



Pharmaceutical Standardisation

Phyto-chemical evaluation of dried aqueous extract of *Jivanti* [*Leptadenia reticulata* (Retz.) Wt. et Arn]

Atanu Pal, Parmeshwar P. Sharma¹, Tarulata N. Pandya², Rabinarayan Acharya³, Bhupesh R. Patel⁴, Vinay J. Shukla⁵, B. Ravishankar⁶

Lecturer, Department of Dravyaguna Vijnana and Rasa Shastra, Biswanath Ayurveda Mahavidyalaya, Kolkata, West Bengal, ¹Ex. Professor and Head, ²Ex. Reader and Head, ³Assistant Professor, ⁴Associate Professor, Department of Dravyaguna, ⁵Head, Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, ⁶Director, Research and Development, SDM College of Ayurveda, Kuthpady, Udupi, Karnataka, India

Abstract

Jivanti (*Leptadenia reticulata* (Retz.) Wt. et Arn) is a well known climber used for its innumerable therapeutic properties like antioxidant, antibacterial, vasodilator, galactogogue, *Jivaniya*, etc., Its use in veterinary practice is tremendous due to its lactogenic effect. The *Ghana* (dried aqueous extract) of the whole plant was prepared and evaluated phyto-chemically by subjecting it to various tests like physico-chemical, qualitative analysis; TLC and HPTLC. Qualitative tests revealed the presence of flavonoids and TLC also inferred positive Rf value (0.30), indicating the presence of quercetin in the *Ghana*.

Key words: Dried extract, *Ghana*, *Jivanti*, *Leptadenia reticulata*, quercetin

Introduction

Plants are preliminary source of chemicals used to treat the various disease conditions of living creature. Chemicals derived from plants fulfill every aspect of our daily requirements, as a source of food as well as medicinal agents.

Leptadenia reticulata (Retz.) Wt. et Arn. is an important plant of Asclepiadaceae family, commonly known as *Jivanti* in Sanskrit and *Dodi* in Hindi and Gujarati, is a climber distributed in tropical and sub-tropical parts of Asia and usually grown as hedges in Gujarat. The botanical source of *Jivanti* is in the state of controversy and Charaka mentioned it as a classical whole some vegetable (*Shreshtha Shaka*)^[1] to be consumed for maintaining the good health. It is also used as *Jivaniya*, *Snehopaga*, *Svasahara*, *Vayahasthaniya*,^[1] *Rasayana*, and *Chakshushya*^[2] in Ayurveda. Various herbs are used under the name of *Jivanti* in the different parts of the country, namely - *Dregia volubilis* Linn,^[3] *Holostemma annulare* Roxb. of Asclepiadaceae family, *Dendrobium normale* Fale (Orchidaceae), etc., However, *L. reticulata* is accepted as a genuine *Jivanti*.^[4]

It is rich in sterols especially stigmasterol and tubers contain fructosan. Physico-chemical parameters, Thin Layer Chromatography (TLC) and High Performance Thin Layer

Chromatography (HPTLC) of the methanolic extract of the leaf and stem has been reported but *Ghana* (dried aqueous extract) of the plant has not been found evaluated yet and hence it was thought to evaluate the *Ghana* of the whole plant of *L. reticulata* in the present study.

Materials and Methods

Materials

The matured *Jivanti* (*L. reticulata*) was collected from Khatia forest of Jamnagar in the month of March and their authenticity was confirmed by referring various floras and with the help of Pharmacognosist of Gujarat Ayurved University. Underground parts of the plant were washed out by running tap water to remove adherent soil, dust, etc., Morphological and microscopical characters of root, stem, leaf, fruit, etc., of the plant were studied separately to confirm the genuinity.

Shade dried plants were subjected to coarse powdering for the preparation of *Ghana* by following standard methods^[5] and converted into tablets of 500 mg weight in the Pharmacy of Gujarat Ayurved University, Jamnagar. The compressed tablets were stored in well closed glass bottles for further study.

Methods

Weight variation, disintegration time, and hardness of the prepared tablets were recorded as per the standard methods preliminarily and then they were subjected to chemical evaluation. Parameters like loss on drying, ash value, water soluble ash, acid insoluble ash, water soluble extractive,

Address for correspondence: Dr. Rabinarayan Acharya, Department of Dravyaguna, I.P.G.T. and R.A., Gujarat Ayurved University, Jamnagar - 361 008, Gujarat, India. E-mail: drnacharya@gmail.com

methanol soluble extractive, hexane soluble extractive, pH value and qualitative tests for tannin, terpenoid/sterols, alkaloid, saponin, flavonoid, glycoside, carbohydrate were carried out by following standard methods.^[6]

In TLC study, methanolic extract of *L. reticulata Ghana* was run on a pre-coated silica gel 60 F 254 plate by using Toluene: Methanol (9:1) as a solvent system and anisaldehyde sulfuric acid as a spray reagent and Quercetin as standard. The solvent was allowed to run up to 8 cm distance and plate was observed as such under long and short ultraviolet (UV) rays and the florescent spots resolved were noted down. Then the plate was sprayed and heated at 110°C in oven. The Rf of the developed colored spots resolved were noted down.

