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Quality Control and Phytochemical Profiling of a Polyherbal Traditional Indian Medicine by GC-MS method

Research Article

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Abstract

Introduction: *Gomutra* (Cow's urine) has been described to be the most effective substance of animal origin. It is useful in treating various diseases due to its pharmacological activities. Nevertheless, due to its pungent smell, palatability, shelf life and fresh availability have always remained a problem. So it is very much essential to modify it into different dosage forms. One of its traditional dosage forms is *Gomutrasava,* an alcoholic fermented product indicated in the management of *Shvitra* (vitiligo). Aim & objective: The study is planned for Pharmaceutical preparation and quality control assessment of *Gomutrasava*. Material & Methods: *Gomutrasava* was prepared according to a reference from *Astanga Hrudaya*. This formulation was tested for organoleptic characters and physicochemical parameters, including Gas Chromatography-Mass Spectrometry (GC-MS). Observation & Results: After fermentation colour of *Gomutrasava* appeared dark brown with specific cow urine smell. pH, specific gravity at 25ºC, refractive index, brix, viscosity, total solid content, alcohol content, reducing sugar, non-reducing sugar of *Gomutrasava* were 5.41, 1.033wt/ml, 1.394, 37º, 1.38/sec, 37%, 6%, 0.96%, 0.07% respectively and test for methanol and microbial contamination were negative. GC-MS revealed some compounds attributed to antimicrobial, anti-cancerous and antifungal activities. Conclusion Thus this data combined with analytical data will be used for clinical study and treatment of *Shvitra* (Vitiligo), *Kushtha* (Leprosy) and various skin diseases.

Keywords: *Gomutra, Gomutrasava, Analytical study, GC-MS.*

Introduction

Ayurveda is the ancient system of medicine practiced in the Indian subcontinent since the Vedic period (1). Ayurveda pharmaceutical products contain several substances of plant origin and a few ingredients of metals and minerals origin. Polyherbal formulations comprise the bulk of Ayurveda drugs and show significant effectiveness due to presence of several phytochemicals. *Ayurvedic* Pharmaceutics, traditionally termed "*Bhaishajya Kalpana*", is the discipline of pharmacy that deals with the formulation, manufacturing and dispensing of herbal drug formulations (2). In Indian culture, the cow is considered highly sacred, and its by-products, especially *Gomutra* (cow urine) have been evaluated for medicinal purposes (3). It is also a vital content of *Panchagavya Chikitsa,* generally termed as Cowpathy(4). Due to its pharmaceutical properties, drinking *Gomutra* distillate has been practiced for thousands of years in India to treat various diseases. However, it has a short shelf life and is pungent, making it unpalatable for many patients (5). *Gomutrasava* is a traditional Ayurveda formulation made from cow urine fermented with ingredients of plant origin shown in Table-1. This classical Ayurveda formulation is unique because it contains self-generated alcohol, which acts as a self-preservative and is palatable. The bioavailability and therapeutic efficacy of plant material is also enhanced in such fermented preparations (6). Hence, this study was planned to pharmaceutically process *Gomutrasava* as per classical Ayurveda literature and establish parameters focusing on phytochemical profiling using GC-MS.

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Material and Methods

Drug collection

Fresh *Gomutra* was collected from Sarvoday Goshala Charitable Trust, Padhegaon, Wardha and all raw drugs were collected from Dattatraya Ayurveda Pharmacy (FDA No. NG/AYU/02/2014), Wardha. The raw drugs were verified and authenticated at

Dravyaguna Department of Datta Meghe Institute of Higher Education (DMIHER), Wardha. Pharmaceutical processing was performed at Rasashastra & Bhaishajya Kalpana Department, DMIHER, Wardha, Maharashtra. Ethical approval vide No. MGACHRC/IEC/July 2021/358 was taken prior to the start of study.

Table 1: List of ingredients used for *Gomutrasava*

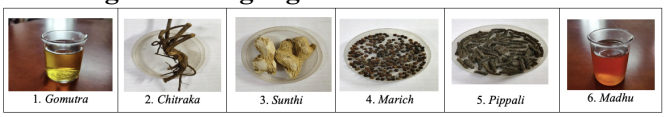
Sr.no	Drug name	Part used	Proportion
1	*Gomutra* (Cow's urine)	Urine	1 *Drona* (12.288 kgs)
2	*Chitraka* (*Plumbago zeylanica* Linn.)	Root	120 gm
3	*Sunthi* (*Zingiber officinale* Roscoe)	Dried Rhizome	120 gm
4	*Maricha* (*Piper nigrum* Linn.)	Fruit	120 gm
5	*Pippali* (*Piper longum* Linn.)	Fruit	120 gm
6	*Madhu* (Honey)	Honey	1/2 *Tula* (2.4 kgs)

