

Scientific Evaluation of *Sarasvata Churna* - An Ayurvedic formulation -

Ashok Kumar Tiwari, Manoj Kumar Tripathi, Neelesh Dwivedi



Abstract:

The present study deals with the standardization of the Ayurvedic formulation *Sarasvata churna* following quality control procedures both for the raw materials and the finished product. The results of physico-chemical parameters viz. loss on drying (7.0%), total ash (14.50%), acid insoluble ash (9.0%), water soluble extractive value (23.0%), alcohol soluble extractive value (37.50%), pH (4.5-5.5) and volatile oil (1.0%) were found. Microbiological limit test, aflatoxins and heavy metals Pb, Cd, As, Hg were also found within the limits set by Ayurvedic Pharmacopoeia of India (API). The obtained values can be adapted to lay down new pharmacopoeial standards with batch to batch consistency. The phytochemical constituents found in the raw material used for the preparation of *Sārasvata cūrna* facilitate the desirable therapeutic efficacy of the medicinal formulations a whole in elements and also could help in knowing the underlying mechanism of pharmacological action.

Key words: Ayurvedic formulation, *Sarasvata churna*, standardization.

Introduction

Ayurveda is an indigenous Indian system of medicine that is mainly plant-based and has gained worldwide attention due to safety and efficacy. However, due to lack of proper quality control methods there are variations in the same product obtained from different sources. Standardization is important for insuring of good quality products as standardized drugs of well defined consistent quality are needed for reliable beneficial therapeutic uses. Thus, there is an urgent need to develop parameters for quality control which are cost effective and can be easily adopted by the manufactures. Efforts are being made in this area that have led to the development of analytical protocols both for single herbal drugs as well as for compound herbal formulations [1 to 8] that can be used as valuable analytical tools in the routine standardization of Ayurvedic drugs and formulations.

Standards such as identity, purity and potency of single drugs as well as formulations are important regarding therapeutic efficacy of herbal medicines. If the raw materials to be used in a medicine and stage by stage processes of manufacturer standardized the final product namely, the compound formulation could be expected to conform to uniform standards [9].

Sarasvata churna is an Ayurvedic compound formulation has been use in *Apasmāra* (Epilepsy), *Unmāda* (Mania/psychosis), retarded intelligence, *Medhya* (brain tonic/nootropic, low intelligence and *Smrtiprada* (memory provider) [10], etc. The preparation of *Sārasvata*

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cūrna is based on traditional methods in accordance with the procedures given in Ayurvedic formulary of India. There is a lack of modern quality standards for such Ayurvedic formulation like *Sarasvata churna* and standard quality methods to get desired quality and batch to batch consistency. Hence there is a need for standardization of Ayurvedic *churna* following modern parameters including taxonomic identification of raw drugs, organoleptic characters, powder microscopic characteristics, phyto-chemical analysis, chromatographic pattern and microbial screening.

The work undertaken is the trust as part of a program of testing and validation of traditional practices of using the Ayurvedic medicine. In this connection, standardization of *Sarasvata churna* becomes imperative. This paper dealt with standardization followed according to GMP guideline. Standardization guidelines to be followed for herbals products provided by World Health Organization [11], and Ayurvedic pharmacopoeia of India have been considered.

Materials and Methods

Preparation of the Churna

All the ingredients used for the preparation of *churna* were collected from forest & market and authenticated by taxonomist [12-15]. The compositions of formulation are given in table 1. The ingredients were washed, dried and ground individually passed through 180 μm mesh separately then weighed separately, mixed in specified ratio and passed through 355 μm mesh to obtain a homogenous blend. It was stored in an airtight container to protect from light and moisture. Three different batches of *Sarasvata churna* were prepared at research laboratory Ayurveda Sadan, Chitrakoot, Madhya Pradesh, India for standardization.

Physico-chemical parameters

Organoleptic characters, particle size and physico-chemical analysis [16-17] of all the samples were carried out. Quantitative analysis for total ash, acid insoluble ash, extractive values in water soluble and alcohol soluble extractive, loss on drying at 105°C, volatile oil and pH of filtrate of 10% w/v aqueous solution were checked in triplicate according to the prescribed Standard methods in Indian Pharmacopoeia

High Performance Thin layer chromatography (HPTLC) profile

Took 5 gm of coarsely powdered drug in 250 ml stoppered conical flask and extracted with 100 ml *ethanol* for 24 hours by maceration technique with occasional shaking. HPTLC of extracts of all the samples were carried out on silica gel 60 F₂₅₄ precoated plates. The mobile phase used was Toluene: Ethyl acetate: Formic acid (7: 2.5: 0.5). The plate was developed and visualized under ultraviolet at 254nm, 366nm and visible light. After spraying with *Dragendorff's* reagent followed by heating at 105°C for 5 min. [18-19].

