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STANDARDIZATION OF JANAMAGHUTTI: A FOLK-LORIC HERBAL FORMULATION OF TRIBALS OF CHITRAKOOT REGION, DISTRICT SATNA, MADHYA PRADESH, INDIA

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Received on: 08/01/13 Revised on: 12/02/13 Accepted on: 17/03/13

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DOI: 10.7897/2277-4343.04309
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ABSTRACT
Janamghutti is used in folklore prcatices as preventive health care need of tribals and rural population of the country since antiquity. This knowledge of medicament transmitted oraly through ancesters. The present study was aimed to validate the traditional medicinal knowledge through pharmacognostical standardization. Physico chemical parameters such as extractive values, ash values, loss on drying and pH were performed as per the WHO guidelines. The microscopic examination of the drug was done to establish characteristic microscopial features. Detailed TLC fingerprint profile of methanolic extracts of the formulation alongwith the corresponding extracts of its individual ingredients were developed. The studied physicochemical parameters were found to be within prescribed limits. TLC fingerprint profiles may ascertain, the quality of the drug. Further studies on pharmacological effects are suggested.
Keywords: Janamghutti, Standardization, Tribals, HPTLC, Pharmacognosy

INTRODUCTION

India has a glorious tradition of arts and science of healing. The origin of Indian medicine is shrouded in "myths" and "inspired history". Myths and folk-lore develop for different reason, but is the folk history of herbs of any value beyond being a source of colorful stories? It can certainly be valuable source of clues to the usefulness of plants and the kinds of relationships that human communities have had with them. History of use in folk-medicine and recently it was found to contain antibiotic, chemicals, effective against the kind of bacteria that cause TB and many other chest infections. Most plant remedies in popular use probably earned their place through this kind of mixer of personal experience, local customs and an added dash of faith. Most of the industrial world's major drugs were originally discovered in plants which had a history of use in traditional medicine or ritual. In the era of globalization, alternative systems of medicine like Ayurveda, Unani, Siddha, Naturopathy etc. are emerging as options for modern system of medicine. Most of the alternative systems of medicine originated from folk medicine. In view of popularity and worldwide increasing interest in plant based medicine; safety, efficacy and quality control of such medicines are becoming important for both health authorities and public. The developing countries are trying to revive and promote their respective native systems, and in turn, make their full use in health care. Even in the developed nations, attempts are afoot to investigate the efficacy and usefulness of such systems[1, 2]. Regulatory bodies have laid down the standardization procedures and specifications for Ayurvedic preparations. In India, the department of AYUSH, Government of India, launched a central scheme to develop a standard operating procedures for the manufacturing process to develop pharmacopoeial standards for Ayurvedic preparations[3].

We have a vast store of oral medical knowledge available in the form of tribal medicine, home remedies and local health traditions, the people living in remote areas who are untouched by modern civilization use plants for their basic health care needs. Chitrakoot is one such area located in Satna District of Madhya Pradesh India. A large section of this population depends on these local health traditions. These people have developed herbal remedies, which they have been using for generation to maintain their health depending on the availability of plants in the area. Janamaghutti is used in almost all the household all over India for centuries to improve the digestive power and for strengthening the immune system of babies. It is used as a churna or as mild paste. In some places it is mixed with mother's milk. Interestingly, all these are part of the knowledge of the elderly women in the house. It can be looked at as a kind of vaccination in modern medicine and took preventive care of various childhood diseases. Due to urbanization and fast changing trends in the life style of the younger generation, they do not want to follow the footsteps of their ancestors. As a consequence, this knowledge is slowly being lost. Traditions of India that continue to provide health care needs to the vast rural masses need to be protected to prevent them from being lost forever. Thus systematic documentation, proper identification and standardization of the drug is essential. Standardization guidelines to be followed for herbals products provided by World Health Organization and Ayurvedic pharmacopoeia of India have been considered[4,5]. Hence physico-chemical studies of a particular drug by making use of various parameters help in standardizing the drug and validate it. Thus the present study was aimed to study microscopical, physicochemical and phytochemical aspects of the drug.