Preparation of *Gomutrasava*

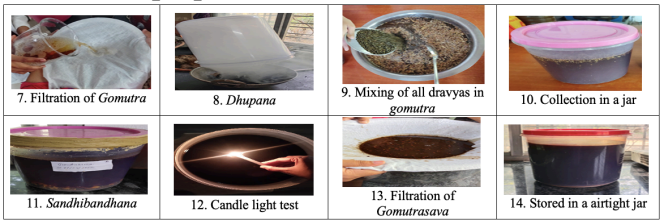
The pharmaceutical processing of *Gomutrasava* was done according to the classical method of fermentation described in the ancient Ayurveda text of *Ashtanga Hrudya* (7). The whole process of *Gomutrasava* manufacturing took thirty days to complete. The unit process is described in Figure 1. Fresh *Gomutra* sourced locally was filtered through muslin cloth to remove foreign matter. A plastic jar was selected as *Sandhanapatra* (fermentation vessel). It was cleaned and checked for cracks, leaks and oozing. It was then fumigated for 10 minutes with ignited drugs of *Commiphora mukul, Aquilaria agallocha, Cinnamomum Camphora, Vateria Indica*, cow dung and cow ghee. The *Gomutra* was poured into the fumigated jar and filled up to 3/4th of the capacity. All the other herbal ingredients (*Prakshep Dravya*) mentioned in Table-1 and showed in image no. 2-5 were coarsely powdered and added to the *gomutra*. Finally, honey was also added, and the contents were stirred thoroughly. The jar was covered with a clean cloth, and a lid was placed on top to seal it temporarily. It was then kept undisturbed in a clean and dry room with no direct exposure to sunlight or air to prevent temperature variations. The arrangement was observed daily for the onset of fermentation. The fermentation started after five days. After the onset of the fermentation process, the jar was sealed using mud clay and cloth. The room temperature was maintained optimally at 370C +/- 20C to support this classical fermentation method. The vessel was examined after a fortnight for the completion of fermentation. Since it was still ongoing, it was again left undisturbed for another fortnight. After thirty days, the fermentation process was completed. The completion was confirmed by evaluating the final product on the classical parameters. The final product was siphoned out and stored in another airtight plastic container.

Figure 1: Unit process of manufacturing of *Gomutrasava* as per the classical method of fermentation described in the ancient *Ayurveda* texts. The whole process was completed in 30 days maintaining the ambient temperature at 37⁰C.

Images showing ingredients of *Gomutrasava*:



Images showing various steps of method of preparation of *Gomutrasava:*



Analytical study

There are no pharmacopeial standards for *Gomutrasava*; hence, an analytical study was conducted to establish basic parameters. The formulation and fresh *Gomutra* was first tested for Organoleptic parameters such as colour, taste and odour. (Table no.3). Samples were analyzed as per API Standards in the analytical lab. The physicochemical analysis includes pH, specific gravity at 25º, refractive index, viscosity, total solid content, alcohol content, reducing sugar, nonreducing sugar, test for methanol (Table no.4), assessment for microbial contamination (8) (Table no.5) and GC-MS.

pH

A glass electrode and pH meter are used to calculate the pH of a liquid. The electrode was submerged in the buffer solution (pH 4, 7 & 9 respectively) in the beaker. The electrode was then submerged in the test sample and pH was noted (9).

Specific gravity

Clean and dry 25ml density bottle was taken and stored in the hot air oven. After that, it was removed, stored in desiccators and weighed empty. The next step was to add water to one bottle and weigh it at room temperature. The same process was used for *Gomutrasava*. By dividing the weight of the liquid in

the density bottle by the weight of the water within, the specific gravity of the liquid was calculated (10).

Refractive index

For refractive index Abbe's refractometer was used. The refractometer should be calibrated against distilled water, which has a refractive index of 1.3325 at 25°C, before using a *Gomutrasava* sample in order to obtain precision. The magnifier in the pointer was used to record the reading (11).

Viscosity

Sample was taken in the 250ml beaker and placed it under the viscometer to get the sample reading (12).

Total Soluble Solid Content

The index of refraction determines the total soluble solids content of a solution. This is measured with an abbe refractometer and is known as the degrees Brix. Before measuring samples, the apparatus was calibrated with deionized water (13).

Alcohol Content

The alcohol content (%, v/v) was measured using an alcohol meter (14).

Reducing & Non-reducing Sugar:

For calculating reducing sugar 10% of HCl solution was prepared. 4ml of *Gomutrasava* was taken in a round bottom flask and added 100ml of 10% HCl to it. Same flask was attached to the condenser and boiled it for 30 mins. Solution was filtered using filter paper. For titration 5-5ml of Fehling's solution A & B, 30ml of distilled water & 3drops of methylene blue wasused as an indicator. Sample was titrated in the boiling condition with the prepared Fehling's solution till it became brick red colour and the burette reading was noted. Fehling dilution factor was calculated by adding 1g of glucose dissolved in 100ml of distilled water and titrated with Fehling's solution A and B with methylene blue. Total sugar was calculated by adding 1ml of

10%HCl in 100ml of filtered solution and titrated with the same fehling's solution. Reading was noted after changed in colour. Non reducing sugar was calculated by the difference obtained between the values of total sugar and reducing sugar (15).

Test for methanol

Asava and *Arishta* that contain a significant quantity of ethanol created during processing are subject to the methanol test. The sample in the test tube received 3 drops of strong sulfuric acid along with a small amount of salicylic acid. The test tube was submerged momentarily in a beaker of boiling water. A test tube holding 5ml of dilute sodium carbonate solution received the contents. Methanol and/or ethanol are present in the sample if the methyl/ethyl salicylate (wintergreen odour) is formed (16).

