DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES

(DEEMED UNIVERSITY)

Mahatma Gandhi Ayurveda College Hospital & Research Centre Salod (H)

Wardha

Year: 2020-21

Synopsis of MD Research Project

On

Pharmaceutico Analytical Study of *Gomutrasava* and *Gomutraghana* and evaluation of its Antimicrobial Activity.

Principal Investigator

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1. Introduction:

Ayurveda means "The Science of life." and is considered to be the oldest healing science of medicine of India. So also called as "Mother of All Healing."[1]

Ayurveda is enhanced with different medicinal plants, metals and minerals. *Ayurvedic* Pharmaceutics is the discipline of pharmacy deals with formulation, manufacturing and dispensing pharmacy. In which *Rasashastra* focus on herbo-minerals preparation and *Bhaishajya kalpana* deals with herbal drug formulations.

In India since ancient times Cow has been worshiped as a God and has been given lot of respect. Cow is beneficial all throughout its lifespan in many ways and her urine is remedy for all diseases.[2] In *SushrutaSamhita and Ashtanga sangraha, Gomutra* has been described to be a most effective substance of animal origin having therapeutic uses. It is called a s "*Amrita*" i.e. beverage of immortality. It is one of the important content of *"Panchagavya Chikitsa"* Drinking of *Gomutra*has been practiced since thousands of years in India. It is useful in treatment of various diseases due to its Bio enhancer, anticancerous, Immuno-stimulant, Anticonvulsant, Antimicrobial and Antioxidant properties.[3]

But due to its pungent smell and to increase palatability and shelf life. It is very much essential to modify it into the different dosage forms like *Gomutraarka, Gomutrasava, Gomutraghana, Gomutraghanavati,* etc.

Sandhana Kalpana (Fermentative preparations) is one of the best dosage forms of Ayurveda. They have unique identity because of the 'Self generated alcohol' in them which acts as a self preservative. These are also called as spirituous liquids. They are known to have infinite shelf life and they get better and better with time in terms of their potency and sharpness. The bioavailability and therapeutic efficacy are more in this preparations.[4]

In *Sandhanakalpana* alcoholic preparation are widely used which consist of *Asav* and *Arishtha* preparation. *Sandhanakalpana* proves more useful due to its nutritive and medicinal values. The self-generated alcohol during *Sandhanaprakriya* acts as a solvent for maximum extraction from the raw drugs and also as a preservatives[5].

Ayurvedic medicine consists of various types of dosage forms. Due to enhanced drug concentration, accelerated therapeutic action and palatability *Asavarishta* are considered to be superior among other dosage form.[6, 7] Due to their better absorption in human body, high

preservative quality and quick action they are more valued and have a special place among all *Madya kalpana*[8]. *Sandhana* is a process of fermentation where *Dravadravya* (*kwatha, Swarasa,* or any other liquid preparations), *Madhura dravya* (Jaggery,Honey, Grapes and Sugar), *Prakshepa dravya* (Fine powders of medicinal drugs) and *Sandhanadravya* (*Dhatakipushpa, Madhukapushpa* as fermentation initiators) are put together in an inert vessel (mud pot) and sealed for specific time period to facilitate the process of fermentations. The *Asava* and *Arishta* are two major products of this process. It is also the process where in only the '*Dravadravya'* or '*Dravadravya*' along with other '*Ausadhadravya*' is put in a selected vessel for a particular period to facilitate the process of fermentation.[9]

During this process deduction of sugar takes place which is present as sweetening substance and converted into the alcohol with released of CO_2. In liquid media alcohol which is produced promotes dissolution of the active principles. Therefore due to the self generated alcohol it get preserved from different microorganisms.[10] By *Hima* and *Swarasa* process preparation of *Asava* takes place.

