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Determination of Lupeol and β-Sitosterol from *Solanum nigrum* L.

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ABSTRACT

During the present study HPTLC analysis of *Solanum nigrum*, a highly medicinal plant has been carried out. Simultaneous determination of lupeol and β-sitosterol was carried out on different accessions of the plant. The leaves of the plant among all the four accessions have been found to be rich in lupeol while the stem samples were found to be rich in β-sitosterol.

Keywords: *Solanum nigrum*, Lupeol, β-sitosterol, HPTLC

INTRODUCTION

S. nigrum is a widely used plant in oriental medicine. It is used in hepatitis, fever, dysentery and bowel disorders. The juice of the plant is used on ulcers and other skin diseases. The fruits are used as a tonic, laxative, appetite stimulant and are also used for treating asthma and diabetes. The juice from its roots is used against asthma and whooping cough. Apart, the plant is considered to be anti-tumorigenic, anti-oxidant, anti-inflammatory, hepatoprotective, diuretic and antipyretic activity.

Berries are used in fevers, diarrhoea, eye diseases and hydrophobia. Juice of the plant is hydragogue cathartic, diuretic, in blood spitting, piles, in enlargement of liver, dysentery, etc. Young shoots are used in treating skin diseases and psoriasis [1]. The plant is a traditional remedy for hepatitis, fever, ulcer and various immunological applications in cancer and others. The plant is beneficial in preventing hepatotoxicity and cytotoxicity thus improving functions of liver and kidney. It also finds use in analgesic, anti-inflammatory, antimicrobial, anti-diabetic, immunostimulant, central nervous system and brain functioning. It can really contribute to medical and pharmaceutical practices [2].

S. nigrum is reported to have many glycoalkaloids like solanine, salsodin, solamajine, solamargine, chaconine, saponins, etc. Out of which solanine, salsodin and chaconine are more commercially exploited. It has been reported that the plant leaves contain the highest concentration of gentisic acid, luteolin, apigenin, kaempferol and m-coumaric acid [3]. The plant has also been reported to contain (+)-pinoresinol, (+)-syringaresinol, (+)-medioresinol, scopoletin, tetracosanoic acid and beta-sitosterol [4]. Therefore, four accessions of *S. nigrum* L. (S_1, S_2, S_3 and S_4) were selected for estimation of lupeol and β-sitosterol.

MATERIALS AND METHODS

In the present study, different parts of the collected plants were subjected to phytochemical screening of pharmacologically important compounds. The plants were air dried, powered in grinder and were stored at room temperature. The powdered plant parts of each sample were dissolved in 20 ml of aqueous methanol for overnight. The extract was concentrated and dried using rotary evaporator under reduced pressure. 2 mg of each dried extract was again dissolved in 2 ml of methanol to obtain 1 mg/1 ml concentration and stored at 4°C till further analysis.

HPTLC instrumentation and conditions

Concentration range from 200-10,000 mg of standard solutions were spotted on silica gel 60 F254 HPTLC plate (Merck, India) using CAMAG Linomat V automatic spotter (Dosage speed: 150 nL/s, Syringe size: 100 µL, Band length: 6.0 mm). Plates were developed in a twin-through chamber (20 × 10 cm) to a distance up to 8 cm. The data regarding the bioactive markers used, composition of the solvent system, derivatizing reagent used and wavelength of the entire marker compounds. After development, the plates were first air dried and then oven dried at 105°C for 3-4 min. Further, the plates were derivatized with p-anisaldehyde sulphuric

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acid. In some cases, the plates were air dried and scanned densitometric using CAMAG TLC Scanner. p-anisaldehyde sulphuric acid was prepared by dissolving 1 ml of p-anisaldehyde solution in 2 ml of concentrated sulphuric acid and 100 ml of acetic acid. Afterwards, measurements were made by win CATS software. Peak areas were recorded and calibration curve of standards was obtained. The plates were scanned at specific wavelength and peak area, peak height, absorption spectra were recorded **(Figures 1 and 2)**.