Test for Aflatoxin

Three samples of *Sarasvata churna* were also checked for mycotoxin, i.e. Aflatoxin with standard markers B₁, B₂, G₁ & G₂ [20].

Test for microbial limits

Following tests were carried out as per WHO to determine the microbial load [21-22] in three batches of *Sarasvata churna*, a formulated compound drug powder of pharmaceutical substances

- (1) Enumeration of *Staphylococcus aureus* /gm
- (2) Enumeration of *Salmonella sp.*/gm
- (3) Enumeration of *Pseudomonas aeruginosa*/gm
- (4) Determination of *E.coli*
- (5) Determination of total bacterial count (TBC)
- (6) Determination of Yeast and mould.

The microbiological tests were determined using specified agar and enrichment media from Himedia and Privet Limited Mumbai.

Heavy metal

Heavy metal analysis [23] (lead, cadmium, arsenic and mercury) were carried out using Atomic absorption spectrophotometry (Shimadzu - Model - AA-7000). All samples are digested with concentrated HNO₃: HClO₄ (4:1). Standards solutions are made for different dilution to get linear calibration (Merck). Pb and Cd were performed using graphite oven method, while As (Arsenic) were determined as hydride method and Hg were determined using cold absorption method.

Assay of Sodium and Potassium

Sodium (Na) and Potassium (K) [24] were carried out using Flame photometer (ELCO - Model-CL361) and results are presented in percent.

Observations and results

The samples of *Sarasvata churna* were found to be a brown coloured, fine powder with pleasant smell. The results of physico-chemical parameters viz. loss on drying (7.0%), total ash (14.50%), acid insoluble ash (9.0%), water soluble extractive value (23.0%), alcohol soluble extractive value (37.50%), pH (4.5-5.5) and volatile oil (1.0%) were found. [Table 2]. The HPTLC plate were examine under ultra violet light at 254 nm; at 366 nm; at visible for both before and after derivatization with *Dragendorff's* reagent [Figures 1, 2, 3 and 4]. The R_f values and colours of the bands obtained were recorded [Tables 3, 4, 5 and 6]. The aflatoxin was absent in the formulated *Sarasvata churna* [Table 7 and Figure 5]. Total bacterial count, Yeast and Moulds counts were reported less than the limit set by API. Pathogenic bacteria, i.e. Salmonella, *Pesudomonas*, *Staphylococcus* and *E.coli* were not detected in samples [Table 8 and Figures 6-6A, 6B, 6C, 6D, 6E and 6F]. In the present study the level of heavy metals viz. Pb (0.9016-1.2951 ppm), Cd (0.3335-3535 ppm), As (0.0013-0015 ppm), Hg (0.0129-0139 ppm) which are within limit set by WHO [Table 9]. The concentration of Na and K are found to ranges 362-380 and 311-320 ppm respectively (Table 9).

Discussion

The present formulation consisted of thirteen plant ingredients, which were proved to be genuine, by assessing the organoleptic characters. Physico-chemical parameters were applied for assessing the prepared formulation. Loss on drying indicates the moisture content of the product because higher value of moisture tends to degradation of the components. The pH of *Sarasvata churna* was found to be 4.5-5.5, showing the acidic nature of the drug. Higher percentage of extractive values shows the solubility of compounds with respective solvents, which is a good sign for efficacy because more solubility tends to good activity. HPTLC showed 11 spots at 254 nm, 10 spots at 366 nm, while for after spraying with *Dragendorff's* reagent. The results of R_f values were observed common for 4 values for both the detection wavelengths well as after derivatisation, which showed that the components were sensitive to both wavelengths and conditions. Aflatoxin are highly dangerous and should be absent. Total bacterial count, Yeast and Moulds counts were reported less than the limit set by API. Pathogenic bacteria, i.e. Salmonella,

Pesudomonas, *Staphylococcus* and *E.coli* were not detected in samples. Heavy metals if present in the drug may carcinogenic and toxic. In the present study the level of heavy metals viz. Pb, Cd, As, Hg are within limit set by WHO.