MATERIALS AND METHODS

Plant materials

The formulation consists of eight ingredients viz., *Zingiber officinale* (Sunthi), *Terminalia chebula* (Harra), *Terminalia bellerica* (Bibhitaka), *Trachyspermum ammni* (Ajwain), *Myristica fragrans* (Jaifal), *Curcuma longa* (Haldi), *Ferula narthex* (Hingu) and Rock salt (Sendha namak). All the ingredients were collected from the forests and procured from local market of Chitrakoot, Madhya Pradesh, India as per availability. The plant materials were authenticated by the botanist at Deen Dayal Research Institute, Chitrakoot, Satna (M.P.) before the study was carried out. All the ingredients were cleaned, washed; dried and powdered separately enough to pass through 180 µm IS Sieve (Sieve number 85). Weighed separately each ingredient, mixed together in specified proportions in a geometrical manner to get uniform mixer and stored under controlled conditions. The formulation is given in Table 1.

Table 1: Composition of the formulation of *Janamghutti*

Ingredients	Scientific name	Part used	Strength
Sunthi	*Zingiber officinale* Rosc.	(Rz.)	1 part
Harra	*Terminalia chebula*	(Fr .)	1 part
Bibhitaka	*Terminalia bellerica*	(Fr.)	1 part
Ajwain	*Trachyspermum ammni*	(Fr.)	1 part
Jaifal	*Myristica fragrans*	(Fr.)	1 part
Haldi	*Curcuma longa*	(Rz.)	1 part
Hingu	*Ferula narthex Boiss.*	(Exd.)	1 part
Saindhava lavana	*Sodium chloride*	-	1 part

Physico-chemical Evaluation

The physico-chemical analysis for total ash, acid insoluble ash, water soluble ash, water soluble and alcohol soluble extractive values, loss on drying at 105^0 C and pH of filtrate of 10% w/v aqueous solution of all the samples were carried out in triplicate according to the prescribed standard methods in Indian Pharmacopoeia[6].

Microscopic Evaluation

For microscopic analysis about 2 g of churna was taken and washed thoroughly with potable water repeatedly to get rid of salt; pour out the water without loss of material and treated the residue as follow; mount a small portion in glycerin; warm few mg with chloral hydrate solution, wash and mount in glycerin; treat few mg with iodine in potassium iodide solution and mount in glycerin. Observed the diagnostic characters in the various mounts[7].

HPTLC profile

Powdered drugs (2g) was extracted by heating under reflux for 15 min with 20 ml methanol and boiled for 5 minutes on a water bath at 105^0C, filtered the solution and concentrated the filtrate to 5ml. 2 µl to 7 µl of this solution were applied to the chromatogram [8,9]. Chromatography was performed on (20x10cm) aluminum packed silica gel $60F_{254}$HPTLC plate (Merck, Darmstadt, Germany). Before use, the plates were dried in an oven at 105^oC for 5 minute. The samples of compound formulation along with single ingredients were applied as 9 mm band by means of sample applicator (Camag Linomat 5, Switzerland) equipped with a 100 ul micro syringe [10-12].

The developing solvent was allowed to ascend to 90 mm with Toluene: ethyl acetate: formic acid (7: 2: 1) as a mobile phase in a Camag glass twin trough chamber, previously saturated for 20 min by lining with thick whatman filter paper. The room temperature was 27^oC and relative humidity 37%. The average development time was 20 min. After development of chromatogram, the plates were removed and completely dried in air at room temperature. Observed the spots produced before and after derivitization with 5% Methanolic-sulphuric acid reagents at day light and ultraviolet light at 254nm and 366nm. Documented the images by means of photo documentation system (Camag Reprostar 3).The R_f values and colors of the resolved bands were recorded[13].

