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Phytochemical screening and HPTLC fingerprinting of different parts of *Solanum indicum* L.: A dashmool species

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Abstract
The objective of the study is to screen phytochemicals and develop chemical fingerprints of medicinally important *Solanum indicum* L. The powdered pant materials of leaf, fruit, stem and roots of *S. indicum* were extracted in methanol by soxhlet apparatus. Extracts were subjected to phytochemical screening and HPTLC fingerprints were developed. For development of fingerprints Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1) was used as mobile phase. Phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins and terpenoids in all the plant parts. Steroids were found present in leaves, fruits and roots whereas tannins were detected in leaves only. HPTLC fingerprinting of methanolic extracts of all plant parts has shown several peaks with different Rf values and peak areas. Phytoconstituents investigated have been described to have tremendous medicinal values in literature. HPTLC fingerprints would be helpful in identification, authentication and quality control of this species.

Keywords: phytochemical screening, chemical fingerprints, caffeic acid, *S. indicum*, HPTLC

Introduction
Medicinal plants have been used as herbal drugs since times immemorial. All plant parts (leaves, flowers, stem, roots, seeds, bark etc) are used as herbal drugs in particular or in combinations of each other. According to WHO, approximately 80% of world population are still relying on traditional system of medicines to cure their diseases in various forms such as teas, decocts or extracts with easily accessible liquids such as water, milk, or alcohol [1, 2]. Due to being safe and effective, the world market for herbal medicines is growing at the rate of 7-15% annually [3, 4]. Standardization of the herbal raw materials is the need of the hour to make the Indian branded drugs most reliable. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical characters. Hence the modern methods describing the identification and quantification of active chemical constituents in the plant material may be helpful for proper standardization of herbs and their formulations [5-7]. World Health Organization (WHO) has also emphasized on the quality assurance of medicinal plants using modern sophisticated techniques and applying suitable standards [8, 9].
High Pressure Thin Layer Chromatography (HPTLC) has emerged as a simple, versatile, accurate, cost effective, rapid and reliable tool for identification, quantification and standardization of herbal materials [5, 10]. Chromatographic fingerprints generated through HPTLC can be visualized and stored as electronic images [11].
Solanum indicum L. is commonly known as Birhata or Badi Kateri or Indian night shade and belongs to the family Solanaceae. It is an erect undershrub of 0.30 to 1.8 m in height (Fig. 1) and found throughout warmer parts of India, Asia and Africa upto an elevation of 1.5 m [12]. The national demand of *S. indicum* is 500-1000 MT per annum [13]. Due to high demand and overexploitation, the herb has become rare in Madhya Pradesh [14, 15]. All plant parts *viz*. berries, leaves, roots, seeds and stem of this species have been utilized in traditional system of medicine and are useful in various diseases such as bronchitis, asthma, dry cough, rhinitis, dysuria, leucoderma, sexual disorders, insomnia, cardiac weakness and pruritis [16-19]. The plant has been documented in Chinese folk medicine as anti-inflammatory and wound-healing agents and as an analgesic for toothache, rhinitis and breast cancer [20]. The species is among the ten medicinal plants whose roots are principally employed in preparation of Dashmularishta, a well-established ayurvedic drug used in the treatment of fatigue, oral sores

and gynecological disorders [21]. The basic ingredient of Dashmoolarishta is utilized in the manufacture of over hundred ayurvedic drugs [22]. This study aimed to screen the phytochemicals, to develop chemical fingerprints of leaves, fruits, stem and roots of *S. indicum* using HPTLC technique which can be utilized for quality standardization of this species. The presence of caffeic acid, a phenolic acid was also checked in the plant samples.

Fig 1: *Solanum indicum* L.

Material and Methods

Collection of plant material

Leaves, fruits, stem and roots (Fig. 2 A, B, C, D) of this species were collected from Tamia range of West Chhindwara Forest Division. The herbarium of plant specimens was deposited in Biodiversity and Sustainable Management Division of Tropical Forest Research Institute, Jabalpur (Identification no. 1761).

Chemicals

Caffeic acid (Fig. 3) was purchased from Sigma Aldrich, India. All chemicals and solvents used were of AR grade.