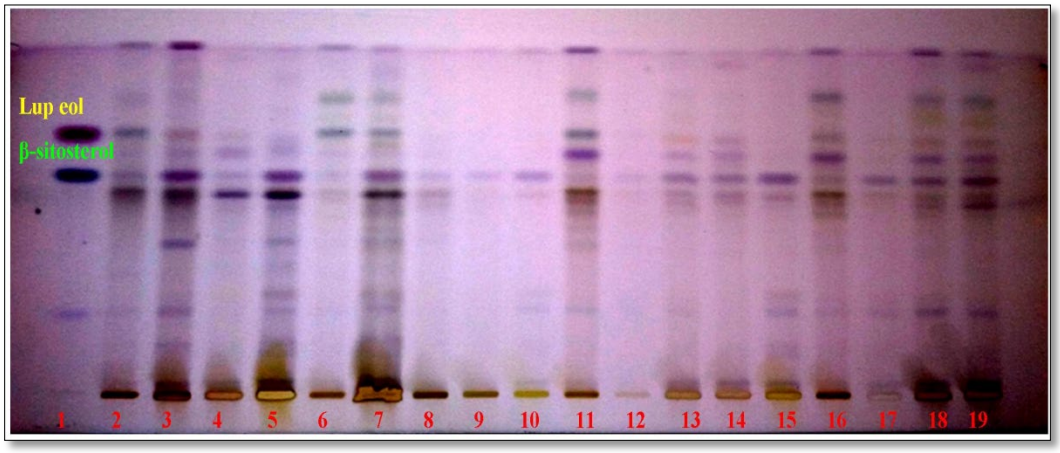

Figure 1. HPTLC plate showing simultaneous determination of lupeol and β-sitosterol (track 1) with all the plant samples (tracks 2-19).
Track 1: Standard (Lupeol and β-sitosterol)
Track 2, 6, 11, 16: S_1, S_2, S_3, S_4 leaf samples
Track 3, 7, 12, 17: S_1, S_2, S_3, S_4 stem samples
Track 4, 9, 14, 19: S_1, S_2, S_3, S_4 mature fruit samples
Track 5, 10, 15: S_1, S_2, S_3, S_4 root samples
Track 8, 13, 18: S_1, S_2, S_3, S_4 raw fruit samples

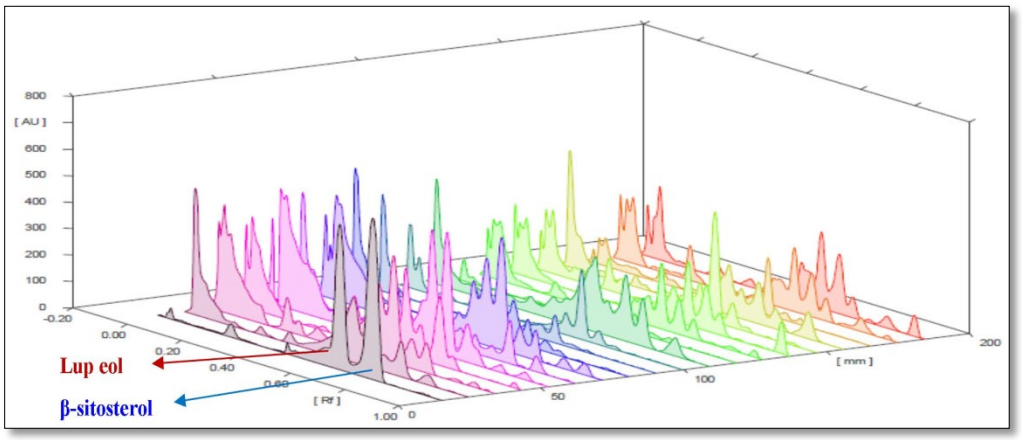

Figure 2. 3D chromatogram of tracks.

RESULTS AND DISCUSSION

In the present study, the leaf regions of the plant among all the four accessions (S_1, S_2, S_3 and S_4) have been found to be rich in lupeol while the reverse trend has been observed in case of stem regions, i.e., the stem region has been found to be rich in β-sitosterol. Otherwise the maximum amount of lupeol has been observed to be present in leaf sample of S_1 while that of β-sitosterol was observed in root sample of S_3. Among the young and mature fruit samples of S_2, S_3 and S_4, the amount of β-sitosterol has been observed to be more in young fruits than the mature ones in both S_2 and S_3 but the opposite of this has been observed in case of S_4.

