European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 11, 2020

PHARMACOGNOSTICAL AND PHRAMACEUTICAL ANALYSIS OF *LAXMINARAYAN RASA VATI*: AN AYURVEDIC HERBOMENERAL FORMULATION

Rahulkumar V. Naika[1], Alankruta R. Dave[2], C.R. Harisha[3], V.J. Shukla[4], Manoj Upadhyay[5]

[1]PhD. Scholar Kayachikitsa Department, ITRA, Jamnagar, Gujarat, India-361008
[2] Associate Professor Kayachikitsa Department, ITRA, Jamnagar, Gujarat, India-361008
[3] Head Pharmacognosy Lab, ITRA, Jamnagar, Gujarat, India-361008
[4] Head Pharmaceutical Lab, ITRA, Jamnagar, Gujarat, India-361008
[5] Professor & HOD, Samhita & Siddhant Department, O.H. Nazar Ayurved College, Surat

ABSTRACT

Introduction: Post Chikungunya Arthralgia is a major health issue in present era as there is persistent Arthralgia seen in many patients of chikungunya fever after treatment. The disease is characterized by a sudden onset of high-grade fever, rash and arthralgia. The current literature reports that CHIKV can result in a severe chronic arthralgia and/or arthritis that can last months to years following the initial infection. In Ayurveda, *Vaatdosha* performs all the activity in body including the movements of the joints. When *Vaatdosha* at *Sandhisthan* is vitiated and mixed with *Aamdosha* it produces symptoms like *Sandhishool, Sandhishoth and Sashoolkriya* or stiffness in joints. There are many formulations mentioned for treatment for such conditions. *Laxminrayan rasa vati* is classicle Ayurvedic herbomineral formulation cited in *Yogratnakara Samhita* in context of *Vaatvyadhi Chikitsa* hence selected for concerned research work of post chikungunya arthralgia.

Material and methods: Formulation is made in tablet form. This paper is made to standardize the formulation through Pharmacognostical and Pharmaceutical measures. The formulation was analysed and standardized scientifically through qualitative and quantitative analysis by physico-chemical parameters and High-Performance Thin Layer Chromatography (HPTLC) and pharmacognostical measures.

Results and Discussion: Pharmacognostical analysis showed characteristics of all the ingredient drugs in the tablet. In Pharmaceuticals analysis, HPTLC was done in Toluene, Ethyl acetate, Acetic acid (7:2:1) in which 10 and 4 spots were distinguished at 254 nm and 366 nm respectively. This study may be used as reference standard in the further researches.

KEY-WORDS: *Laxminrayan rasa vati,* Pharmacognostical, Pharmaceutical, Post Chikungunya arthralgia

INTRODUCTION

Chikungunya is a mosquito borne alpha virus illness caused by CHIK virus and transmitted by the bite of *Aedes egypti* mosquito. Chikungunya virus was first isolated in 1953 in the Newala district of Tanzania[1]. The disease so named from Makonde root verb kungunyala meaning that "which bends up" which refers to postures adopted by the affected person due to musculoskeletal manifestations of the disease. Chikungunya usually starts with sudden onset of fever, chills, headache, nausea, vomiting, and joint pain with or without swelling and rash which is very similar to that of Dengue fever. Unlike dengue there is no haemorrhagic or shock syndrome.

The first epidemic in India occurred at few regions of south India in 1963. The Massive outbreak which occurred in 2005 affected more than 1.4 million people spread over 13 states. According to WHO there is no specific antiviral drug against CHIK virus as there is no specific management for Chikungunya and vaccine is under investigation and not available, presently symptomatic treatment is recommended. The line of management usually the rest, IV fluids, antipyretic, anti-inflammatory and analgesic agents.