Assessment of Microbial Contamination

The sample was dissolved in water (100% soluble) and then inoculated onto sterilized agar plates. A clean loop was taken and heated on the flame before being dipped in the sample solution. This loop was streaked on the surface of the agar plates and covered with lids. These plates were incubated at 38-40°C for 24 hours. The growth, if any, was discovered on the plate after 24 hours. These were reincubated in another plate using the same process to achieve a clear difference between found, counted and measured. Thus, the number of organisms in a unit concentration of sample was identified. Microbes were distinguished based on their organism's nature and typical characteristics. As a result, overall bacterial count, yeast and mould, Salmonella and E. coli were identified (17).

Gas chromatography Mass Spectrometry (GC-MS) (18):

Specifications:

- Analyzer: Quadrupole with prefilter
- Mass Range: 20-620 Daltons (amu)
- Mass Stability: ± 0.1 m/z mass accuracy over 48 hrs
- Ionization Modes: EI/CI Ionization
- Vacuum Pump: Turbo molecular pump 250L/Sec
- Software: Turbo Mass
- Model: Autosystem XL GC with Turbomass
- Make: Perkin Elmer

GC-MS is a technique that separates ions or atoms of a substance by mass. By ionizing the sample molecules and accelerating them with an electric field, this is achieved. Then, a magnetic field is applied to these charged particles, causing them to deflect at various angles depending on their mass to charge (m/z) ratio. This will show the sample's mass spectra. Since GC-MS is capable of performing both positive and negative ionization, it uses electron ionization (EI) and chemical ionization (CI) to determine the molecular weight of more complicated materials. By comparing the obtained spectra with reference data, mass spectra are helpful in confirming the atomic structure of known compounds. Results from GC-MS can be used to predict the probable structure of an unknown chemical.

Observations and Results

The observations were noted at three levels during the process of fermentation. The initial observations were made at Day-1, followed twice fortnightly till the process of fermentation was completed at Day-30. The observations are shown in table 2

Table 2: Observation made through out the process of fermentation

Before the onset of fermentation		
Sr.no	**Parameters**	**Observation**
1	Colour of liquid	Dark brown
2	Powder of herbal	Floating
3	Temperature	Same as room
After the onset of Fermentation		
1	Odour	Mild alcoholic

2	Effervescence	Present
3	Powder of herbal	Floating
4	Hissing sound	Not present
At completion of fermentation		
1	Duration for fermentation	30 days Date of initiation-7.04.2022 Date of completion-
2	Alcoholic smell	Present
3	Effervescence	Not present
4	Hissing sound	Not present
5	Candle light test	Positive
6	Prakshepa dravya	Settled down
7	Colour	Dark brown

Table 3: Results of organoleptic characters of *Gomutra* and *Gomutrasava*

Organoleptic characters	***Gomutra***	***Gomutrasava***
***Rupa* (Appearance)**	*Pita Varni, Paradarshi, Swaccha*	*Madhuvarni*
***Rasa* (Taste)**	*Katu, Tikshna*	*Madhur, Lavan, Kashaya*
***Gandha* (Odour)**	*Mutragandhi*	*Madhu Mutragandhi*

Table 4: Results of physiochemical parameters of *Gomutrasava* and *Gomutra*

S.N.	Parameters	Values of *Gomutra*	Values of *Gomutrasava*
1	pH	7.94	5.41
2	Specific gravity at 25ºC	1.023wt/ml	1.033wt/ml
3	Reftractive Index	1.393	1.394
4	Brix value	-	37^0
5	Viscosity	1.23/sec	1.38/sec
6	Total Solid Content	36.5%	37%
7	Alcohol content	-	6%
8	Total sugar	-	1.03%
9	Reducing sugar	-	0.96%
10	Non reducing sugar	-	0.07%
11	Test for methanol	-	Negative

Table 5: Results of microbial load/ microbial specification in *Gomutra* and *Gomutrasava*

Sr.no	**Specification**	**Parameter as per CCRAS**	**Observation in *Gomutra***	**Observation in *Gomutrasava***
1	Total viable count	Maximum 10^5/g	No growth	No growth
2	Enterobacteriaceae	Maximum 10^3/g	No growth	No growth
3	Total fungus count	Maximum 10^3/g	No growth	No growth
4	E-coli	Maximum 10^5/g	No growth	No growth
5	Salmonella	None	No growth	No growth
6	Staphylococcus aureus	Absent	No growth	No growth
7	Pseudomonas aeruginosa	Absent	No growth	No growth

Results of Gas chromatography Mass Spectrometry (GC-MS) analysis of *Gomutra*

Figure 2. area percent report of *gomutra*

Area Percent Report

Peak List File : D:\GCMS1\SICART2013.PRO\PeakDB\020223DRPAYAL-2.a
Last Modified : Thursday, February 02, 2023 2:58:48 PM
Printed : Thursday, February 02, 2023 2:58:58 PM