Bhaishajya kalpana has five fundamental basic dosage forms or *Kalpanas* namely *Swarasa, Kalka,Kwatha,Hima* and *Phanta.* Among all these *Kwatha kalpana* is one of the most useful and acts as base *kalpana.* So to increase its potency, to prolong shelf life and for greater palatability different dosage forms of *Kwathakalpana*are used. *Rasakriya* and *Ghanakalpana,* Granules, *Pravahikwatha,* Powder/ Tablets/ Capsules, Syrups are used as a modified dosage forms. *Rasakriya* and *Ghana Kalpana* is one of the modified, concentrated dosage form of *Kwathakalpana.* It is prepared by heating *kwatha* till it becomes concentrated and comes in semisolid state and then it is dried to solid form.[11]

The *Gomutra*is widely used but no commercial preparation is available. Availability of fresh Cow urine (*Gomutra)* has always remained a problem. So, in this study an effort will be taken to prepare *Gomutra Ghana* and *Gomutrasava* and to study its Antimicrobial activity along with Analytical parameters.

2. Review of literature:

A] Properties of *Gomutra*:

According to *Sushruta*: [12]

- *Rasa – katu, tikshna*

- *Guna – Laghu*
- *Virya – Ushna*
- *Karma – Agnidipaka, Medhya*
- *Doshagnata– Vatanashaka (*due to *kshara)*
  Pittajanaka
  Kaphanashaka
- *Prayogya – Shoola,Gulma, Udara roga*
- *In Panchkarma -Aanaha, Virenchan karma, Aasthanpanbasti.*

According to *Charaka*: 13

- *Rasa – Slightly Madhur*
- *Doshagnata – Tridoshanashaka*
- *Prayogya – Krumi, Kushtha, Kandu, DoshajanyaUdarroga*

According to *Ashtanga Samgraha: 14*

- *Rasa – Tikshna*
- *Guna – Ruksha*
- *Virya – Ushna*
- *Anurasa – Lavana, Katu*
- *Karma – Deepana, Pachana, Bhedana*
- *Doshagnata – Pittavardhaka*
- *Prayogya – Krumi(worms), Shofa(oedema), Udarroga(abdominal disease), Aanaha(tympanitis), Shoola(pain), Pandu(anaemia), Gulma(tumour), Aruchi, Visha(poison), Shvitra(leucoderma), Kushta(skin diseases), Arsha(piles)*
- *In Panchkarma - LaghuVirechan, Aasthapanbasti, lepa, Swedana karma*

B] Composition of Cow Urine: [15]

Water, Urea, Uric acid, Minerals, Salts, Hormones like growth hormone, Gonadotropins. Erythtopoetine, Enzymes, Bioactive substance, Urokinase, Epithelial growth factors, trypsin inhibitor, H-11 beta-iodole-acetic acid, allantoides, anti-neoplastic.

It contains vitamins – A, B, C, D, E.

Calcium, nitrogen, sulphur, sodium, copper, phosphate, potassium, manganese, lactose sugar, Acids like carbolic and hippuric, creatinine, aurum hydroxide, etc.

Fresh cow urine and it's distillate shows antimicrobial activity and it suppressthe free radicals by scavenging DPPH radicals and superoxide. Comparatively fresh cow urine was found to be more active than cow urine distillate. Cow urine and ofloxacine have some comparable antimicrobial activity.

The fresh cow urine has great amount of phenols so it has a potent antimicrobial activity as phenols are bactericidal to gram positive and gram negative bacteria.

C] *Gomutrasava*:

Gomutrasava is an alcoholic fermented product. This formulation contains *Gomutra, Chitraka, Vyosha(Sunthi, Marichi, Pippali)* and *Madhu.* This preparation is appreciated in the treatment of *Shvitra* and *Kushtha.* In this study *Gomutrasava* will be prepared according to reference cited in *Astanga Hrdaya.*[16]

D] *Gomutraghana :*

Gomutraghana is *rasakriya* and *ghanasara* product. It contains *Gomutra.* It will be prepared according to *rasakriya* general method cited by *Dalhana*-the commentator of *Susruta Samhita.*[17]