Conclusion

The present work deals with the physicochemical, aflatoxins, microbiol and heavy metals study of the *Sarasvata churna*. Observed heavy metals data for *Sarasvata churna* presence of Pb, Cd, As, Hg which are within limit set by WHO. The concentration of Na and K are found to ranges 362-380 and 311-320 ppm respectively. HPTLC profile shows 11 spots at 254 nm, 10 spots at 366 nm and after spraying 12 spots at 366 nm, 7 spots at visible light. All the above physicochemical, aflatoxins, microbial and heavy metals data can be useful as diagnostic tool for identification and play an important role in quality control for further research.

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References

- [1] Rastogi S, Khatoon S, Rai V, Agnihotri AK, Rawat AKS, Mehrotra S. Evaluation of Ayurvedic compound formulations-Naraca Cūrṇa, Indian J Tradit Knowle, 2005; 42(2): 185-189.
- [2] Simha KRG, Laxaminarayan V. Standardization of Ayurvedic polyherbal formulation, Nyagrodhadi churna, Indian J Tradit Knowle, 2007; 6(4): 648-652.
- [3] Rastogi S, Khatoon S, Pandey MM, Rathi A, Rawat AKS, Mehrotra S. Evaluation of Ayurvedic compound formulations 2 - Palashbijadi Cūrṇa, Indian J Tradit Knowle, 2008; 7(3): 384-388.
- [4] Rathi A, Rai V, Khatoon S, Rastogi S, Rawat AKS, Mehrotra S. Evaluation of Ayurvedic compound formulations 3 - Laghugangadhar Cūrṇa, Indian J Tradit Knowle, 2010; 9(3): 576-580.
- [5] Tiwari AK, Tripathi MK, Dwivedi N, Jaiswal A, Shukla P, Mishra R, Tripathi SP. Quality Control of Musali Curna A Traditional Ayurvedic Formulation, Life Sci Bull, 2012; 9(2): 417-424.
- [6] Gupta P, Tiwari AK, Chaturvedi A. Standardization of Ayurvedic formulation- Kutajadi Kashay Curna, J Chem Pharmaceut Res, 2013; 5(10): 34-38.

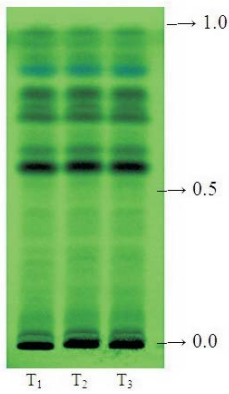


Figure 1 TLC profile of Sārasvata cūrna observed under 254 nm

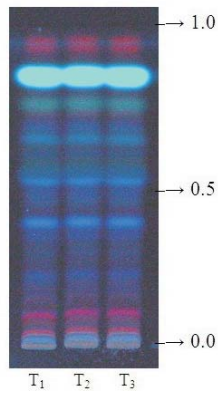


Figure 2 TLC profile of Sārasvata cūrna observed under 366 nm

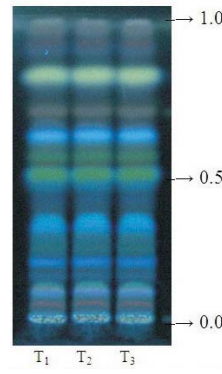


Figure 3 TLC profile of Sārasvata cūrna after spraying with Dragendorff's reagent observed under 366 nm

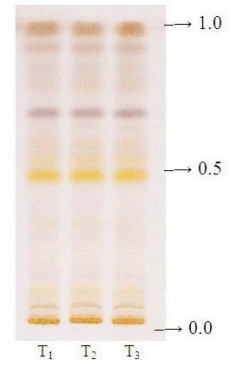


Figure 4 TLC profile of Sārasvata cūrna after spraying with Dragendorff's reagent observed under visible light