A mixture of Toluene:Ethyl acetate:Glacial Acetic Acid (14.5:4.5:1.0 v/v/v) was used as mobile phase for the separation of lupeol. The compound is detected at 525 nm **(Figure 2)** with R_f value 0.61 **(Figure 3 and Table 1)**. HPTLC densitometric chromatogram of standard tracks and sample tracks of four accessions of *S. nigrum* L. has been given in **Figures 1 and 2**.

Table 1. Showing parameters of the developed HPTLC method.

S. No.	Marker compounds	Linearity range (mg/spot)	Linear equation (y)	R_f	Correlation coefficient ($R^{2)}$
1.	Lupeol	2-10	y=908.31x	0.61	0.9953
2.	β-sitosterol	2-10	y=1641.6x	0.54	0.9952

In the present study maximum amount of Lupeol was detected in S_1 leaf sample (13.84 ± 0.21 mg/g of DW), followed by S_2 leaf sample, S_3 leaf sample, S_1 stem sample, young fruit sample of S_3, S_4 leaf sample, S_2 root samples, S_2 stem sample, S_4 young fruit sample, S_4 mature fruit sample, S_3 mature fruit sample, S_1 mature fruit sample, S_4 stem sample, S_2 young fruit sample and S_3 stem sample (0.21 ± 0.04 mg/g of DW).

Lupeol was not detected in mature fruit samples of S_2 and in root samples of both S_3 and S_4. Among the leaf samples of all the four accessions of *S. nigrum* L. maximum concentration of Lupeol was detected in S1 leaf sample, followed by S_2 leaf sample, S_3 leaf sample, and S_4 leaf sample. Similarly, among the stem samples of the studied accessions, maximum amount has been detected in S_1 stem sample, followed by S_2 stem sample, S_4 stem samples and S_3 stem samples. Since Lupeol was not detected in the mature fruit sample of S_2 therefore, the maximum concentration of Lupeol among rest of the mature fruit samples was found to be maximum in S_4 mature fruit sample, followed by S_3 mature fruit sample and S_1 mature fruit sample while among the raw fruit samples of S_2, S_3 and S_4, maximum concentration of Lupeol was detected in S_3, followed by S_4 and S_2 **(Table 2)**.

Table 2. Table showing amount of lupeol and β-sitosterol in different plant parts of four accessions of *S. nigrum* L.

Sample code	Plant part	Lupeol (mg/g of DW)	β-sitosterol (mg/g of DW)
S_1	Leaf	13.84 ± 0.21	5.77 ± 0.39
	Stem	5.77 ± 0.09	32.81 ± 0.22
	Mature fruit	0.404 ± 0.06	2.64 ± 0.12
S_2	Roots	1.88 ± 0.02	7.65 ± 0.02
	Leaf	9.338 ± 0.14	2.47 ± 0.2
	Stem	1.65 ± 0.03	11.27 ± 0.05
	Young fruit	0.28 ± 0.15	5.34 ± 0.19
	Mature fruit	-	2.56 ± 0.08
S_3	Roots	-	60.38 ± 0.51
	Leaf	7.74 ± 0.11	0.36 ± 0.21
	Stem	0.21 ± 0.04	1.53 ± 0.11
	Young fruit	3.42 ± 0.12	23.04 ± 0.27
	Mature fruit	0.41 ± 0.015	2.79 ± 0.03
S_4	Roots	-	15.26 ± 0.05
	Leaf	2.48 ± 0.072	1.26 ± 0.14
	Stem	0.37 ± 0.06	10.16 ± 0.11
	Young fruit	1.47 ± 0.043	7.17 ± 0.09
	Mature fruit	1.074 ± 0.063	9.58 ± 0.11

Separation of β-sitosterol has been done using Toluene: Ethyl acetate: Glacial Acetic Acid (14.5: 4.5: 1.0 v/v/v) as mobile phase. The bands were visualised at 525 nm **(Figures 1 and 2)**. The Rf value of β-sitosterol is observed to be 0.54 **(Table 1)**. HPTLC densitometric chromatogram of standard tracks and sample tracks of four accessions of *S. nigrum* L. has been given in **Figure 2**. In the present studies, β-sitosterol was detected in all plant parts of presently studied four accessions. Maximum amount of β-sitosterol was detected in S_3 root sample (60.38 ± 0.51 mg/g of DW), followed by S_1 stem sample, S_3 young fruit sample, S_4 root sample, S_2 stem sample, S_4 stem sample, S_4 mature fruit sample, S_2 root sample, S_4 young fruit sample, S_1 leaf sample, S_2 young fruit sample, S_3 mature fruit sample, S_1 mature fruit sample, S_2 mature fruit sample, S_2 leaf sample, S_3 stem sample, S_4 leaf sample and the least was detected in S_3 leaf sample (0.36 ± 0.21 mg/g of DW).