E*]* ***Rasapanchaka* chart :**

Sr. no	***Dravya***	***Rasa***	***Guna***	***Virya***	***Vipak***	***Karma***	***Doshagnata***
1.	*Gomutra*[18] (Cow's urine)	*Tikshna*	*Ruksha*	*Ushna*	-	*Dipan, Pachana, Bhedana*	*Pittavardhaka*
2.	*Chitraka*[19,20] (Plumbago Zeylanica Linn.)	*Katu*	*Laghu, Ruksha, Tikshana*	*Ushna*	*Katu*	*Dipan, Pachana, Lekhana, Grahi, Krimighana, Swedjana, Rasayan, Rochaka*	*KaphaVataShamak*
3.	*Sunthi*[21] (Zingiber officinate)	*Katu*	*Laghu, Snighdha*	*Ushna*	*Madhura*	*Ama pachana, Agni vardhana*	*KaphaVataShamak*
4.	*Maricha*[21] (Piper nigrum)	*Katu*	*Laghu, Tikshna*	*Ushna*	*Katu*	*Ama pachana,*	*VataKaphaShamak*

						Agni vardhana	
5.	*Pippali*[21] (Piper longum)	*Katu*	*Katu*	*Ushna*	*Madhura*	*Ama pachana, Agni vardhana*	*VataKaphaShamak*
6.	*Madhu*[22] (Honey)	*Madhura, Kashaya*	*Laghu, Ruksha, Sukshma*	*Sheeta*	*Katu*	*Lekhana, Sangrahi, Shodhana, Kaphavilayana, Etc*	*Tridoshahara*

3. Previous work done:

S.N.	Studies	Conclusion	Research Gap
1.	Clinical evaluation of the efficacy of *Gomutraaasava* in Shvitra vis-a-vis vitiligo by Raman S.Belgeet al., Dept.of RS BK, Shri SaptshrungiAyurved college, Nashik, MS-2012	A clinical experiment with oral administration of *Gomutra Aasava* having *shunthi ,maricha, pippali,chitrak,madhu* and *gomutra* proved that it is a safe remedy with significant repigmentation property.	The pharmaceutical and analytical study of *Gomutrasava* is not done. It's antimicrobial activity is not studied.
2.	Clinical evaluation of cow urine extract special reference to *Arsha* (Hemorrhoids) by Dr.Talokar, Dr.ArchanaR.Belge et al ,dept of RSBK R.T.AyurvedMahavidyala,Akola-2013	The oral supplementation with the cow urine prevents expensive, time consuming and painful complication of Hemorroids of grade I and grade II.	The pharmaceutical and analytical study of *Gomutraghana* is not done. It's antimicrobial activity is not studied.
3.	Anti cancer activity of cow urine: current status and future directions by K.Dhama et al, Indian veterinary research institute,Izatnagar(U.P),2005	Anticancer properties has been claimed to present in Cow urine therapy. In the field of cancer treatment the cow urine have been supported recently by the grand of US patent by it's goodness of bioenhancing	The Antimicrobial activity of *Gomutrasava* and *Gomutraghana* is not studied.

		the activity of anti cancer drug.	
4.	Antioxidant and antimicrobial activities of cow urine by Edwin Jarald et al,dept of Natural Drug Research,B.R.Nahata College of Pharmacy and Research Center,Mandsaur,(M.P),2008	Fresh cow urine and its distillate shows antimicrobial activity. Comparatively fresh cow urine was found to be more active than cow urine distillate. Cow urine and ofloxacine have some comparable antimicrobial activity.	The Antimicrobial activity of *Gomutrasava* and *Gomutraghana* is not studied.

4. Need of study:

The present study is planned to prepare *Gomutrasava* described as per *Astanga Hrudaya* in *Shvitrakrumi chikitsa* and to prepare *Gomutra Ghana* by *Rasakriya* general method described by *Dalhana* the commentator of *Susruta Samhita.* As *Gomutra* is having prime importance in Ayurveda pharmaceutics with respect to *Shodhana, Marana, Bhavna* and so many processes. Therapeutically also it is used in various disorders either as a medicine or as an adjuvant successfully. But still, proper preparative, analytical and antimicrobial activity of *Gomutra sava* and *Gomutra Ghana* are not established yet and this study will fulfil the gap.

