Standardisation of *Kuvalayaghrta*: A Potential Nootropic Supplement

Research Article

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Abstract

Nootropics are groups of medicines which are known to act as memory enhancers. There is a growing need for such medicines from alternate systems of medicine to prevent or delay memory loss and related disorders. Classical textbooks in Ayurveda describe a group called as *Medhya Rasayana*, which act as neuro-nutrients. *Chathushkuvalaya Rasayana* is one such ghee preparation mentioned in *Ashtangahrdaya*. In it, *Kuvalaya*, botanically identified in Kerala as *Monochoria vaginalis* Presl. is the only herbal ingredient. Though *Kuvalaya* has been considered widely as a memory enhancer, there is scarce published literature regarding standardisation aspects of *Kuvalaya ghrta*. In this work, we have phytochemically standardized the ingredients of *Kuvalayaghrta* including its Atomic Absorption spectroscopy, Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) profiling followed by the preparation of the *ghrta*(ghee) according to the standard procedure mentioned in Ayurvedic Formulary of India. *Kuvalayaghrta* was also standardised physicochemically and was found to be safe for internal administration. All the observations obtained in this study can be used for future reference.

Keywords: *Chathushkuvalaya Rasayana, Kuvalaya Ghrta, Monochoria vaginalis Presl., Medhya Rasayana,* Thin Layer Chromatography, Nootropics.

Introduction

Nootropics are often known to act as memory enhancers, intelligence enhancers etc. This group includes drugs, supplements, nutraceuticals, and functional foods that improve one or more aspects of mental function. (1,2) Poor memory, lower retention capacity, and slow recalling ability are among the common problems faced in the present era. (3) Hence, several works in contemporary medical literature have focused primarily on understanding the neurobiological principles behind learning and memory, and in exploring agents that can prevent memory loss or enhance memory capacity. (4)

Classical ayurvedic texts describe a set of rejuvenative measures to impart biological sustenance to body tissues known as '*Rasayana*' and those specific to brain tissue are called as '*Medhya Rasayana*'. They help in slowing down brain ageing and help in the regeneration of neural tissues thereby enhancing memory. (5) There are many medicated ghee preparations such as *Brahmighrta* (6) are found to have memory enhancing effects. The Ayurvedic text *Ashtangahrdaya in Utharasthana* describes the preparation of a potent memory enhancer namely *Chathushkuvalaya Rasayana*, where *Kuvalaya* is the only herbal ingredient.(7)The plant *Kuvalaya* has been botanically identified as *Monochoria vaginalis* Presl. belonging to the aquatic plant family Pontederiaceae.

Monochoria vaginalis is commonly found in marshy regions and alongside paddy fields throughout the state of Kerala, especially during the rainy season. It is a herb having a short sub erect, spongy rootstock, variable leaves, and flowers with centripetal inflorescence. There are studies proving its antioxidant and anti-inflammatory potential (8), antioxidant and curative effect of its methanolic extract against carbon tetrachloride induced acute liver injury in rats (9), and its nutritional assessment. (10) However, there is only scarce published literature regarding its standardization aspects. Hence, the present study intends to standardize the raw materials and *Kuvalayaghrta*.

Aims

- The standardization of raw materials (rhizome, flower, leaf, and stem of *Monochoria vaginalis* Presl.) and their preliminary phytochemical and physicochemical evaluations.
- Preparation of *Kuvalayaghrta* and its preliminary physicochemical evaluation.

Materials and Methods
Plant Materials

Genuine samples of the drug *Monochoria vaginalis* Presl. (Around 10kg) of Pontederiaceae family was collected from the marshy lands of Oachira,

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Kerala. They were thoroughly washed in running water. Then the rhizome, leaf, stem, and flower of the plant were separated, cut into small pieces, shade dried, powdered, and stored in airtight containers. These were checked for purity and authenticated (DGAVC195/18). at the Department of Dravyaguna Vijnana, Government Ayurveda College, Thiruvananthapuram, Kerala. About 500g was kept for the standardization of raw material and rest was used for the preparation of *ghrta*.

Preparation of Ghrta

The *ghrta* was prepared according to the standard procedure mentioned in the Ayurvedic Formulary of India. (11) After attaining the *paakalakshana* (signs of proper preparation), the *ghrta* was filtered while hot through muslin cloth and allowed to cool. It was packed in tightly closed container to protect from moisture.