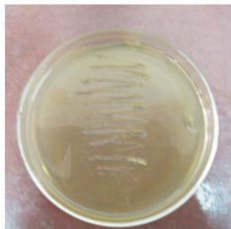


Figure 6A -Plate showing negative result for Staphylococcus

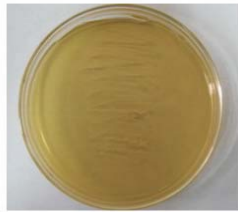


Figure 6B- Pseudomonas aeruginosa

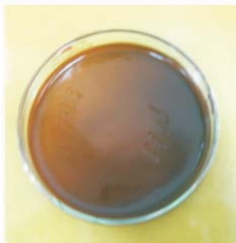


Figure 6C: Plate showing negative results for Salmonella sp

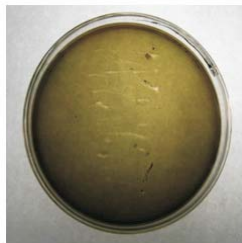


Figure 6D -Plate showing negative results for E. Coli



Figure 6E -Plate showing results for Total bacterial counts

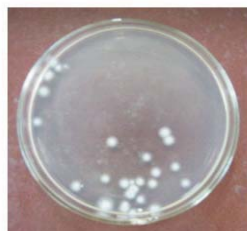


Figure 6F- Plate showing results for Yeast & Moulds

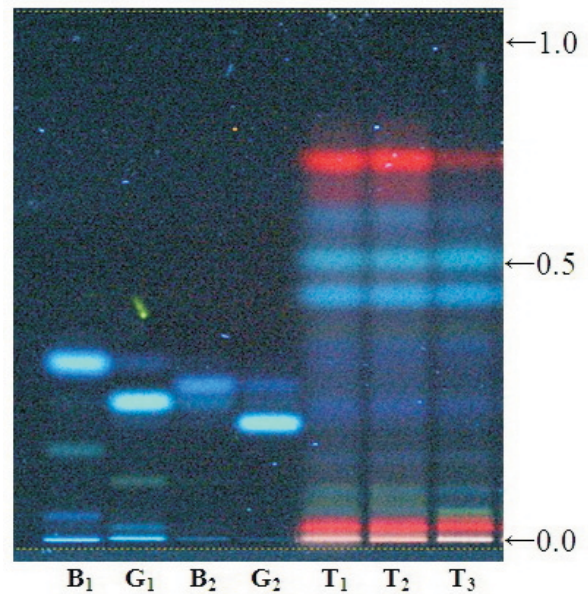


Figure 5 TLC Finger prints of test solution for Aflatoxins in Sārasvata cūrna at 366 nm

Table 1: Formulation composition of Sarasvata churna

Sanskrit name of ingredients	Botanical name	Part Used	Proportion
Kuṣṭha	<i>Saussurea lappa</i>	Root	1 Part
Aśvagandhā	<i>Withania somnifera</i>	Root	1 Part
Lavaṇa (Saindhava lavaṇa)	Rock Salt	-	1 Part
Ajamodā	<i>Apium leptophyllum</i>	Fruit	1 Part
Sveta jīraka	<i>Cuminum cyminum</i>	Fruit	1 Part
Ḳṛṣṇajīraka	<i>Carum carvi</i>	Fruit	1 Part
Śunṭhī	<i>Zingiber officinale</i>	Rhizome	1 Part
Marica	<i>Piper nigrum</i>	Fruit	1 Part
Pippalī	<i>Piper longum</i>	Fruit	1 Part
Pātha	<i>Cissampelos pareira</i>	Root	1 Part
Maṅgalyapusṭī (Śāṅkhapusṭī)	<i>Convolvulos pluricaulis</i>	Whole plant	1 Part
Vacā	<i>Acorus calamus</i>	Rhizome	11 Parts
Brāhmarasa (Brāhmī)	<i>Bacopa monnieri</i>	Whole plant	3 bhāvanās

Table 2: Physico-chemical analysis of Sarasvata churna

Parameters	Results
LOD at 105° C	7.0 %
Total Ash	14.50 %
Acid-insoluble ash	9.00 %
Water-soluble extract	23.00 %
Alcohol-soluble extract	37.50 %
pH (10% aqueous solution)	4.5 - 5.5
Volatile oil (w/v)	1.00

Table 3: R_f values in test solution of Sarasvata churna at 254 nm

R _f values	Sarasvata churna		
	Batch 01	Batch 02	Batch 03
R _f 1 (black)	0.05	0.05	0.05
R _f 2 (green)	0.26	0.26	0.26
R _f 3 (green)	0.34	0.34	0.34
R _f 4 (green)	0.44	0.44	0.44
R _f 5 (black)	0.54	0.54	0.54
R _f 6 (black)	0.58	0.58	0.58
R _f 7 (black)	0.68	0.68	0.68
R _f 8 (black)	0.76	0.76	0.76
R _f 9 (blue)	0.80	0.80	0.80
R _f 10 (black)	0.86	0.86	0.86
R _f 11 (black)	0.93	0.93	0.93

