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Phytochemical comparison and evaluation of anti-inflammatory and anti-diabetic activity of three source plants of Jivanti-an important Ayurvedic drug

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Abstract

Background: In Ayurveda, Jivanti is an important Rasayana drug that increases the energy level of the body. The botanical source of Jivanti is in a situation of controversy. The root of *Leptadenia reticulata* is the genuine source plant for Jivanti as per Ayurvedic Pharmacopeia of India. However, other species such as *Holostemma ada-kodien* and *Flickingeria nodosa* are also used as source plants for Jivanti in various parts of the country. The objective of this study is to identify a scientifically validated alternative source plant for Jivanti by phytochemical and pharmacological evaluation.

Results: In this study, anti-inflammatory and anti-diabetic activities of various source plants for Jivanti were evaluated along with their phytochemical comparison. *H. ada-kodien* showed significant anti-inflammatory and anti-diabetic activity when compared to *L. reticulata*.

Conclusion: The study concluded the possibility of using *Holostemma ada-kodien* as a substitute for Jivanti in the Ayurvedic drug industry.

Keywords: Jivanti, HPTLC, LC/MS, Anti-diabetic activity, Anti-inflammatory activity

Background

In Ayurveda, *Jivanti* is an important rejuvenating drug that boosts the energy level of the body [1]. The botanical identity of *Jivanti* is in a situation of controversy. Identification of an Ayurvedic drug from classical texts is difficult through etymological analysis of its terminology as it does not disclose identity. Identity needs detailed morphological descriptions in the literature and such descriptions in classical texts are unclear. The root of *Leptadenia reticulata* (Retz.) Wight & Arn. (*Apocynaceae*) is accepted as the genuine source of *Jivanti* as per Ayurvedic Pharmacopeia of India, but various herbs are used under the name of *Jivanti* in different parts of the country. *Holostemma ada-kodien* (*Apocynaceae*) and *Flickingeria nodosa* (*Orchidaceae*) are the commonly used species as source plants for *Jivanti*. In the central and western part of India, *L. reticulata* is generally used as *Jivanti*, but in some regions, *Flickingeria nodosa* is also considered as a type of *Jivanti* which is commonly known as *Suvarna Jivanti*. In the southern part of India, *Holostemma ada-kodien* is considered as a source plant for *Jivanti* [2–5].

L. reticulata is a twining shrub that grows in sub-Himalayan tracts of Punjab, Gujarat, Uttar Pradesh, and throughout peninsular India [6]. *L. reticulata* is used for treating various ailments such as hematopoiesis, emaciation, cough, dyspnea, fever, burning sensation, night blindness, and dysentery [2]. *H. ada-kodien*, a species indigenous to India, is a twiny, laticiferous perennial medicinal shrub that is endangered. The tuberous root, which is the official part, is used for diabetes [7]. *F.*

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nodosa belonging to the family *Orchidaceae* is known as *Suvarna Jivanti* due to its golden yellow color [8]. The aim of the study is to compare the selected source plants for *Jivanti* by evaluating their phytochemical and pharmacological properties.

Methods

Collection of plant materials

Various plant materials were collected during September 2016. *H. ada-kodien* was collected from the nursery of the Centre. Dried roots and stems of *L. reticulata* were obtained from Dabur India Ltd, New Delhi. *Flickingeria nodosa* was obtained from Khari Baoli market, New Delhi. All the materials were authenticated by the Plant Systematics and Genetic Resources Division of the Centre and voucher specimens were deposited in the herbarium (CMPR 9684, CMPR 9685, and CMPR 9998).

Extraction of materials

Five grams each of the shade dried materials of *L. reticulata* root (LRR), *L. reticulata* stem (LRS), *H. ada-kodien* root (HAR), and *F. nodosa* whole plant (FNW) were successively extracted with various solvents like petroleum ether, chloroform, and methanol using reflux extraction method. Crude extracts with methanol, water, and hydro alcohol were also prepared separately by reflux extraction method.

One hundred fifty grams of each sample was individually extracted in water for pharmacological evaluation using the Soxhlet extraction method. The extracts were dried under vacuum by a rotary evaporator (Heidolph, Germany). All the extracts were stored under a refrigerator until phytochemical and pharmacological evaluation.

Estimation of total polyphenolics

Total phenolic content (TPC) was estimated spectrophotometrically [9, 10] and was expressed as mg gallic acid equivalents (mg GaE).

