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Research Article

STANDARD OPERATING PROCEDURE OF *PANCHATIKTA GHRITA* AND STUDY OF ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Sneha kalpana is the specialized pharmaceutical procedure to prepare oleaginous medicine from *Kalka* and *Drava- dravya*. While reviewing ancient texts variations are found regarding preparatory procedures, ingredients used, confirmatory tests advised, indication of *Panchatikta Ghrita*. All the information available is in scattered form in different texts. To overcome these ambiguities it is necessary to develop Standard Operating Procedure (SOP). In this SOP the Sequential, Scientific and Logical illustration and documentation of each and every step is given. In Pre-Operative phase *Shodhana* of *Guggulu* and *Murchchana* of *Ghrita* was carried out. In operative phase *Kwath*-4L, *Kalka*-90gm, *Guggul*-70gm, *Murchchit ghrita*-240ml were taken together and subjected *Snehasadhan vidhi* for 3 days giving 2 hours of heat daily keeping the temp. in between 50^0 to 90^0 C till *Snehasiddhi lakshana* obtained. 175gm of *Panchatikta ghrita* was prepared according to this SOP. It took 9 days to complete entire procedure. Quality parameter assessment was done by carrying physicochemical analysis viz. Sp. Gravity, Ref. index, Congealing point Sap value, Iodine value etc. Further antimicrobial study of *Panchatikta ghrita* against E-coli, Streptococcus Pyogens, Staphylococcus Aurens, S.typhi, C. Albicans etc was carried out. But no zone of inhibition was seen for any of the selected micro-organisms. The in vitro negative results didn't entirely reveal its therapeutic efficacy. In *Ayurvedic* therapeutics the Pharmacokinetics and Pharmacodynamics of the drug entirely depends upon *Rasa, Veerya, Veepaka, Prabhava* and balance of *Tridosha* in the body. The ingredients of *Panchatikta Ghrita* with their *Rasa, Veerya, Veepak* are very much competent to exhibit antimicrobial property from *Ayurvedic* perspective.

KEYWORDS: Antimicrobial property, in vitro, *Panchatikta Ghrita, Sneha kalpana,* SOP**.**

INTRODUCTION

Rasashastra & Bhaishajyakalpana (Ayurvedic Pharmaco-therapeutics) is one of the branch of *Ayurveda* enriched with wide range of dosage forms. *Sneha kalpana* is one of the commonly prescribed dosage form in day-to-day practice. It is the specialized pharmaceutical procedure to prepare oleaginous medicine from substances like *Kalka* and *Dravadravya.* **[1]** They are prepared by subjecting this mixture to uniform heat for a specific duration to fulfill certain pharmaceutical parameters as per the standard guidelines prescribed in texts.[2]

While reviewing ancient texts variations are found regarding preparatory procedures, ingredients used, confirmatory tests advised, indication of *Panchatikta Ghrita*. All the information available is in scattered form in different texts. To overcome these ambiguities it is necessary to develop Standard Operating Procedure (SOP) of *Panchatikta Ghrita*. SOP is a set of instructions having the force of a directive, covering those features of operation that lend themselves to a definite or standardized procedure without loss of effectiveness.

Increased Antibacterial Resistance and untoward effects of antibiotics is the major problem in present era. The ingredients of *Panchatikta Ghrita*, shows their strong antibacterial activity which would presumably act against the causative principle present in the bacteria responsible for *Bhagandar* (fistula in ano), *Kushtha* (psoriasis) etc. For instance, the protien and carbohydrate content of Neem have antimicrobial activity against S.aurens, E.coli, S.typhi, C. albicans, etc. Vasicine and alkaloids present in *Vasaka* show anti-oxidant and anti-inflammatory property. Also the essential oil show antimicrobial activity against Streptococcus pyogens, Stephylococcus aurens, etc. Issra Abdul wahed et al in their study isolated E Coli, Bacteroides supp, Streptococci, Staphylococus Aureus from the pus discharge of Anorectal Abscess[3] Another study conducted by Chien-Kuo Liu et al states that the pus discharge from anorectal abscess contain E Coli, Bacterio fragillis, Klebsiella pnumoniae, Streptococcus etc pathogens.[4]

Keeping above considerations in mind and multidimensional activity of *Panchatikta Ghrita*, the Antimicrobial study of *Panchatikta Ghrita* against Ecoli, Streptococcus Pyogens, Staphylococcus Aurens, S.typhi, C. Albicans etc will be carried out. This study will definitely prove to be helpful in benchmark in the preparation of *Panchatikta Ghrita* and providing alternative to fast resisting antibiotics and their side effects.

