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Original Research Article

Physico-analytical study of *ghrita* formulation indicated in *Vataj Artava Dushti* with special reference to hypomenorrhoea and oligomenorrhoea

Ajay Kumar[1]*, Neelam[1], Sunita Suman[1], Riden Saxena[2]

[1]Department of Prasuti Tantra and Stri Roga, Faculty of Ayurveda, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

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***Correspondence:**
Dr. Ajay Kumar,
E-mail: dr.ajayayu84@gmail.com

ABSTRACT

Background: To establish safety profile for oral use of a classical Ayurvedic *Ghrita* formulation indicated in menstrual disorder known as *Vataj Artava Dushti* recent scientific techniques were used to fulfill the criteria of WHO guidelines for herbal drug formulations like heavy metal content and microbial load estimation, chromatography and others physic- analytical characteristic.
Methods: In the present study formulated *ghrita* sample was selected for estimation of refractive index, total ash value acid, iodine, saponification, loss on drying, PH, fat content, sugar content, heavy metals namely lead (Pb), cadmium (Cd), mercury (Hg), acid value, iodine value, by ICP-MAS, TLC and HPTLC.
Results: SEM- EDX shows presence of elements like Carbon, Oxygen and Aluminum, Lead, Calcium, Magnesium, Zinc. Refractive index 1.459, total ash 12%, saponification technique 55.26, Iodine value 4.32, acid value 6.6, PH 5.23 Loss on drying 0.32 fat 76.58% and sugar content 13.37%, Mercury <10 ppb, Lead 2.33 ppm, Cadmium 0.20 ppm. Total bacterial count 0.5×103, total fungal count <103. TLC and HPTLC results reveals presence of active compound.
Conclusions: The Physico-analytical, heavy metals, microbial and chromatographic study of the formulated *ghrita* follows the standard parameters. This study can be helpful for the future research regarding establishment of safety profile and therapeutic efficacy of *ghrita* formulations.

Keywords: Acid value, Ayurveda, Hypomenorrhoea, Iodine value, Oligomenorrhoea, Siddha *ghrita*, Saponification value, Vataj artava dushti

INTRODUCTION

Ghrita is originated from its root word "ghr" which means bright or to make bright. Ghrita is Sanskrit name for clarified buttermilk, *ghrita* has great medicinal and nutritional value that's why Ayurvedic text has mentioned ghrita under *ajasrik rasyan* to be used in daily routine diet.[1] *Ghrita* has unique property of pacifying the vitiated *vata and pitta dosha* on the other hand it causes *agnidipana* means stimulate the digestive enzyme to increase appetite, digestion and absorption.[2] Modern researches have proved that lipid based formulation are more efficient carrier for drug delivery to the target organ system. One of the oral formulation consisting of herbs like Clerodendrum serratum (Bharangi) *Kapha vata Shamak*, Cedrus deodar (Bhadradaru) *Kapha vata shamak* and glycyrrhiza glabra (Madhuk) *Vata-pitta shamak*, working synergistically in a formulation as *tridosh shamak* was mentioned for the treatment of *vataj artava dushti*.[3,4] *Vataj artava dushti* is one of the eight type of menstrual disorder that is caused by vitiated *vata dosha*.[5,6] Clinically it can be correlated with

Oligomenorrhoea and Hypomenorrhoea as mentioned in modern Gynaecology.[7] With the objective to access safety and efficacy of the formulation physico- analytical and microbial studies were done and result was discussed.

METHODS

Scanning Electron Microscope (SEM) and Energy Dispersive X-ray spectroscopy (EDX) techniques was used for characterization of dried raw drug samples.[8] *Ghrita* based formulation was prepared by standard procedure mentioned in Ayurvedic formulary of India.[9] Estimation of acid value, iodine value, saponification value, Fat content, sugar content, PH, loss on drying, refractive index.[10] AOAC- official methods of analysis 18th edition 2005 for Heavy metal analysis for lead and cadmium, Mercury Analyser MA 5840-D for estimation of mercury.[11,12] WHO guidelines (QAS/05,131/Rev-1) was used as lab method for microbial load estimation.[13] For TLC photo documentation toluene and ethyl acetate (9:1) was used as solvent system. For HPTLC E. Merck. Aluminium plate pre-coated with silica gel60 f_{254} of 0.2 mm thickness. CAMAG TLC scanner densitometric system with wincats software and linomet-5 as an automatic applicator were used.[14,15]

RESULTS

Identification and characterization of dried raw samples shows presence various elements like Carbon, Oxygen and Aluminum, Lead, Calcium, Magnesium, Zinc (Figure 1, Figure 2, Figure 3).