Extraction of phenolics

Phenolic extraction was done by the previously reported method [11] with slight modification. Briefly, 10 g of materials was extracted with 100 ml of 80% of ethanol at room temperature for 5 h using an incubator shaker. It was filtered and concentrated under vacuum at 40 °C using rotary evaporator (Heidolph, Germany). The pH was adjusted to 11 by adding sodium hydroxide. It was then extracted with $CHCl_3$ to remove the chloroform soluble fraction. The residue was acidified with hydrochloric acid to pH 2 and was extracted with 150 ml of ethyl acetate. The ethyl acetate extract was concentrated and hydrolyzed with 50 ml of 4 N NaOH, stirred at 50 °C for 5 h and pH was adjusted to 2 by adding hydrochloric acid. It was then extracted with ethyl acetate, concentrated to dryness, and it was dissolved in LC/MS grade methanol.

High-performance thin-layer chromatographic (HPTLC) analysis

Various extracts of *L. reticulata*, *H. ada-kodien*, and *F. nodosa* were subjected to chemical profiling by Camag HPTLC system (Switzerland). Samples were applied using an auto sampler (Camag ATS 4) on HPTLC plates ($60F_{254}$, Merck India). The mobile phase was optimized as toluene, ethyl acetate, and methanol in the ratio of 7:2:1 for n-hexane and chloroform extracts and in the ratio of 7:3:1 for alcoholic and aqueous extracts. The chromatogram was developed in a saturated Twin Trough chromatographic chamber (Camag, Switzerland). The developed plate was visualized under UV at 254 nm and 366 nm and in visible light after derivatizing with anisaldehyde sulfuric acid reagent followed by heating at 105 °C for 5 min [12].

LC–ESI/MS-MS analysis

Mass spectroscopic characterization was done by electrospray ionization (ESI) technique in negative mode using Agilent 6520 accurate mass Q-TOF-LC/MS system with Agilent 1200 liquid chromatography having an extend-C18 column of 1.8 μm, 2.1 × 50 mm. Gradient elution was performed with LC/MS grade acetonitrile (A) and 0.1% acetic acid in methanol (B) at a constant flow rate of 0.7 ml/min, with an increase in the volume of B%: 5–20%, 12–30%, 19–40%, 26–50%, and 30–40%. The conditions for mass spectrometry were drying gas (nitrogen) flow 5 l/min, nebulizer pressure 40 psig, drying gas temperature 325 °C, capillary voltage 3000 V, and fragmentor volt 125 V. The mass fragmentation was performed by collision-induced dissociation with varying collision energy 4 V/100 DA with an offset of 8 V [12].

Anti-inflammatory activity

Induction of paw edema using carrageenan

Anti-inflammatory activity was evaluated using the carrageenan-induced rat paw edema model [13]. The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA, Government of India (Approval No: KAHE/IAEC/2018/21-04/009). The rats were challenged by subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw after 30 min of 5 ml of water (controls) by stomach tube or the test drug dissolved in the same volume of water.

Experimental setup

Male Sprague–Dawley rats with the body weight between 150 and 180 g were used. The animals were

Table 1 Experimental design for anti-inflammatory activity

S.No	Groups	Experiment design
1	Group I (control)	Water 5 ml (each rat)
2	Group II (induction)	Induction of 0.05 ml of carrageenan
3	Group III (FNW)	Treated with 5 ml of FNW extract and after 30 min induced with 0.05 ml of 1% carrageenan
4	Group IV (LRR)	Treated with 5 ml of LRR extract and after 30 min induced with 0.05 ml of 1% carrageenan
5	Group V (LRS)	Treated with 5 ml of LRS extract and after 30 min induced with 0.05 ml of 1% carrageenan
6	Group VI (HAR)	Treated with 5 ml of HAR extract and after 30 min induced with 0.05 ml of 1% carrageenan

divided into six groups of 4 rats in each group and starved overnight (Table 1).

The paw volume was measured using a vernier caliper immediately after injection and 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after injection. The increase of paw volume after 3 to 6 h is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal.

Evaluation of anti-diabetic activity by streptozotocin-induced diabetes

Animals and diet

Adult Wistar albino rats weighing about 150–180 g were procured from the animal house of Karpagam Academy of Higher Education, Coimbatore, India. The animals were under standard conditions and were housed four per cage in polypropylene cages with a wire mesh top and a hygienic bed of husk in a specific pathogen-free animal room under controlled conditions of 12-h light and 12-h dark cycle, with a temperature of 24 ± 2 °C, relative humidity of 50 ± 10%, and fed with rodent diet and water ad libitum (Table 2). The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA, Government of India, with approval no. KAHE/IAEC/2018/21-04/008 [14].

Induction of diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg b.wt) in 0.1 M citrate buffer (pH 4.5) [14]. Diabetes was identified in rats by moderate polydipsia and marked polyuria. After 48 h of streptozotocin administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose ranging between 200 and 400 mg/dl were considered diabetic and used for the experiments.

