

Standardisation of Vettumaran Gulika A herbomineral formulation

*Sudarsan Nair P.K., **Gayathridevi

Abstract

Vettumaran Gulika is a herbomineral formulation widely used by the Ayurvedic Physicians of Kerala and Tamil Nadu for treating various clinical conditions especially fever with chills, rheumatism, vomiting, retention of urine and abdominal disorders. Since there is no uniform standard for the preparation of this drug, the study has been conducted to formulate a standard in preparation and quality control procedures to ensure maximum therapeutic efficacy, safety and authenticity of the drug. The raw drugs used in the preparation were properly identified and authenticated. The TLC profile and Rf values obtained are important parameters for standardisation. HPTLC chromatographic studies of the formulation gave characteristic HPTLC pattern which can be used to establish the proper identity of the drug.

Introduction

Ayurveda, the traditional Indian system of medicine is the outcome of, experiments, constant observations and practices of eminent scholars of India carried out through centuries. According to their studies and observations, new combination of drugs-herbal, animal and mineral origin-were formulated for various clinical conditions and incorporated in the therapeutic programme. The practising physicians of various parts of the country have formulated special combinations according to the availability of drugs, regional requirements based on the predominant clinical conditions. The majority of formulations developed by such models are documented in regional languages and become popular in that part of the country.

The ancient Ayurvedic Physicians of Kerala have developed many herbal and herbo mineral combinations being used in various diseased conditions. These are time tested and its efficacy have been clinically proved. The documentation of such formulations and its details is available in Malayalam language. The books Yogamrutham, Chikitsamanjari, Arogyakalpadrumam etc are famous and very popular among Ayurvedic physicians of

Kerala. Vettumaran gulika is a herbomineral formulation described in Sahasrayogam, the most popular handbook of Ayurvedic practitioners, which is also considered as Ayurvedic pharmacopoeia of Kerala.

The description of the medicine is given in the form of sloka which is a blend of Tamil and Malayalam language.

*Kararkuzhli porikaram kanal mulagu
nabhichatilingam
Varayomam evayanchum
pankottachattiludan
Nerayoru yamam mudangatharaithu
cherupayaralavay thiruttu
Mundai ttheerachuravum vasuriyum pokkum
nalvayu sanniyarum*

(Sahasrayogam-gulika yogangal)

*Ponkaram (Tankanam),
Mulagu (Maricham),
Nabhi (Vatsanabhi)
Jatilinga (Hingula),
Omam (Ajamoda) are the ingredients.*

Vettumaran gulika is used in clinical practice in various conditions with different anupana (adjuvants).

Jwara /Amavatha -	Ardraakaswarasa
Vayu vaigunya -	Jeerakwatha
Chardi -	Kachura kwatha
Mutraghata -	Narikel jala
Soola -	Ajamodakwatha
Gulma -	Lasuna swarasa

*Asst. Director/IC,(Rtd)National Research Institute for Panchakarma Cheruthuruthy, Kerala. ** Research Officer (Chem),Siddha Regional Research Institute, Thiruvananthapuram, Kerala.

This medicine has been manufactured in large scale by the Ayurvedic drug manufacturing companies in Kerala. Since it contains minerals like hingula (Cinnabar) and toxic drug, vatsanabhi (Aconite), proper purification procedures of these drugs and classical methods of preparation are to be followed to nullify the toxic effect and to ensure the safety of the medicine. But the medicine available in the market manufactured by various pharmaceuticals are not having a uniform standard either in preparation or even in the size and weight of a tablet. Hence it does not provide the expected therapeutic efficacy to the prescribing physician and relief to the patients. Instead, it may produce certain adverse effect also. So, standardisation of procedures and quality control methods for this drug to ensure maximum therapeutic efficacy, safety and authenticity of the product are justified.

Ingredients and review of properties

1. Vatsanabhi

Botanical name : Aconitum ferox Wall (rhizome)
Family : Ranunculaceae

Distribution

The drug growing at high altitudes, higher than 600 metres is less potent. The drug grows wild in the alpine Himalayas and Kashmir even at an altitude of 3600 m.