#	Name	RT	Area	Height	BL	Conc	Units	Area/Conc	m/z	Area %
1	1	10.121	44,082,136.0	63,722,264	MM	0.00		0.00	TIC	29.20
2	2	10.637	27,060,666.0	83,964,808	MM	0.00		0.00	TIC	17.93
3	3	11.202	74,550,880.0	52,553,196	MM	0.00		0.00	TIC	49.39
4	4	18.435	5,260,618.5	10,243,567	MM	0.00		0.00	TIC	3.48

Figure 3. chromatogram generated by a GC of *gomutra*

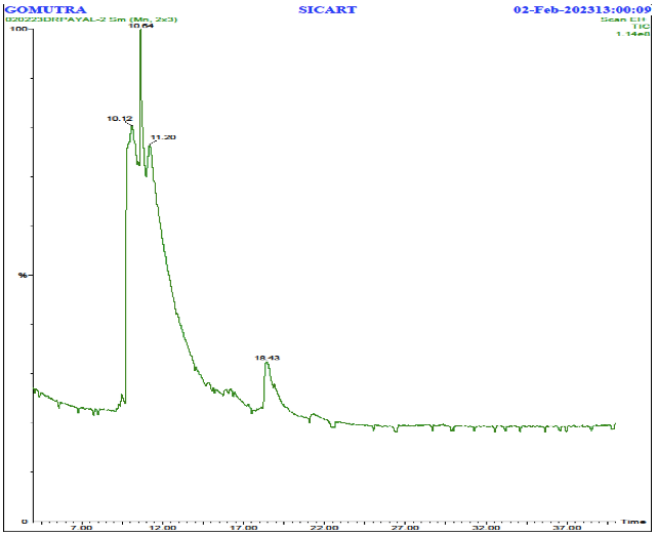
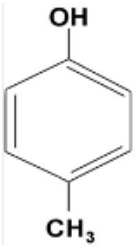
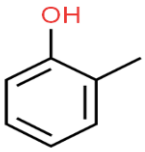
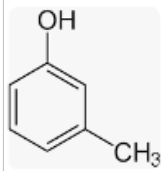
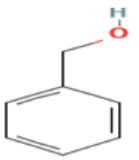
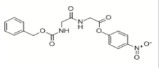
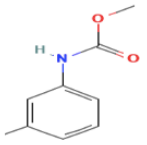
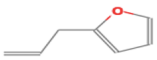
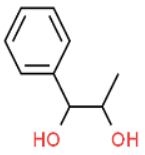
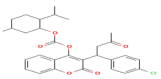
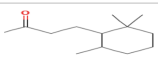
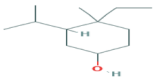
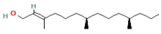
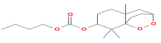
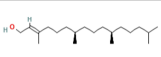
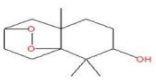
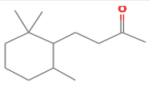
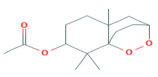
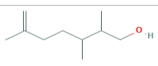
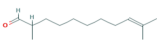
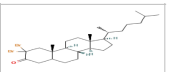
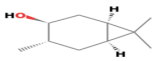
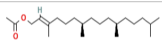
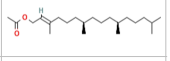
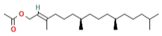
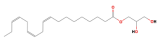
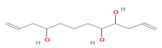
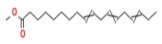
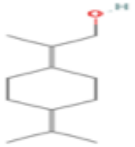
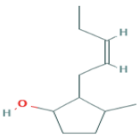
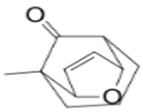
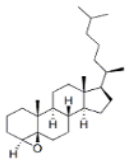
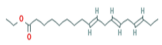
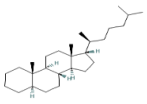
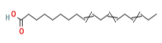
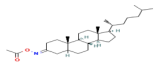
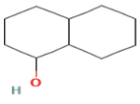
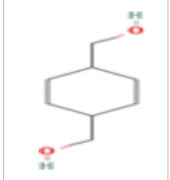
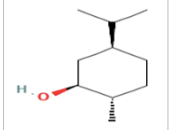
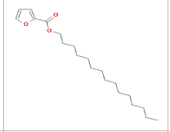
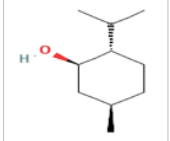
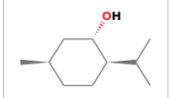
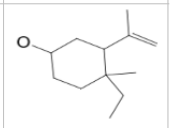
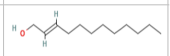
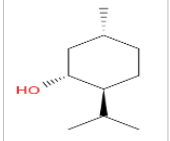
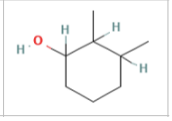


Table 6: Showing compounds obtained from GC-MS analysis of *Gomutra*

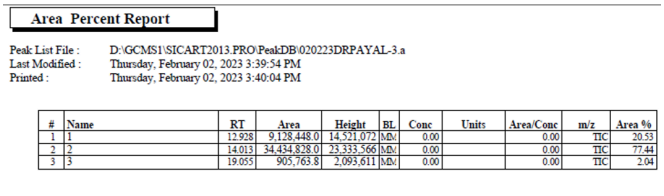
Sr. no	**Compound name**	**Formula**	**Structure**
1	P-Cresol	C7H8O	
2	Phenol, 2-Methyl-	C7H8O	
3	Phenol, 3-Methyl-	C7H8O	
4	Benzyl Alcohol	C7H8O	