After completion of treatment course there is persistent arthralgia or arthritis have been seen in most of patients which is in later stage ends in chronic arthralgia/arthritis[2,3]. Chikungunya infection may be divided into an acute phase (3 months). The acute phase may be further subdivided into viraemic (5–10 days) and sub-acute post-viraemic (6–21 days) phases[4]. Acute CHIKV infection may also exacerbate underlying rheumatic diseases with studies showing CHIKV infection causing relapses of autoimmune arthritis in patients who were in remission prior to infection.[5] The chronic phase of the disease may follow a pattern similar to rheumatoid arthritis, peripheral spondyloarthritis, undifferentiated arthritis and fibromyalgia. The ankles, knees, hips, wrists, elbows and metacarpo-phalangeal joints are mainly involved[6].

In Ayurveda, the concept of epidemics is very well defined and established. *Acharya Charaka* had mentioned epidemic condition under the head '*Janapadodhwansa*'. It has also been mentioned that the natural course of the disease and its treatment may vary according to the period, geographical region, eco climatic condition, psychosomatic constitution of an individual and so on. Certain new disease entities may also appear in the course of time that has no identity and they may be managed through suitable drugs and procedures based on the sign and symptoms, taking clues from the authentic literature.

Though there is no precise term for this condition in Ayurveda, yet many Scholars tried to distinct nomenclature for this like *Upastambhita Sandhigatvaat, Saam jwara, Dhatugata jwara, Amvaat* or *Sandhig jwara*. As described in Ayurveda; sign and symptoms of *Sandhig jwara* strikingly resembles Post Chikungunya Arthralgia[7]. Treatment of Post Chikungunya arthralgia is generally symptomatic and supportive, and the efficacies of potentially more specific therapies are currently unknown.

Even after taking treatment, in majority of cases there are persistent arthralgia and myalgia. Because this condition will become chronic, it has to be managed by long term medicine consumption with no or minimal adverse effect since it needs regular management. Though many theses have been worked out for this burning problem, still there is need of evaluation of certain drugs clinically on various scientific parameters which could be safe, effective, cheap and readily available in the management of Post Chikungunya arthralgia. Hence, for this clinical trial drug *Laxminarayan Rasa Vati* is selected. This article is made to standardize the current herbomeneral preparation through Pharmacognostical and Pharmaceutical procedures.

MATERIALS AND METHODS

Materials: All raw materials were collected from pharmacy of ITRA, Jamnagar. All materials were listed in table no. 1.

Table no. 1: Ingredients of *Laxminarayan Rasa Vati.*

Sr.no	Drug Name	Botanical / English Name	Part used	Proportion
1	Shuddha Hingula	*Cinnebar*	-	1 part

2	Abhrak bhasma	*Biotite mica*	-	1 part
3	Shuddha gandhaka	*Sulphur*	-	1 part
4	Shuddha Tankana	*Borax*	-	1 part
5	Shudhha vatsanabha	*Aconitum ferox wall.ex.ser*	Root	1 part
6	Nirgundi bija	*Vitex nigundo Linn.*	Seed	1 part
7	Ativisha	*Aconitum heterophyllum wall.*	Root	1 part
8	Pimpali	*Piper longum linn.*	Fruit	1 part
9	Katuki	*Picrorriza kuroran Royle ex.benth*	Root	1 part
10	Saindhav	*Rock salt*	-	1 part

PHARMACOGNOSTICAL STUDY

All the raw drugs were identified and authenticated by the Pharmacognosy department, ITRA, Jamnagar. The identification was carried out on the basis of organoleptic features, structural features and powder microscopy of individual drugs by following the standard protocol in standard books. Pharmacognostical assessment of prepared drug was also carried out. Drug dissolved in small quantity of distilled water, filtered through filter paper, filtrate studied under the microscope attached with camera. The microphotographs were also taken under the microscope [8].

METHOD OF PREPARATION

All the raw drugs mixed thoroughly with each other, than 3 bhavana of *Dantimool kwatha* and *triphala kwatha* each has been given as per *Sharangdharacharya*. After total 6 *Bhavana* of *kwatha* (decoction) *Laxminarayan rasa vati* is made in the form of tablet.