5. Aim and Objectives:

A]Aim of study:

Pharmaceutical and Analytical Study of *Gomutrasava* and *Gomutra ghana* and evaluation of its Antimicrobial activity.

B] Objectives of the study:

1. To prepare *Gomutrasava* as per the reference *Astanga Hrudaya.*
2. To prepare *Gomutraghana*
3. To study the quality control parameters of *Gomutrasava and Gomutraghana*
4. To Study Antimicrobial activity of *Gomutrasava Gomutraghana*

Research question:

Whether traditionally prepared *Gomutrasava and Gomutraghana* show significant *Antimicrobial* activity?

Null hypothesis: Traditionally prepared *Gomutrasava and Gomutraghana* will not show significant *Antimicrobial* activity

Alternate hypothesis: Traditionally prepared *Gomutrasava and Gomutraghana* will show significant *Antimicrobial* activity

6. Material and Methods :

Collection of Material:

1. Fresh *Gomutra* will be collected from *Goshala* and other drugs will be collected from Dattatraya Ayurveda *Rasashala* , Mahatma Gandhi Ayurveda College Hospital & research Centre Salod(H) Wardha.
2. Raw drugs will be verified and primarily authenticated by *Dravyaguna* Department, MGACH and RC.
3. Authentication will be done by Taxonomist (FRLHT, Banglore).
4. Raw drugs will be standardized as per API.

Table: List of ingredients used for *Gomutrasava*:

Sr.no	Drug name	Part to be used	Proportion
1	*Gomutra*(Cow's urine)		1 *Drona*(12.288 kgs)
2	*Chitraka* (Plambagozeylanica Linn.)	Root	480 gm
3	*Sunthi* (Zingiber officinale	Dried Rhizome	480 gm
4	*Maricha*(Piper nigrum Linn.)	Fruit	480 gm
5	*Pippali*(PiperlongumLinn.)	Fruit	480 gm

6	*Madhu*(Honey)		1/2 *Tula* (2.4 kgs)

7. Method of *Gomutrasava :*

All the drugs will be procured and authenticated

⇩

Fresh *Gomutra* will be collected from Goshala

⇩

Gomutra will be filtered (to remove foreign matter) through clean and dry muslin cloth

⇩

Sandhanapatra (plastic jar) will be selected and *Dhupana* of *Sandhanapatra* will be carried out with *Guggulu* and *Vidanga* powder and jar will be checked for cracks, leaks and oozing

⇩

The *Lepana* with *Goghrita* will be done to the jar

⇩

The *Gomutra* will be taken into the jar (contents will be transferred into the vessel to it's $3/4^{th}$ of the capacity)

⇩

All the dravyas – *Chitraka, sunthi, marich, pippali* will be added in either coarse form

⇩

Madhu will be added

⇩

The pot will be kept undisturbed by temporarily closing with lid and cloth

⇩

It will be observed from the onset of fermentation, daily for 3 to 5 days

⇩

Soon after fermentation starts, the *sandhi bandhan* of the pot will be done The vessel will be left undisturbed for 15 day

⇩

After 15 days confirming of fermentation, the vessel will be opened and the preparation is siphoned out

⇩

It will be stored in airtight glass container and checked for continued fermentation in the liquid for a fortnight or two

8. Method of *Gomutra ghana*:

Fresh *Gomutra* will be collected from *Goshala* & will be filtered (to remove foreign matter) through clean and dry muslin cloth

⇩

Gomutra will be taken in stainless vessel & will be heated on *Mandagni* (mild heat over low flame)

⇩

Continuous stirring will be done to avoid adhesiveness and burning

⇩

Consistency will be checked time to time as required

⇩

The filtrate will be reduced till it attains to a 'thicker consistency'

⇩

This will kept for drying in sunlight and will be stored in a clean sealed pack glass jar

Antimicrobial study[23]

There is various methods to test antimicrobial activity. In the present study antimicrobial activity of *Gomutrasava* and *Gomutra Ghana* will be tested by Agar well diffusion method.