5	N-Cbz-Glycylglycine P-Nitrophenyl Ester	C18H17O7N3	
6	Carbamic Acid, Methyl-, 3-Methylphenyl Ester	C9H11O2N	
7	Furan, 2-(2-Propenyl)-	C7H8O	
8	1,2-Propanediol, 1-Phenyl-	C9H12O2	
9	2-Isopropyl-5-Methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-Co	C30H33O6CL	
10	2-Butanone, 4-(2,2,6-Trimethylcyclohexyl)-	C13H24O	
11	Cyclohexanol, 4-Ethyl-4-Methyl-3-(1-Methylethyl), (1.Alpha., 3.Alpha.,4.	C12H24O	
12	Phytol	C20HC40O	
13	2-Butyloxycarbonyloxy-1, 1, 10-Trimethyl-6,9-Epidioxydecalin	C18H30O5	
14	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	C20H40O	
15	4-Methyl-Z-4-Hexadecen-1-Ol	C17H34O	
16	6,11-Undecadiene, 1-Acetoxy-3,7-Dimethyl-	C16H28O2	
17	2-Hydroxy-1,1,10-Trimethyl-6,9-Epidioxydecalin	C13H22O3	
18	2-Dodecen-1-Yl(-)Succinic Anhydride	C16H26O3	
19	Tetrahydroionone	C13H24O	
20	2-Acetoxy-1,1,10-Trimethyl-6,9-Epidioxydecalin	C15H24O4	
21	2,3,6-Trimethylhept-6-En-1-Ol	C10H20O	
22	9-Undecenol, 2,10-Dimethyl-	C13H26O	
23	(9z,12z)-3,7-Dimethyloct-6-En-1-Yl Octadeca-9,12-Dienoate	C28H50O2	
24	Neophytadiene	C20H38	
25	2,2-Dibromocholestanone	C27H44OBR2	
26	Bicyclo[4.1.0]Heptan-3-Ol, 3,7,7-Trimethyl-, [1s (1.Alpha.,3.Alpha.,6.Alpha.)]	C10H18O	
27	Phytol, Acetate	C22H42O2	
28	1,5-Cyclooctadiene, 1-T-Butyl-	C12H2O	
29	8-Methylenecyclooctene-3, 4-Diol	C9H14O2	
30	Linolenic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester (Z,Z,Z)-	C21H36O4	
31	4,5,9-Trihydroxy-Dodeca-1,11-Diene	C12H22O3	
32	9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-	C19H32O2	
33	Cyclohexaneethanol, 4-Methyl-.Beta.-Methylene-	C10H18O	
34	Cyclopentanol, 3-Methyl-2-(2-Pentenyl)-	C11H20O	
35	11-Oxatricyclo[4.3.1.1(2,5)] Undec-3-En-10-One	C10H12O2	
36	Cholestane, 4,5-Epoxy-, (4.Alpha.,5.Alpha.)-	C27H46O	
37	9,12,15-Octadecatrienoic Acid, Ethyl Ester, (Z,Z,Z)-	C20H34O2	
38	9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-	C19H32O2	
39	Silane, Tetraethenyl-	C8H12Si	
40	9,12,15-Octadecatrienoic Acid, (Z,Z,Z)-	C18H30O2	
41	Cholestanone Oxime Acetate	C29H49O2N	
42	1.Alpha., 4a.Alpha., 8a.Beta.-Decehydro-1-Naphthalenol	C10H18O	

43	11,14,17-Eicosatrienoic Acid, Methyl Ester	C21H36O2	
44	1,4-Cyclohexanedimethanol	C8H16O2	
45	Cyclohexanol, 2-Methyl-5-(1-Methylethyl)-	C10H20O	
46	2-Furancarboxylic Acid, 4-Pentadecyl Ester	C20H34O3	
47	Levomenthol	C10H20O	
48	1-Octadecyne	C18H34	
49	2-Furancarboxylic Acid, 3-Pentadecyl Ester	C20H34O3	
50	1-Hexadecyne	C16H30	
51	Cyclohexanol, 5-Methyl-2-(1-Methylethyl). [1s-(1.Alpha.,2.Alpha.,5.Beta.	C10H20O	
52	Cyclohexanol, 4-Ethyl-4-Methyl-3-(1-Methylethenyl), (1.Alpha.,3.Alpha.	C12H22O	
53	Trans-2-Dodecen-1-Ol	C12H24O	
54	Dl-Menthol	C10H20O	
55	Cyclohexanol, 2,3-Dimethyl-	C8H16O	

Results of Gas chromatography Mass Spectrometry (GC-MS) analysis of *Gomutrasava*:

Figure 4: Area percent report of *gomutrasava*



Area Percent Report

Peak List File : D:\GCMS1\SICART2013.PRO\PeakDB\020223DRPAYAL-3.a
Last Modified : Thursday, February 02, 2023 3:39:54 PM
Printed : Thursday, February 02, 2023 3:40:04 PM