Preliminary physicochemical and Phytochemical evaluation

The preliminary physicochemical and phytochemical evaluation of rhizome, leaf, stem, and flower of the genuine sample of *Monochoria vaginalis* Presl. were done according to the standard procedure described in Ayurvedic Pharmacopeia of India (API). (12) Various parameters like foreign matter, moisture content, volatile oil content, different extractive values etc. were evaluated. TLC and HPTLC profile were also done. Qualitative tests for the detection of steroids (Liebermann-Burchard test), phenols (with neutral ferric chloride), alkaloids (Dragendorff test), flavonoids (Shinoda test), glycosides, and tannins were also done. (13) All the chemicals used were of the highest commercial grade.

Physicochemical evaluation of *Kuvalaya Ghrta*

The organoleptic evaluation was performed for sample of *Kuvalayaghrta*. The colour and odour of the sample were noted. The physico-chemical analysis of the sample was performed in order to compare the quality, which included Refractive index, Acid value, Saponification value, Rancidity test, and Iodine value.

Results and Discussion
a) Physicochemical Evaluation of *Kuvalaya* (*Monochoria vaginalis* Presl.)

Table no. 1: Physicochemical evaluation of *Kuvalaya* (*Monochoria vaginalis* Presl.)

Sl.no	Experiment	Value
1	Foreign matter (%)	Nil
2	Moisture content (%)	10 ± 1.58
3	Volatile oil (%)	Nil
4	Total ash (%)	16.8±0.006
5	Acid insoluble ash (%)	17.11±0.11
6	Water soluble extractive (%)	5.3±0.023
7	Alcohol soluble extractive (%)	6.5±0.042
8	Total Sugar	
	a) Reducing sugar (%)	0.2±0.0004
	b) Total sugar (%)	0.3±0.001
9	Fibre content (%)	15.2±0.026

b) Qualitative Tests Done in *Kuvalaya* (*Monochoria vaginalis* Presl.)

Table no 2: Qualitative Tests Done in *Kuvalaya* (*Monochoria vaginalis* Presl.)

Sl.No	Experiment	Sample
1	Steroids (Liebermann-Burchard test)	+++
2	Flavonoids (Shinoda test)	++
3	Phenol –with neutral Ferric Chloride	+
4	Alkaloids (Dragendorff test)	++
5	Tannin	++
6	Saponin	Absent

These tests were only done as this was a preliminary study of a potential nootropic supplement, the major constituents having role in protection against neuro-inflammation and having neuro-protective actions were done. Plant steroids also act as potential anti-inflammatory agents thus help in neuroprotection. (14) Flavanoids help in maintaining the quality of neurons and number thus exhibiting neuro-protective effects.(15) Alkaloids have shown neuro-protective activities in many diseases like memory loss, dementia, etc.(16) Tannins exert their neuroprotective effects by virtue of its anti-oxidative, anti-inflammatory, and anti-cholinesterase activities.(17) Phenolic compounds are among widely distributed bioactives in the human diet and act as a promising source of compounds for the treatment of age-related cognitive decline and the risk of developing neurodegeneration due to their antioxidant and anti-inflammatory properties.(18)

c) Chromatography
i) Thin Layer Chromatography (TLC)

The plate in which best separation achieved was first observed through UV fluorescence viewing cabinet(365nm) and the Rf values of the spots were noted. Then, the plates were developed in the iodine chamber and no changes were observed.

Table no 3: Rf value of spots obtained in TLC with solvent system- Toluene:Ethyl acetate in the ratio 6:1 with 6 drops of formic acid.

Solvent system with ratio	Number of spots	Colour of spots	Rf value
Toluene:Ethyl acetate (6:1) with 6 drops of formic acid	4	Fluorescent blue	0.10
		Fluorescent Blue	0.21
		Fluorescent blue	0.39
		Flourescent blue	0.51

Figure no 1: TLC plate with solvent system Toluene: Ethyl acetate 6:1 with 6 drops of formic acid

ii) HPTLC Study of *Monochoria vaginalis* Presl.

