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Pharmaceutico Analytical Study of Saindhava Hinguvadi Ghrita

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ABSTRACT:Bhaishajya Kalpana is a branch of Ayurveda which deals with the preparation of many formulations.Sneha Kalpana is a unique method of preparation which coming under Bhaishajya Kalpana.Sneha Kalpana helps to utilise fat soluble and water soluble properties of drugs.Among four snehas Ghrita is the best one.It is stated as Sarvasnehottama and Samskarasyanuvartanam. The present study is concerned with the formulation Saindhava Hinguvadi Ghrita which is mentioned in Charaka Apasmara Chikitsa.In classics many ghrita preparations are mentioned under Apasmara Chikitsa.Many are not explored for its therapeutic efficacy.Till now no scientific studies have been carried out in this formulation.The aim of the study is to scientifically establish the anti epileptic effect of Saindhava Hinguvadi Ghrita. Experimental study revealed as follows:-Saindhava Hinguvadi Ghrita showed statistically nonsignificant results in experimental study.

KEYWORDS: Bhaishajya kalpana, Snehakalpana, Saindhava HinguvadiGhrita, Antiepileptic effect, Experimental study.

I. INTRODUCTION

Ayurveda is a system of healing unlike any other,offering a unique approach to health care in the form of self discovery with its roots in ancient India.Ayurveda is a tradition thought to be over five thousand years old [1] .The name Ayurveda is derived from two words in Sanskrit,`ayuh‘ meaning` life‘or longevity and veda meaning` science‘ or sacred knowledge.So it is the science of longevity.The earliest known references of Ayurveda appeared in scholarly texts from the time called vedic era[2] . The word Bhaishajya Kalpana is composed of two words- `bhaishajya‘ or `bheshaja‘ and kalpana.The word bheshaja or bhaishajya means that which wins the fear of disease or restores the health of a person by stabilizing the doshas.The word kalpana means the process or the method employed for the preparation of pharmaceutical products.

Sneha Kalpana is a unique dosage form in Ayurveda.It may be defined as ―A pharmaceutical process to prepare oleaginous medicaments from the substances like kalka,kwatha,dradravya taken in specific proportion and by subjecting them to unique heating pattern and duration to fullfill certain pharmaceutical parameters,according to the need of therapeutics[3] .

Sneha Kalpana helps to utilise the fat soluble and water soluble properties of drugs.In classics many sneha preparations are mentioned under Apasmara Chikitsa.Saindhava Hinguvadi Ghrita is such a preparation mentioned by Charakacharya in Apasmara Chikitsa.

Approximately fifty million people currently live with epilepsy world wide.Globally estimated 2.4million people are diagnosed with epilepsy each year[4].Prevalance in India is 5.59-10/1000.Prevalance is higher in rural compared to urban.There is high of premature mortality in people with epilepsy[5] . Epilepsy contributes a variety of medical,social,physiological and economic burdens.The impact of disease is experienced in all aspects of patient‘s life and also some extend tothe family[6] . In classics it is mentioned that among four snehas, Ghrita is the best one and it is stated as sarvasnehottama,samskarasya anuvartanam[7].Its daily usage increases dhi,smriti,medha[8].Many ghrita preparations mentioned in classics are still not proved for its therapeutic efficacy.Analysis of Saindhava Hinguvadi Ghrita using modern parameters is of much importance in the present era,With out analytical studyresearch of a drug is incomplete.It provides some standards to judge quality it helps us to prove the authenticity of the study. Analysis becomes the core portion of this study.The analysis work was carried out at Quality control lab of M V R Ayurveda Medical College.

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II. MATERIALS AND METHODS

Preparation of Saindhava Hinguvadi Ghrita:

- Ghrita was taken in a clean dry vessel and kept over medium flame.
- Add mutra and kalka to the ghrita.
- Whole mixture is stirred until gets a homogenous mixture.
- Continuous stirring was done through out the procedure.
- Medium flame was maintained through out the procedure.
- Heating will be continued till sneha sidhi lakshanas were obtained.
- After attaining sidhi lakshanas,ghrita was filtered through a double layered cloth,when it is in hot stage and stored in an air tight glass bottle after cooling.
- Paka sidhi lakshanas assessed as per the reference in Sarangadhara samhita.