REVIEW OF LITERATURE

After reviewing through *Ayurvedic* texts *Panchatikta Ghrita* has been found mentioned in various

Samhitas with slight differences in ingredients and indications. The details are summarized in following table.

Table I: *Panchatikta Ghrita* In Ayurvedic texts

S. No.	Name of Text	Indication
1	*Bhaishajyaratnavali, Kushtharog chikitsa prakaran (sutra 257)*	*Kushtha*
2	*Chakradatta, Kushthadhikar, ch.49*	*Kushtha, Visarpa*
3	*Yogtarangini Ch.66 Sutra no. 4*	*Kushtha*
4	*Gadanigraha Ch.1 Sutra 239-241*	*Kushtha*
5	*Sharangadhar Samhita Ch.9 Sutra 91,92*	*Pandu, Kushtha, Visarpa, Krimirog, Arsha*
6	*Yogratnakar Sutra 3*	*Vatavyadhi, Kushtha, Visarpa, Bhagandar, Vidradhi, Vatarakta*

Previous work done on *Panchatikta Ghrita*

- Haldar Pronab et al carried out a clinical study of *Panchatikta Ghrita* in respect of its Pharmaceutical, Hematological & Biochemical Evaluation.[5]
- Gond Pushpa et al studied the role of *Panchatikta Ghrita* and leech therapy in the management of psoriasis. [6]
- Shamkuvar Manoj et al studied the effect of *Matra Basti* Of *Panchatikta Ghrita* in psoriasis.[7]
- Zala Upendra et al assessed the Analgesic and Anti-Inflammatory Activity of *Panchatikta Ghrita*. [8]
- Dr. Sateesh Kumar N carried out Comparative Clinical Study To Evaluate Effect Of *Pnchatikta Ksheera Basti And Panchatikta Guggulu Ghrita Basti* In The Management of *Asthi Kshay* w.s.r. to Osteoporesis. [9]
- Sandeep Agrawal et al conducted a review study on Efficacy Of *Ayurvedic* Therapies On Psoriasis: A Review On Researches Conducted At Gujarat Ayurved University, Jamnagar. [10]

AIM

Study of Standard Operating Procedure (SOP) of *Panchatikta Ghrita Nirman* and Study of its Antimicrobial Activity.

OBJECTIVES

1) To develop a Standard Operating Procedure of *Panchatikta Ghrita.*
2) Physicochemical Analysis of *Panchatikta Ghrita* according to Ayurved Pharmacopeia of India (API).
3) In Vitro study of *Panchatikta Ghrita*.

MATERIAL AND METHODS

Present study has been done in following three steps

1) Standard Operating Procedure of *Panchatikta Ghrita* according to selected reference of *Bharat Bhaishajyaratnakar.* [11]
2) Physicochemical Analysis of *Panchatikta Ghrita* according to Ayurved Pharmacopeia of India (API).
3) In Vitro study of *Panchatikta Ghrita.*

1) Standard Operating Procedure of *Panchatikta Ghrita* according to selected reference

The raw materials were procured from local market. They were identified by experts from *Dravyaguna* and authenticated according to acceptable qualities and quality control parameters prescribed in *Ayurved* classics viz. *Panchabhautik Parikshan and Rasa, Veerya, Veepak* etc.

Ingredients

The ingredients of *Panchatikta Ghrita* are as follows.