Processing and extraction of plant material

The different plant parts of above said species were separated, packed in jute bags and brought to the laboratory. These were washed thoroughly in running water to remove soil and other foreign particles. The stem and roots were cut into small pieces. All plant parts of *S. indicum* were dried in shade and subsequently dried in a hot air oven at 40 ^{0}C for 48 hours. All the plant parts were powdered using grinder and the powdered plant materials were used for making extracts. 100 mg of powdered plant materials of leaves, fruits, stem and roots were separately soaked overnight in 25 ml of methanol. Different extracts were filtered and filtrates were used for qualitative phytochemical analysis.

Fig 2: *S. indicum* A. Leaves B. Fruits C. Stem D. Roots

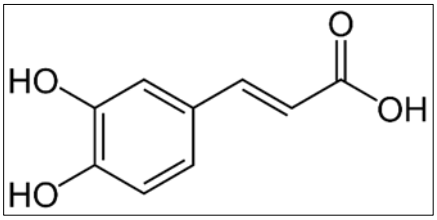


Fig 3: Chemical structure of caffeic acid

Phytochemical screening of plant extracts

The preliminary phytochemical screening of methanolic extracts of selected plant parts was carried out according to the methods described by Edeoga (2005) [23], Harborne (1998) [24], Sofawara (1993) [25] and Trease and Evans (1989) [26].

Preparation of caffeic acid solution

A solution of 0.1 mg/ml of caffeic acid in methanol was prepared to compare the presence of caffeic acid in different crude extracts.

Development of chemical fingerprints using HPTLC

Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1) mobile phase was standardized for better resolution of peaks. 10 µL of each solution and 3 µL of caffeic acid solution were applied in the form of bands of width 8 mm using a 100 µL CAMAG syringe on 10 x 10 cm aluminum packed TLC plate prewashed with methanol and coated with 0.2 mm layer of silica gel 60F 254 (E. Merck Ltd., Darmstadt, Germany) with the help of a 100 µL Hamilton syringe and LinomatV applicator attached to CAMAG HPTLC system, which was programmed through WinCATS software. Samples loaded TLC plate was developed by the ascending technique using 10 ml of standardized mobile phase in a CAMAG twin-through glass chamber (10 cm x 10 cm) saturated with mobile phase and covered with a stainless-steel lid. The developed plate was dried by hot air to evaporate solvents from the plate and kept in photo – documentation chamber and captured the images at 254 and 366 nm. The image of plate was also taken in visible light. Densitometric scanning was then performed with a CAMAG TLC Scanner4 equipped with Win CATS software at λ_{max} = 330 nm using deuterium and tungsten light source. Rf values, peak tables and HPTLC chromatograms were recorded.

Results

The phytochemical characters of leaves, fruits, stem and roots of *S. indicum* are summarized in Table 1. The results showed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins and terpenoids in all the plant parts. Steroids were detected in leaves, fruits and roots whereas tannins in leaves only.

HPTLC profiles of methanolic extracts of leaves, fruits, stem and roots of *S. indicum* under visible light, UV 254 nm and 366 nm were recorded and presented in Fig. 3. The corresponding chromatograms of HPTLC profiles of extracts obtained after densitometric scanning at 330 nm are given as Fig. 4 which showed several peaks of various phytochemicals. Number of peaks observed in chromatogram of all extracts along with their Rf values, maximum height and area % are defined in Table 2. It can be observed in Table 2 that HPTLC chromatogram of stem extract revealed 9 peaks with maximum Rf values in the range of 0.04 to 0.84 (Table 2, Fig. 4, Track 2), leaf extract showed 13 peaks with Rf values in the range of 0.06 to 0.98 (Table 2, Fig. 4, Track 3), fruit extract revealed 7 peaks with Rf values in the range of 0.04 to

0.83 (Table 2, Fig. 4, Track 4) and root extract revealed 9 peaks with Rf values in range of 0.04 to 0.84 (Table 2, Fig. 4, Track 5). Caffeic acid appeared at Rf 0.47 in all extracts. The presence of caffeic acid in samples was confirmed by comparing the absorption spectra at start, middle and end position (Fig. 5).