▪ Analytical study includes :

Analysis of plain ghrita and three samples of Saindhava Hinguvadi ghrita :

- Organoleptic characters: Colour, Odour etc.
- Physical methods: Specific gravity,Refractive index,Viscosity,Loss on drying.
- Chemical methods: Acid value,Iodine value,Saponification value,Peroxide value.
- Instrumental techniques: HPTLC

- Organoleptic characters :

Organoleptic characters are the preliminary tests that can be performed with out the use of any equipment.These characters are perceived by the sense organs and give a basic knowledge about the nature and form of the material to be evaluated and are the first step in assessing the quality of any material.

▪ Physical methods:

❖ Specific gravity: Procedure – A thoroughly cleaned and dried pycnometer was selected and weighed.Fill the pycnometer with distilled water and record the weight.Then again clean the pycnometer and made it dry.Fill the same pycnometer with the sample of ghrita to be tested,in such a manner to prevent entrapment of air bubbles inside.Record the weight with sample. Specific gravity of a given sample = A-B C-B Where, A = weight in gram of the specific gracity bottle with ghrita B = weight in gram of the specific gravity bottle C = weight in gram of the specific gravity bottle with water.

❖ Refractive index : The refractive index was read on an Abbe refractometer which gives the true refractive index or on a butyro-refractometer which reads on an arbitrary scale at constant temperature near 40℃as possible.

Procedure[1]: • Open the prism of Abb type refractometer and clean with soft cotton.•Place a drope of sample to be tested on a lower part of the prism and close the refractometer.
Observe through the eye piece and turn the depression correction compensator knob until the coloured indistinct boundary seen between the light and dark field becomes a sharp line. • Adjust the knurled knob until the sharp line exactly intersects the midpoint of the crossed wires in the image.Read the refractive index from the magnifier in the pointer and record the reading. • Record the ambient temperature.

❖ Viscosity :Viscosity measured by Ford Cup Viscometer.

Procedure: • Close the orifice of viscometer with a finger and fill the cup completely with ghrita. • Place glass plate on the cup,the negative pressure will hold the liquid inside. Slide the glass plate from the top of cup and simultaneously start timing the efflux. Confirm the desired temperature of the sample directlty in the efflux stream.Collect the sample in a vessel. • Measure the time to the nearest 0.2 sec from the moment efflux commences until the first break in the stream occurs below the orifice. Relative viscosity of a liquid compared to a known liquid is calculated by formula Ƞ1= p1 t1 x η2 P2 t2 Where,η1 and η2 are viscosities of unknown & known liquids P1&P2 are densities of unknown & known liquids t 1& t 2 are their time to pass. Here relative viscosity of samples compared to that of water.

❖ Loss on drying Procedure: • About 5 gm of accurately weighed substance was placed in a tarred evaporating dish.The sample was evenly distributed in the dish. • Dried the sample at 105℃ for 5 hrs. • After 5 hrs,placed the evapourating dish in a desicator to bring down the temperature of china dish to room temperature. • Weighed the sample.Drying and weighing was continued at 1 hr interval till the difference between two successive weighing corresponds to not more than 0.25%. • Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desicator show not more than 0.01 gm

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difference[2] . • Calculate loss on drying with the formula LOD = W2-W3 ×100 (W2-W1) Where, W1 = empty weight of evaporating dish W2 = weight of evapourating dish with sample W3 = weight of evapourating dish after drying.

- Chemical methods

❖ Acid value:

Procedure: • Dissolved about 10g of accurately weighed sample,in a mixture of equal volumes of ethanol (95%) and ether (50ml), previously neutralized with 0.1M potassium hydroxide to phenolphthalein solution. • If the sample does not dissolve in the cold solvent,connect the flask with a reflux condenser and warm slowly, with frequent shaking,until the sample dissolves.

1ml of phenolphthalein solution was added and titrated with 0.1M potassium hydroxide until the solution remained faintly pink after shaking for 30 seconds. Calculated the acid value from the given expression. Acid value = a×56.1×normality of KOH W = a×5.61 W

Where,a is the number of ml of 0.1N potassium hydroxide required and w is the weight in gram of the substance taken[2,3] .

❖ Iodine value:

Procedure: • An accurately weighed quantity of the sample was placed in a dry 500ml iodine flask. 10ml of carbon tetra chloride was added and dissolved. • 20 ml of iodine monochloride solution was added,inserted the stopper and allowed to stand in dark at a temperature between 150 and 250 for 30 minutes. • 15ml of potassium iodide solution was placed in the cup top,carefully removed the stopper and rinsed the stopper and the slides of the flask with 100ml of water. • Shake and titrated with 0.1M sodium thiosulphate using starch solution as indicator. • Noted the number of ml required(a) • Repeat the operation omitting the substance being examined and noted the number of ml required(b)[4] . • Calculate iodine value from the expression, (b-a)×0.01269×100 Iodine value = W = (b-a)×1.269 W Where W is the weight in gram of the substance taken.