Table II: Ingredients with proportion

Sr. No.	Ingredients	Quantity
	Kwatha dravya	
01.	*Neem twak (Azadirachta indica)*	100 gm
02.	*Guduchi (Tinospora cordifolia)*	100gm
03.	*Vasa (Adhatoda vasica)*	100 gm
04.	*Patol(Trichosanthes dioica)*	100 gm
05.	*Kantakari (Solanum surattense)*	100 gm
	Kalka Dravya	
06.	*Rasna (Pluchea lanceolata)*	3 gm
07.	*Vidanga (Embelia ribes)*	3 gm
08.	*Devdaru (Cedrus deodara)*	3 gm
09.	*Gajapippali (Piper chaba)*	3 gm
10.	*Yava kshar*	3 gm
11.	*Sajji kshar*	3 gm
12.	*Shunthi (Zingiber officinale)*	3 gm
13.	*Haridra (Curcuma longa)*	3 gm
14.	*Mishreya (Foenieulum vulgare)*	3 gm

15.	*Chavya (Piper retrofractum)*	3 gm
16.	*Kushtha (Saussurea lappa)*	3 gm
17.	*Tejovati (Zanthoxylum armatum)*	3 gm
18.	*Marich (Piper nigrum)*	3 gm
19.	*Kutaj twak (Holerrrhena antidysenterica)*	3 gm
20.	*Ajmoda (Carum roxburghianum)*	3 gm
21.	*Chitraka (Plumbago zeylanica)*	3 gm
22.	*Katuka (Picrorhiza kurroa)*	3 gm
23.	*Pushkar mool (Inula recemose)*	3 gm
24.	*Vacha (Acorus calamus)*	3 gm
25.	*Pippali mool (Piper longum)*	3 gm
26.	*Manjishtha (Rubia cordifolia)*	3 gm
27.	*Ativisha (Aconitum heterophylum)*	3 gm
28.	*Trivrutta (Operculina turpethum)*	3 gm
29.	*Guggul (Camiphora mukul)*	200 gm
30.	*Ghrita*	250 ml
31.	*Jala (Water)*	8000

Methods

Pre- Operative

a) *Guggulu Shodhana*

b) *Ghrita Murchchna*

Procedure of *Guggulu Shodhana* [12]

Ingredients

1) *Guggulu* – 200gm
2) Water – 800ml

- *Guggulu* was taken in given proportion in a vessel and was washed thoroughly.
- It was weighed and then soaked in 4 times water for overnight.
- Next day, the dissolved *Guggulu* was filtered out.
- It was again weighed and taken in a vessel and heated till it became 1/4th of its earlier proportion.
- Later, it was poured in a plate to dry.
- It was dried in 4 days and was then scrapped out and weighed.
- Thus, in this way pure *Guggulu* was obtained.

Ghrita Murchhchana [13]

Ingredients

1) *Ghrita – 250ml*
2) *Haritaki churna – 15gm*
3) *Bibhitak churna – 15gm*
4) *Amalaki churna – 15gm*
5) *Musta churna – 15gm*
6) *Haridra churna – 15gm*
7) *Nimbu swaras* – as required
8) Water – 1L

Method

1) All *Kalka dravyas* i.e. *Churnas* were taken in given proportion and a complete blend or '*Kalka*' was prepared by adding *Nimbu swaras*.
2) After that, *Ghrita* was taken in a steel vessel in given proportion and heated over moderate flame, till complete evaporation of moisture.
3) Then, the prepared *Kalka* was added to *Ghrita* after it cooled down slightly.
4) When the *Kalka* became light brown in colour, water was added in given proportion.
5) After addition of water, it was heated in *Mandagni* with intermediate stirring to complete the *Snehapaka* till *Sneha siddhi lakshana* appeared.
6) After the *Sneha siddhi lakshana* were seen, the *Ghrita* was taken out from flame and filtered through clean cloth in its mild hot stage.

   Thus, *Murchchit Ghrita* was obtained.

Operative Phase

This stage consists of actual preparation of "*Panchatikta Ghrita*". It consists of following steps.