RESULTS AND DISCUSSION

The formulation and respective ingredients (numbered T^1toT^7) were evaluated for physico-chemical parameters like pH value, total ash, acid-insoluble ash, alcohol soluble extractives, water soluble extractives and volatile oil contents. The results were placed at Table 2.

Table 2: Physico-chemical parameters of *Janamghutti* and used single ingredients

Parameters	Samples							
	JG	T_1	T_2	T_3	T_4	T_5	T_6	T_7
pH	5.7	5.9	5.3	5.3	4.7	4.8	5.2	6.7
Loss on drying (% w/w)	10.29	9.25	9.44	9.63	11.43	6.99	22.45	5.71
Total Ash (% w/w)	7.25	5.7	2.0	4.4	8.69	2.2	7.2	8.21
API limits		NMT6	NMT5	NMT7	NMT9	NMT3	NMT9	NMT15
Acid-Insoluble Ash (% w/w)	0.93	2.0	1.2	0.99	0.18	0.22	0.76	1.85
API limits		NMT3	NMT5	NMT 1	NMT0.2	NMT 0.5	NMT 1	NMT 3
Alcohol Soluble Extractive (% w/w)	28.7	5.90	60.80	33.11	26.84	22.0	7.54	61.31
API limits		NLT 3	NLT40	NLT 8	NLT 2	NLT 11	NMT 8	NLT 9
Water Soluble Extractive (% w/w)	36.64	16.65	60.17	54.62	26.37	9.90	8.78	64.0
API limits		NLT10	NLT 60	NLT 35	NLT 13	NLT 7	NLT 12	NLT 50
Volatile oil (% w/w)	-	2.2	-	-	13.9	11.22	3.9	13
API limits		2-6	-	-	10-17	6-16	5-8	10-17

T_1= Sunthi , T_2= Harra, T_3= Bibhitaka, T_4= Ajwain, T_5= Jaifal, T_6= Haldi, T_7= Hingu, NMT: Not More Than; NLT: Not Less Than

Table 3: TLC profile of methanolic extracts of Janamghutti and its ingredients in Toluene: Ethyl acetate: Formic acid (7: 2: 1v/v)

R_{fs}↓	Janamghutti	Colour	Single Ingredients						
	JG		**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**
R_{f1}	0.11	black	0.11	–	–	–	–	–	–
R_{f2}	0.16	black	–	–	–	0.16	0.15	–	0.16
R_{f3}	0.32	black		–	0.33	–	-	0.32	0.32
R_{f4}	0.41	black	–	–	–	–	–	0.41	0.41
R_{f5}	0.59	black	0.58	–	0.59	–	–	0.59	–
R_{f6}	0.82	black	0.82	–	–	–	–	0.82	–
R_{f7}	0.87	black	–	–	–	–	0.86	0.87	–

Detection-at 254nm

Table 4: TLC profile of methanolic extracts of Janamghutti and its ingredients in Toluene: Ethyl acetate: Formic acid (7: 2: 1v/v)

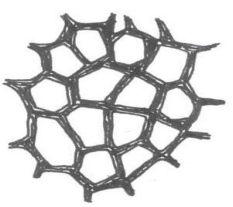
R_{fs}↓	Janamghutti	Colour	Single Ingredients						
	JG		**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**
R_{f1}	0.16	blue		–		0.17	–	–	0.16
R_{f2}	0.22	red	0.22	–	0.22	0.22	–	–	0.21
R_{f3}	0.36	whitish brown	–	–	0.36	–	0.36	–	–
R_{f4}	0.41	blue	–	–	–	–	–		0.41
R_{f5}	0.48	blue	–	–		0.48	–	–	–
R_{f6}	0.53	reddish pink	0.53	–	0.54	0.54	–	0.54	–
R_{f7}	0.64	reddish pink		–	0.64	0.63	–	–	–
R_{f8}	0.69	reddish pink	0.71	–	–	0.69	–	–	0.69

Detection-at 366nm

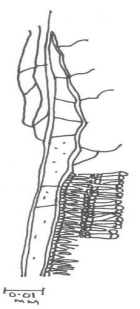
Table 5: TLC profile of methanolic extracts of Janamghutti and its ingredients after development in Toluene: Ethyl acetate: Formic acid (7: 2: 1v/v).