❖ Saponification value:

Procedure: • Weigh accurately about 2g of the sample in a tarred 250ml flask. • Add 25ml of 0.5M ethanolic solution of potassium hydroxide and attached a reflux condenser and boiled in water bath for 30 minutes. • Frequently rotated the contents of the flask,cooled and added 1ml of phenolphthalein solution and titrated excess of alkali with 0.5N hydrochloric acid. • Noted the number of ml required(a). • Repeat the experiment with same quantities of same reagent without the substance.Noted the number of ml required(b)[4] . Saponofication value = (b-a)×1×(56.11×0.5)×1 W = (b-a)×28.05 W Where,W is the weight in gram of the substance.

❖ Peroxide Value : Peroxide value is the number of milli equivalents of active oxygen that expresses the amount of peroxide contained in 1000g of the substance.

Procedure : Unless otherwise specified in the individual monograph, weigh 5g of the substance being examined,accurately weighed,250 ml glass-stoppered conical flask, add 30ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform,swirl until dissolved and add .5ml volumes of saturated potassium iodide solution.Allow to stand for exactly 1 minute,with occasional shaking,add 30ml of water and titrate gradually,with continuious and vigorous shaking,with .01M sodium thiosulphate until the yellow colour almost disappears.Add 0.5ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a Methodology Pharmaceutico-Analytical and Experimental Study of Saindhava Hinguvadi Ghrita WSR to Its Antiepileptic Effect 160 ml).Repeat the operation omitting the substance being examined (b ml).The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1ml[5] .
Calculate the peroxide value from the expression,
Peroxide value = 10(a-b)/w Where w = weight in gram of the substance.

❖ Instrumental technique: HPTLC

HPTLC of three samples of Saindhava Hinguvadi ghrita were carried out at Centre for Medicinal Plants Research,Arya Vaidya Sala,Kottakkal.

The specification of the procedure done as mentioned below,

Stationary phase Plate size(x ×y) :8.0×10.0cm
Material :HPTLC plates silics gel 60F254
Manufacturer :E.MERCK KGaA
Sample application – CAMAG Automatic TLC Sampler 4(ATS4)
Sample solvent name :Methanol
Application volume : 5.0µl

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III. OBSERVATION AND RESULTS

- Analysis of plain ghrita : Plain ghrita is tested for its organoleptic and physico chemical parameters.The procedure used is standard analytical procedure used for the analysis of fats and oils, mentioned under analytical procedures.The tests were done under the supervision of laboratory technician.

Each test was repeated for three times and its average was taken into consideration.This was done to minimize manual mistakes.

- Organoleptic characters: Colour – Yellowish in colour. Odor - Ghrita had characteristic peculiar smell.

Table: Showing physio-chemical parameters of plain ghrita

Parameter	Value
Refractive index	1.4580
Specifi gravity	.9216
Viscosity	.9678
Loss on drying	.3959
Acid value	4.6214
Iodine value	18.6542
Saponification value	220.5162
Peroxide value	.80

❖ Analysis of Saindhava Hinguvadi Ghrita

- Organoleptic parameters

Colour of samples of Saindhava Hinguvadi ghrita determined by matching it with Asian Paints colour chart.
Colour – Ginger root,Odor – Characteristic,Taste – Bitter

- Physio-Chemical parameters of Saindhava Hinguvadi Ghrita

Table: Specific gravity(in mg/g)

Sample	Values	Values	Values	Mean value
I	.9106	.9110	.9120	.9112
II	.9108	.9128	.9130	.9122
III	.9125	.9132	.9128	.9128

Specific gravity of plain ghrita was.9216,compared to this all samples having slight increased values.

Table: Refractive index

Sample	Values	Values	Values	Mean Value
I	1.4590	1.4590	1.4590	1.459
II	1.4590	1.4590	1.4590	1.459
III	1.4590	1.4590	1.4590	1.459

Refractive index of plain ghrita was 1.4580.All samples having same value as that of plain ghrita.

Table71.Viscosity

Sample	Values	Values	Values	Mean Value
I	.9840	.9842	.9840	.9840
II	.9838	.9840	.9836	.9838
III	.9836	.9838	.9840	.9838

Vicosity of plain ghrita is .9645,all the samples having less viscosity ranges than that of plain ghrita.

