

PHYTO-PHARMACEUTICAL ASSAY OF DASHAMOOLADI RASAYANA COMPOUND: A NOVEL COMPOUND FOR DIABETIC POLYNEUROPATHY

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ABSTRACT

At least 171 million people worldwide have diabetes; among them 47% suffer from symptom of diabetic sensorimotor peripheral polyneuropathy (DSPN). Despite advances in understanding metabolic causes of neuropathy, treatments aimed at interrupting these pathological processes have been limited by side effects and lack of efficacy. Even though it is not mentioned directly in Ayurvedic literature, critical analysis of the lakshanas of DSPN gives certain insights to the role of Avarana of Vata by Dushyas. Ideally, Rasayanas are preferred in such multi-faceted disorders, which not only disintegrate the clutches of Samprapti but also energize the glucose starved tissues. The objective of current article is to explore and understand pathogenesis of DSPN in the light of Ayurveda and to propose a novel herbal compound preparation- Dashamooladi Rasayana Compound for treatment of the same. This compound was analyzed and standardized scientifically through qualitative and quantitative analysis by Physico-chemical parameters, Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC). This approach will signal a new era towards the use of Rasayanas in such neurodegenerative disorders and help millions in desolation.

KEYWORDS: Diabetic Polyneuropathy; Rasayana; Dashamooladi Rasayana Compound; Phyto-pharmaceutical Assay, Ayurveda

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INTRODUCTION

At least 171 million people worldwide have diabetes; among them 47% suffer from symptom of diabetic sensorimotor peripheral polyneuropathy (DSPN) ¹. Despite advances in understanding metabolic causes of neuropathy, treatments aimed at interrupting these pathological processes have been limited by side effects and lack of efficacy². Even though it is not mentioned directly in Ayurvedic literature, critical analysis^{3, 4, 5} of the lakshanas of DSPN give certain insights to the role of Avarana of Vata by Dushyas. Ideally, Rasayanas are preferred in such multi-faceted disorders, which not only disintegrate the clutches of Samprapti but also energize the glucose starved tissues. This paper is a product of these thoughts and the efforts to modify the disease progression of this degenerative condition. The formula is time tested and regularly used in the out-patient departments of Ayurveda institutions and practitioners worldwide. This effort signifies two aspects; one, to

establish quality parameters, two, to make the formulation more user friendly in a convenient tablet form instead of cumbersome choorna and Kwatha forms. But no concrete efforts have been made towards exploring the preparation and to analyze it scientifically. The basis of this formula is derived from Vagbhata⁶ and modified based on the available researches on individual drugs and views of the senior physicians.

Aims and objectives

Pharmacognostical and Phytochemical analysis of Dashamooladi Rasayana Compound for DSPN

MATERIALS AND METHODS

- The study involved the following operating procedures:
- Collection, identification and authentication of raw drugs.
- Preparation of the drug at Pharmacy.
- Phytochemical analysis of the compound drug.

Collection, Identification and Authentication of Raw Drugs

The raw drugs for the study were procured from the Pharmacy of IPGT&RA, Gujarat Ayurved University, Jamnagar. The ingredients and the part used are given in the **table 1**. The raw drugs are identified and authenticated by the department of Pharmacognosy of IPGT &RA, Gujarat Ayurved University, Jamnagar. The identification was carried out based on the morphological features, organoleptic features and powder microscopy of the individual drugs. The API⁸ standards were used for the authentication.

Preparation of the Drug at Pharmacy

The ingredients enlisted from 1 – 4 are made into fine powder and sieved in mesh no.80. The ingredients are mixed well in equal quantity in mass mixing machine till a homogenous mixture was obtained. The mixture was given two bhavanas of Dashamoola kwatha, which was prepared according to classical Kwatha kalpana⁷. Thus obtained drug was in the form of thick paste, which was dried in shade. The dried material is fed in to the tablet-pressing machine. The final product was in the form of tablets weighing approximately 500mg.

Phytochemical Assay of Dashamooladi Rasayana Compound

Dashamooladi Rasayana Compound was analyzed by using qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory of I. P. G.T & R. A., Gujarat Ayurved University, Jamnagar.

RESULTS AND DISCUSSION

Organoleptic Parameters

The characters of the sample are tabulated in **table 2**.

Physico-Chemical Parameters

Dashamooladi Rasayana Compound was evaluated for various physico chemical parameters like average weight, Hardness, Disintegration time, Loss on drying, Water soluble extract, Alcohol soluble extract, Total Ash Value, pH value. The results were placed at **table 3**. The Common parameters mentioned for compressed tablets in Ayurvedic Pharmacopoeia of India⁹ and CCRAS guidelines¹⁰ are total ash, pH Value, water and alcohol soluble extractives. On its basis the parameters were selected. Presence of more moisture content in a sample can create preservation problem. Hence loss on drying was also selected as one of parameters. The water-soluble extractive and methanol soluble extractive values were found to be 21.4% and 14.4 % respectively.

