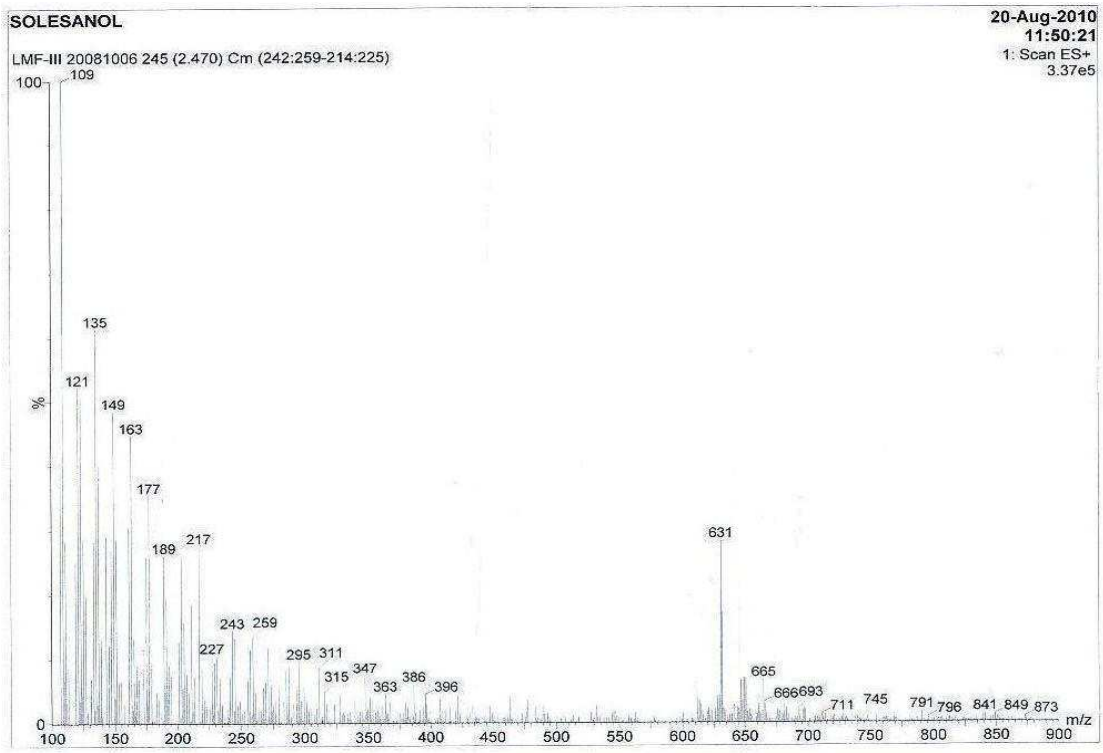

Fig. 8: ESI-MS spectrum of solanesol

RESULTS AND DISCUSSIONS

1. n-hexane has good extracting ability of solanesol compared to other solvents like toluene, isopropanol and methanol.
2. The quantity of solanesol in extract with n-hexane is higher compared to the other solvents.
3. The percentage of impurities are also low with n-hexane extraction.
4. The yield besides the percentage purity is also significant.

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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