Among the leaf samples, maximum amount of β-sitosterol has been found to be present in S_1, followed by S_2, S_4 and S_3 while among the stem samples the highest concentration of β-sitosterol in the present study has been recorded in S_1, followed by S_2, S_4 and S_3. The concentration of β-sitosterol in the root samples of S_2, S_3 and S_4 has been observed to be present in enormous amount with maximum in S_3 root sample, followed by S_4 root sample and S_2 root sample. Similarly, in young fruit samples, the highest concentration of β-sitosterol has been recorded in S_3, intermediate in S_4 and the lowest being detected in S_2 while among the mature fruit samples, maximum concentration of β-sitosterol has been found to be present in S_4, followed by S_3, S_1 and S_2 **(Table 2)**. Overall, lowest amount of β-sitosterol has been observed to be present in the leaf sample of the plant among all the four accessions. The maximum amount of lupeol has been observed to be present in leaf sample of S_1, while that of β-sitosterol was observed in root sample of S_3. Among the young and mature fruit samples of S_2, S_3 and S_4, the amount of β-sitosterol has been observed to be more in young fruits than the mature fruits in both S_2 and S_3 but the opposite of this has been observed in case of S_4 where mature fruit sample contained more amount of β-sitosterol than the young fruit sample **(Figure 3)**.

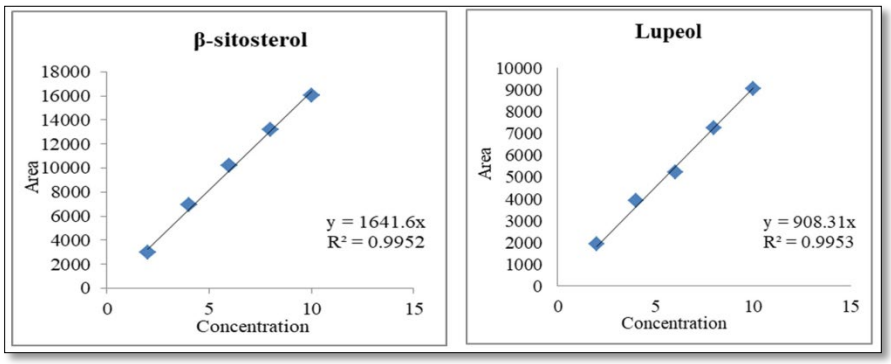

Figure 3. The linear regression equation with the correlation coefficient.

Earlier, Jagtap et al. [5] carried out pharmacognostic and phytochemical investigation of root of *S. nigrum* Linn. The preliminary phytochemical screening of three extracts of the root powder revealed the presence of alkaloids, tannins and saponins along with other phytoconstituents and HPTLC studies, the alcoholic extract showed presence of ten and seven phytoconstituents at 254 nm and 366 nm.

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REFERENCES

1. Chopra RN, Nayar SL, Chopra IC, Asolkar LV, Kakkar KK, Chakre OJ, et al. (1956) Glossary of Indian medicinal plants. Council of Scientific and Industrial Research, New Delhi.
2. Nyeem MAB, Rashid AMU, Nowrose M, Hossain MA (2017) *Solanum nigrum* (Maku): A review of pharmacological activities and clinical effects. IJAR 3: 12-17.
3. Huang HC, Syu KY, Lin JK (2010) Chemical composition of *Solanum nigrum* Linn extract and induction of autophagy by leaf water extract and its major flavonoids in AU565 breast cancer cells. J Agric Food Chem 58: 8699-8708.
4. Zhao Y, Liu F, Lou HX (2010) Studies on the chemical constituents of *Solanum nigrum*. Zhong yao cai 33: 555-556.
5. Jagtap CY, Prajapati PK, Rudrappa HC, Shukla VJ (2013) Pharmacognostic and phytochemical investigation of root of *Solanum nigrum* Linn.: An ethnomedicinally important herb. Int J Green Pharm 7: 46-49.