R_{fs}↓	Janamghutti	Colour	Single Ingredients						
	JG		**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**
R_{f1}	0.31	dark brown	–		0.31	–	–	–	–
R_{f2}	0.37	dark brown	–	–			0.36	0.38	0.38
R_{f3}	0.43	dark brown		0.43		0.43	–		0.43
R_{f4}	0.60	dark brown	–	–	0.60	–	–	0.60	0.60
R_{f5}	0.65	orange	0.65	–	–	–	–		–
R_{f6}	0.82	dark red	0.82	–	–	–	–	0.82	–
R_{f7}	0.87	dark red	–	–	–	–	–	0.87	–

Detection-at visible light (AD)



Thick walled cork cells



Septet fiber attached with reticulate and spiral vessels

Starch grains

Figure 1(A): Microscopical characteristics of Sunthi (Thick walled cork cells, Septet fiber attached with reticulate and spiral vessels, Starch grains)

Figure 1(B): Microscopical characteristics of Haritaki (Rosette crystals of calcium oxalate, Sclerieds, Vessels with reticulate and Unicellular trichomes)

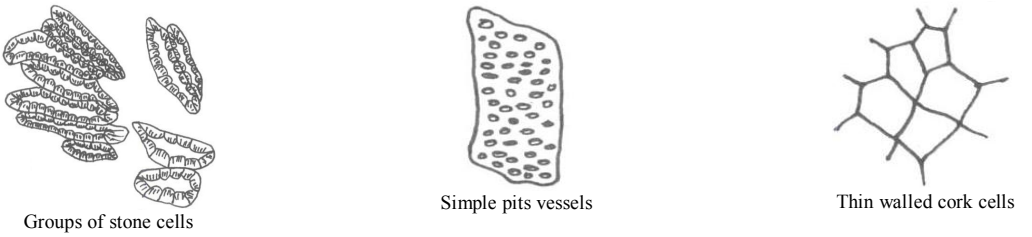


Figure 1(C): Microscopical characteristics of Bhibhitaki (Groups of stone cells, Simple pits vessels and Thin walled cork cells)

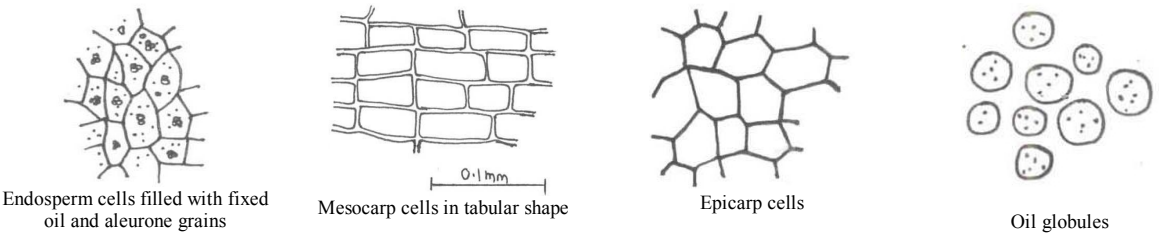


Figure 1(D): Microscopical characteristics of Ajwain (Endosperm cells filled with fixed oil and aleurone grains, Mesocarp cells in tabular shape, Epicarp cells and Oil globules).