Table 2 Experimental design for in vivo anti-diabetic study

Groups	Treatment
Group 1	Control
Group 2	Diabetic control (streptozotocin 45 mg/kg b.wt)
Group 3	Diabetic rats treated with glibenclamide (2 mg/kg b.wt)
Group 4	Diabetic rats treated with FNW
Group 5	Diabetic rats treated with LRR
Group 6	Diabetic rats treated with LRS
Group 7	Diabetic rats treated with HAR

The animals were weighed and dosed through an oral intragastric tube every day. The test drug and standard were fed orally for 30 days. The study was completed in overnight fasted rats at the end of 30 days.

Collection of blood

After the experimental period of 30 days, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by decapitation and plasma was separated by centrifugation for 10 min at 3000 rpm and used for the estimation of various biochemical estimations.

High-fat diet and streptozotocin-induced diabetes

Animals and diet

Adult Wistar albino rats weighing about 150–180 g were procured from the animal house of Karpagam Academy of Higher Education, Coimbatore, India. The animals were under standard conditions and were housed four per cage in polypropylene cages with a wire mesh top and a hygienic bed of husk in a specific pathogen-free animal room under controlled conditions of 12-h light and 12-h dark cycle, with a temperature of 24 ± 2 °C, relative humidity of 50 ± 10%, and fed with rodent diet and water ad libitum (Table 3). The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA, Government of India (Approval No. KAHE/IAEC/2018/21-04/009).

Induction of diabetes

The rats were fed with a high-fat diet (HFD) ad libitum, respectively, for the initial period of 2 weeks. After the 2 weeks of dietary manipulation, the dietary group was injected intraperitoneally (i.p.) with a low dose of STZ (35 mg/ kg b.wt), while the respective control rats were given vehicle citrate buffer (pH 4.5). Diabetes was

Table 3 Experimental design for in vivo anti-diabetic study with high-fat diet

Groups	Treatment
Group 1	Control
Group 2	HFD + STZ-induced diabetic control (35 mg/kg b.wt of STZ)
Group 3	HFD + STZ-induced diabetic rats treated with metformin (50 mg/kg b.wt)
Group 4	HFD + STZ-induced diabetic rats treated with FNW
Group 5	HFD + STZ-induced diabetic rats treated with LRR
Group 6	HFD + STZ-induced diabetic rats treated with LRS
Group 7	HFD + STZ-induced diabetic rats treated with HAR

identified in rats by moderate polydypsia and marked polyuria. After 48 h of streptozotocin administration, blood glucose levels were estimated in rats following overnight fasting [14].

The animals were weighed and dosed through an oral intragastric tube every day. The test drug and reference standard drugs were fed orally for 3 weeks. The study was completed in overnight fasted rats at the end of 3 weeks.

Collection of blood

After the experimental period of 3 weeks, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by decapitation and plasma was separated by centrifugation for 10 min at 3000 rpm and used for the estimation of various biochemical estimations (glucose, triglycerides, and cholesterol in plasma-Kit Method).

Statistical analysis

Graphpad Prism-6 software (Graphpad software, Inc., USA) was used for the statistical analysis.

Results

Total phenolic content

Total phenolic content (TPC) of selected source plants of *Jivanti* was estimated in different extracts such as methanol, water, and hydro alcohol. TPC was calculated from the calibration curve of gallic acid (R^2 = 0.992). TPC of various species is presented as Fig. 1. The highest phenolics were observed for the hydro alcoholic extract of FNW (18.87 mg Eq Ga/g). TPC of LRR showed 3.77, 9.07, and 7.33 for various extracts like methanol, water, and hydro alcohol, respectively. The stem extracts of *L. reticulata* showed higher phenolics for methanol and hydro alcohol extracts compared to that of its root. Phenolic contents of HAR were calculated as 3.10, 5.70, and 4.83 for methanol, water, and hydro alcohol, respectively.

HPTLC profiling

Comparative chemical profiling of various species was done by HPTLC analysis. HPTLC profiles of sequential *n*-hexane and chloroform extracts were presented in Fig. 2. On visualizing under 254 nm, *n*-hexane extract showed bands at R_f 0.26, 0.32, 044, 0.55, 0.69, and 0.73 as common for *L. reticulata* root, *L. reticulata* stem, *H. ada-kodien* root, and *F. nodosa*. A band at 0.67 was found common for *L. reticulata* and *H. ada-kodien*. Compound with R_f 0.16 was observed in the root and stem of *L. reticulata*. A band at 0.20 was found to be common for *L. reticulata* root and *F. nodosa*. Chloroform extracts showed a