Table 1: Phytochemical characters of leaves, fruits, stem and roots of *S. indicum*

S. No.	Phytochemical constituents	Plant parts	Methanol extract
1.	Alkaloids	Leaves	+
		Fruits	+
		Stem	+
		Roots	+
2.	Cardiac glycosides	Leaves	+
		Fruits	+
		Stem	+
		Roots	+
3.	Flavonoids	Leaves	+
		Fruits	+
		Stem	+
		Roots	+
4.	Phenols	Leaves	+
		Fruits	+
		Stem	+
		Roots	+
5.	Saponins	Leaves	+
		Fruits	+
		Stem	+
		Roots	+
6.	Steroids	Leaves	+
		Fruits	+
		Stem	-
		Roots	+
7.	Tannins	Leaves	+
		Fruits	-
		Stem	-
		Roots	-
8.	Terpenoids	Leaves	+
		Fruits	+
		Stem	+
		Roots	+

(+) = detected and (-) = not detected

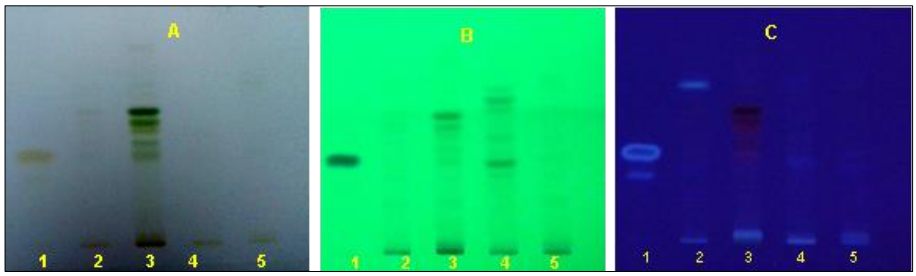
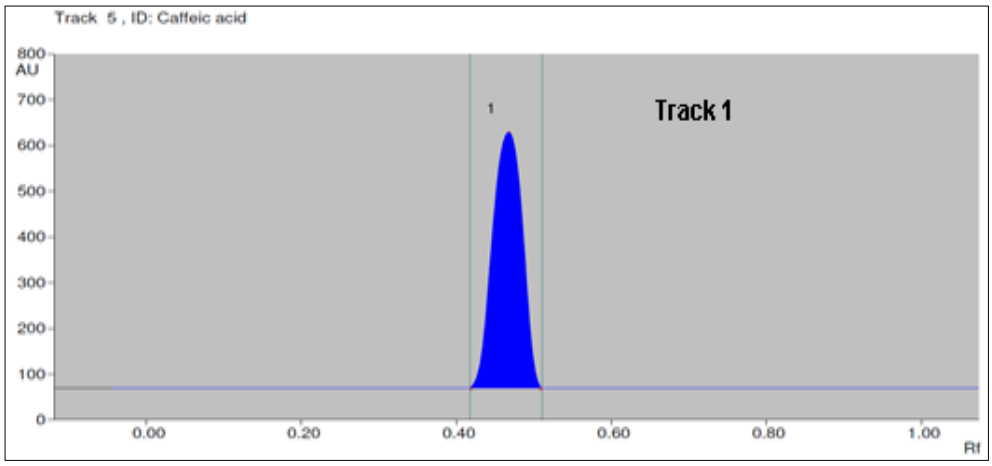


Fig 3: HPTLC fingerprint profiles of methanolic extracts of different parts of *S. indicum*, A. Visible light, B. 254 nm, C. 366 nm (Track 1: Caffeic acid, Track 2: Stem extract, Track 3: Leaf extract, Track 4: Fruit extract, Track 5: Root extract)