#	Name	RT	Area	Height	BL	Conc	Units	Area/Conc	m/z	Area %
1	1	12.928	9,128,448.0	14,521,072	MM	0.00		0.00	TIC	20.53
2	2	14.013	34,434,828.0	23,333,566	MM	0.00		0.00	TIC	77.44
3	3	19.055	905,763.8	2,093,611	MM	0.00		0.00	TIC	2.04

Figure 5: chromatogram generated by a GC of *gomutrasava*

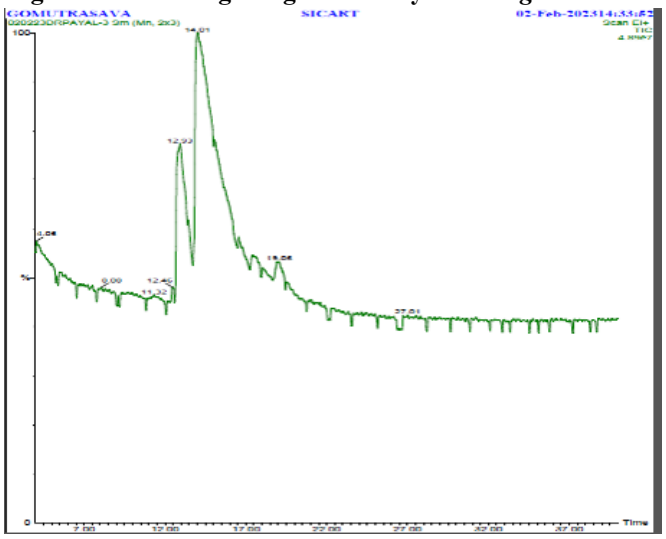
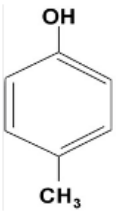
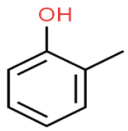
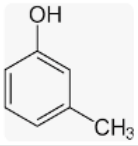
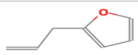
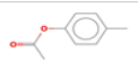
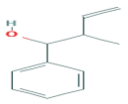
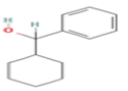
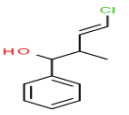
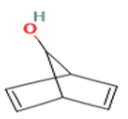
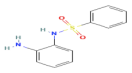
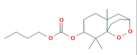
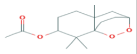
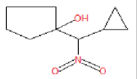
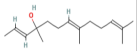
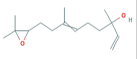
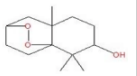
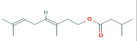
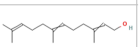
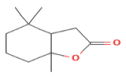
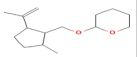
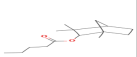
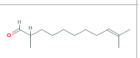
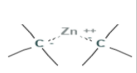
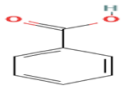
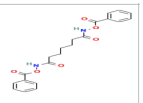
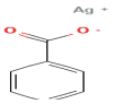
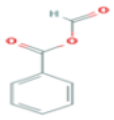
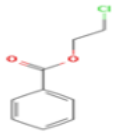
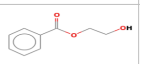
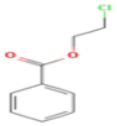
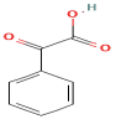
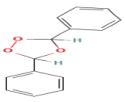


Table 7: Showing compounds obtained from GC-MS analysis of *Gomutrasava*

Sr.no	Compound name	Formula	Structure
1	P-Cresol	C7H8O	
2	Phenol, 2-Methyl-	C7H8O	
3	Phenol, 3-Methyl-	C7H8O	
4	Furan, 2-(2-Propenyl)-	C7H8O	
5	Acetic Acid, 4-Methylphenyl Ester	C9H10O2	
6	2-Methyl-1-Phenylbut-3-En-1-Ol	C11H14O	
7	Methanol, Cyclohexylphenyl-	C13H18O	
8	3-Buten-1-Ol, 4-Chloro-2-Methyl-1-Phenyl-	C11H13OCl	
9	Bicyclo[2.2.1]Hepta-2,5-Dien-7-Ol	C7H8O	
10	Benzenemethanol, .Alpha.-2-Cyclohexen-1-Yl-	C13H16O	