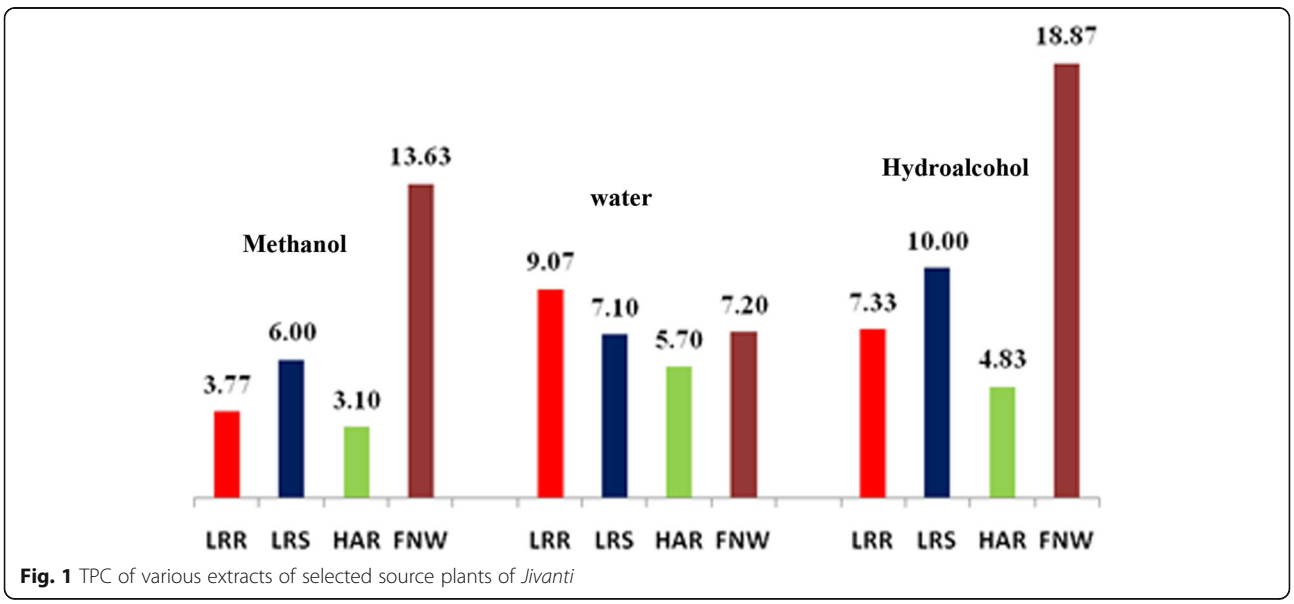

Fig. 1 TPC of various extracts of selected source plants of *Jivanti*

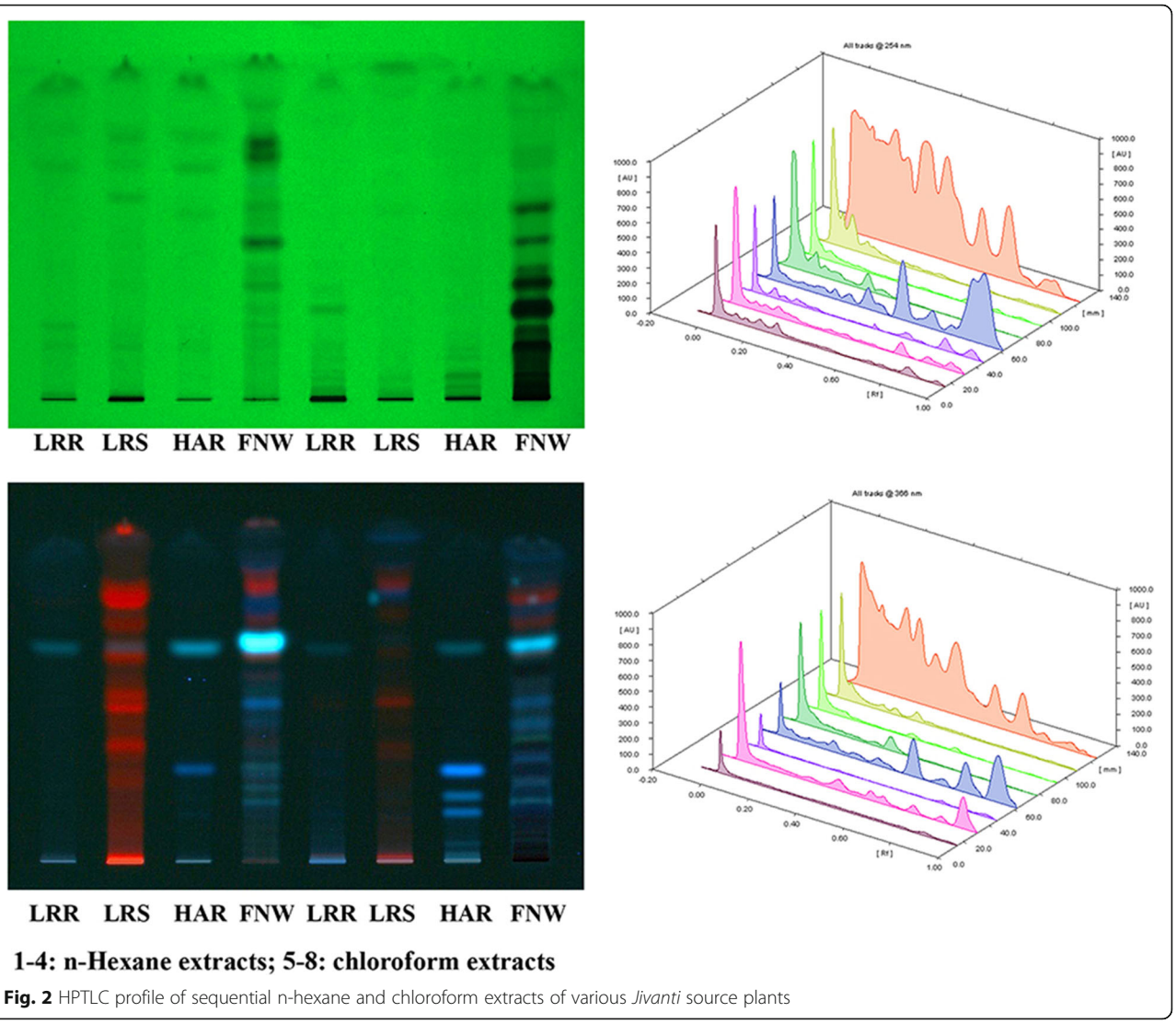

Fig. 2 HPTLC profile of sequential n-hexane and chloroform extracts of various *Jivanti* source plants

common band at 0.04 for the root of *L. reticulata* and *H. ada-kodien*. A compound at 0.08 is common for three extracts except *L. reticulata* stem. *L. reticulata* root and *F. nodosa* showed a common band at 0.26. Some specific bands were observed for *F. nodosa* at 0.15, 0.33, 0.45, and 0.54. At 366 nm, n-hexane extract of *L. reticulata* root showed a blue fluorescent band at 0.61 and it is common for all the selected four samples. The stem of *L. reticulata* showed an entirely dissimilar banding pattern with its root. In the case of chloroform extracts, 0.61 (blue) is a common band for all the selected species. Methanol and water extracts also showed different chromatographic patterns (Fig. 3). Only a few compounds are found to be common for methanolic extract.

LC/MS analysis of phenolic fractions

The phenolic fractions of various samples were subjected to LC/MS analysis for the detailed characterization. LC/MS analysis was done in ESI negative mode. Total ion chromatogram (TIC) was integrated to record all the molecular ion peaks. The molecular ions with different [M-H] values were evaluated and further characterization was carried out by MS/MS analysis. On MS/MS analysis, molecular ions were fragmented on collision-induced dissociation (CID). The tentative structure was assigned by comparing mass fragmentation patterns of previously reported values (Table 4).

Flavonoids such as 7,3′-dihydroxy flavones, 3,7-dihydroxy-3′, 4′-dimethoxyflavone, and 3,4-dimethoxycinnamic acid were identified from HAR, and LRR showed the presence of protocatechuic acid. Catechin, dihydroxyquercetin, and 2′6′-dihydroxyflavone were detected in FNW [15–22].

Pharmacological studies

Anti-inflammatory activity

Oxidative agents such as reactive oxygen species and reactive nitrogen species are produced in abundant