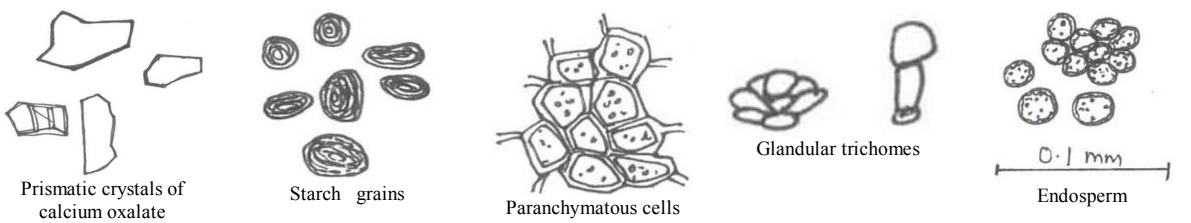


Figure 1(E): Microscopical characteristics of Jaifal (Prismatic crystals of calcium oxalate, Starch grains, Paranchymatous cells, Glandular trichomes and Endosperm).

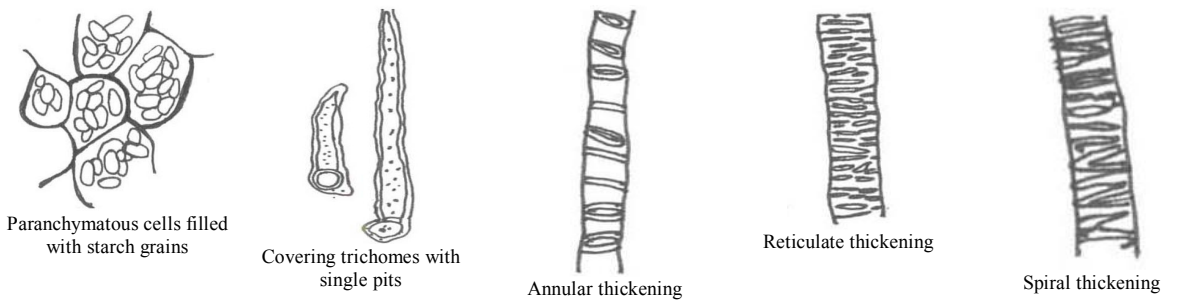


Figure 1(F): Microscopical characteristics of Haldi (Paranchymatous cells filled with starch grains, Covering trichomes with single pits, Annular thickening, reticulate thickening and Spiral thickening)

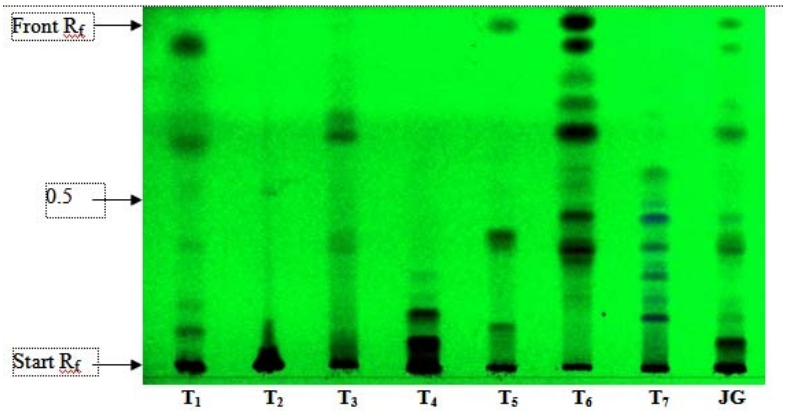


Figure 2 (A): HPTLC fingerprints of methanolic extracts of the formulation and its ingredients at 254 nm. Solvent system: Toluene: Ethyl acetate: Formic acid (7: 2: 1v/v) Tracks: T_1: Ajowan; T_2: Bahera; T_3: Sunthi; T_4: Harra; T_5: Haldi; T_6 : Jaifal; T_7 : Hingu and JG: Compound formulation of Janamghutti