- The ingredients of *Panchatikta* i.e., the *Kwath dravyas* were taken in coarse powdered form and given quantity of water was added to it. It was then heated and reduced to 1/4th of its volume and filtered with muslin cloth to obtain *Panchatikta kwath*.
- The other ingredients (*Kalka dravyas*) were taken in fine powder form. These were then transferred to wet grinder and ground with sufficient quantity of water to prepare a homogeneous blend (*Kalka*).
- Then, the *Murchita Ghrita* was taken in a stainless steel vessel and heated mildly. Next, the increments of *Kalka* were added and the mixture was stirred thoroughly while adding *Kwath.*
- It was heated for some time with constant stirring and maintaining the temperature between 50^0 C and 90^0C during the first hour of heating. The heat was stopped and it was allowed to stand overnight.
- Next day, it was heated again and observed for the *Sneh Siddhi lakshana*.
- Finally, it was filtered while hot through a muslin cloth and was allowed to cool.

Post-Operative

In this stage the *Ghrita* was tested for the *Sneha Siddhi lakshana* and was then filtered and packed.

***Sneh Siddhi lakshana* (Confirmatory Tests)** [14]

1. *Phen Pariksha*- Subsidence of froth in case of *Ghrita* i.e., *Phen shanti*.

2. *Varti Pariksha* - Formation of *Varti* from *Kalka*.

3. Agni *Pariksha* - On exposing the *Varti* to flame, the absence of crackling sound indicates absence of moisture.

4. *Gandha Pariksha* - The *Sneha* smells of the *Dravyas* used.

3) Physicochemical Analysis of *Panchatikta Ghrita* according to Ayurved Pharmacopea of India (API) [15]

Table III: Physicochemical Analysis of *Panchatikta Ghrita*

Sr No	Test	Results
1.	Specific Gravity at 40^0	0.914
2.	Refractive Index	1.4600
3.	Congealing Point	38^0c
4.	Saponification Value	220.13 mgKOH/g
5.	Iodine Value	38.21 gl/100g
6.	Peroxide Value	8.43 meq/1000gm
7.	Acid Value	0.28 mgKOH/g

3) In Vitro study of *Panchatikta Ghrita* [16]

Method

The antimicrobial study of prepared *Panchatikta Ghrita* was done by 'CUP PLATE method', which is as follows:

- Sterile borer was used to prepare 4 cups of 6 mm in the agar medium spread with the microorganisms and 0.1ml of inoculums.
- These cups were spread on agar plate by spread plate techniques.
- Accurately measured 0.05ml solution of each concentration and reference standards were added to the cups with micro pipette
- All the plates were kept in a refrigerator at 2-8^0 C for period of 2 hrs for effective diffusion of test compounds and standards. Later, they were incubated at 37^0 C for 24 hrs
- The presence of definite zone of inhibition of any size around the cup antibacterial activity.
- The solvent control was run simultaneously to assess the activity of Dimethyl sulphoxide and water was used as a vehicle
- The experiment was performed 3 times
- The diameter of zone of inhibition was measured.

OBSERVATIONS

Table IV- *Guggulu Shodhana*

Day	Task	Time Required	Quantitative Analysis	Remarks
1st	Soaking of *Guggulu*	overnight	*Guggulu* =200gm Water=800ml	*Guggulu* smelled of *Gomutra*
2nd	Filtration of dissolved *Guggulu*	2:00 pm to 2:30 pm	Wt. Of water containing *Guggulu* =880gm	Filtered liquid was muddy in appearance
3rd	Heating of filtrate	1:00 pm to 4:30 pm	Wt. Of *Guggulu* after heating=190gm	It became dark brown and sticky
4th to 8th	Kept for drying	4 days	Wt. Of dry and pure *Guggulu* =70gm	It was dark brown in colour and aroma was gone

Ghrita Murchchhana

- Time required: 15- 20 min
- Wt. of *Ghrita* taken= 250ml
- Wt. of *Murchchhit Ghrita*= 240ml
- Colour of *Murchchhit Ghrita* – Mustard yellow
- Temp. Maintained = 100^oC

