Research Article

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QUANTITATIVE DETERMINATION OF THREE CONSTITUENTS OF RASAYANA CHURNA (A CLASSICAL AYURVEDIC FORMULATION) BY A REVERSED PHASE HPLC

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ABSTRACT

India has long tradition of using Ayurvedic medicines as therapeutic agent. In comparison to rapid growth of herbal market, export rate of traditional Indian medicine is quite insignificant. It might be due to lack of proper quality control measures of raw materials and formulation. So, it is essential to ensure quality, safety and efficacy of raw materials and formulation. Rasayana Churna is a classical Ayurvedic formulation, which comprises dried powders of three well known rejuvenating drugs viz. dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur (*Tribulus terrestris* Linn.) and dried pericarp of Amalaki (*Embllica officinalis* Gaertn.) in equal proportion. It is used in Ayurveda as Rasayana to enhance general body resistance, promote longevity, as anti-stress and adaptogen. Therapeutic activity of Rasayana Churna may be attributed to cordifolioside A, diosgenin and gallic acid. Cordifolioside A is an active constituent of Guduchi (*Tinospora cordifolia* Miers.). Diosgenin is steroidal saponin present in Gokshur (*Tribulus terrestris* Linn.) and gallic acid is a phenolic compound present in Amalaki (*Embllica officinalis* Gaertn.). Several research work have reported the quantitative estimation of active constituent in individual drug but no established method was found for quantitative analysis of Rasayana Churna. This is the first attempt ever regarding quantitative estimation of Rasayana Churna by RP-HPLC. Various validated methods were used for estimation of cordifolioside A, diosgenin and gallic acid. These RP-HPLC methods can be used for routine quality control of raw materials and Rasayana Churna.

Keywords: Rasayana Churna, Reversed-phase HPLC, Cordifolioside A, Diosgenin, Gallic acid

INTRODUCTION

India has long tradition of using Ayurvedic medicines as therapeutic agent. In recent years, a renewed interest has been shown by many people throughout the world to know more about the contributions that have been made or that can be made in future by the Ayurvedic Medicine (Indian System of Medicine). The World Health Organization (WHO) estimated that 80 % of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs^{1,2}. In comparison to rapid growth of herbal market, export rate of traditional Indian medicine is quite insignificant. It might be due to lack of proper quality control measures of raw materials and formulation. So, it is essential to ensure quality, safety and efficacy of raw materials and formulation³. Instrumental analytical methods play a significant role in assessing the authenticity and quality of herbal drugs hence these methods are widely used for quality control. The persistent and excessive stress of modern life and the explosion of conventional therapeutic drugs (chemical stressor) have made us recognize with new class of diseases, which we call the stress-related diseases⁴. In Ayurveda stress related diseases and their treatment with vitalizes and rejuvenators is well documented under the heading of Rasayana by charak⁵. The main purpose of Rasayana therapy is to prevent the process of ageing, minimize problems of elderly and enhance the immunity of the body. Rasayana Churna is a classical Ayurvedic formulation, which comprises dried powders of three well known rejuvenating drugs viz. dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur

(*Tribulus terrestris* Linn.) and dried pericarp of Amalaki (*Embllica officinalis* Gaertn.) in equal proportion^{6,7}. As name suggested, it is used in Ayurveda as Rasayana to enhance general body resistance, promote longevity, as anti-stress and adaptogen⁷⁻⁹. The therapeutic efficacy of Rasayana Churna might be due to presence of secondary metabolites like alkaloids, glycosides, steroids, flavonoids, tannins, terpenoids and phenolic compounds. As its evident that quantification of active constituents can play important role to ensure quality of raw materials and formulation. In the present study three active constituent's viz. cordifolioside A, diosgenin and gallic acid were taken to establish quality of Rasayana Churna. Cordifolioside A is an active constituent of Guduchi (*Tinospora cordifolia* Miers.)¹⁰. Diosgenin is steroidal saponin present in Gokshur (*Tribulus terrestris* Linn.)¹¹ and gallic acid is a phenolic compound present in Amalaki (*Embllica officinalis* Gaertn.)¹². Several research work have reported the quantitative estimation of active constituent in individual drug but no established method was found for quantitative analysis of Rasayana Churna. This is the first attempt ever regarding quantitative estimation of Rasayana Churna by RP-HPLC (Reversed Phase – High Performance Liquid Chromatography). We have used validated RP-HPLC methods for the estimation of above mentioned active components respectively¹⁰⁻¹⁴. In previous research publication, Quality of raw materials and Rasayana Churna was already ensured by different physico-chemical parameters, heavy metal analysis and microbial load¹⁵.