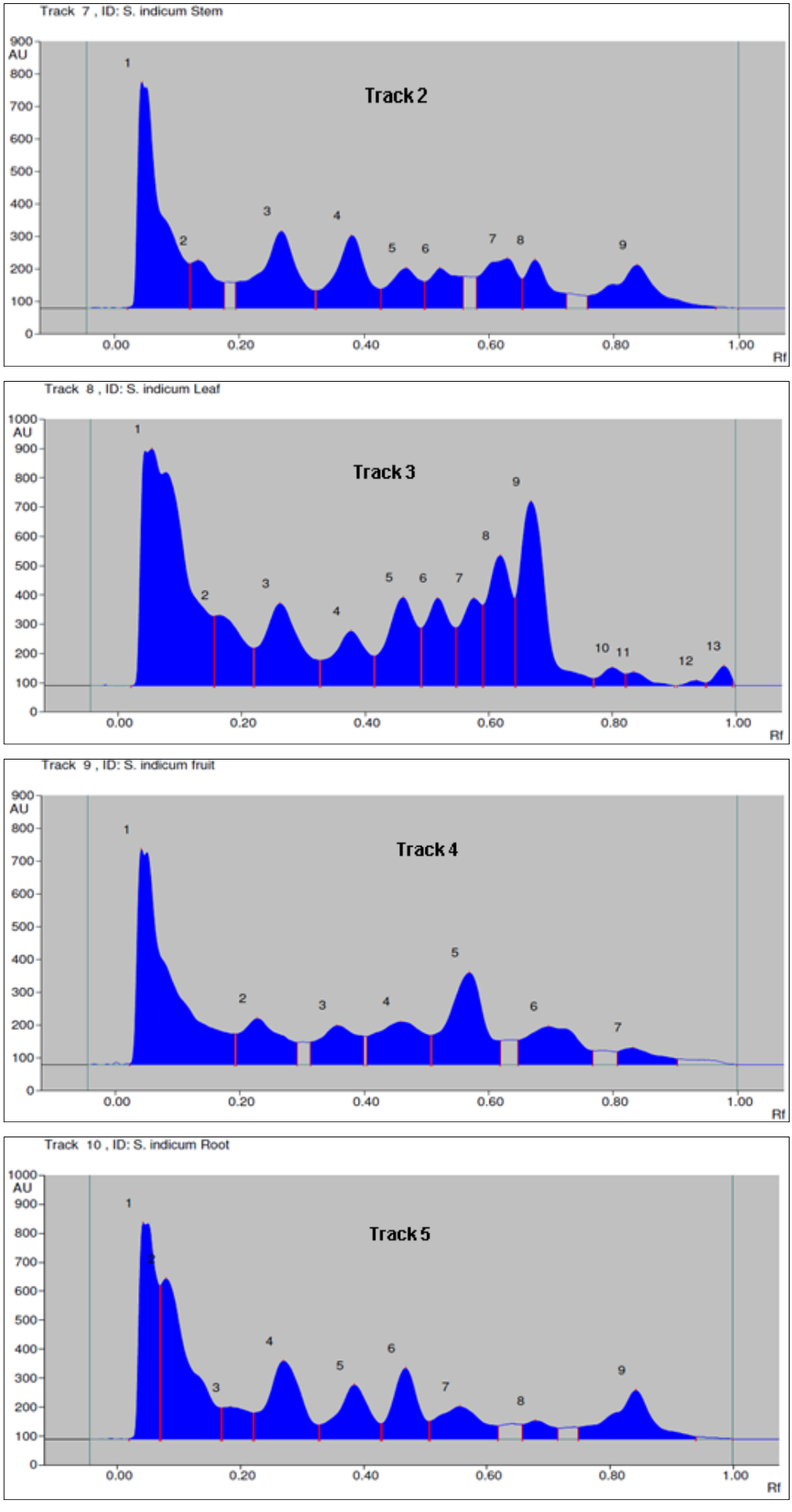


Fig 4: HPTLC chromatograms of caffeic acid (Track1), methanolic extracts of stem (Track 2), leaves (Track 3), fruits (Track 4) and roots (Track 5) of *S. indicum* showing peaks of phytochemicals at 330 nm

Table 2: Peak list and Rf values of HPTLC chromatograms of methanolic extracts of stem, leaves, fruits and roots of *S. indicum* at 330 nm