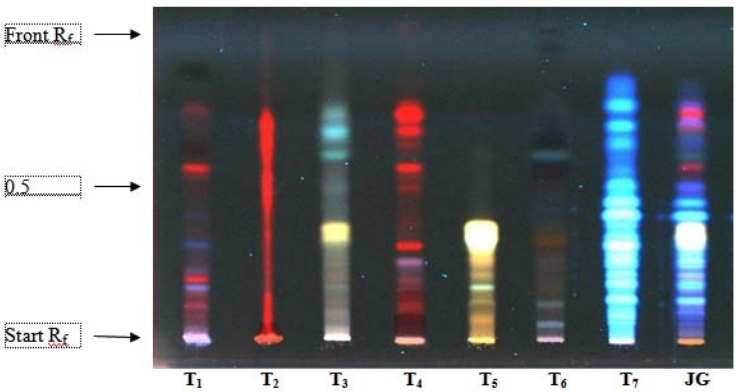


Figure 2 (B): HPTLC fingerprints of methanolic extracts of the formulation and its ingredients at 366nm. Solvent system: Toluene: Ethyl acetate: Formic acid (7: 2: 1v/v) Tracks: T_1: Ajowan; T_2: Bahera; T_3: Sunthi; T_4: Harra; T_5: Haldi; T_6 : Jaifal; T_7 : Hingu and JG: Compound formulation of Janamghutti

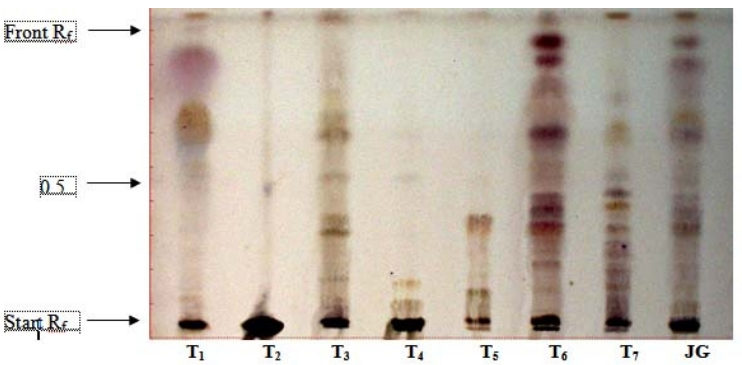


Figure 2 (C): HPTLC fingerprints of methanolic extracts of the formulation and its ingredients after derivatization with 5% methanolic sulphuric acid reagent at visible light. Solvent system: Toluene: Ethyl acetate: Formic acid (7: 2: 1v/v) nTracks: T_1: Ajowan; T_2: Bahera; T_3: Sunthi; T_4: Harra; T_5: Haldi; T_6 : Jaifal; T_7 : Hingu and JG: Compound formulation of Janamghutti

The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value obtained is an indicative of silicate impurities, which might have arise due to improper washing of crude drugs. Extractive values indicate the amount of active constituents in given amount of plant material. Loss on drying is the loss of mass expressed as percent w/w. Loss on drying was determined to find out any increase in weight caused by moisture absorptions. The improper value of moisture content could present bacterial, fungal or yeast growth. The quantitative data obtained from single ingredients for total ash, acid -insoluble ash, alcohol soluble extractive, water soluble extractives and volatile oil contents were compared with API standards and were found to be within standards.

The microscopic examination of the formulation (Figures 1 A-F) showed various diagnostic characteristics of the

ingredients present in it. Thick walled cork cells, septate fibre attached with reticulate and spiral thickening, fragment of septate fibre, starch grains (Sunthi); Groups of stone cells,simple pits vessels,thin walled cork cells (Bibhitaka); Rossette crystals of calcium oxalate, sclerieds, unicellular trichomes,vessels with reticulate thicknings (Harra); Endosperm cells filled with fixed oil and aleurone grains, mesocarp cells in tubular shape, epicarp cells, oil globules (Ajwain); Prismatic crystalls of calcium oxalate, starch grains, paranchymatous cells, glandular trichomes, endosperm (Jaifal); Parenchymatous cells filled with starch grains, covering trichomes with simple pits, annular thickning, reticulate thickning and spiral thickning (Haldi).