MATERIALS AND METHODS

Chemicals

HPLC grade solvents and other analytical grade solvents were purchased from Merck Ltd., Mumbai, India. Cordifolioside A was purchased from Chromodex; India. Diosgenin was purchased from Sigma Aldrich; India. Gallic acid was purchased from Himedia Laboratories Pvt. Ltd.; India. Purity of Cordifolioside A was found to be > 60 %, diosgenin and gallic acid were found to be > 98 %.

Test materials

Collection and authentication of raw materials

Raw materials were collected from Atarsumba forest department, Vireshwar, Sabarkantha, Gujarat, India during the winter season. Authentication of raw materials was carried out at Department of Botany, The H.N.S.B. Ltd. Science College, Himmatnagar, Gujarat, India.

Preparation of Rasayana Churna

The Rasayana Churna was prepared as per the general method describe in Ayurvedic Formulary of India. All the ingredients were shade dried and powdered separately, passed through 80# sieve and then mixed together in equal proportions to get uniformly blended Churna. Rasayana Churna was finally passed through 80# sieve.

Preparation of standard solution

For Cordifolioside A: 10 mg Cordifolioside A was dissolved in 10 mL methanol.

For Diosgenin: 2.4 mg diosgenin was dissolved in 10 mL Acetonitrile: Methanol (8:2 v/v) solution.

For Gallic acid: 2.5 mg Gallic acid was dissolved in 25 mL Water: Acetonitrile: Glacial Acetic Acid (90: 8: 2 v/v/v) solution.

Sample Preparation

For Guduchi powder and Rasayana Churna

500 mg of sample was weighed and transferred to refluxing flask. 25 mL methanol was added and refluxed it for 1 h. Content was filtered through 0.22 mm filter paper. Final volume was made up to 25 mL with methanol.

For Gokshur powder and Rasayana Churna

5 g of sample was refluxed with 50 mL of 10 % w/v sulphuric acid for 4 h. Content was transferred to separating funnel and extracted with 50 mL ethyl acetate (3 times). Collected ethyl acetate was passed through sodium sulphate bed. Dry residue was obtained after evaporation of ethyl acetate. 500 mg residue was dissolved in 50 mL of methanol. Solution was filtered using 0.22 mm filter paper and used as test solution.

For Amalaki powder and Rasayana Churna

100 mg sample was dissolved in 25 mL Water: Acetonitrile: Glacial Acetic Acid (90: 8: 2 v/v/v) solution. Solution was filtered using 0.22 mm filter paper and used as test solution.

Chromatographic conditions for HPLC

The liquid chromatography was performed on a Shimadzu LC-20AT, SPD-20A HPLC instrument equipped with UV detector UV-20A with wavelength range 190-700 nm and Spinchrom LC Solution software. Phenomenex Luna C-18 column (250 × 4.6 mm, 5 µm particle size) was used as stationary phase. Rheodyne manual injector with 20 µL capacity loop was used.

For Cordifolioside A: Mobile phase consisted of Acetonitrile: Water (25:75 v/v) and detection wavelength was set at 210 nm¹⁰.

For Diosgenin: Mobile phase consisted of Acetonitrile: Methanol (80:20 v/v) and detection wavelength was set at 210 nm¹³.

For Gallic acid: Mobile phase consisted of Water: Acetonitrile: Glacial Acetic Acid (90: 8: 2 v/v/v) and detection wavelength was set at 272 nm¹⁴.