Plant parts	Peak	Max Rf	Max Height (AU)	Area (AU)	Area (%)
Caffeic acid	1	0.47	808.4	34262.6	85.99
Stem	1	0.04	692.7	20298.7	28.80
	2	0.13	146	4281.6	6.08
	3	0.27	235.5	10863.3	15.41
	4	0.38	222.4	8380.5	11.89
	5	0.47	122.3	4390.6	6.23
	6	0.52	122	4307.6	6.11
	7	0.63	151.3	6357.8	9.02
	8	0.67	147.5	4306.5	6.11
	9	0.84	131.8	7286.4	10.34
Leaves	1	0.06	808.2	43635.2	31.85
	2	0.16	239.8	8323.5	6.07
	3	0.26	280.3	12662.6	9.24
	4	0.38	185.9	7752.2	5.66
	5	0.47	300.7	10786	7.87
	6	0.52	298.4	9254.5	6.75
	7	0.58	297.8	7558.3	5.52
	8	0.62	444	12871	9.39
	9	0.67	628.2	19912.2	14.53
	10	0.80	60.9	1596.6	1.17
	11	0.84	45.9	1147.5	0.84
	12	0.94	17.1	326.9	0.24
	13	0.98	66.6	1196	0.87
Fruits	1	0.04	654.9	27292.7	39.38
	2	0.23	140	6928	10.00
	3	0.36	118.4	5599.9	8.08
	4	0.47	130	7632.3	11.01
	5	0.57	279.2	12124	17.49
	6	0.70	115.9	7414.5	10.70
	7	0.83	50.7	2311.2	3.33
Roots	1	0.04	743.8	15741.5	19.31
	2	0.08	552	20091.1	24.64
	3	0.18	109.2	3594.1	4.41
	4	0.27	268.6	10986.5	13.48
	5	0.39	185.4	7211.5	8.85
	6	0.47	243.2	7370.1	9.04
	7	0.56	111.5	5971.2	7.32
	8	0.68	62.6	2069.3	2.54
	9	0.84	166.8	8488.8	10.41