The methanolic extract was also tested by HPTLC with the help of CAMAG Linomat-5 instrument to detect the presence of quercetin in *Jivanti Ghana* by following standard method.^[7]

Results

Analysis on routine physico-chemical parameters

Prior to physico-chemical tests, the prepared tablet of *Ghana* were tested for the parameters, results show its disintegration time as 37 minutes and hardness as 6.5 kg/cm². The physico-chemical analysis revealed not less than 11.00% W/W as moisture content and not more than 32.50% W/W as ash value. The water soluble ash was not more than 19.50% W/W and acid insoluble ash was not more than 1.50% W/W. There was wide variation in all the extractive values. Water soluble extractive was not less than 56.00% W/W, whereas methanol and hexane soluble extractive were not less than 28.00% and 2.00% W/W, respectively [Table 1].

Analysis on routine qualitative parameters

Qualitative tests revealed the presence of tannin, terpinod/sterols, alkaloids, saponin, flavonoids, carbohydrate, and glycoside [Table 2].

Analysis on thin layer chromatography separation of the methanol extract

TLC analysis of the extract showed four resolved spots (Rf – 0.30, 0.44, 0.60, and 0.73) and Rf value (0.30) and the color of the quercetin were matching with one of the resolved spot of the extract, indicating the presence of quercetin in *Ghana* [Table 3 and Figure 1].

Table 1: Analytical data of physico-chemical parameters of *Jivanti Ghana* tablet

Parameter	Results (%)
Determination of loss on drying	11.00 W/W
Ash value (% of total ash)	32.50 W/W
Water soluble ash	19.50 W/W
Acid insoluble ash	1.50 W/W
Water soluble extractive value	56.00 W/W
Methanol soluble extractive value	28.00 W/W
Hexane soluble extractive value	2.00 W/W
pH value (5% aqueous solution)	5.88

Analysis on HPTLC identity test

In the HPTLC study, same Rf value (0.86) was noticed in all three tracks at 700 nm in track I and track II and at 260 nm in track III [Table 4 and Figure 2].

Discussion

As the drug is in tablet form, it should first be dissolved in the stomach in right time for its proper absorption. Since the drug, having moderate disintegration time and very high water and

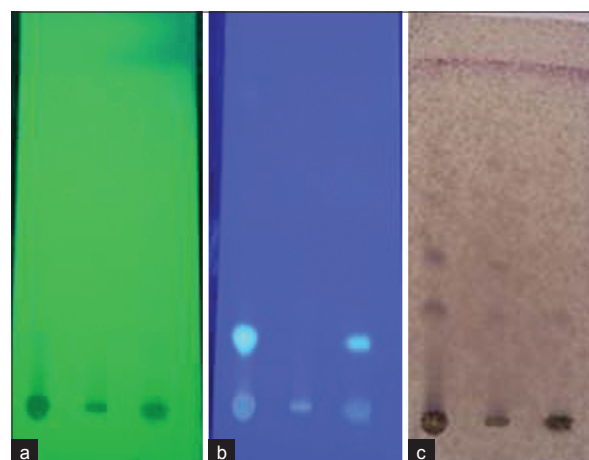


Figure 1: TLC plates of methanolic extract of *Jivanti Ghana* a = Under short UV, b = Under long UV, c = After anisaldehyde sulfuric acid spray

Table 2: Analytical data of qualitative tests of *Jivanti Ghana* tablet

Components	Tests	Results
Tannin	With dilute solution ferric chloride	Positive
	With 5% lead acetate and KOH	Positive
Terpenoid/sterols	Libermann–Buchard test	Positive
	Salkowski reaction	Positive
Alkaloid	Dragendroff reagent	Positive
	Mayer's reagent	Positive
	Wagner's reagent	Positive
Saponin	With lead acetate	Positive
	Froth test	Positive
Flavonoid	With neutral lead acetate	Positive
	With sulphuric acid	Positive
	With NaOH and sulphuric acid	Positive
Glycoside	Molish test	Positive
Carbohydrate	Fehling A and B solution test	Positive