11	Benzenesulfonamide, N-(2-Aminophenyl)-	C12H12O2N2S	
12	Toluene,3-Cyclopropylmethoxy	C11H14O	
13	2-Butyloxycarbonyloxy-1,1,10-Trimethyl-6,9-Epidioxydecalin	C18H30O5	
14	2-Acetoxy-1,1,10-Trimethyl-6,9-Epidioxydecalin	C15H24O4	
15	1-(Cyclopropyl-Nitro-Methyl)-Cyclopentanol	C9H15O3N	
16	2,6,11-Tridecatrien-10-Ol, 2,6,10-Trimethyl-	C16H28O	
17	4-Methyl-Z-4-Hexadecen-1-Ol	C17H34O	
18	1,3-Dioxolan-2-One, 3-Methyl-3-(4,8-Dimethylnona-3,7-Dienyl)-4-Methylen	C16H24O3	
19	9-(3,3-Dimethyloxiran-2-Yl)-2,7-Dimethylnona-2,6-Dien-1-Ol	C15H26O2	
20	2-Hydroxy-1,1,10-Trimethyl-6,9-Epidioxydecalin	C13H22O3	
21	10-Methyl-E-11-Tridece-1-Ol Acetate	C16H30O2	
22	Undec-10-Ynoic Acid, 4-Methyl-2-Pentyl Ester	C17H30O2	
23	Geranyl Isovalerate	C15H26O2	
24	Trans-Farnesol	C15H26O	
25	2(3H)-Benzofuranone, Hexahydro-4,4,7a-Trimethyl-	C11H18O2	
26	2,2,4-Trimethyl-3-(3,8,12,16-Tetramethyl-Heptadeca-3,7,11,15-Tetraenyl)-	C30H52O	
27	2-(2-Isopropenyl-5-Methylcyclopentylmethoxy)Tetrahydropyran	C15H26O2	
28	Pentanoic Acid, 1,3,3-Trimethylbicyclo[2.2.1]Hept-2-Yl Ester	C15H26O2	
29	9-Undecenol, 2, 10-Dimethyl-	C13H26O	
30	Zinc, Bis[2-(1,1-Dimethyl-2-Propenyl)-3,3-Dimethylcyclopropyl], [1.Alpha	C20H34Zn	
31	Benzoic Acid	C7H6O2	
32	Cyclobutane-1, 1-Dicarboxamide, N,N'-Di-Benzoyloxy-	C20H18O6N2	
33	Heptanediamide, N,N'-Di-Benzoyloxy-	C21H22O6N2	
34	Benzoic Acid, Silver(1+) Salt	C7H5O2Ag	
35	Methanol, Oxo-, Benzoate	C8H6O3	
36	2,4-Dinitrophennylhydrazone Of Ribose Tetrabenzoate	C39H30O12N4	
37	4-Piperidinepropanoic Acid, 1-Benzoyl-3-(2-Chloroethyl), Ethyl Ester	C19H26O3NCI	
38	Cyclopropanecarboxamide, N-Benzoyloxy-	C11H11O3N	
39	2-Chloroethyl Benzoate	C9H9O2CI	
40	1,2-Ethanediol, Monobenzoate	C9H10O3	
41	3(1h)-Imino-2-(2,3,5-Tris-O-Benzoyl-Beta-D-Ribofuranosyl)-2,3-Dihydro-S	C29H24O7N6	
42	2-Chloroethyl Benzoate	C9H9O2C1	
43	(+)-Dibenzoyl-L-Tartaric Acid Anhydride	C18H12O7	
44	Butanedioic Acid, 2,3-Bis(Benzoyloxy)-, (2r,3r)-	C18H14O8	
45	1-0-Monoacetyl-2,3-O-Dibenzoyl-D-Ribofuranose	C21H20O8	
46	Benzoylformic Acid	C8H6O3	
47	1,2,4-Trioxolane, 3,5-Diphenyl-	C14H12O3	

Discussion

Asava-Arishta are traditional liquid dosage forms of *Ayurveda* medicine in which an infusion or decoction of natural components is allowed to ferment, resulting in a product containing self-generated alcohol (19). It is a method of preparing formulations in which the therapeutic properties of a group of herbal ingredients are extracted from either freshly obtained plant juice or dissolving them in water or by preparing a decoction in water. The substratum dissolved in water allows for biochemical or microbial fermentation in an anaerobic environment leading to the generation of alcohol content into the liquid (20). Because of its ease of administration, efficacy, and long-term usage, *Asava-*

Arishta is a preferred dosage form prescribed for many diseases (21-22).

Standardization of *Ayurvedic* medicines is necessary to ensure their quality. However, no pharmacopoeial standards are currently available for *Gomutrasava*; hence, this study was planned. The pH of Gomutra (pH 7.94) indicates that it is alkaline, whereas the pH of Gomutrasava (pH 5.41) indicates that it is slightly acidic (Table no.4). As it is weak acidic, it can be better bioavailable. According to research, acids had increased oral bioavailability, which was most likely due to better solubility and reduced clearance (23). *Asavaristas* are fermented products having self-generated alcohol content up to 8%v/v. Yet, the legal Act forbids a value of 12% or above among the self-generated alcoholic preparations (24). The alcohol content in *Gomutrasava* was 6% which is within the permissible limit. Total solids are a measure of the amount of active ingredients that can be extracted from aqueous medium. All the phytochemical components of the mixture were found to be uniformly dissolved. The process of fermentation facilitates several chemical and biochemical reactions with bond breaking and reunion. These herb-related suspended or dissolved particles may add to the total solids (25). In this study the total solid content of *Gomutrasava* (37.5%) was nearly same to that of *Gomutra* (36.5%) (Table 4). Specific gravity is the weight of given volume of the liquid specific temperature compared with the weight of equal volume of water of the same temperature. There was not much variation in the specific gravity of *Gomutra* (1.023 wt/ml) and *Gomutrasava* (1.033 wt/ml). Methanol is not recommended for human consumption. It is also routinely added to ethanol produced for industrial usage as a denaturant. There was no methanol content in *Gomutrasava,* making it fit for consumption. The viscosity of a liquid is a measure of its composition and is constant at a given temperature. The viscosity of *Gomutrasava* is 1.38/sec. The refractive index can be used to calculate the amount of sugar in a solution. It may also be used to estimate medication concentration (26). The refractive index of *Gomutrasava* and *Gomutra* was found to be same i.e 1.394 and 1.393 respectively. Reducing sugars are any sugars that can act as a reducing agent because they have a free aldehyde or ketone group, which includes all monosaccharides and some disaccharides such as galactose, glucose, fructose, ribose, and xylose. Reducing sugars can alter the color or flavor of food by interacting with other components, such as amino acids. Non-reducing sugar, on the other hand, lacks any free aldehyde or ketone group. Sucrose is the most common cyclic disaccharide and a non-reducing sugar (27). Reducing sugar and non-reducing sugar of *Gomutrasava* was found to be 1.03% and 0.07% respectively which is within limit. The source of reducing sugar in *Gomutrasava* is honey (28). The samples of *Gomutra* and *Gomutrasava* were assessed for microbial contamination. The results showed no microbial contamination due to Escherichia coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa, (Table no.5), thus making it fit for therapeutic administration. It also indicated that formulation was prepared in best hygienic conditions and sterilized instruments.