RESULTS AND DISCUSSION

Bauer and Tittel¹⁶ and Springfield *et al.*¹⁷ reported that HPLC fingerprinting is the best way for chemical characterization. The present RP-HPLC methods were conducted to quantify three active constituents (viz. cordifolioside A, diosgenin and gallic acid) of Rasayana Churna which also play important role in therapeutic efficacy. Quantification of cordifolioside A was carried out in Guduchi powder and Rasayana Churna. Cordifolioside-A isolated from *Tinospora cordifolia* stems have been identified as active principles responsible for immune stimulant action¹⁸ and potential *in-vivo* radio-protective effect as well as *in-vitro* cytoprotective activity¹⁹. In study 0.57 % and 0.24 % cordifolioside A was found in Guduchi powder and Rasayana Churna respectively (Table 1). HPLC chromatograms of cordifolioside A standard, Guduchi powder and Rasayana Churna are shown in Figure 1, Figure 2 and Figure 3 respectively. Diosgenin is a naturally occurring steroidal saponin abundantly present in many medical plants and one of the active constituent of *Tribulus terrestris*. Diosgenin could stimulate lymphocyte transformation and enhance phagocytic capability of macrophages *in-vitro*, and remarkably promoted the secretion of nitric oxide and TNF-α in macrophages. It could improve both specific and non-specific cellular immune responses²⁰. Gokshur powder and Rasayana Churna was standardized by estimation of Diosgenin through HPLC. Diosgenin was found to be 1.69 % and 0.58 % in Gokshur powder and Rasayana Churna respectively (Table 2). HPLC chromatograms of diosgenin standard, Gokshur powder and Rasayana Churna are shown in Figure 4, Figure 5 and Figure 6 respectively. The anti-oxidant and pro-oxidant mechanism of gallic acid is most likely due to the strong reducing power and weak metal chelating ability²¹. Amalaki powder and Rasayana Churna was standardized by estimation of gallic acid through HPLC. Gallic acid was found to be 11.02 % and 4.13 % in Amalaki powder and Rasayana Churna respectively (Table 3). HPLC chromatograms of gallic acid standard, Amalaki powder and Rasayana Churna are shown in Figure 7, Figure 8 and Figure 9 respectively.

Table 1: Percentage of cordifolioside A in Guduchi powder and Rasayana Churna

S. No.	Name of the sample	Retention time (Min.)	Area (mV. s)	Percentage of cordifolioside A (% w/w)
1	Cordifolioside A (Standard)	9.724	4781	---
2	Guduchi powder	9.723	864	0.57 %
3	Rasayana Churna	9.713	375	0.24 %

Table 2: Percentage of diosgenin in Gokshur powder and Rasayana Churna

S. No.	Name of the sample	Retention time (Min.)	Area (mV.s)	Percentage of diosgenin (% w/w)
1	Diosgenin (Standard)	2.297	4924	---
2	Gokshur powder	2.327	3421	1.69 %
3	Rasayana Churna	2.314	1174	0.58 %

Table 3: Percentage of gallic acid in Amalaki powder and Rasayana Churna

S. No.	Name of the sample	Retention time (Min.)	Area (mV.s)	Percentage of gallic acid (% w/w)
1	Gallic acid (Standard)	4.620	1081	---
2	Amalaki powder	4.690	4639	11.02%
3	Rasayana Churna	4.850	1759	4.13%

Figure 1: HPLC chromatogram of cordifolioside A (Standard)

Figure 2: HPLC chromatogram of Guduchi (*Tinospora cordifolia* Miers.) powder

Figure 3: HPLC chromatogram of Rasayana Churna for cordifolioside A

Figure 4: HPLC chromatogram of diosgenin (Standard)

Figure 5: HPLC chromatogram of Gokshur (*Tribulus terrestris* Linn.) powder

Figure 6: HPLC chromatogram of Rasayana Churna for Diosgenin

Figure 7: HPLC chromatogram of gallic acid (Standard)

Figure 8: HPLC chromatogram of Amalaki (*Emblca officinalis* Gaertn.) powder

Figure 9: HPLC chromatogram of Rasayana Churna for gallic acid

CONCLUSION

Rasayana Churna was standardized by quantification of cordifolioside A, diosgenin and gallic acid. These RP-HPLC methods may be used for routine quality control of raw materials and Rasayana Churna.