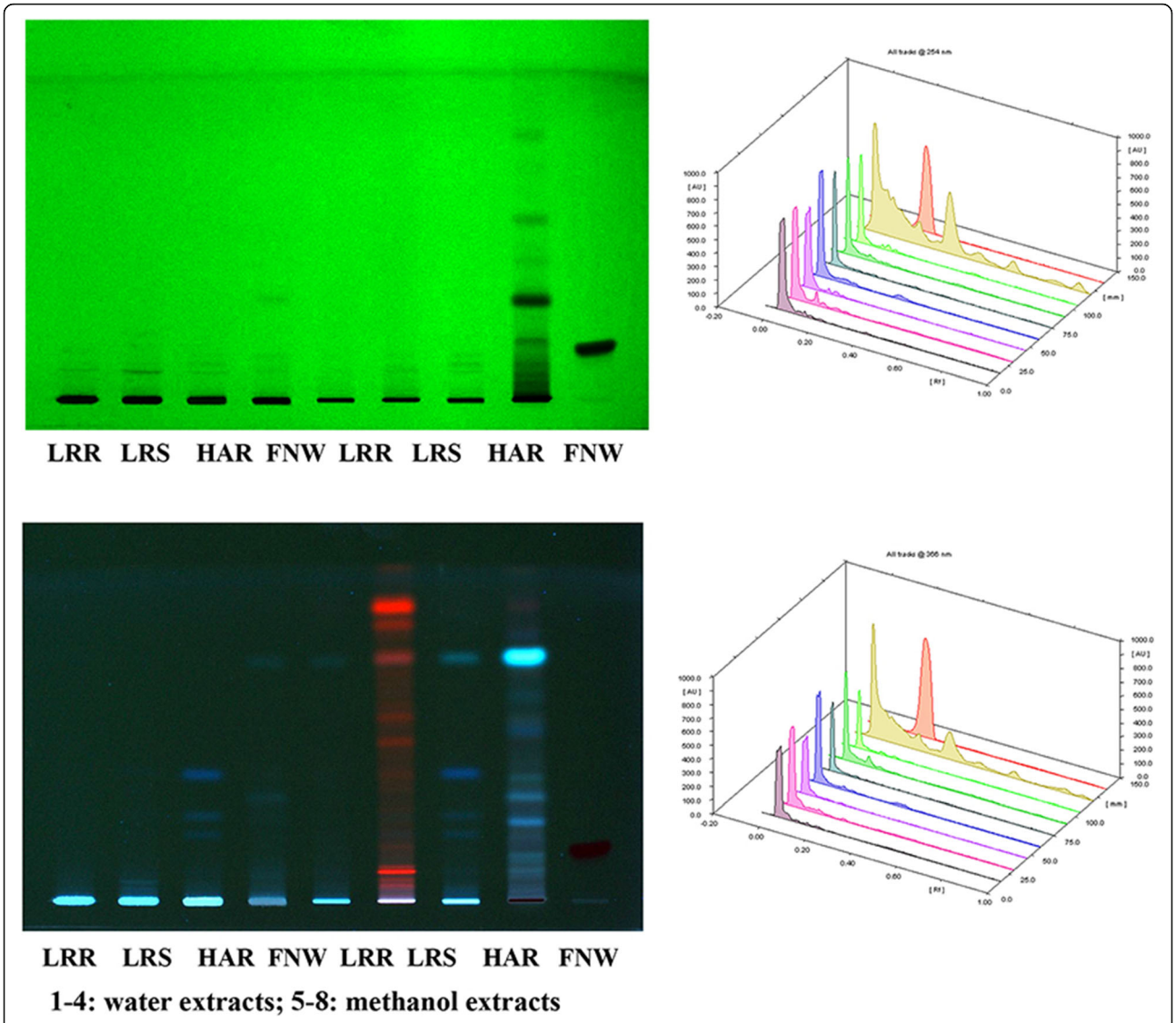

Fig. 3 HPTLC profile of crude methanol and aqueous extracts of various *Jivanti* source plants

Table 4 LC-MS/MS analysis of the phenolic fraction of selected *Jivanti* species

Compound	Molecular formula	*m/z* [M-H]	MS/MS	Present in	Reference
Protocatechuic acid	$C_7H_6O_4$	153.0103	108.02, 109.02	LRR	[11]
7,3′-Dihydroxy flavones	$C_{15}H_{10}O_4$	253.1013	210.03,165.07,163.0	LRS, HAR	[12]
3,7-Dihydroxy-3′, 4′-dimethoxyflavone	$C_{17}H_{14}O_6$	313.2256	295.01,227.17, 183.04	LRS, HAR	[13]
Kaempferol	$C_{15}H_{10}O_6$	285.0492	163, 108.02	HAR	[14]
3,4-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	207.0206	163.07, 118.99	HAR	[15]
Pinocembrine	$C_{15}H_{12}O_4$	255.1127	211.08, 184.05,183.08	HAR	[16]
Glabranine	$C_{20}H_{20}O_4$	323.1735	178.07, 139.05	HAR	[17]
Catechin	$C_{15}H_{14}O_6$	289.1318	201.04, 158.03	FNW	[11]
Dihydroxyquercetin	$C_{15}H_{12}O_7$	303.1472	259.36, 199.04, 145.02	FNW	[18]
2′6′-Dihydroxyflavone	$C_{15}H_{10}O_4$	253.1014	211.04, 181.06, 147.01, 105.03	FNW	[19]

Table 5 Effect of different extracts on carrageenan-induced paw edema

% Swelling					
Group I Control	Group II Induced	Group III FNW	Group IV LRR	Group V LRS	Group VI HAR
0	100 ± 0	80.45 ± 0.009	83.91 ± 0.06	58.09 ± 0.95	32.69 ± 0.37

The data were represented as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests

quantities during every inflammatory response [23]. The effect of test drug on paw edema induced by carrageenan is presented in Table 5. Different plant extracts such as FNW, LRR, LRS, and HAR at a concentration of 150 mg/kg showed swelling of 80.45%, 83.91%, 58.09%, and 32.69% respectively when compared to the 100% swelling of the induced group. *H. ada-kodien* showed comparatively good anti-inflammatory activity among the tested extracts.