Qualitative Tests

The methanol extract of the sample was analyzed qualitatively for different functional groups. Details are placed at **table 4**.

Thin Layer Chromatography Study

Thin layer chromatography of Methanol Extract of Dashamooladi Rasayana Compound: Powder of Dashamooladi Rasayana Compound weighing 5 gm are taken with 100 ml of alcohol and kept for twenty-four hours. Filtrate was prepared and evaporated till it gets dried in a flat-bottomed shallow dish and concentrated on water bath to volume of requirement.

TLC is mentioned as a primary tool for identification as part of monographs on all medicinal plants. Alkaloid fraction was used for the spotting of the TLC plate (Silica gel G Pre-coated plates). Then the spotted TLC was run with the solvent systems Toluene (7 ml), Ethyl acetate (3 ml), Glacial acetic acid (0.5 ml) separately. And the resulting TLC pattern was viewed under long wave ultra violet light at 366 nm and Short wave ultra violet light at 254 nm (**Table 5**). Then after spraying with the Anisaldehyde Sulphuric acid reagents and drying in a hot air oven and the number of spots viewed under daylight (**Table 6**). (**Figure 1**)

TLC of alcoholic extract of drug on silica gel "G" plate using Toluene (7 ml): Ethyl acetate (3 ml): Glacial acetic acid (0.5 ml) shows 8 spots under 366 nm U.V. at hRf 37,44,51,56,63,75,84 and 95. Where as in 254 nm UV three zones visible at hRf 32, 48 and 63. On running mobile phase over stationary phase, well distributed, distinct, clear spots were observed without clumping. Thin Layer Chromatography of Dashamooladi Rasayana Compound (Methanol extract) after spraying Anisaldehyde Sulphuric acid followed by heating and then visualized in day light shows 4 prominent spots at hR_f 20, 60, 87 and 95.

High-Performance Thin Layer Chromatography Study

Methanol extract of Dashamooladi Rasayana Compound were spotted on pre-coated silica gel GF 60₂₅₄ aluminium plate as 5mm bands, 5mm apart and 1cm from the edge of the plates, by means of a Camag Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene (7 ml), Ethyl acetate (3 ml), Glacial acetic acid (0.5 ml) (v/v) (20ml) was used as a mobile phase. The development distance was 6.4 cm (development time 30 min.). After development, Densitometric scanning was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of win CATS software (V 1.2.1 Camag) (**Fig 2**). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 20 mm s⁻¹. Then the plate was sprayed with Anisaldehyde Sulphuric acid followed by heating and then visualized in day light shows 7 prominent spots.

Visual observation under UV showed few spots, but on analyzing under densitometer much more was observed. This may be due to the limitations of the integrative system Savitsky – Golay 7 with following specifications like Slope – 5; Minimum height – 10AU; Minimum area – 50 AU and Maximum height was 990 AU. However, chromatogram shows 7 prominent spots at hR_f 3, 16, 42, 61, 68, 84, 98 in short wave uv 254 nm and 2 prominent spots at hR_f 3, 98 in long wave UV 356 nm.

CONCLUSION

The Rasayana Choorna is known for the treatment of peripheral and central nerve dysfunctions since ancient time. This formula is modified and fortified with Dashamoola Kwatha and Ashwagandha for better clinical efficacy. To overcome the unwieldy situation of Kwatha preparation and consumption of dry powder an effort is made to convert it in to convenient tablet form. This not only enhances patient compliance but also prolongs shelf life. In the present study, the tablets were evaluated for pharmaceutical feasibility and for patient palatability.

The ingredients were identified and authenticated pharmacognostically and were used for the preparation. The formulation was subjected to phytochemical, physico-chemical, TLC and 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.

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Table 1: Ingredients of Dashamooladi rasayana compound

Sl. No.	Drug	Botanical Name	Parts Used
1	Gokshura	<i>Tribulus terrestris</i> Linn.	Fruit
2	Amalaki	<i>Emblica officinalis</i> Gaertn.	Dried fruit
3	Amruta	<i>Tinospora cordifolia</i> (Willd.)Miers.	Stem
4	Ashwagandha	<i>Withania somnifera</i> Dunal.	Root
5	Bilva	<i>Aegle marmelos</i> Carr.	Aerial parts
6	Agnimantha	<i>Clerodendrum phlomidis</i> Linn.	
7	Shyonaka	<i>Oroxylum indicum</i> Vent.	
8	Patala	<i>Stereospermum suaveolens</i> DC.	
9	Gambhari	<i>Gmelina arborea</i> Roxb.	
10	Shalaparni	<i>Desmodium gangeticum</i> DC.	
11	Prushniparni	<i>Uraria picta</i> Desv.	
12	Brihati	<i>Solanum indicum</i> Linn.	
13	Kantakari	<i>Solanum surattense</i> Burm. F.	
14	Gokshura	<i>Tribulus terrestris</i> Linn.	