Table 3: TLC details of test solution of *Jivanti Ghana* tablet and quercetin

Track	Sample	No. of spot	Rf value
Track I	<i>L. reticulata</i>	4	0.30, 0.44, 0.60, and 0.73
Track II	<i>L. reticulata</i>	4	0.30, 0.43, 0.60, and 0.70
Track III	Quercetin	1	0.30

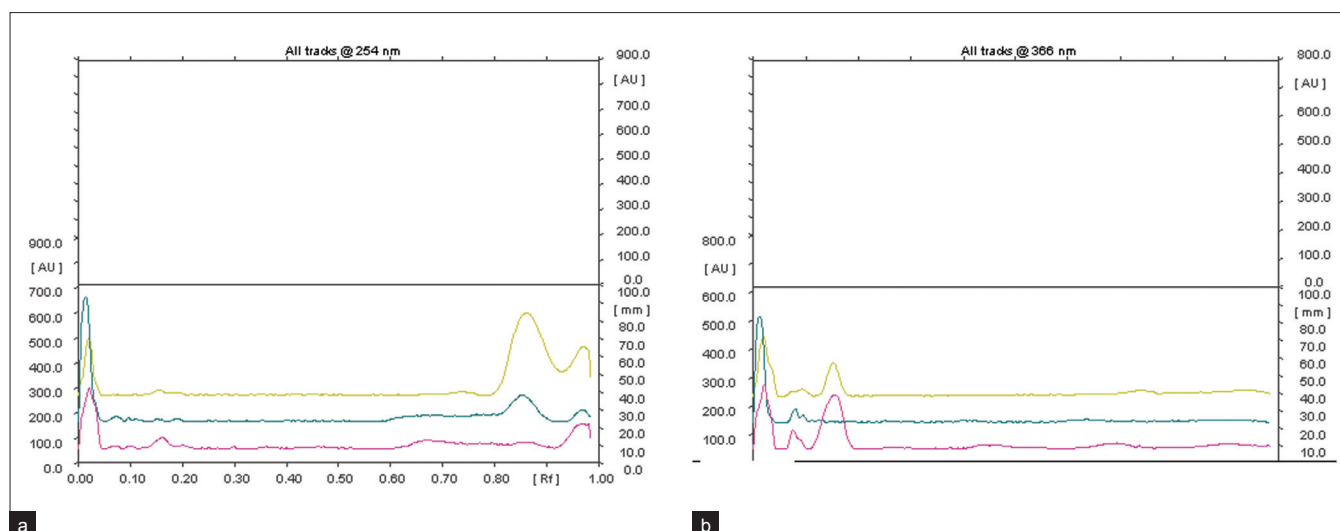


Figure 2: All tracks in HPTLC of methanolic extract of *Jivanti Ghana* and quercetin at 254 nm (a) and 366 nm (b)

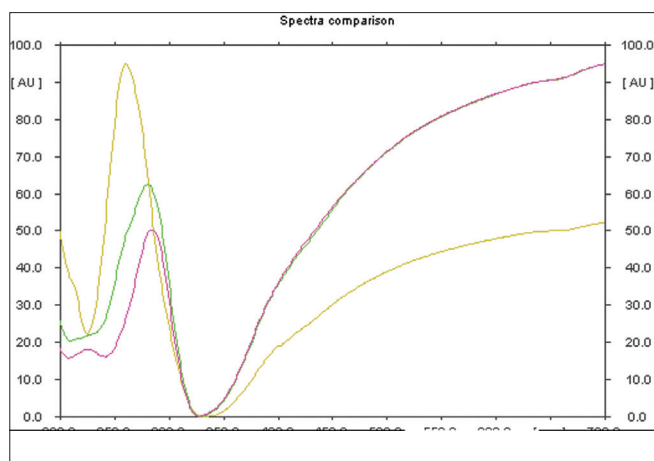


Figure 3: Positive Rf value (0.86) at 700 nm in track I and track II and at 260 nm in track III

Table 4: HPTLC details of test solution of *Jivanti Ghana* and quercetin at 254 and 366 nm

Track	Sample	No. of spots at 254 nm	Rf value	No. of spot at 366 nm	Rf value
I	<i>L. reticulata</i> MeOH	8	0.02, 0.07	8	0.02, 0.07
			0.10, 0.16		0.10, 0.16
			0.20, 0.68		0.20, 0.68
			0.86, 0.97		0.86, 0.97
II	<i>L. reticulata</i> MeOH	9	0.01, 0.07,	3	0.01, 0.08
			0.10, 0.11,		0.10
			0.15, 0.19,		
III	Quercetin	5	0.02, 0.16	4	0.02, 0.09
			0.74, 0.86		0.15, 0.74
			0.97		

alcohol soluble extractive value, is indicative of proper dissolution and absorption in the acid media of stomach and intestine. Any drug must first pass into solution before it can be absorbed and so the alcohol soluble extractive of *Ghana* has physiological importance. Hence, it may be inferred that chemical constituents of *Jivanti* will be physiologically more available in the form of *Ghana*. Furthermore, other parameters like pH (5.88) and ash value (32.50% W/W) of the *Ghana* also support its better efficacy.