Evaluation of anti-diabetic activity by streptozotocin-induced diabetes

A pool of β-cell destruction in STZ-induced diabetic rats results into severe insulin deficiency followed by elevation in fasting blood glucose level beyond the normal value. Metformin was used as a standard drug. In the diabetic control rats, glucose, cholesterol, triglycerides, and LDL values were elevated to a high level during the study whereas HDL value was decreased. All the extracts tested showed anti-diabetic activity. HAR and LRS showed significant anti-hyperglycemic activity against STZ-induced diabetes. LRS and HAR showed 35% and 36% of decrease in glucose level. Metformin caused 20% lowering of blood glucose in the same period of the experiment (Table 6).

High-fat diet and streptozotocin-induced diabetes

The high-fat-fed STZ-treated diabetic rat model was also validated using various extracts (Table 7). The animals treated with LRS and HAR showed a significant decrease in glucose level. Lipid profiling showed that LRS and HAR decreased the elevated cholesterol level.

Discussion

In Ayurvedic classical texts, the descriptions of medicinal plants are given by synonyms. These synonyms have caused controversy in the identification of plants. It is very important to set methods for identifying the exact source plant for a particular drug among different plants being used under the same name. Phytochemical and pharmacological evaluation of such medicinal plants is the important steps to resolve the controversy. The botanical identity of *Jivanti* is in a situation of controversy as the various plants such as *L. reticulata*, *H. ada-kodien*, and *F. nodosa* are being used as its source plant.

The present study showed that certain chemical constituents are similar for both *L. reticulata* root and *H. ada-kodien*. HPTLC profiles showed the presence of some specific common compounds which was confirmed by matching R_f values and densitometry scanning. The quantitative estimation of polyphenolics showed that *F. nodosa* contains a high amount of phenolics among the three species studied. The phenolic content of *H. ada-kodien* and *L. reticulata* is in a moderate level and is in agreement with the previous reports [24]. The variation of phenolic content with respect to the nature of extraction solvents such as methanol, water, and hydro alcohol was observed for all the species studied and it might be due to the difference in the polarity of various solvents. The same has been reported for many other species previously [25, 26]. The literature survey showed that no comparative evaluations of these species are available.

Tandem mass spectroscopic analysis showed the presence of various polyphenolics in the studied *Jivanti* species. Flavonoids such as 7,3′-dihydroxy flavones, 3,7-dihydroxy-3′, 4′-dimethoxyflavone, and 3,4-dimethoxycinnamic acid were identified from *H. ada-kodien*, and the root of *L. reticulata* showed the presence of protocatechuic acid. Catechin, dihydroxyquercetin, and 2′6′-

Table 6 Anti-diabetic activity in STZ-induced diabetic rats

Groups	Parameters							
	Glucose	Hb	HbA1c	Cholesterol	Triglycerides	HDL	LDL	VLDL
Control	98 ± 0.5	9 ± 0.76	5.9 ± 0.56	198 ± 1.22	121 ± 0.89	60 ± 1.67	113.8 ± 2.01	24.2 ± 1.67
Diabetic control	126 ± 1.55	5.2 ± 0.36	7.9 ± 0.73	265 ± 2.06	221 ± 1.53	31 ± 1.78	189.8 ± 1.96	44.2 ± 0.98
Standard	100 ± 1.57	8.5 ± 0.51	6.7 ± 0.98	205 ± 0.88	110 ± 0.86	57 ± 0.97	126 ± 1.22	22 ± 1.45
FNW	116 ± 1.42	7.1 ± 0.2	6.1 ± 0.8	207 ± 1.00	119 ± 0.68	55 ± 1.23	128.2 ± 0.98	23.8 ± 1.29
LRR	87 ± 1.02	6.4 ± 0.26	6.8 ± 0.79	205 ± 1.64	124 ± 1.25	**63 ± 1.87**	**117.2 ± 1.32**	24.8 ± 1.12
LRS	**81 ± 1.25**	**7.1 ± 0.19**	6.3 ± 0.79	**202 ± 1.89**	**110 ± 1.1**	60 ± 0.79	120 ± 1.01	**22 ± 1.23**
HAR	82 ± 0.67	6.1 ± 0.45	**5.1 ± 1.12**	207 ± 1.68	115 ± 1.26	61 ± 1.34	123 ± 0.67	23 ± 0.96