Vernacular names

Bengali : Katbish
English : Indian aconite
Hindi : Bachnaag, Bish
Kannada : Vatsanabhi
Malayalam : Valsanabhi
Sanskrit : Vatsanabhi
Tamil : Vasanaavi, Karunaabhi
Telugu : Vatsanabhi

The drug is extremely poisonous. But the toxicity may be reduced by suitable processing. In small doses (0.023 to 0.018 g) they are beneficial in nasal catarrh, tonsillitis, sore throat, gastric disorders, debility and fever of inflammatory origin,. In large doses it acts as narcotic poison and powerful sedative. Though the drug as such is a cardiac depressant poison, after mitigation or purification, the active principles lose their depressant action on the heart and it comes to possess marked cardiac stimulant property, as testified from pharmacological experiments. The drug finds application in the formulation of many useful Ayurvedic and Unani medicines.

The rhizome on analysis gave the following physico-chemical values.

Foreign matter	: Nil
Moisture content	: 10.00-12.00%
Total ash	: 3.00-3.50%
Acid-insoluble ash	: 1.45-1.75%
Water-soluble ash	: 1.20-1.40%
Alcohol soluble extractive	: 4.50-5.00%
Water-soluble extractive	: 8.70-9.30%
Volatile oil	: Nil
Fibre content	: 6.00%

Important plant constituents

The drug contains toxic alkaloids such as aconitine, neopline, pseudoaconitine, chasmaconitine, indaconitine, bikhaconitine, veratroyl pseudoaconitine and diacetyl pseudoaconitine.

2 Maricha

Botanical name : Piper nigrum Linn.
(fruit)
Family : Piperaceae

Vernacular names

Sanskrit : Maricha, Maricham
Hindi : Golmirch
English : Black Pepper
Malayalam : Kurumulak
Tamil : Nallamulagu, Milagu

Black pepper is the dried mature but unripe berries of Piper nigrum, a branching vine or a perennial climbing shrub mostly found in hot and most parts of southern India. Notably, Kerala alone is contributing about 96% of India's total production, Karnataka 3.5% and the rest in Tamil Nadu and Pondicherry.

Analysis of black pepper gave the following ranges of values:

Moisture content	: 8.7 – 14.1%
Total nitrogen	: 1.55 – 1.60%,
Nitrogen in non-volatile ether extract	: 2.70 4.22%,
Volatile ether extract	: 0.3 – 4.2%,
Starch (by acid hydrolysis)	: 28 – 49%,
Crude fibre	: 8.7–18.0%,
Crude piperine	: 2.8-9.0 %,
Piperine (spectrophotometrically)	: 1.7 – 7.4%,
Total ash	: 3.9 – 5.7%,
Acid insoluble ash	: 0.03 – 0.55

Chemical constituents

Phytochemical investigation on the plant revealed the presence of a volatile alkaloid piperine, 5-9%, piperidine, 5%, piperettine, piperolein A and piperolein B, trichostachine (-piperylin), N – trans – feruloyl piperidine, feruperine, dihydroferuperine, N-trans- feruloyl tyramine, citronellol, p-cymene, α - thujene, d – (α) sabinene, (α) gamotene, cryptone, (α) – terpinene, dl – limonene, dihydrocarveol, α - pinene, β - pinene, piperonal, camphene, β -caryophyllene, β - alanine, artinine, piperolic acid, serine, threonine, ascorbic acid, carotene, β - methyl pyroline , (2E, 4E)-N- isobutyl – 2,4, decadienamide, and piperide. Chavicine, a resinous isomer of piperine is said to be the strong ingredient of pepper, and on hydrolysis, it yields piperidine and isochavicine acid (an isomer of piperic acid). Piperine is stated to occur in pepper in amounts usually ranging from 4 to 10% but this amount represents crude piperine. The true piperine content being lower. Among the methods described in literature, the spectrophotometric methods gives the best results for the quantitative estimation of piperine.

Therapeutic uses

Pepper is used for a variety of purposes. The Ayurvedic classical books describe it as a powerful remedy for various disorders and prescribed it as an effective cure for dyspepsia, malaria, delirium, tremors, hemorrhoids etc. It is carminative and aphrodisiac and used in colic. The combination of Black pepper, long pepper and dry ginger constitute the group by the name, trikatu.