PHARMACEUTICAL EVALUATION

Assessment of the various physicochemical parameters for Laxminarayan rasa vati such as foreign matter, moisture content, ash value, acid insoluble ash, water soluble ash, water-soluble extractive, alcohol-soluble and pH, etc. was carried out by following standard common parameters mentioned for compressed tablets in Ayurvedic Pharmacopeia of India[9] and CCRAS[10] at Pharmaceutical Laboratory, ITRA, Jamnagar. Presence of more moisture content in a sample can create preservation problem. Hence loss on drying was also selected as one of the parameters.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY STUDY (HPTLC)

Methanol extract of Laxminarayan rasa vati were spotted on precoated silica gel GF 60254 aluminium plate as 5mm bands, 5mm apart and 1 cm from the edge of the plates, by means of a Camag Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene: Ethyl acetate: Acetic acid (7:2:1) was used as mobile phase. After Development scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 Camag)[7]. The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm s-1[11].

RESULTS AND DISCUSSION

PHARMACOGNOSTIC STUDY

The primary purpose of the study was to confirm the authenticity of the drugs used in the preparation of Laxminarayan rasa vati. For that coarse powder of all the ingredients were subjected to organoleptic and microscopic evaluation separately. The tablet is reddish brown in colour, slightly aromatic and hard in touch, weigh about 250mg each.

Organoleptic evaluation:

Organoleptic features like colour, odour and taste of Laxminarayan rasa vati were recorded and are placed in table no.2.

Table No. 2 Results of Organoleptic evaluation

Sr.no.	Characteristics	Observations
1	Form	Tablet
2	Colour	Reddish brown
3	Odour	Slightly aromatic
4	Taste	Pungent
5	Touch	Hard, rough
6	Weight (each tablet)	250mg

Microscopic evaluation:

Microscopic assessment of *Laxminarayan rasa vati* was conducted by powdering the tablet and dissolving it in the distilled water and studied under microscope for the presence of the characteristics of the ingredient drug and for the possible changes in the structures if any. The microphotographs were taken by using Carl Zeiss trinocular microscope. Characteristics of all the ingredient drugs were identified in *Vati* also. Microscopic characters of Laxminarayan rasa vati are oil globules & lignified fibres of *Katuki*, starch grains, fibres, lignified pitted stone cells & annular vessels of *Ativisha*, stone cells of *pippali*, unicellular trichome & epidermal cells of *nirgundi beej*, epicarb cells of *pippali* along with oleoresin content and brown content of *katuki*. (figure 1A-L)