Table 4: R_f values in test solution of Sarasvata churna at 366nm

R _f values	Sarasvata churna		
	Batch 01	Batch 02	Batch 03
R _f 1 (pink)	0.07	0.07	0.07
R _f 2 (pink)	0.12	0.12	0.12
R _f 3 (blue)	0.24	0.24	0.24
R _f 4 (blue)	0.40	0.40	0.40
R _f 5 (blue)	0.52	0.52	0.52
R _f 6 (blue)	0.64	0.64	0.64
R _f 7 (blue)	0.67	0.67	0.67
R _f 8 (grey)	0.73	0.73	0.73
R _f 9 (florescent)	0.82	0.82	0.82
R _f 10 (pink)	0.93	0.93	0.93

Table 5: R_f values in test solution of *Sarasvata churna* at 366nm (after derivatization)

R _f values	<i>Sarasvata churna</i>		
	Batch 01	Batch 02	Batch 03
R _f 1 (blue)	0.07	0.07	0.07
R _f 2 (grey)	0.10	0.10	0.10
R _f 3 (blue)	0.15	0.15	0.15
R _f 4 (blue)	0.20	0.20	0.20
R _f 5 (sky blue)	0.32	0.32	0.32
R _f 6 (blue)	0.48	0.48	0.48
R _f 7 (green)	0.53	0.53	0.53
R _f 8 (grey)	0.55	0.55	0.55
R _f 9 (blue)	0.59	0.59	0.59
R _f 10(brown)	0.69	0.69	0.69
R _f 11(light yellow)	0.78	0.78	0.78
R _f 12 (blue)	0.87	0.87	0.87
R _f 12 (grey)	0.93	0.93	0.93

Table 6: R_f values in test solution of *Sarasvata churna* at visible light (after derivatization)

R _f values	<i>Sarasvata churna</i>		
	Batch 01	Batch 02	Batch 03
R _f 1 (grey)	0.07	0.07	0.07
R _f 2 (light yellow)	0.48	0.48	0.48
R _f 3 (light yellow)	NA	NA	NA
R _f 4 (grey)	0.66	0.66	0.66
R _f 5 (light yellow)	NA	NA	NA
R _f 6 (brown)	0.87	0.87	0.87
R _f 7 (red)	0.93	0.93	0.93

Table 7: R_f values in test solutions for Aflatoxins in *Sarasvata churna* at 366 nm

R _f values	Aflatoxin Standards				<i>Sarasvata churna</i>		
	B ₁	G ₁	B ₂	G ₂	Batch 01	Batch02	Batch 03
R _f 1	Absent	Absent	Absent	0.27	Absent	Absent	Absent
R _f 2	Absent	Absent	0.35	Absent	Absent	Absent	Absent
R _f 3	Absent	0.33	Absent	Absent	Absent	Absent	Absent
R _f 4	0.39	Absent	Absent	Absent	Absent	Absent	Absent

Table 8: Microbiological limit test in compound formulation of *Sarasvata churna*

Parameters	<i>Sarasvata churna</i>			Permissible Limits (API)
	Batch 01	Batch 02	Batch 03	
<i>Staphylococcus aureus</i> /g	Absent	Absent	Absent	Absent
<i>Salmonella sp.</i> /g	Absent	Absent	Absent	Absent
<i>Pseudomonas aeruginosa</i> /g	Absent	Absent	Absent	Absent
<i>E.coli</i>	Absent	Absent	Absent	Absent
Total bacterial count. (TBC)	800 cfu/g	850 cfu/g	875 cfu/g	10 ⁵ cfu/g
Total Yeast and mould.	29 cfu/g	22 cfu/g	25 cfu/g	10. ³ cfu/g

Table 9: Heavy metals and Na and K assay of Sarasvata churna

Parameters	Sarasvata churna			Actual conc. unit	WHO Limits
	Batch 01-Result	Batch 02 -Result	Batch 03-Result		
Lead (Pb)	0.9016	1.0328	1.2951	ppm	10 ppm
Cadmium (Cd)	0.3535	0.3441	0.3312	ppm	0.3 ppm
Arsenic (As)	0.0015	0.0015	0.0013	ppm	3 ppm
Mercury (Hg)	0.0130	0.0139	0.0129	ppm	01 ppm
Sodium	380.0	362.0	368.3	ppm	-
Potassium	320.4	311.6	319.9	ppm	-