Table:72.Loss on drying (%)

Sample	Values	Values	Values	Mean Value
I	.3306	.3243	.3885	.3478
II	.3496	.2392	.2016	.2873
III	.1607	.3871	.1978	.2485

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Loss on drying of plain ghrita was.3959,compared to this all the samples having more LOD values.

- Chemical parameters:

Table:Acid value(mg/g)

Sample	Values	Values	Values	Mean Value
I	5.8437	5.8434	5.8435	5.8435
II	5.8435	5.8434	5.8434	5.8434
III	5.8432	5.8434	5.8435	5.8433

Acid value of plain ghrita was 4.6214,compared to this all the samples having slight increase in value.

Table:74.Iodine value(mg/g)

Sample	Values	Values	Values	Mean Value
I	20.1236	20.1240	20.1280	20.1252
II	20.1310	20.1320	20.1250	20.1293
III	20.1320 20.1280	20.1280	20.1360	20.1320

Iodine value of plain ghrita was 18.6542,compared to this all samples having slight increase in values.

Table: Saponification value(mg/g)

Sample	Values	Values	Values	Mean Value
I	251.6635	256.4571	254.0377	254.0527
II	269.28	252.8450	250.3528	257.4926
III	252.8450	251.5281	252..7146	252.3625

Saponification value of plain ghrita was 220.5162,all the samples having increase in value compared to plain ghrita.

Table : Peroxide value(mg/g)

Sample	Values	Values	Values	Mean Value
I	1.1210	1.1214	1.1220	1.1214
II	1.1220	1.1230	1.1220	1.1223
III	1.1310	1.1218	1.1220	1.1249

Peroxide value of plain ghrita was.8.All the samples having slight increase in value compared to plain ghrita.

Table: Showing mean values of analytical report of Saindhava Hinguvadi ghrita

S NO	Parameters	Sample I	Sample II	Sample III
1	Specific gravity	.9112	.9122	.9128
2	Refractive index	1.459	1.459	1.459
3	Viscosity	.9840	.9838	.9838
4	Loss on drying	.3478	.2873	.2485
5	Acid value	5.8435	5.8434	5.8433
6	Iodine value	20.1252	20.1293	20.1320
7	Saponification value	254.0527	257.4926	252.3625
8	Peroxide value	1.1214	1.1223	1.1249

❖ HPTLC results

- Comparison of Rf values and area percentages of the three samples.

The plus(+)‘ sign indicates presence of a peak in the range mentioned.The percentage values with respect to their concentrations have been provided in brackets.

Table: HPTLC Results

Rf Value	Sample I	Sample II	Sample III	Plain ghrita
.04-.07	+(.34)		+(.58)	
.08-.12	+(1.12)	+(.63)		
.13-.17	+(2.27)	+(2.26)		+(1.64)
.17-.23	+(4.27)	+(2.63)		

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.25-.30	+(1.75)	+(1.09)	+(1.99)	
.30-.34	+(1.20)	+(.83)	+(.97)	
.40-.44	+(3.03)	+(1.75)	+(2.39)	
.45-.52	+(5.74)	+(3.44)	+(5.21)	
.52-.69	+(41.47)	+(37.74)	+(42.93)	+(48.02)
.67-.73		+(5.02)	+(4.14)	+(6.46)
.74-.78	+(3.27)		+(3.85)	
.79-.84	+(3.97)	+(7.13)	+(5.48)	+(6.17)
.88-.99	+(17.63)	+(29.13)	+(17.06)	

IV. DISCUSSION

Organoleptic characters are perceived by the sense organs and give a basic knowledge about the nature and form of the material to be evaluated.It is the first step in assessing the quality of any material.
Colour-Ginger root according to Asian paints colour chart,Odor-Characteristic odor,Taste-Bitter.

- Specific gravity : Here all the samples having slightly increased specific gravity compared to ghrita.The weight of lipid material is affected by basic constitution,dissolved constituants used in the processing of formulation or any other compound which may be formed during the process etc.In case of fat it may also with thermal effects.This slight increase in density can be due to addition of certain compounds from other ingrediants.
- Refractive index : Refractive index is found to be same in all samples.
- Viscosity : Viscocity values of all samples are slightly decreased compared to viscocity value of ghrita.The viscosity of fats and oils decreases slightly with an increase in unsaturation.Oils and fats containing a greater proportion of fatty acids of relatively low molecular weight are less viscous than ones of an equivalent degree of unsaturation,but containing a higher prpportion of high molecular weight acids8.
- Loss on drying : Indicates total moisture content in a sample.On analysing it was found that when compared to ghrita loss on drying value increased in all the samples.This increase is due to escaping of moisture on heating.
- Acid value : Acid number indicates the measure of amount of carboxylic acid groups in a chemical compound such as fatty acids or in a mixture of compounds.Acid value indicates the free fatty acid present in ghrita which is related to its stability.It affects the shelf life,flavour and the stability of the ghrita.Here all the samples showed slight increase in the value compare to that of plain ghrita.This indicates that during the process of snehapaka,hydrolysis of ghrita takes place,which may be promoted by the reaction of triglycerides in the ghrita with the active ingrediants in ghrita samples resulting in glycerol and free fatty acids.