Fig 1 A. Laxminarayan rasa tablet

Fig 1 B. Oil globules of Katuki

Fig 1 C. Unicellular trichome of nirgundi beej

Fig 1 D. Fibres of Ativisha

Fig 1 E. Annular vessels of Ativisha

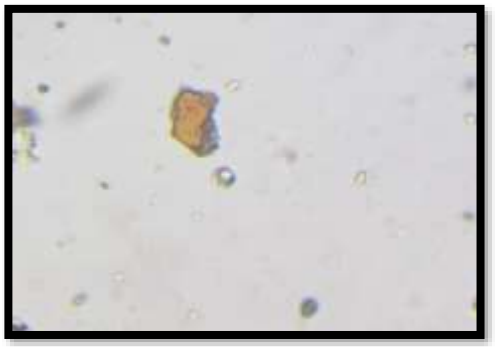

Fig 1 F. Brown content of katuki

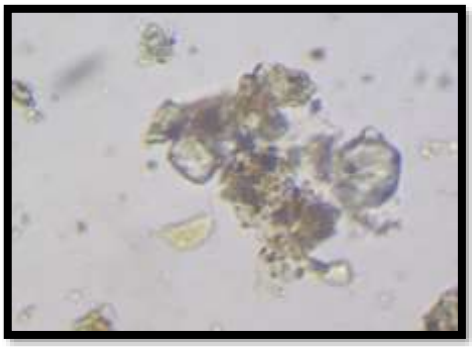

Fig 1 G. Starch grains of ativisha

Fig 1 H. Lignified pitted stone cells of Ativisha

Fig 1 I. Lignified fibres of katuki

Fig 1 J. Epidermal cells of nirgundi beej

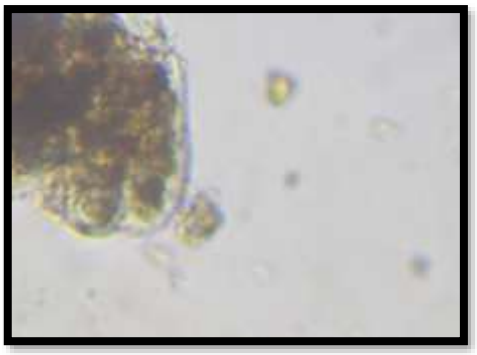

Fig 1 K. Epicarb cells of pippali along with oleoresin content

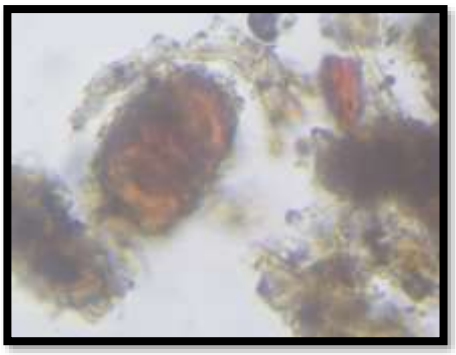

Fig 1 L. Stone cells of pippali

PHARMACEUTICAL STUDY

Physicochemical parameters:

Physicochemical Parameters of the tablet like Uniformity, Disintegration time, Hardness, Loss on Drying were all found to be within the normal range. The water-soluble extractive and methanol soluble extractive values were found to be 35.25 % w/w and 5.77 % w/w respectively. Details are placed at table 3.

Table 3: Physicochemical parameters of Laxminarayan rasa vati

Uniformity of tablet	**Average**	442mg
	Highest	364mg
	lowest	403.35mg
Hardness		1.6kg/ cm^2
Loss on drying		4.221% w/w
Ash value		27.31 % w/w
Water soluble extract		35.25%w/w
Methanol soluble extract		5.77 % w/w
pH value		6.5

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY STUDY

Densitometric scanning of the HPTLC pattern showed 10 spots corresponding to Rf values 0.02, 0.14, 0.18, 0.27, 0.31, 0.4, 0.49, 0.64, 0.7, 0.9 in short wave UV 254 nm and 4 spots corresponding to Rf values 0.02, 0.16, 0.27, 0.31 obtained in long wave UV 366nm (Table 4, Figure 2). Though it may not be able to identify particular chemical constituent from the spots obtained, the pattern may be used as a reference standard for further quality control researches.

Table 4: HPTLC of Laxminarayan rasa vati

254nm		**366nm**	
Peak	**Rf**	**Peak**	**Rf**
1	0.02	1	0.02
2	0.14	2	0.16
3	0.18	3	0.27
4	0.27	4	0.31