Fruit is aromatic with a strong pungent taste due to the presence of an oleoresin. Pepper is employed as an aromatic stimulant, for body weakness following fevers, as a stomachic and as antiperiodic in malarial fever.

3 Ajamoda

Botanical name	: Trachyspermum ammi (Linn.) Sprague (seed) Trachyspermum roxburghianum(DC)carib syn
Synonyms	: Carum copticum Benth. & Hook. f. Ptychotis ajowan DC
Family	: Umbelliferae

Vernacular names

Bengali	: Jowan
Marathi	: Ajwan
English	: Ajowan
Hindi	: Ajowan
Malayalam	: Ayamodakam, Omam
Sanskrit	: Ajamoda
Tamil	: Omum
Telugu	: Omamu

The plant is cultivated extensively in Indian gardens from Punjab, West Bengal to the Deccan Peninsula.

The seed on analysis gave the following physico-chemical values.

Foreign matter	: Nil
Moisture content	: 9.00-12.00%
Total ash	: 7.60-8.80%
Acid-insoluble ash	: 0.70-1.10%
Water-soluble ash	: 3.60-4.90
Alcohol soluble extractive	: 10.00-12.00
Water-soluble extractive	: 13.00-15.00
Volatile oil	: 4.00-6.00
Fibre content	: 34.00-39.00

Chemical constituents

Camphene, Δ^3 -carene, carvacol, p-cymene, dipentene, myrcene, α - and β - phellandrenes, γ - terpinene, thymine, thymol, linoleic, oleic, $\Delta^5,6$ - octadecanoic, palmitic, petroselenic acids, resin acids, nicotinic acid, riboflavin, thiamine, protein, sugars, tannins, flaone,sterol and a phenolic glycoside.

Therapeutic uses

The plant possesses multiple pharmacological properties: tranquilising, anticonvulsant, antibacterial, antifungal, regenerative of liver, antifertility, hypotensive, anthelmintic, anticancer and anti inflammatory.

Fruit is antiseptic, stomachic, carminative, stimulant, and tonic, used in diarrhoea, dyspepsia, colic, flatulence, indigestion. Root is diuretic and carminative.

4 Hingula

Vernacular names

Arab	: Sinjraph
Gujarathi	: Hingaloo
Hindi	: Hingula
Kannada	: Ingleeyaka
Malayalam	: Chayilyam
Sanskrit	: Lingam
Tamil	: Elingam
Telugu	: Ingileekam
English	: Cinnabar

Cinnabar is the red sulphide of mercury (HgS). It is natural and could also be prepared. Specific gravity is 8.00. It is restorative, alterative and tonic. It is used in syphilis, diarrhoea, fever and asthma.

This sulphide occurs in nature as a fine grained, dark-red, very heavy mineral ore in many parts of the world.

5 Tankana

Vernacular names

Arab	: Buraekes-sagah
Bengali	: Sohaga, Tinkar
Hindi	: Tinkal, Sohaja
Kashmiri	: Vavut
Malayalam	: Ponkaram
Sanskrit	: Tankana, Rasashodhan
Tamil	: Venkaram, Vengaram
Telugu	: Velligaram, Elegaram
Tibetan	: Chusal
English	: Borax, Sodium biborate, Sodium pyroboate

Tankana occurs as a natural deposit. Crude borax is found in masses by evaporation of water, on shores of dried up lakes in India and Tibet. It is also obtained from the mud of lakes surrounded by hills in Nepal.

MATERIALS AND METHODS

1. Standard Operational Procedure (SOP) of Vettumaran Gulika

Vettumaran gulika was prepared in the pharmacy of National Research Institute of Panchakarma, Cheruthuruthy as per the methods described in Sahasrayogam. The ingredients of preparation are given in Table-1. The ingredients were devoid of any foreign matter and microbial contamination which were identified and authenticated by Survey and Pharmacognosy Section, Regional Research Institute, Thiruvananthapuram and checked for their quality and used for the preparation. The raw drugs were washed, dried, coarsely powdered and weighed as per the formula.

a. Purification of single drugs (Sodhana) and preparation of gulika

Vatsanabhi, Tankana and Hingula are purified prior to the preparation.