Qualitative assessment in *Jivanti Ghana* by different tests reveals the presence of tannin, terpinod/sterols, alkaloids, saponins, flavonoids, carbohydrates, and glycosides-like constituents. Presence of carbohydrate denotes *Jivanti* having *Madhura Rasa*, may confirms classical reference.

The resolution of four spots on TLC indicates the presence of four different chemical constituents one of them being quercetin resolved at 0.30 Rf.

In the HPTLC study, same Rf value (0.86) was noticed in all three tracks at 700 nm. in track I and track II and at 260 nm. in track III. Positive Rf value indicate the possibility of the presence of quercetin-like flavonoid in *Jivanti Ghana* and variation in covered area due to presence of iso-quercetin, a nearer flavoniod glycoside-like quercetin [Figure 3].

Conclusion

The absorption and bioavailability of *Jivanti* (*L. reticulata*) may be better in the form of *Ghana*. Presence of carbohydrate may confirm classical reference regarding *Rasa* of *Jivanti*. Positive R_f value at 0.86 confirm the possibility of the presence of quercetin/iso-quercetin in the *Ghana*.

Acknowledgment

The authors express deepest sense of gratitude to The Director, I.P.G.T and R.A., G.A.U., Jamnagar, Prof. M.S. Baghel Whose blessing at every step have inspired not only to accomplish this work but also in all aspects.

References

1. Agnivesh, Charaka, Dridhabala, Charaka Samhita, Sutrasthana, 25/38, edited by Shastri K, Pandey GS. 4th ed. Chaukhambha Sanskrit Sansthan, Varanasi 1994; p. 317.
2. Bhavamishra, Bhavaprakash Nighantu, edited by Chunekar KC, Pandey GS. Guduchyadi Varga. 1st ed. Chaukhambha Bharati Academy, Varanasi, 2004. p. 295-6.
3. Kumar RK. Comparative Phyto-chemical Analysis of *Leptadenia reticulata* W. and A. and *Dregia volubilis* Linn. (Source of Jivanti). M.Sc. Med. Plants, IAMPS, GAU. Jamnagar, 2006. p. 47-59.
4. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants Used in Ayurveda Volume 2, CCRAS, Dept. of ISM and H, Ministry of Health and Family welfare, Government of India, 1st ed. 2001. p. 270-3.
5. Anonymous. The Ayurvedic Formulary of India. Kwath Churna Kalpana. Part I, 2nd Revised English ed. New Delhi, The Controller of Publications Civil Lines, 2003. p. 51-62.
6. Baxi AJ. Methods of Qualitative testing of phyto-chemical constituents. In: Shukla VJ, Bhatt UB (prepared). Methods of Qualitative Testing of Some Ayurvedic Formulations, 1st ed. Jamnaga: Gujarat Ayurved University, 2003; p. 3-15.
7. Gupta AK, Tendon N, Sharma M. *Leptadenia reticulata* W and A. In: Gupta AK editors. Quality Standards of Indian Medicinal Plants. Volume 3, 1st ed. New Delhi: ICMR, 2005. p. 236-45.

हिन्दी सारांश

जीवन्ती घन का विश्लेषणात्मक अध्ययन

अतनु पाल, परमेश्वर पी. शर्मा, तरुलता एन. पंड्या, रबिनारायण आचार्य, भूपेश आर. पटेल,
विनय जे. शुक्ला, बी. रविशंकर

जीवन्ती एक सर्वविदित लता है जिसका प्रयोग रसायन, जीवाणुघ्न, सिरा विस्फारक, दुग्धवर्द्धक एवं जीवनीय के रूप में किया जाता है। इसका प्रयोग दुग्धवर्द्धक क्रिया के लिये पशु चिकित्सा में होता है। इस लता के पत्रांग से घन बना कर विविध रासायनिक तत्वों की अनुसन्धान परीक्षा (फिजिकोकेमिकल, क्वालिटेटिव, टी.एल.सी. एवं एच.पी.टी.एल.सी.) की गयी है। गुणात्मक परीक्षा में फ्लेवेनॉयड की उपस्थिति पायी गयी है। इसके घन की टी.एल.सी. परीक्षा में क्रेसिटिन की उपस्थिति पायी गयी है।