HPTLC fingerprint profile of the formulation and respective ingredients along with their R_f values and color of the band resolved were recorded and the developed chromatogram images documented. The data of R_f values are given in Table 3-5 and developed chromatogram are given in Figures 2(A), (B), (C). The methanol extracts of the Janamaghutti (JG) and the respective ingredients viz- *Trachyspermum ammni* (T_1), *Terminalia belerica* (T_2), *Zingiber officinale* (T_3), *Terminalia chebula* (T_4), *Curcuma longa* (T_5), *Myristica fragrans* (T_6) and *Ferula northex* (T_7) were spotted parallel. JG shows major spots at R_{fs} 0.11, 0.16, 0.32, 0.41, 0.59, 0.82 & 0.87 (all black). The corresponding spots from the respective ingredients for T_1 at R_f 0.11; 0.58; 0.82, for T_2 no spot was found, for T_3 at R_f 0. 33; 0.59, for T_4 at R_f 0.16, for T_5 at R_f 0.15; 0.86, for T_6 at R_f 0.32; 0.41; 0.59; 0.82; 0.87 and for T_7 at R_f 0.16; 0.32; 0.41 were resolved under ultraviolet light at 254nm (Table 3, Figure 2A). Similarly at 366 nm JG shows major spots at R_{fs} 0.16 (blue); 0.22 (red); 0.36 (whitish brown); 0.41(blue); 0.48 (blue); 0.53; 0.64; 0.69 (all reddish pink). The corresponding reference R_{fs} values from the ingredients for T_1 at R_f 0.22; 0.53; 0.71, for T_2 no spot, for T_3 at R_f 0.22; 0.36; 0.54; 0.64, for T_4 at R_f 0.17; 0.22; 0.48; 0.54; 0.63; 0.69, for T_5 at R_f 0.36 for T_6 at R_f 0.54 and for T_7 0.16; 0.21; 0.41; 0.69 (Table 4, Figure 2B). All the values from formulation are compared with ingredients R_f values and found almost coinciding.

Plate after derivatization with 5% methanolic sulphuric acid reagent and heated for 10 minutes at 105^0C and examined under visible light JG shows major spots at R_{fs} 0.31, 0.37, 0.43, 0.60 (all dark brown), 0.65 (orange), 0.82 and 0.87 (both dark red). The corresponding spots from the single ingredients for T_1 at R_f 0.65; 0.82, for T_2 0.43, for T_3 0.31; 0.60, for T_4 at R_f 0.43, for T_5 at R_f 0.36, for T_6 0.38; 0.60; 0.82; 0.87 and for T_7 at R_f 0.38; 0.43; 0.60 and 0.38 were observed (Table 5, Figure 2C). On running mobile phase over stationary phase, well distributed, distinct and clear spots were observed without clumping and represented similarity or almost coinciding with corresponding ingredients R_f values.

CONCLUSION

The drugs are used from ancient time for its medicinal values. The ingredients present in it have been shown to have various pharmacological activities. Efficacy of any drug depends on the genuineness of the raw material used for its preparation. Adulteration of the genuine raw material is the main cause for deterioration of the desired therapeutic effect of a particular drug. The results of the present study revealed that the characteristic microscopic features and the distinguished fingerprints in the HPTLC profiles may be utilized as marker parameters for identity and monitoring the quality of the drug. The results of physicochemical parameters were found to be significant and encouraging towards the goal for standardizing Janamghutti churna.

ACKNOWLEDGEMENT

Authors are grateful to Dr. Bharat Pathak, General Secretary, Deendayal Research Institute, Arogyadham, Chitrakoot, Satna, Madhya Pradesh, India for providing the necessaries research facilities and are also thankful to Prof. K.B. Pandeya, Vice-chancellor, MGCGV, Chitrakoot, Satna (M.P.) for providing excellent research environment to carry out the study.

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Cite this article as:

Indra P Tripathi and Neelesh Dwivedi. Standardization of Janamaghutti: A folk-loric herbal formulation of tribals of Chitrakoot region, District Satna, Madhya pradesh, India. Int. J. Res. Ayurveda Pharm. 2013;4(3):349-354

Source of support: Nil, Conflict of interest: None Declared