i. Purification of Vatsanabhi (Vatsanabhi sodhana)

Vatsanabhi is made into small pieces and soaked in 500 ml gomutra and kept in sunlight. The process is carried out for three days by changing Gomutra everyday. On the fourth day, Vatsanabhi is taken out from the Gomutra and dried in sunlight.

ii. Purification of Tankana (Tankana sodhana)

Tankana is made into small pieces and fried in a pan on fire and collected when dehydrated and powdered. (AFI, Part I pp304)

iii. Purification of Hingula (Hingula sodhana)

Hingula is made into powder and kept in 300 ml Ajaksheera (goats milk) for one day. On the second day it is taken from goat's milk, put into a grinder and ground with fresh lemon juice (Nimbu swarasa) prepared from 400 gm lemon for three hours. The process was repeated for six days, each day 300 gm lemon bring used to prepare juice.

Table I: Ingredients of Vettumaran gulika

Sl. No.	Sanskrit name	Scientific name	Quantity used
1	Vatsanabhi	Aconitum ferox Wall.	150 gm
2	Maricha	Piper nigrum Linn.	150 gm
3	Ajamoda	Trachyspermum ammi (Linn.) Sprague	150 gm
4	Hingula	Cinnabar (Mercuric sulphide)	150 gm
5	Tankana	Borax (Sodium biborate)	150 gm
6	Gomutra	Urine of Cow (Bos Taurus Linn.)	1.5 lit
7	Ajaksheera	Milk of Goat (Capra aegagrus Linn.)	300 ml
8	Nimbu	Citrus lemon (Linn.) Burm.f.	2.5 kg
9	Fresh ginger juice	Zingiber officinale Rosc.	3 kg

b. Preparation of the gulika

To the purified Hingula obtained as above, powders of Vatsanabhi, Maricha and Ajamoda are added and ground with ginger juice for four days. 1 kg Ginger was used to prepare ginger juice on first day and 300 gm Ginger was used on second, third and fourth day. On the fifth day Tankana bhasma was added and ground with juice of 500 gm ginger and again ground for two days using juice of 300 gm ginger. After proper and fine mixing the ingredients were taken out from the grinder. The weight of the product obtained is 1.160 kg. Tablets of 120mg were made adding 30mg of binding material by using tablet making machine.

2. Chemical analysis of Vettumaran gulika

The chemical analysis of vettumaran gulika was carried out in Captain Srinivasa Murti Drug Research Institute for Ayurveda, (CCRAS, Ministry of Health &FW, New Delhi) Arumbakkam, Chennai.

a. Organoleptic characters

Organoleptic characters such as colour, touch, taste and odour were noted.

b. Physico-chemical parameters

Physico-chemical parameters such as ash content, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, loss on drying at 105°C, uniformity of weight of tablets etc. were determined by standard methods (WHO, 1998).

Table II : Physico-chemical values

S. No	Parameter	Obtained Value*
1	Ash content (w/w)	22.38 %
2	Acid insoluble ash(w/w)	01.46 %
3	Water soluble extractive(w/w)	26.60 %
4	Alcohol soluble extractive (w/w)	04.9 %
5	Loss on drying at 105 o C (w/w)	10.62 %
6	Borax (w/w)	15.42 %
*n=2		

c. Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC)

2 gm of the sample was soaked in 25 ml 90% ethyl alcohol for 18 hours and boiled for 10 minutes and filtered. The filtrate was concentrated and made up to 50 ml in a standard flask. 15 µl of the solution was applied on Aluminium plate pre-coated with Silica gel 60 F254 of 0.2 mm thickness (Merck) using Linomat IV applicator. The plate was developed in Toluene : Ethyl acetate (5:2 v/v). The plate was visualized in UV 254 and 366 nm. The

plate was then scanned at 254 nm and 366 nm using Deuterium and Mercury lamp in Camag HPTLC instrument provided with Cats 4.05 software. The plate was then dipped in Vanillin- Sulphuric acid and heated in air oven at 105°C till the spots appeared.

Results

Vettumara gulika is brownish red in colour with pungent taste and no specific odour. The physico-chemical parameters are given in Table II.