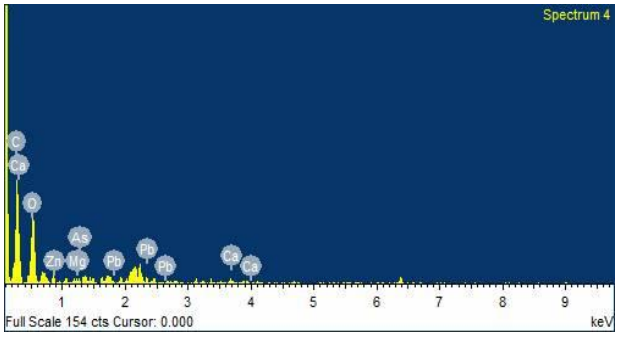

Figure 1: EDS spectrum *Glycirrhyza glabra* root.

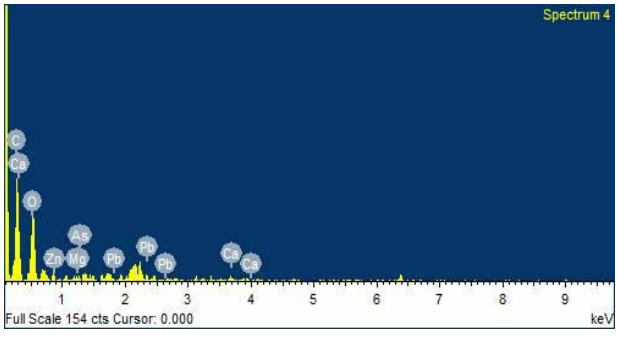

Figure 2: EDS spectrum *Cedrus deodar*.

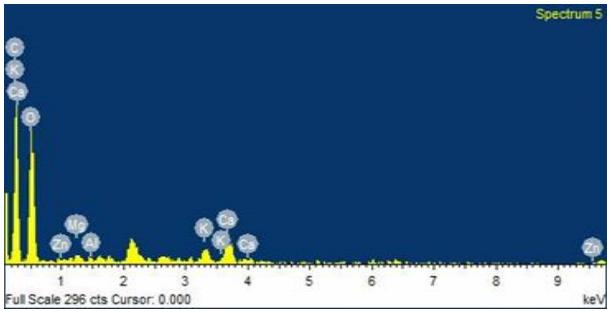

Figure 3: EDS spectrum *Clerodendrum serratum*.

The organoleptic study of the *ghrita* based formulation shows light brown colour, fragrant odour and sweet taste which is mentioned in Table 1.

Table 1: Result of organoleptic study formulated *ghrita*.

Organoleptic parameters	Results
Colour	Light brown
Odour	fragrant
Taste	Sweet

Refractive index 1.459, is the ratio of velocity of light in the vaccum and velocity of light in substance. Total ash 12% shows presence of inorganic content in the formulation. Saponification value of formulated *ghrita* 55.26 represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify 1g of fat normal value is not more than 225.

The Iodine value of the formulated *ghrita* is 4.32 is the weight of iodine absorbed by 100 parts by weight of the substance, normal iodine value is not more than 35. PH 5.23. Acid value is the measure of mg of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance acid value of *ghrita* formulation is 6.6. Loss on drying 0.32 % indicates moisture content of the substance it should be less than 1%. Formulated *ghrita* contains 76.58% fat content and sugar content 13.37% (Table 2).

Table 2: Results physico-analytical study of formulated *ghrita*.

Analytical parameters	Results
Refrective index	1.459
Total ash value	12%
Saponification value	55.26
Iodine value	4.32
Acid value	6.6191
PH Value	5.23
Loss on drying	0.32 %
Fat content	76.58%
Total sugar	13.37

Heavy metal analysis reveals mercury <10 PPB, cadmium 0.20 ppm, lead 2.33 ppm mentioned in Table 3.

Table 3: Results of heavy metal analysis formulated *ghrita.*

Heavy metal	Result
Mercury	<10 PPB
Lead	2.33 ppm
Cadmium	0.20 ppm

Microbial load estimation shows total bacterial count 0.5×10^3 and total fungal count $<10^3$. Test for other specific pathogen is negative defined in Table 4.

TLC shows maximum 5 spots in chloroform extract at 254 nm depicted in Table 5, Table 6 and Table 7.

Table 4: Microbial load estimation of formulated *ghrita.*

Name of the drug	Total bacterial count(cfu/g)	Total fungal count (cfu/g)	*Entero-bacteriaceae*	*Escherichia-coli*	*Salmonella spp*	*Staphylococcus aureus*	*Pseudomonas aeroginosa*
Formulated *Ghrita*	0.5×10^3	$< 10^3$	Absent	Absent	Absent	Absent	Absent

Table 5: Rf value of formulated *ghrita* in hexane-extract.

Wave length	Colour	R_f values
At 254 nm	Green	0.05,0.36,0.47,0.65
At 366 nm	Blue	0.06,0.40,0.56,0.64
Dipped in vaniline sulphuric acid	Grey	0.04,0.40,0.49,0.59

Table 6: Rf value of formulated *ghrita* in choloroform-extract.