Table 2: Organoleptic parameters of Dashamooladi rasayana compound

S.No	Parameters	Sample - DRC
1	Appearance	Compressed Tablet
2	Color	Greyish white
3	Taste	Bitter, Astringent
4	Odor	Non Specific

Table 3: Physico - chemical parameters

S. No.	Parameters	Sample - DRC
1.	Weight	Average - 448 mg Minimum - 420 mg Maximum - 468 mg
2.	Hardness	0.9kg/cm ²
3.	Disintegration Time	8 minutes
4.	Loss on drying	7.44 % w/w
5.	Water soluble Extract	21.4 % w/w
6.	Alcohol soluble Extract	14.4 % w/w
7.	Total Ash	9.4 % w/w
8.	pH Value (5% of Aqueous solution)	3.88

Table 4: Qualitative tests of Dashamooladi rasayana compound for different functional groups

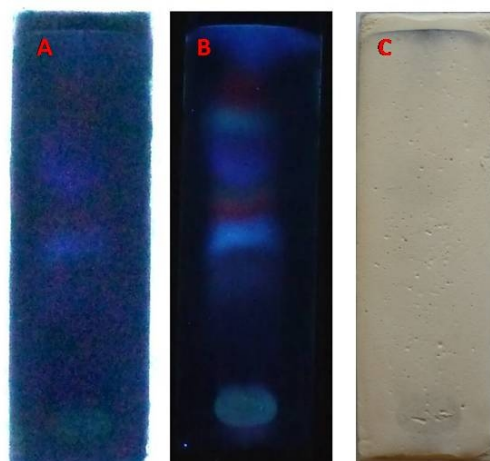
S.No	Functional Group	Name of Test/ Reagents	Results
1	Carbohydrates	Molisch's test	Positive
2	Steroids	Liebermann – Burchard test	Positive
3	Saponin Glycosides	Foam Test	Negative
4	Flavonoids	NaOH test	Positive
5	Tannins	Lead Acetate test	Positive
6	Alkaloids	Dragendroff's test	Positive
7	Glycosides	Legal's test	Positive

Table 5: TLC of Dashamooladi rasayana compound (Methanol extract)

Extract	Solvent System	Wavelength	No. of spots	R_f value	Observation under UV Light
Methanol Extract	Toluene (7 ml): Ethyl acetate (3 ml): Glacial acetic acid (0.5 ml)	366 nm UV	8	37 44 51 56 63 75 84 95	Light bluish green Blue spot Red spot Yellow spot Sky Blue spot Yellow spot Red spot Sky Blue spot
		254 nm UV	3	32 48 63	Light Blue spot Light Blue spot Light Blue spot

Table 6: TLC of Dashamooladi rasayana compound (Methanol extract) after spray

S. No.	Spray	Number of spots	R_f value	Observation
1.	Anisaldehyde Sulphuric acid	4	20	Dark Blue spot
			60	Dark Blue spot
			87	Dark Blue spot
			95	Dark Blue spot



A. 256nm
B. 366nm
C. After spraying with Anisaldehyde Sulphuric acid

Figure 1: TLC of methanol extract of Dashamooladi Rasayana Compound

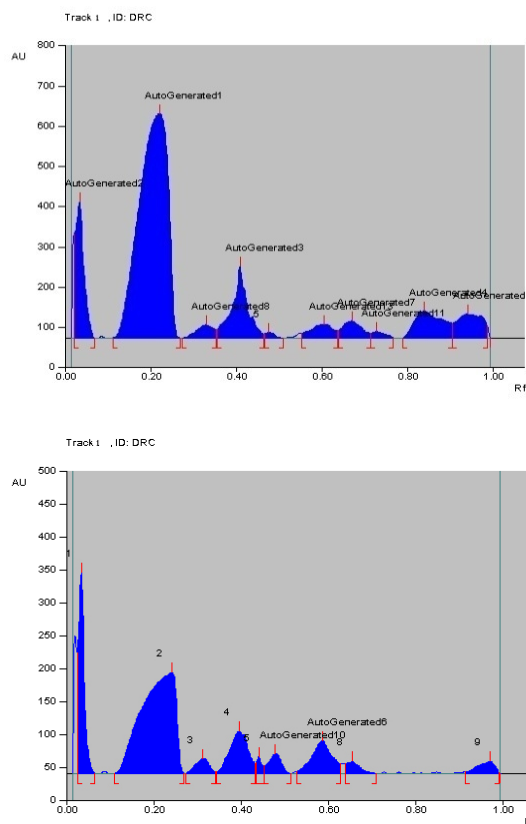


Figure 2: Densitogram curve of methanol extract of Dashamooladi Rasayana Compound at 254nm and 366nm