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REFERENCES

- Dobriyal RM and Narayana DBA. Ayurvedic herbal raw material. The Eastern Pharmacist; 1998. p. 31-5.
- Venkata Rao. Modern approaches to herbal medicine. The Eastern Pharmacist 2000; 12: 35-9.
- Palav YK, D'mello PM. Standardization of selected Indian medicinal herbal raw materials containing poly phenols as major phytoconstituents. Indian J Pharm Sci 2006; 68(4): 506-9. <http://dx.doi.org/10.4103/0250-474X.27829>
- Singh N, Kumar P, Ahmad S, Singh RP, Kohli RP, Bhargava KP. Anti-stress plant drugs - A new approach in the treatment of stress diseases. Planta med 1982; 45(3): 138. <http://dx.doi.org/10.1055/s-2007-971282>
- Charak. Charak Samhita Chikitsa Sthana, published by Ayurvedic Society of India, 3rd edition; 1949. p. 1278.
- Vagbhata. Ashtanga Hridayam, with the commentaries, 'sarvangasundara' of Arunadatta and 'Ayurvedarasayana' of Hemadri collated by Dr Anna Moreshvara kunte, and Krishna Ramachandra Shastri Navre, edited by pt. Harishastri Paradakar Vaidya, Gopal Mandir Lane, Uttar sthan, chapter 39/160. Varanasi: Krishanadas Academy; 2000. p. 937.
- Anonymous. The Ayurvedic Formulary of India. part-III, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, New Delhi, 1st edition; 2011. p. 120.
- Deole YS, Chavan SS, Ashok BK, Ravishankar B, Thakar AB, Chandola HM. Evaluation of anti-depressant and anxiolytic activity of Rasayana Ghana Tablet (A compound Ayurvedic formulation) in albino mice. AYU 2011; 32: 375-9. <http://dx.doi.org/10.4103/0974-8520.93918>
- Deole YS, Chavan SS, Ashok BK, Ravishankar B, Thakar AB, Chandola HM. Experimental study on adaptogenic and antiulcer activity of Rasayana Ghana tablet (a tri-herbal formulation) in albino rats. Indian J Nat Prod Resour 2013; 4(1): 73-80.
- Alam P, Ali M, Singh R, Ahmad S, Shakeel F. A validated HPLC method for estimation of cordifolioside A in *Tinospora cordifolia*, Miers and marketed formulations. J Chromatogr Sci 2009; 47(10): 910-3. <http://dx.doi.org/10.1093/chromsci/47.10.910>
- Gupta P, Nagore D, Kuber V, Purohit S. A validated RP-HPLC method for the estimation of diosgenin from Polyherbal formulation containing *Tribulus terrestris* Linn. Asian J Pharm Clin Res 2012; 5(4): 91-4.
- Biswas A, Chatterjee A, Bahdhopadhyay S. Quantitative estimation of gallic acid in Amla extract by gradient RP-HPLC method. Int J Pharm Inno 2012; 2(1): 72-76.
- Anonymous. Indian Pharmacopeia. Government of India, Ministry of Health and Family Welfare, New Delhi 2007; 3: 2034.
- Magdalena K, Agnieszka K, Ronald B. Content of Gallic acid in selected plant extracts. Pol J Food Nutr Sci 2006; 15/56(1): 55-8.
- Soni H, Bhatt S, Patgiri B. Standardization of Rasayana Churna – A classical Ayurvedic formulation. Int J Res Pharm Sci 2013; 4(2): 283-9.
- Bauer R, Tittel G. Quality assessment of herbal preparations as a precondition of pharmacological and clinical studies. Phyto med 1996; 2: 193-8. [http://dx.doi.org/10.1016/S0944-7113\(96\)80041-9](http://dx.doi.org/10.1016/S0944-7113(96)80041-9)
- Springfield EP, Eagles PKF, Scott G. Quality assessment of South African herbal medicines by means of HPLC fingerprinting. J Ethno pharmacol 2005; 101: 75-83. <http://dx.doi.org/10.1016/j.jep.2005.03.012>
- Maurya R, Wazir V, Kapil RS. Cordifolioside-A and B, two new phenylpropene disaccharides from *Tinospora cordifolia* possessing immunostimulant activity. Nat Prod Lett 1996; 8: 7-10. <http://dx.doi.org/10.1080/10575639608043231>
- Patel A, Bigoniya P, Singh CS, Patel NS. Radio protective and cytoprotective activity of *Tinospora cordifolia* stem enriched extract containing cordifolioside-A. Indian J Pharmacol 2013; 45(3): 237-43. <http://dx.doi.org/10.4103/0253-7613.111919>
- Zhongmei He, Yudan Tian, Xiantao Zhang, Bai Bing, Lianxue Zhang, Huaisheng Wang *et al.* Anti-tumour and immune modulating activities of diosgenin, a naturally occurring steroidal saponin. Nat Prod Lett 2012; 26(23): 2243-6. <http://dx.doi.org/10.1080/14786419.2011.648192>
- Gow Chin Yen, Pin Der Duh, Hui Ling Tsai. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem 2002; 79(3): 307-13. [http://dx.doi.org/10.1016/S0308-8146\(02\)00145-0](http://dx.doi.org/10.1016/S0308-8146(02)00145-0)

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