The TLC profile of the alcohol extract of Vettumaran gulika was carried out using Toluene: Ethyl acetate (5:2v/v) as mobile phase and Silica gel 60 F254 as adsorbent.

The Rf values recorded in different detecting reagents are given in Table III and IV.

Discussion

The physico-chemical parameters help to a great extent for the purpose of standardization. The test for loss on drying determines both water and volatile matter. The total ash is the total amount of material remaining after ignition. This includes both “physiological ash” which is derived from the plant tissue itself, and “non- physiological” ash, which is the residue of the extraneous matter (eg: sand and soil) adhering to the plant surface. Acid-insoluble ash measures the amount of silica present, especially as sand and siliceous earth. Alcohol soluble extractives & water soluble extractives determine the amount of active constituents extracted with solvents from the drug. Borax is an ingredient of the formulation and the value obtained is as expected to be present in the formulation. For the TLC study, various solvent systems were tried and the solvent which gave maximum separation is reported. The TLC profile and Rf values obtained are important parameters for standardisation. HPTLC chromatographic studies of the formulation gave characteristic HPTLC pattern which can be used to establish the identity of the drug.

The above discussed parameters organoleptic, physico-chemical, TLC and HPTLC data will help us to determine the quality of the gulika. These results can be used in turn to check and ensure the quality of the medicine.

Table III : HPTLC profile of the alcohol extract of Vettumaran gulika at 254 nm

Peak	Starting		Maximum			Ending		Area	
#	Rf	H	Rf	H	%	Rf	H	F	(%)
1	0.01	120.1	0.01	122.1	4.93	0.02	0.0	526.9	0.56
2	0.04	2.7	0.06	32.9	1.33	0.08	0.2	572.1	0.61
3	0.09	0.1	0.12	122.9	4.97	0.13	95.4	2571.2	2.74
4	0.13	95.4	0.14	108.0	4.36	0.17	48.4	2569.6	2.74
5	0.18	49.9	0.19	51.1	2.06	0.21	26.9	1141.8	1.22
6	0.21	26.9	0.23	37.0	1.50	0.24	3.1	779.2	0.83
7	0.25	0.1	0.32	462.0	18.67	0.36	214.6	20365.4	21.72
8	0.36	214.6	0.38	301.4	12.18	0.41	156.9	10899.1	11.62
9	0.41	156.9	0.43	193.0	7.80	0.46	66.8	5910.8	6.30
10	0.46	66.8	0.49	182.2	7.36	0.55	89.2	9592.5	10.34
11	0.55	89.2	0.59	335.0	13.54	0.63	34.8	13215.6	14.09
12	0.68	19.7	0.72	62.9	2.54	0.77	36.0	3209.6	3.42
13	0.79	37.5	0.86	188.0	7.60	0.88	167.0	8737.3	9.32
14	0.90	177.0	0.95	276.2	11.16	1.00	0.8	13591.6	14.49
Total Height – 2474.6								Total area - 93782.9	

Table IV : HPTLC profile of the alcohol extract of Vettumaran gulika at 366 nm

Peak	Starting		Maximum			Ending		Area	
#	Rf	H	Rf	H	(%)	Rf	H	F	(%)
1	0.01	84.9	0.01	415.3	28.12	0.06	0.2	7101.3	15.16
2	0.06	0.0	0.10	27.5	1.86	0.12	0.1	592.6	1.27
3	0.13	0.2	0.15	29.1	1.97	0.18	24.1	961.1	2.05
4	0.18	24.1	0.22	53.3	3.61	0.24	50.5	1988.0	4.25
5	0.24	50.5	0.28	76.6	5.19	0.33	0.1	3240.0	6.92
6	0.43	0.0	0.53	67.5	4.57	0.54	66.8	4258.9	9.09
7	0.60	57.3	0.65	173.8	11.77	0.69	70.8	7406.6	15.82
8	0.69	70.8	0.72	191.7	12.98	0.77	34.3	7213.6	15.40
9	0.77	34.3	0.81	415.0	28.10	0.86	17.3	12958.1	27.67
10.	0.93	16.4	0.97	26.9	1.82	1.01	5.7	1110.5	2.37
Total height – 1476.8								Total area-46830.8	

Conclusion

Organoleptic, physico-chemical, TLC and HPTLC studies were carried out on Vettumaran Gulika – a herbo mineral preparation with a view to establish the standardization of the drug. The standardisation procedure based in the present study will be highly useful for detecting substandard/adulterated formulations selling in the market under the name of Vettumaran Gulika.