- [7] Tiwari AK, Tripathi MK, Dwivedi N. Evaluation of Ayurvedic Compound Formulation 5- Sama Śarkar C rna, Res J Pharmacog Photochem, 2014; 6(1): 9-15.
- [8] Tiwari AK, Tripathi MK, Dwivedi N, Jaiswal A, Mishra R, Tripathi SP. Evaluation of Ayurvedic Compound Formulation Vidangadi Chūrna, J Ayurveda, 2013; 7(3): 111-117.
- [9] Anonymous: The Ayurvedic Formulary of India, Government of India, Ministry of Health and Family Welfare, [The Controller of Publications, Delhi; Part- I, 2nd ed. 2000. p.130.
- [10] Misra B, Misra BS. Bhavaprakasa Unmadadhikara, Chaukhambha Sanskrit Sansthan, Varanasi (India); Part- II, 5th ed. 1993. p. 214.
- [11] Anonymous: Quality Control Methods for Medicinal Plant Materials, A.I.T.B.S. Publishers & Distributors (Regd.) New Delhi; 2002. p. 68-70.
- [12] Anonymous: The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health and Family Welfare, The Controller of Publications, Delhi; Part- I, 1st ed. vol.-1, 2001. p.2-3, 15-16, 73-74, 76-77, 92-93, 103-104, 106.
- [13] Anonymous: The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health and Family Welfare, The Controller of Publications, Delhi; Part- I, 1st ed. vol.-2, 1999. P.147-149.
- [14] Anonymous: The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health and Family Welfare, The Controller of Publications, Delhi; Part- I, 1st ed. vol.- 3, 2001. p.115-117.
- [15] Anonymous: The Ayurvedic Pharmacopoeia of India, Government of India, Ministry of Health and Family Welfare, The Controller of Publications, Delhi; Part- I, 1st ed. vol.- 4, 2004. p.91-92.
- [16] Lohar DR, Singh R, Protocol for Testing of Ayurvedic, Siddha & Unani Medicines. Government of India Department of AYUSH, Ministry of Health and Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad; 2008. p.21-24.
- [17] Anonymous: Laboratory Guide For the Analysis of Ayurveda and Siddha Formulation.: Government of India, Department of AYUSH, Ministry of Health and Family Welfare, New Delhi, Printed at Pearl Offset Press Pvt. Ltd.; 2010. p.27.
- [18] Anonymous: Quality Control Methods for Medicinal Plant Materials, A.I.T.B.S. Publishers & Distributors (Regd.) New Delhi; 2002. p.25-27.
- [19] Wall PE, Thin-Layer Chromatography, Modern Practical Approach Series Editor Smith, R.M., Published by The Royal Society of Chemistry, Thomas Graham House Cambridge-UK; 2005. p. 1-15.
- [20] Anonymous: The Ayurvedic Pharmacopoeia of India (Formulation), Government of India, Ministry of Health and Family Welfare; Department of Ayurveda Yoga & Naturopathy, Unani, Siddha & Homoeopathy, The Controller of Publications, Delhi; Part- II, 1st ed. vol.-3, 2010. p. 188-190.
- [21] Anonymous: The Ayurvedic Pharmacopoeia of India (Formulation), Government of India, Ministry of Health and Family Welfare; Department of Ayurveda Yoga & Naturopathy, Unani, Siddha & Homoeopathy, The Controller of Publications, Delhi; Part- II, 1st ed. vol.-3, 2010. p. 165-180.
- [22] Pelczar M.J, Chan ECS, Krieg NR, Microbiology, 5th ed, Tata McGraw-Hill Publishing Company Limited, New Delhi; 1993. p. 265-299, 333-364.
- [23] Anonymous: The Ayurvedic Pharmacopoeia of India (Formulation), Government of India, Ministry of Health and Family Welfare; Department of Ayurveda Yoga & Naturopathy, Unani, Siddha & Homoeopathy, The Controller of Publications, Delhi; Part- II, 1st ed. vol.-3, 2010. p. 161-164.
- [24] Anonymous: The Ayurvedic Pharmacopoeia of India (Formulation), Government of India, Ministry of Health and Family Welfare; Department of Ayurveda Yoga & Naturopathy, Unani, Siddha & Homoeopathy, The Controller of Publications, Delhi; Part- II, 1st ed. vol.-3, 2010. p. 240-241.