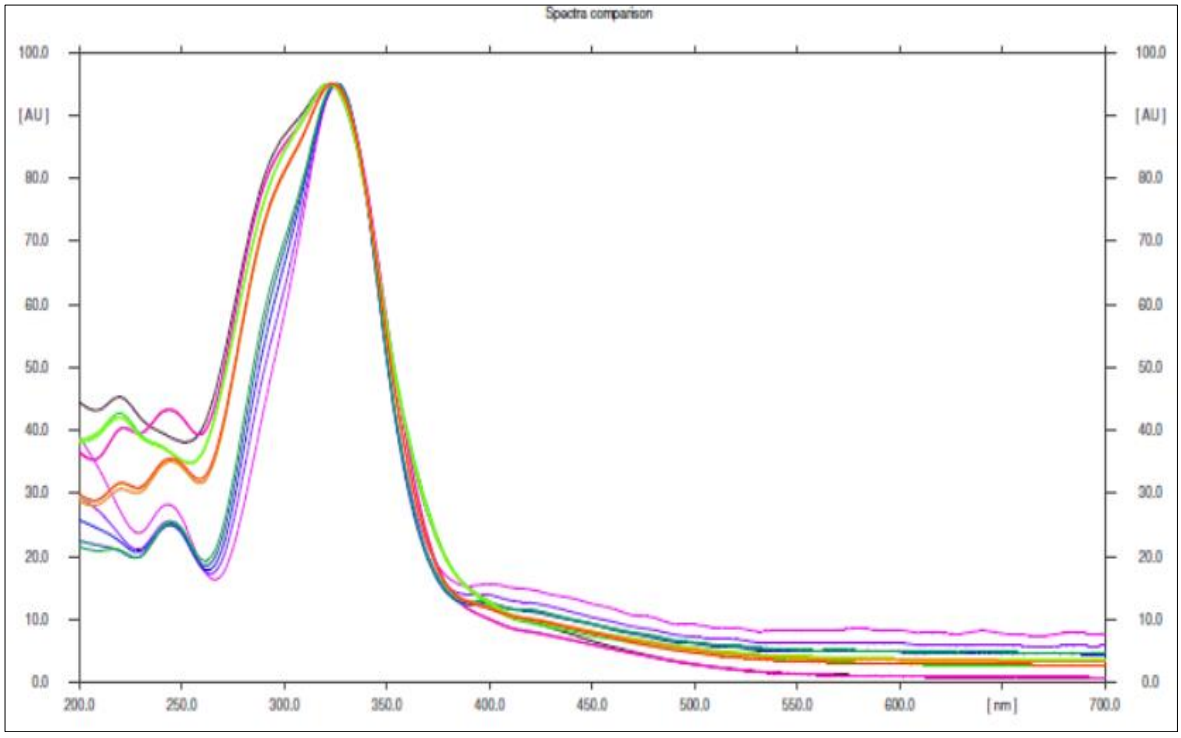


Fig 5: Spectra overlay of CA standard and test samples, scanned at 330 nm

Discussion

Preliminary phytochemical screening of plant extracts plays a vital role for determination, isolation and characterization of active secondary metabolites. This knowledge of secondary metabolites of plants is very essential to understand plant drugs and their formulations and also in discovering the actual value of folkloric rehabilitations [27]. The study showed the richness of secondary metabolites in methanolic extracts of leaf, fruit, stem and root parts of *S. indicum*. Secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins and terpenoids have the established pharmacological activities and provide protection from most of the chronic diseases [28, 29]. The pharmacological effects of the investigated secondary metabolites in methanolic extracts of targeted parts of *S. indicum* are defined in Table 3.

Table 3: Pharmacological effects of investigated secondary metabolites

Secondary metabolites	Pharmacological effects
Alkaloids	Antiarrhythmic, anticholinergic, analgesic, antitumor, antihypertensive, antipyretics, antimalarial, stimulant, anti-HIV, antileukmic and many more [30] and often used as medications and recreational drugs [31]
Cardiac glycosides	Effective in treatment of congestive heart failure and cardiac arrhythmia [32]
Flavonoids	CNS activity, cardiotonic, lipid lowering, antiulcer, hepatoprotective, anti-inflammatory, antineoplastic, antimicrobial, antioxidant and hypoglycemic activity [33]
Phenols and phenolic compounds	Antimicrobial, effective in atherogenesis and cancer, strong antioxidant and antimutagenic activities and essential health promoter [34, 35]
Saponins	Dietary supplements and nutraceuticals [36], hypocholesterolemic and antidiabetic properties [37]
Steroids	Analgesic and central nervous system activities [38, 39]
Tannins	To stimulate glucose uptake and exhibit insulin like activity acting as glucose transport activators of fat cells [40]
Terpenoids	Analgesic and hypoglycemia effects [38, 41]

Chemical fingerprinting emerged as an effective tool to resolve problems in standardization of plant-based drugs. With the help of chemical fingerprinting, species, strain and geographical origin of plants can be delineated [42]. HPTLC fingerprinting is proved to be a better, linear, precise and accurate method for herbal identification, authentication, quality standardization and characterization of medicinal plant species [10]. HPTLC profiles of methanolic extracts of leaf, fruit, stem and root of *S. indicum* were developed in order to find out various chemical moieties which will be helpful in further isolation and structure elucidation of active compounds [43]. There profiles will also be useful in quality control and standardization of herbal preparations. The developed chromatograms will be specific with standardized solvent system Cyclohexane: Ethyl acetate: Formic acid (6: 4: 1), v/v) and Rf values. Caffeic acid, an important phenolic compound found effective in various chronic diseases was also investigated in all selected plant parts of *S. indicum*. A large number of biological activities have been reported for caffeic acid and its phenethyl ester in the literature such as strong antioxidant, antimitogenic, anti-allergic, immuno-modulatory, anti-inflammatory and anti-carcinogen activities both *in-vitro* and *in-vivo* [44]. The presence caffeic acid in plant parts of *S. indicum* is adding towards its therapeutic potential and utilization in Ayurvedic preparations.

Conclusion

It can be concluded from the study that methanolic extracts of leaves, fruits, stem and roots of *S. indicum* are rich source of phytoconstituents which are conferred with huge therapeutic values. On the basis of present investigation, further studies can be planned in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. Besides, HPTLC finger print images would be helpful in identification and authentication of this dashmool species. These fingerprints will serve as biochemical markers to distinguish between authentic drug and adulterants, thus will be of utmost importance for quality control purpose.

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