References:

1. Anonymous. (1999) The Ayurvedic Pharmacopoeia of India – Part 1, Volume II Govt. of India, Dept. of Ayush, New Delhi
2. J. S. Pruthi. (1998) Spices and condiments, 5th edition
3. R. N. Chopra, S. L. Nayar & I. C. Chopra. (1996) Glossary of Indian Medicinal Plants, 4th reprint.
4. Ram. P. Rastogi & B. N. Mehrotra (1970-1979) Compendium of Indian Medicinal Plants, Volume II.
5. Ram. P. Rastogi & B. N. Mehrotra. (1980-1984) Compendium of Indian Medicinal Plants, Volume III.
6. Ram. P. Rastogi & B. N. Mehrotra. (1985-1989) Compendium of Indian Medicinal Plants, Volume IV.
7. Ram. P. Rastogi & B. N. Mehrotra. (1990-1994) Compendium of Indian Medicinal Plants, Volume V
8. Dr. K. M. Nadkarni. (1991). Indian Materia Medica
9. Anonymous. (1976) The Wealth of India
10. Lt. Colonel K. R. Kirtikar & Major. B. O. Basu. And I.C.S. (1994) Indian Medicinal Plants. Volume IV.
11. S. Sadasivam & A. Manickam. (1997) Bio-Chemical methods, 2nd edition First reprint.
12. Asima Chatterjee & Satyesh Chandrapakrashi. (1997) The Treatise on Indian Medicinal Plants, Volume I, 3rd reprint.
13. J.B. Harborne. (1973) Phytochemical Methods. (1973)
14. Anonymous. (1998) Quality control methods for medicinal plant materials, World Health Organisation, Geneva.
15. Dr. O.P. Agarwal. (1992) Chemistry of organic natural products, Volume-I, 15th edition, Goel Publishing House, Meerut.
16. Govt of India-Ayurvedic formulary of India, Part-I, Second revised edition, Ministry of health and F&W, Dept of Aush
17. Ambasta. S.P. (2000) The Useful plants of India, forth edition, National Institute of Science Communication, CSIR, New Delhi
18. Anonymous. (1995) Sahsrayogam-sujanapriya commentary Edited by K.V. Krishnan Vaidyar, S. Gopalapillai, Vidyarambham Publishers Alleppy-20th Edition,

STATEMENT OF OWNERSHIP AND OTHER PARTICULARS ABOUT "VAIDYARATNAM JOURNAL OF AYURVEDIC MEDICINE"

Title	: Vaidyaratnam Journal of Ayurvedic Medicine	Editor Name	: Dr. P.C. Muraleemadhavan
Regn No.	: KERENG/2019/78329	Editor Address	: Aparna, Vidya Nagar Ayyanthole P.O., Thrissur Kerala - 680003
Language	: English	Printing Press Address	: Ebnezer Print Pack(P) Ltd Industrial Development Plot, Velakkode, Mundur Thrissur - 680 541, Kerala.
Periodicity	: Four Monthly	Owner Name	: Vaidyaratnam Ayurveda Foundation
State	: Kerala	Place of Publication	: Vaidyaratnam Ayurveda Foundation, Vaidyaratnam Road, Thaikkattussery P.O., Ollur, Thrissur - 680 306, Kerala.
Publication District	: Thrissur		
Price	: Rs. 175/- Per copy		
Publisher Name	: E.T. Neelakandhan Mooss		
Publisher Address	: Eledath Thaikkattu Mana Thaikkattussery P.O., Ollur - Thaikkattussery Dist. - Thrissur-680 306, Kerala.		
Printer Name	: E.T. Neelakandhan Mooss		
Printer Address	: Eledath Thaikkattu Mana Thaikkattussery P.O., Ollur - Thaikkattussery Dist. - Thrissur-680 306, Kerala.		
		I E.T. Neelakandhan Mooss, hereby declare that the particulars given above are true to the best of my knowledge and belief	
			Sd/-