Principle

The antimicrobial present in the *Gomutrasava* and *Gomutra Ghana* are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organism .The resulting zone of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeter. The size of the well will be 10 mm and 0.5 mm of *Gomutrasava* and *Gomutra Ghana* will be introduced. Ofloxacin 10 µg ml will be used as the standard for the study. The petri dishes will then be incubated at 37ºC for a period of 24 hours and the zone of inhibition will be measured[24].

Selection of test microorganism

a) Gram Positive bacteria: 1. Staphylococcus aurous (NCIM 2079)

2. Streptococcus epidermis's (NCIM 2493)

b) Gram Negative bacteria: 1.Escheria coli (NCIM 2931)

2. Klebsiella pneumoni (NCIM 2957)

3. Proteus vulgaris (NCIM 2027)

Table no. : Antimicrobial activity of *Gomutrasava and Gomutra Ghana*

Microorganism	**Positive control(zone of Inhibition in mm) Ofloxacin 10 µg/ml**		**Sample (Testing drug)**			
			100 ul	**150ul**	**200 ul**	**mean**
1Staphylococcus aurous						
2Streptococcus epidermis's						
3. Proteus vulgaris						
4 Escheria coli						
5 Klebsiella pneumoni						

7.i)Place of study :

1. Department of Rasashastra and Bhaishajyakalpana MGACH and RC Salod(H), Wardha.
2. Analytical study will be carried out at Quality control Lab,DattatrayAyurveda Rasashala MGACH and RC Salod(H), Wardha.
3. Antimicrobial study will be carried out at Jawaharlal Nehru Medical College ,Sawangi (M) Wardha.

4. According to the need, study will be carried out at Certified or Standard Institute/Laboratory/Organization of National Repute and as Recognized or Recommended by DMIMS (DU).

ii)Study design: Pharmaceutical

Iii) Study type: Pharmaceutical, Analytical and Anti-microbial.

iv) Study period: 2 Yrs

8. Standardization:

A] Standardization parameters for *Gomutrasava*:

- **Organoleptic characters**
  1. Sparsha (Touch)
  2. *Rupa* (Appearence)
  3. *Rasa* (Taste)
  4. *Gandha* (odour)
- **Physiochemical parameters**[25]
  1. pH
  2. Specific gravity at 25ºC
  3. Total solid
  4. Alcohol content
  5. Reducing sugar
  6. Non reducing sugar
  7. HPTLC
  8. Test for methanol

- **Microbial load:**Assessment for microbial contamination

B]Standardization parameters for Ghana:

- **Organoleptic characters:**
  1. Sparsha (Touch)

2. *Rupa* (Appearence)
3. *Rasa* (Taste)
4. *Gandha* (odour)

- **Physiochemical paramaters:**[26]
  1. Loss on drying at 105ºC
  2. Total ash/Acid insoluble ash
  3. pH
  4. Particle size
  5. Water/Alcohol soluble extractives
  6. Test for heavy /toxic metals
  7. Test for specific pathogens
  8. Pesticide residue
  9. Test for Aflatoxins ($B_1B_2G_1G_2$)
  10. TLC/HPTLC
- **Microbial load:** Assessment for microbial contamination

Observation and Results: will be noted and presented in the form of tables, chart, photographs etc.

Methods of statistical analysis: Statistical analysis will be done by applying Paired and unpaired T test and one way ANOVA test.

Discussion: The discussion will be done on the basis of observation and analyzed data.

Conclusion: Conclusion will be drawn on the basis of observations and results.