- Iodine value : Iodine value is a measure of degree of unsaturation of fat.The more the iodine number,more the unsaturated fatty acid bonds present.The product will be more reactive,less stable and more susceptible to oxidation and rancidity.Here the samples are having a slight increase in value compared to that of plain ghrita.

- Saponification value :The saponification value indicates the average molecular weight/chain length of all fatty acids present.The longer chain fatty acids have a low saponification value and shorter chain fatty acids have a high saponification value.Shorter chain fatty acids have faster rate of absorption than longer chain fatty acids.Here samples are having more saponification value than that of plain ghrita.

- Peroxide value :It is used to assess the stability or rancidity of fats by measuring lipid peroxides and hydro peroxides formed during initial stages of oxidation.The peroxide value of all samples are slightly increased compared to that of plain ghrita.It may be due to exposure of moisture content during storage etc.

- High Performance Thin Layer Chromatography(HPTLC) : HPTLC gives the knowledge regarding components of the formulations.The prepared drug was subjected to HPTLC fingerprinting at different wavelengths(254nm &366nm).The colour spots observed indicates the presence of different components in the sample.

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Total 8 peaks obtained for plain ghrita.
Total 13 peaks obtained for all the samples.

Illumination instrument CAMAG Visualizer : 170205 (Visualizer_170205) Digital camera type : snr & Lens DXA252: 441480210, Computer, 12 mm, f4.0 Created by : on avscmpr:Friday,November01,201912:02:59PM

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Save mode Lossy (JPG)
Exposure mode Automatic,digitallevel:80%, Band

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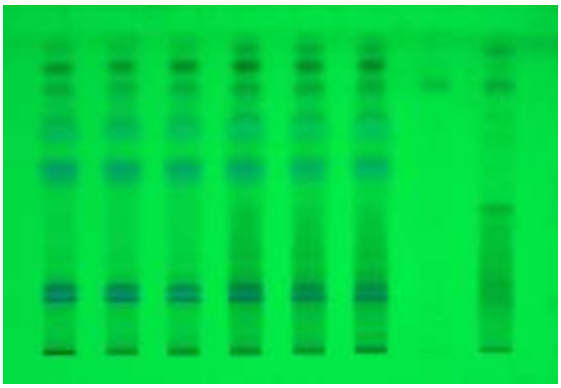

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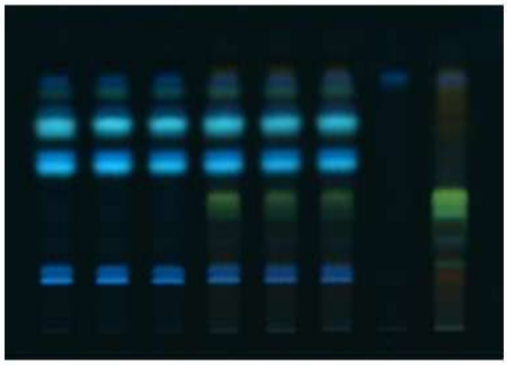

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Contrastenhancement: 1.00
Brightness: 0.00
Accentuation: 0.80
Colorsaturation: 1.30
Blankplatecompensation: N/A

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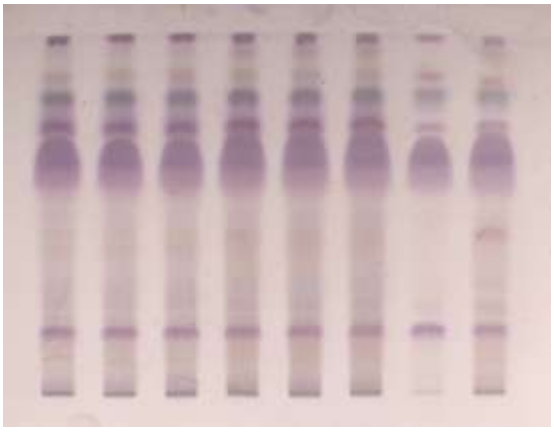

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