The procedure was performed at the Drug Standardisation Unit of Government Ayurveda College, Trivandrum, Kerala, India. The ethanolic extract of *Monochoria vaginalis* Presl. was used as the test solution for HPTLC analysis. 2µl of each ethanolic extract solution was applied as 8mm band length in the 10×200 silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The sample applied plate was kept in TLC twin through developing chamber (after saturated with solvent vapor) with respective mobile phase up to 70mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo documentation chamber (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV254nm and UV366nm. The peak table and peak densitogram were noted. The software used was WinCATS 1.3.4 version.

HPTLC analysis of the alcohol extract of *Monochoria vaginalis* Presl. was done with the solvent system Toluene:Ethyl acetate-6:1 with 6 drops of formic acid.

Figure no 2: Densitogram with different peaks with their Rf values and Peak areas 8 peaks were identified in the densitogram with Rf values 0.08, 0.15, 0.22, 0.57, 0.68, 0.77, 0.95, 0.98
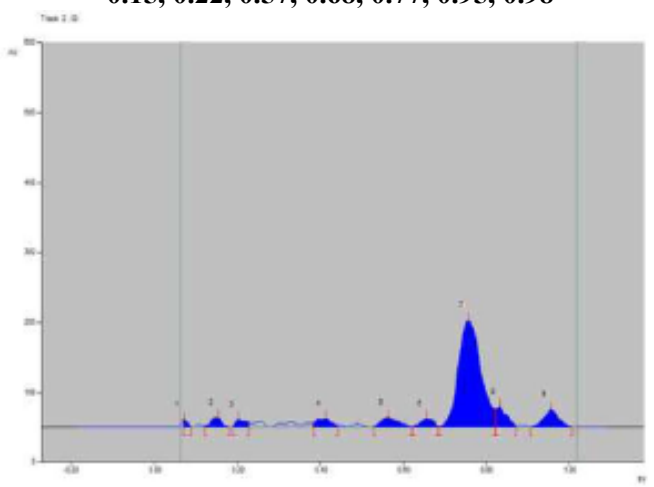
d) Atomic absorption spectroscopy (in parts per million-ppm)

The determination of Lead, Cadmium, Iron and Zinc in ppm levels in test drug were carried out using standard procedure at DSU, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India. The instrument (Thermo Electron Corporation M-Series AA Spectrophotometer) was set as per the optimum conditions as per the guidelines given in the instruction manual (provided along with the instrument).

Preparation of test sample: The plant material was incinerated to ash and it was dissolved in 2N HNO_3 and boiled for 2 minutes and filtered into 100ml volumetric flask and made up to the volume.

Sample feed- the sample solution was then nebulized and mixed with the fuel and oxidant prior to introduction into the flame with appropriate technique.

The absorbance was measured with the help of Photo Multiplier Tube (PMT).

Read out-The measured signals from the PMT then amplified and processed with the help of software.

Heavy metal content was screened in the samples of Kuvalaya (Monochoria vaginalis Presl.). The results observed were as follows

Table no 4: Results of Atomic absorption spectroscopy

Heavy metal concentration in ppm	Sample (in ppm)	Permissible limits (API)ppm	Permissible Limits (ICH Guidelines)
Lead	$0.16\pm2\times10^{-3}$	10	0.5
Cadmium	$0.027\pm2\times10^{-4}$	0.3	0.5
Iron	51.48 ± 0.77	-	-
Zinc	$0.45\pm4\times10^{-3}$	-	-
Copper	$0.22\pm6\times10^{-3}$	-	300
Nickel	$0.15\pm9\times10^{-4}$	-	20

The limits of Fe and Zn according to EMEA (European Medicine Agency) is 1300ppm.The heavy metal analysis revealed values within normal limits for the raw drug.

Results and Discussion

Preparation of *KuvalayaGhrta*

The formulation composition included *Kuvalaya Kalka* (Fine paste of the drug)1/8th part i.e., 64.32gm, *Ghrta* (Ghee) - 1 Part – 514.5 ml, *Kuvalaya Swarasa* (Fresh juice) - 4 Parts – 2058.2 ml. The final yield of the product was 216.4 ml.