5	0.31	
6	0.4	
7	0.49	
8	0.64	
9	0.7	
10	0.9	

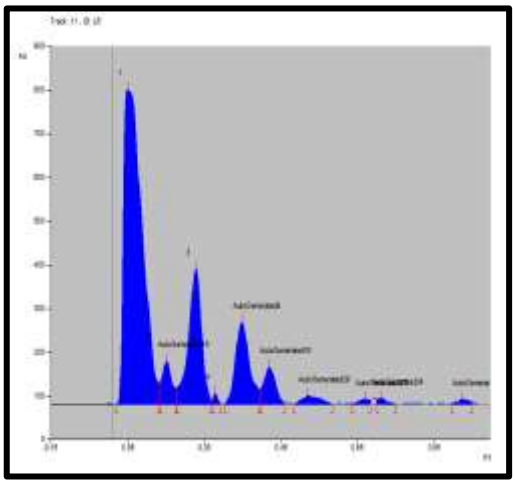

Fig 2 A. Densitogram curve of Methanol extract of Laxminarayan rasa vati 254 nm

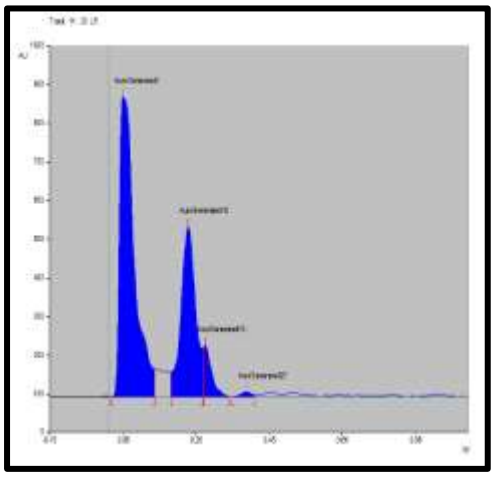

Fig 2B. Densitogram curve of Methanol extract of Laxminarayan rasa vati 366nm

CONCLUSION

The chikungunya virus (CHIKV) infection epidemic has emerged as a significant public health concern in the last 10–15 years, especially in Asian and south American countries. V The infection should always be suspected in a returning traveller presenting with fever, skin rash and arthralgia. Ayurvedic system of medicine is being relied upon more and more for the various health issues particularly lifestyle diseases.

Laxminarayan rasa vati is used for Post Chikungunya arthralgia and various joint related problems in Ayurveda. Since Vati is handmade, which is very time taking and inconvenient too. Also, it is tough to maintain the proper & exact dose of Vati due to mode of preparation. Hence to overcome these problems along with the problems of palatability, feasibility and to increase the self-life, Vati form is converted in to tablet form.

The ingredients were identified and authenticated pharmacognostically and were used for the preparation. The formulation was subjected to pharmacognostical, physicochemical, HPTLC studies. It is inferred that the formulation meets the minimum qualitative standards as reported in the API at a preliminary level. The inference from this study may be used as reference standard in the further quality control researches. Further clinical evaluation of the compound is in progress.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of ITRA, Jamnagar for providing facilities to carry out research work.

REFERENCES

1. World Health Organization . Chikungunya . Geneva : WHO . www.who. int/news-room/fact-sheets/detail/chikungunya [Accessed 10 April 2020]
2. Clinical guidelines on management of Chikungunya fever, research paper of WHO, WHO press, Geneva, October 2008
3. Management of Chikungunya through Ayurveda and Siddha, technical report published by CCRAS 2009, Dept. of AYUSH, on behalf of Govt. of India.
4. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond). 1956; 54: 177 – 9 .
5. Blettery M, Brunier, Polomat K et al. Brief report: management of chronic post-chikungunya rheumatic disease: the Martinican experience. Arthritis Rheumatol. 2016 ; 68 :2817 – 24
6. Rodríguez-Morales AJ, Cardona-Ospina JA, Fernanda UrbanoGarzón S et al. Prevalence of post-chikungunya infection chronic inflammatory arthritis: a systematic review and meta-analysis. Arthritis Care Res (Hoboken). 2016; 68:1849 – 58
7. Agnivesha, Charaka, Dhridhabala, Charaka S, Edited by Vd.yadavji Trikamji Acharya, published by Chaukhambha Surbharati Publications, Varanasi, reprint 2000, Chikitasthana 3/85-87.
8. Wallis TE, Text book of Pharmacognosy, 5th edition, New Delhi: CBS Publisher & Distributors, 2002; 123-132, 210-215.
9. Anonymous, Protocol for testing of Ayurveda, Siddha & Unani medicines, Pharmacopoeial laboratory for Indian medicines, Ghaziabad, Ministry of AYUSH, Government of India.
10. Anonymous, Parameters for qualitative assessment of Ayurveda, Siddha drugs, CCRAS, New Delhi, 2005.
11. Stahl E; Thin layer chromatography, 2nd Ed. Springer-Verlag New York, Inc. 175 5th Ave. New York, NY, 1969; 125-133.