The computer creates a graph from the signal while the instrument is working, as shown in Fig.3 & 5. This type of graph is known as a chromatogram. The signal created when the compound passes from the GC column to the detector is shown by the height of each chromatogram. The x-axis represents Retention time (RT), whereas the y-axis represents signal strength (% area). Several peaks are marked with their RTs in Fig. 3 &5. Total 4 prominent peaks were obtained in *Gomutra* and 3 peaks were obtained in *Gomutrasava*. Fig. 2 & 4 shows the peaks RT, area covered, peak height and area percentage. Each height indicates a different compound that is separated by a sample combination. GC–MS analysis of *Gomutra* and *Gomutrasava* identified the presence of 55 and 47 components (Table no.6 & 7) respectively at varying concentrations at specific retention time. Phenols are present in *Gomutra* and *Gomutrasava.* Phenolic compounds have antimicrobial, anti-aging and anti-inflammatory properties (29). p-Cresol present in both are antioxidants and valued because they are relatively low in toxicity (30). 9,12,15 Octadecatrienoic acid, ethyl ester (Z,Z,Z) acts as Anti-inflammatory, Cancer preventive, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, and Antiacne (31). *Gomutra* shows the presence of benzyl alcohol while it gets oxidized to benzoic acid in *Gomutrasava*. In many multidose pharmaceutical vials and parenteral solutions, benzyl alcohol (also known as -hydroxytoluene), an aromatic alcohol, is employed as an antibacterial preservative at concentrations ranging from 0.9% to 2.0%. Hippuric acid is formed when it is conjugated with glycine and is easily oxidized in living tissue to benzoic acid. Benzyl alcohol and benzoic acid can accumulate in the body after repeated doses and because neonates have a reduced ability to metabolize benzoic acid, poisoning may develop. Neonatal patients who unintentionally received benzyl alcohol at doses of 100 to 240 mg/kg/day (mostly in bacteriostatic catheter flush solutions) experienced a syndrome known as the "gasping baby syndrome" that included CNS depression, severe metabolic acidosis, gasping respiration, thrombocytopenia, hepatorenal failure, seizures, intracranial hemorrhage, bradycardia, cardiovascular collapse, and death (32). Zinc is present in *Gomutrasava*, which may be due to the honey, which contains several vitamins and minerals. (33). The physiological functions of zinc are well known. Catalytic, structural, and regulatory roles are the three broad functional groupings that best describe zinc's role in biology (34). *Gomutrasava* contain farnesol, an effective anti-microbial, anti-inflammatory, anti-allergic, anticancer and anti-obesity agent (35). Geranyl isovalerate present in *Gomutrasava* triggers apoptosis through oxidative stress-mediated mechanisms. Oxidative stress-mediated cell death was supported by increased

ROS (reactive oxygen species) production, MMP (matrix metalloproteinase) loss, decreased antiapoptotic gene expression, and enhanced proapoptotic gene expression (36). Menthol found in *Gomutra* used as a

topical antipruritic and in cough drops and inhalers (37). Some new compounds were also observed in *Gomutrasava that were ab*sent in *Gomutra*. Interestingly, *Gomutrasava* contains numerous compounds whose activities are unknown. It would be interesting to research these molecules to shed further light on the medicinal potential of *Gomutrasava*.

Conclusion

Asava and *Arishta* are labelled as one of the greatest formulations in Ayurveda as they possess self generated alcohol which acts as self-preservative. *Gomutra* & *Gomutrasava* has several compounds that can exhibit antibacterial, anticancer, and antifungal activity, according to results from gas chromatography mass spectrometry (GC-MS), therefore this information will be used for clinical research and the treatment of leprosy, vitiligo, and other skin conditions. Additional study in this field will be helpful in the creation of effective *Gomutra* formulations to treat a variety of microbial diseases while boosting the human immune system. The pharmaceutical standards for *Gomutrasava* are not available hence the analytical parameters of present study may act as a reference towards establishing analytical standards for *Gomutrasava*.

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Conflict of interest

Conflict of interest declared none.

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