Figure no 3: Preparation of *Kuvalayaghrta*

Figure no 4: Prepared *ghrta* just after *Ghrta* filtration

Results of Physicochemical Analysis of *Kuvalaya Ghrta*

Kuvalayaghrta was subjected to physicochemical evaluation. The organoleptic evaluation was performed followed by the preliminary chemical analysis of the sample. The observations have been tabulated below:

Table 5: Organoleptic evaluation of *Kuvalaya Ghrta*

Organoleptic Evaluation	*KuvalayaGhrta*
Colour	Green
Smell	Characteristic
Appearance	Viscous
Touch	Oily

Table 6: Physico-chemical evaluation of *Kuvalaya Ghrta*

Physico-chemical parameters	*Kuvalaya Ghrta*	API Standards of Plain *ghrta*	AGMARK Standards (Plain *ghrta*)
Refractive index	1.463±4x104	-	1.454
Acid value	2.8±0.02	-	3.0
Saponification value	196.907±1.23	Not more than 225	
Iodine value	23.68±0.28	Not more than 35	
Specific gravity	0.91±1x103	At 25 degree 1.01995	
Rancidity	Not rancid	-	

The values obtained were compared with the physico-chemical parameters of *Go-ghrta* mentioned in API. All the obtained values were within normal limits thus confirming the genuineness of the sample.

Chathushkuvalaya Rasayana is a potent Ayurvedic formulation mentioned by Acharya Vaghbhata in *Ashtangahrdya*. The formulation is widely considered as a potent memory enhancer. *Kuvalaya ghrta* may also have such properties. The plant is widely available throughout the marshy regions especially during rainy season. Though widely used in clinical practice, there is scarce published literature regarding its standardization aspects. Standardization of formulations is utmost important for the science to gain global acceptance.

Physico-chemical analysis revealed that it had 10±1.58 moisture content (%), Nil volatile oil content, 16.85±0.006 total ash (%), 17.11±0.11 acid insoluble ash (%), 5.3±0.023 water soluble extractive (%), 6.5±0.042 alcohol soluble extractive (%), a total sugar (%) of 0.3±0.001 and reducing sugar (%) of 0.2±0.0004. The fibre content (%) in the sample was found to be 15.2±0.026. (Table no 1)

Qualitative analysis of Ethanolic extract of the sample revealed that it had the presence of steroids, flavanoids, alkaloids and tannins. Saponins were absent in the sample (Table no 2). Thin Layer Chromatography of the ethanolic extract of the sample was done using different solvent systems and the best separation of the spots was obtained with the solvent system Toluene: Ethyl acetate in the ratio 6:1 with 6 drops of formic acid. Four well defined spots were observed which were photographed, Rf value calculated and recorded for further reference (Table no3) (Figure no 1). High Performance thin layer chromatography was also done using the same solvent system of Toluene:Ethylactetate in the ratio 6:1 with 6 drops of formic acid and the peak values were recorded. 8 peaks were observed and their Rf values were noted (Figure no 2).

Heavy metal contamination in medicinal plant material may be attributed to environmental pollution, soil contamination etc. Hence, in the present study Atomic absorption spectroscopy of the sample was done for 4 heavy metal namely lead, cadmium, zinc and iron which revealed that all the heavy metals were within permissible limits. This indicated the purity of the samples used for the study and safety in internal administration (Table no 4).

The safety parameters of the preparations to be used for the study namely *KuvalayaGhrta*(KG). The *ghrta* was prepared according to the standard procedure of preparation of *ghrta* as mentioned in Ayurvedic Formulary of India. The results revealed that the prepared sample was genuine and was safe for internal administration. All the above values obtained may be used for further studies as reference (Table no 5 &6).

Conclusion

Kuvalaya ghrta which is considered as a potent nootropic agent, is prepared by taking 1 part of the genuine sample of *Monochoria vaginalis* Presl. and 4 parts of *ghrta*. In this work the ingredients for the preparation of the *ghrta* were phytochemically and physico-chemically standardized. This can be used as a preliminary reference standard for further preparations.