Table V: *Panchatikta Ghrita Nirman*

Day	Task	Time Required	Quantitative Analysis	Remarks
1st	Preparation of *Kwath*	2:40 pm to 4:35 pm	*Kwath dravya*= 500gm, Water=8L, *Kwath obtained*=4L	-
1st	Preparation of *Kalka*	3:00 pm to 3:15 pm	Wt. = 90gm	Homogeneous blend was prepared
2nd	Mixing of all the contents and preparation of *Ghrita*	2:00 pm to 5:30 pm	*Kwath=4L, Kalka=90gm, Guggul=70gm, Murchchit ghrita=240ml*	Temperature kept between 82^0– 95^0.C
3rd	Confirmatory tests and filtering prepared *Ghrita*	12:30 pm to 1:45 pm	Net wt of *Panchatikta ghrita* obtained= 175gm	The colour of *Ghrita* changed to greenish yellow and it smelled of its ingredients

RESULTS

I. Preparatory Aspect

Thus 175gm of *Panchatikta ghrita* was prepared according to the Standard Operating Procedure. It took 9 days to complete the entire procedure.

I. Physicochemical Analysis

a) Sp. gravity at 40^0C = 0.914
a) Refractive index = 1.4600
b) Congealing point = 38^0C
c) Saponification value = 220.13 mg KOH/g
d) Iodine value = 38.21 gI/100g
e) Peroxide value = 8.43 meq/1000g
f) Acid value = 0.28 mg KOH/g

II. Antimicrobial activity

a) Against *E.coli* - No zone of inhibition was seen.
b) Against *S.aureus*- No zone of inhibition was seen.
c) Against *Salmonella abony*- No zone of inhibition was seen.
d) Against *Candid albicans*- No zone of inhibition was seen.

DISCUSSION

Present research work has been done by aiming at developing a standard manufacturing procedure of *Panchatikta Ghrita* and evaluating its antimicrobial property in vitro. *Panchatikta Ghrita* is commonly used formulations by *Ayurvedic* Physicians for *Kushta, Visarpa, Arsha, Bhagandar* etc. Many *Ayurvedic* Physicians prepare it on their own and dispense it to patients. Due to lack of comprehensive preparatory procedure and non mentioning of quality control parameters the self prepared *Panchatikta Ghrita* is compromised on many issues while preparation and quality control assessment which results in substandard medicine having lesser therapeutic value. To resolve this issue each and every step i.e. Raw Material Identification, Preparatory Procedure, Precautions & observations during procedure, In process quality control assessment, Prepared drug quality assessment were done in logical and sequential manner and documented. This SOP of *Panchatikta Ghrita* will serve as guideline for *Ayurvedic* Physicians to prepare the genuine *Panchatikta Ghrita* on their own.

The antimicrobial study of prepared *Panchatikta Ghrita* was done by 'CUP PLATE method in reputed quality control lab having NABL accreditation. But no zone of inhibition was seen for any of the selected micro-organisms. The in vitro negative results didn't entirely reveal its therapeutic efficacy. In *Ayurvedic* Therapeutics the Pharmacokinetics and Pharmacodynamics of the drug entirely depends upon *Rasa, Veerya, Veepaka* and balance of *Tridosha* in the body. The ingredients in *Panchatikta Ghrita* like *Rasna, Vidanga, Gajapippali, Devadaru, Shunthi* etc are having *Katu, Tikta Rasa* and *Ushna Veerya* which pacifies alleviated *Vata-kapha Dosha*. Other ingredients like *Guggulu, Neem, Vidanga* etc are well known for their *Krmighna* (wormicidal) property which in turn reveals its antimicrobial property from *Ayurvedic* perspective

CONCLUSION

The Sequential, Scientific and Logical illustration and documentation of each and every step viz. Raw Material Identification, Preparatory Procedure, Precautions & observations during procedure, In process quality control assessment, Prepared drug quality assessment will make the preparation of *Panchatikta Ghrita* easy to execute and accurate. It will serve as a small step toward standardization of *Panchatikta Ghrita*. The *Tridoshaghna* (Pacification of alleviated *Tridosha*) and *krimighna* (wormicidal) property of ingredients in *Panchatikta Ghrita* demonstrates its antimicrobial property from Ayurvedic point of view.

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