Ethical clearance: The study will be conducted after obtaining ethical clearance from Institutional Ethical Committee (IEC) Mahatma Gandhi Ayurveda College Hospital & Research Centre, Salod(H) Wardha.

Gannt Chart:

Title: -: Pharmaceutico Analytical Study of Gomutrasava and Gomutraghana and evaluation of its Antimicrobial Activity						
Name:- Payal Raut PG Scholar 1st year						
Steps	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**
IEC Approval						
Drug Collection						
Literature Review						
Analytical Study						
Bioavailability study						
Data Analysis						
Writing rest of thesis submission						

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Budget:

SR.NO	ITEM/PARAMETER	CONTENTS	UNIT COST	TOTAL NUM.OF UNITS	TOTAL
1	Ingredients/drugs/raw materials	Gomutra Chitraka Sunthi Maricha Pippali madhu	Rs.40/L Rs.536/kg Rs.715/kg Rs.1436/kg Rs.1565/kg Rs.400/kg	24L 480gm 480gm 480gm 480gm 2.4kg	Rs.960/- Rs.270/- Rs.343/- Rs.689/- Rs.751/- Rs.960/-
2	Special assistant for service outside DMIMS (Analytical parameter)	HPTLC Particle size distribution Pesticide residue Test of aflatoxins(B1B2G1G2)	Rs.2500/test Rs.2000/test Rs.2000/test Rs.2000/test	2 1 1 1	Rs.5000/- Rs.2000/- Rs.2000/- Rs.2000/-
3	Antimicrobial Parameter	Staphylococcus aureus Streptococcus epidermitis Proteus vulgaris Escheria coli Klebsiella pneu	Rs.3500	2	Rs.7000/-
4	Other	Glass container	Rs.200	4	Rs.800/-
	TOTAL				**Rs.22,773/-**

Justification:

- *Gomutra, Trikatu,madhu* -Drug preparation
- HPTLC –Analytical Study
- Particle size distribution- Analytical Study
- Pesticide residue- Analytical Study
- Test of aflatoxins(B1B2G1G2)- Analytical Study
- Antimicrobial Study

DATTA MEGHE INSTITUTE OF MEDICAL SCIENCES
(Deemed to be University)
Re-accredited by NAAC (3rd Cycle) with 'A+' Grade
MAHATMA GANDHI AYURVED COLLEGE, HOSPITAL & RESEARCH CENTRE
NABH Accredited Hospital
INSTITUTIONAL ETHICS COMMITTEE
Salod (H), Wardha-442004, Maharashtra, India
Email: iecmgachrc@gmail.com

Ref. No. MGACHRC/IEC/July-2021/ 358

Date:30.07.2021

Letter of Approval

The Institutional Ethics Committee in its meeting held on 24-07-2021 has approved the following research work proposed to be carried out at Mahatma Gandhi Ayurved College, Hospital & Research Centre, Salod (H),Wardha.

This approval has been granted on the assumption that the proposed research work will be carried out in accordance with the ethical guidelines prescribed by Central ethics committee on human research (C.E.C.H.R.)

The details of the proposed research work approved by the committee are as follows.

Sl. no.	Principal Investigator (Co-Investigator/Guide)	Category	Topic of Research
1	Dr. Payal Raut (Dr. Bharat Rathi)	**Post Graduate Dissertation** **MD(Ayu) Rasashastra andBhaishajya Kalpana**	Pharmaceutical Analytical Study of GomutraAsava &Gomutra Ghana and Evaluation of its Antimicrobial Activity.

M.G.A.C.H. & R.C. – APPROVED – INSTITUTIONAL ETHICS COMMITTEE

MEMBER SECRETARY
IEC, MGACH &RC

Copy to

1 Dr. Payal Raut,PG Scholar, Dept.of Rasashastra and Bhaishajya Kalpana,MGACH&RC, Salod (H), Wardha

2 Dr. Bharat Rathi,Dept.of Rasashastra and Bhaishajya Kalpana,MGACH&RC, Salod (H), Wardha.