Conflict of Interest: Nil

Abbreviations

AAS - Atomic Absorption spectroscopy
AFI – Ayurvedic Formulary of India
AGMARK – Agricultural Marketing
API – Ayurvedic Pharmacopoeia of India
EMEA – European Medicine Agency
HPTLC - High Performance Thin Layer Chromatography
ICH – International Conference on Harmonisation
KG – *KuvalayaGhrta*
Rf – Retardation factor
TLC - Thin Layer Chromatography
UV – Ultra Violet

References

1. Frati P, Kyriakou C, Del Rio A,Marinelli E,Vergallo GM,Zaami C,Busardo FP, Smart drugs and synthetic androgens for cognitive and physical enhancement: revolving doors of cosmetic neurology. Curr Neuropharmacol. January,2015;13(1); 5-11.

2. Lanni C, Lenzken SC, Pascale A, Del Vecchio I, Racchi M, Pistoia F, Govoni S. Cognition enhancers between treating and doping the mind. Pharmacol Res. March 2008; 57(3); 196- 213

3. Parle M, Vasudevan M. Memory enhancing activity of Abana an Indian ayurvedic poly herbal formulation. J Health Sci.February,2007; 53(1); 43-52.

4. Harshad Onkarrao Malve. Exploring Bhavanasamskara using Tinospora cordifolia and Phyllanthus emblica combination for learning and memory in mice. J Ayurveda. Integr Med. December 2015;6(4); 233-240.

5. Singh, R.H, Narasimhamurthy, K, Singh, G. Neuronutrient impact of Ayurvedic Rasayana therapy in brain aging. Biogerontology. December, 2008;9(6); 369-374.

6. Yadav D K, Reddy K R C, Kumar V. Beneficial effect of Brahmi Ghrita on learning and memory in normal rat. AYU. September, 2014; 35(3); 325-329.

7. Anna Moreshwar Kunte& Krishna Ramachandra Sastri Navre; Ashtangahrdaya of Vaghbhata.9th edition.Varanasi;Chaukhamba Orientalia;1995.p926.

8. Chandran R, Thangaraj P, Thankarajan S, Karuppusamy A Antioxidant and anti-inflammatory potential of Monochoria vaginalis (Burm. F.) C. Presl.: A Wild edible Plant. J. Food Biochem. August ,2012 ; 36(4) ; 421-431

9. Latha B, Latha M S. Antioxidant and curative effect of Monochoria vaginalis methanolic extract against carbon tetrachloride induced acute liver injury in rats. Der Pharma Chemica. January , 2013; 5(1); 306-312

10. Chandran R, Thangaraj P. Nutritional Assessment of Monochoria vaginalis, a Wild Edible Vegetable Supplement to the Human Diet, Int. J. Veg. Sci. April 2012 18(2); 199-207

11. Anonymous. Ayurvedic Formulary of India.1st edition. New Delhi: The Controller of Publications civil lines; 2003.97p.

12. Anonymous. The Ayurvedic Pharmacopoeia of India.1st edition, Vol 9, New Delhi, Pharmacopoeia Commission for Indian Medicine & Homoeopathy; 2001.111p

13. Kokte C K.Pharmacognosy. 50ed.Pune; Nirali Prakashan; 2014.A.22p

14. Patel SS,Patel JK,Savjani JK. Systematic review of plant steroids as potential anti-inflammatory agents: Current status and future perspectives.J. Phytopharmacol.April, 2015,4(2);121-125

15. Ayaz M,Sadiq A,Junaid M,Ullah F,Ovais M,Ullah I,Ahmed J,Shahid M. Flavonoids as Prospective Neuroprotectants and Their Therapeutic Propensity in Aging Associated Neurological Disorders. Front Aging Neurosci.June, 2019;11;404705

16. Hussain G, Rasul A, Anwar H, Aziz N, Razzaq A, Wei W,Ali M,Li J,Li X. Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. Int J Biol Sci. Int J Biol Sci. March, 2018; 14(3);341–57.

17. Hussain G, Huang J, Rasul A, Anwar H, Imran A, Maqbool J,Razzaq A,Aziz N, Makhdoom U,Konuk M, Sun T, Putative Roles of Plant-Derived Tannins in Neurodegenerative and Neuropsychiatry Disorders: An Updated Review. Molecules.June, 2019,24(12);2213

18. Rojas-García A, Fernández-Ochoa Á, Cádiz-Gurrea M de la L, Arráez-Román D, Segura-Carretero A, Neuroprotective Effects of Agri-Food By-Products Rich in Phenolic Compounds. Nutrients.January, 2023;15(2).

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