Wave length	Colour	R_f values
At 254 nm	Green	0.03,0.06,0.34,0.38,0.45
At 366 nm	Blue	0.06,0.41,0.52
Dipped in vaniline sulphuric acid	Grey	0.04,0.05,0.40,0.48

Table 7: Rf value of formulated *ghrita* ethanol-extract.

Wave length	Colour	R_f values
At 254 nm	Green	0.07,0.33,0.42
At 366 nm	Blue	0.04,0.60
Dipped in vaniline sulphuric acid	Grey	0.34,0.42,0.52,0.88

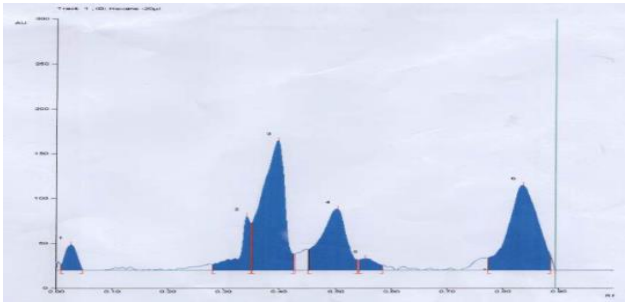

Figure 4: HPTLC finger print profile of DTL 1605378 -hexane extract-20µL. (scanning at 254 nm).

HPTLC fingerprint shows 6 peaks with average Rf (Retention factor) value 0.37 at which maximum concentration 35.32% present (Figure 4, Figure 5, Figure 6).

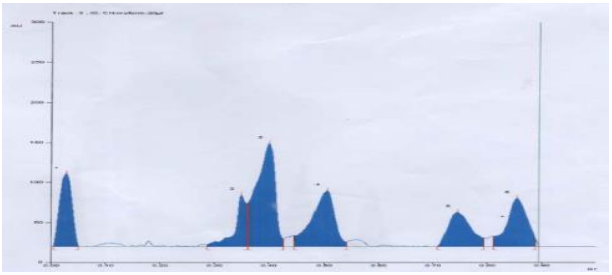

Figure 5: HPTLC finger print profile of DTL 1605378 -chloroform extract-20µl (scanning at 254 nm).

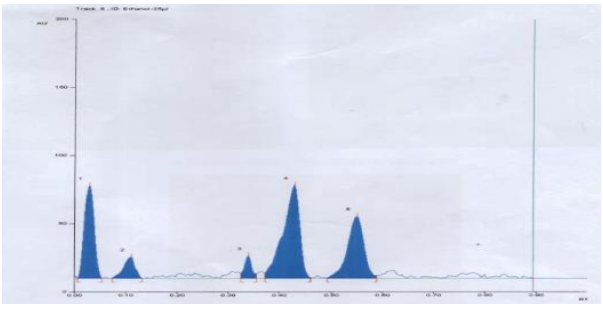

Figure 6: HPTLC finger print profile of DTL 1605378 -ethanol extract-25µl (scanning at 254 nm).

DISCUSSION

Ayurvedic formulation have been used traditionally since very long time in Indian subcontinent. Although demands of Ayurvedic product as dietary supplements and medicinal purposes throughout the globe are increasing.[16] But unfortunately, herbal preparations are considered only as dietary supplement unlike pharmaceutical

preparations hence preparations due to lack of herbal with evidence based scientific study regarding their safety and efficacy in most of the developed countries.[17] Increasing environmental pollution, soil pollution, use of artificial fertilizers and industrial affluent have serious impact to the quality and safety of herbal drugs. Lead, arsenic cadmium, mercury are the nonessential toxic heavy metal have no biological role and are toxic to the microorganism.[18-20] Maximum permissible limit for heavy metal recommended by the Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives maximum permissible daily intake *of* Cadmium (Cd) 0.3 µg/g, 250 µg/d of Lead, 50 µg/d of Mercury, and 150 µg/d of Arsenic for a 70-kg adults.[21,22] Some fungi and Gram-negative bacteria contaminating dairy foods which includes *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter* may constitute a health risk if pathogenic species are present.[23,24] Therefore, highest level of hygiene should be maintained from the beginning of the process till the manufacturing and packaging of finished product. To maintain lowest possible level of pathogenic organism as per WHO guideline for safe internal use of herbal preparations.[11]

CONCLUSION

The formulated *ghrita* mentioned in Ayurveda for the management of *vataja artva dushti* (menstrual disorder). Study based on various parameters results at conclusion that the formulated *ghrita* is palatable to the patients and have stable shelf life at room temperature, presence of heavy metals below permissible limit, microbial contamination is within the permissible limit. Chromatographic study results suggest the presence and incorporation of active constituents of herbal drugs into lipid formulations. For the prospective research, study will be helpful to the establishment of safety profile, efficacy and acceptance of classical Ayurvedic *ghrita* formulation.

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