The data were represented as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests

Table 7 Anti-diabetic activity of HFD with streptozotocin-induced rats

Groups	Parameters								
	Glucose (mg/dl)			Triglycerides (mg/dl)			Cholesterol (mg/dl)		
	HFD	STZ	Treatment	HFD	STZ	Treatment	HFD	STZ	Treatment
Control	73 ± 0.76	82 ± 1.45	85 ± 0.98	118 ± 2.01	124 ± 0.87	120 ± 1.87	103 ± 0.98	117 ± 1.98	107 ± 1.34
Diabetic control	102 ± 0.89	237 ± 2.09	249 ± 0.85	246 ± 1.67	261 ± 0.91	282 ± 1.65	200 ± 0.76	227 ± 1.45	251 ± 1.65
Standard	100 ± 0.87	195 ± 1.56	115 ± 1.12	209 ± 1.45	231 ± 1.54	163 ± 1.76	134 ± 0.97	205 ± 1.34	116 ± 1.33
FNW	111 ± 1.34	200 ± 1.31	142 ± 1.56	219 ± 1.76	**270 ± 1.43**	**139 ± 1.98**	139 ± 1.02	227 ± 1.98	123 ± 1.76
LRR	101 ± 1.23	192 ± 1.11	138 ± 1.38	207 ± 1.45	285 ± 1.35	146 ± 1.34	124 ± 0.89	229 ± 1.47	120 ± 1.65
LRS	**98 ± 1.11**	**182 ± 1.67**	**112 ± 0.99**	**176 ± 1.47**	274 ± 1.98	155 ± 1.21	127 ± 1.12	239 ± 1.34	124 ± 1.55
HAR	**99 ± 0.96**	**193 ± 1.22**	**104 ± 1.01**	211 ± 1.32	245 ± 1.67	175 ± 1.67	**119 ± 1.43**	**185 ± 1.87**	**111 ± 1.11**

The data were represented as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests

dihydroxyflavone were identified from *F. nodosa*. Most of the compounds identified are biologically active molecules possessing numerous pharmacological properties such as anti-oxidant, anti-diabetic, anti-inflammatory, etc. [27–30].

Anti-inflammatory and anti-diabetic activity of *L. reticulata* has been reported earlier [23]; however, comparative evaluation of the same for various source plants of *Jivanti* is not yet reported. *H. ada-kodien* showed comparatively good anti-inflammatory activity at a dose level of 100 mg/kg among the tested species. Type 2 diabetes has become a global health problem. According to the World Health Organization, more than 176 million people are suffering from this disease globally [31]. Both stems of *L. reticulata* and *H. ada-kodien* showed significant anti-diabetic activity. The same was also confirmed with a high-fat diet streptozotocin-induced diabetic model in albino rats.

The results of the present study concluded the possibility of using *Holostemma ada-kodien* as an alternative source plant for *Jivanti* in Ayurvedic drug industry as it possessed significant anti-inflammatory and anti-diabetic activity when compared to the genuine source drug, *L. reticulata*.

Conclusion

Jivanti is a controversial Ayurvedic drug as it has more than one botanical source. Leptadenia reticulata is the genuine source plant of Jivanti according to Ayurvedic Pharmacopeia of India, although Holostemma ada-kodien and Flickingeria nodosa are also considered as source plants for Jivanti in some parts of the country. In this study, comparative phytochemical and pharmacological evaluation has been carried out to identify alternative source plants for Jivanti. Phytochemical and pharmacological evaluation of various source plants of Jivanti revealed that the root of H. ada-kodien possesses significant anti-inflammatory and anti-diabetic activity exploring the possibility of using the same as a validated substitute of Jivanti.

Abbreviations

LRR: *Leptadenia reticulata* root; LRS: *Leptadenia reticulata* stem; HAR: *Holostemma ada-kodien* root; FNW: *Flickingeria nodosa*; STZ: Streptozotocin; HPTLC: High-performance thin-layer chromatography; LC-MS/MS: Liquid chromatography-mass spectroscopy

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Plant authentication

Plant authentication was done by Plant Systematics and Genetic Resources Division of the institute.

Authors' contributions

All authors have read and approved the final manuscript. SCT: Designed the study, carried out LC/MS analysis and pharmacological evaluation, and drafted the manuscript. JCK: Carried out the phytochemical analysis. JKU: Participated in the phytochemical analysis. PKM: Collected the plant materials. IB: Participated in study designing and edited the manuscript

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Availability of data and materials

All data and material are available upon request.

Ethics approval and consent to participate

The study protocol was approved by the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No. KAHE/IAEC/2018/21-04/008 and KAHE/IAEC/2018/21-04/009).

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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