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Pharmaceutical Standardization

Ayurvedic hydro-alcoholic anti-asthmatic medicine Vasarishta built upon Mritasanjeevani Sura: Development and evaluation

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Abstract

Introduction: Vasarishta built upon Mritasanjeevani Sura (MS) is a polyherbal hydro-alcoholic anti-asthmatic formulation which is administered in a dose of I ml instead of standard dose 40 ml, generally advocated for any "Asava-Arishta" in Ayurveda. Aim: The present study was aimed at finding out rationale for the peculiar distillation process to manufacture MS followed by Sthapana process to make Vasarishta. It was further aimed to find out difference in Vasarishta samples manufactured by purely fermentation process and the peculiar method mentioned above. Materials and Methods: Three batches of MS and subsequently three batches of Vasarishta were prepared. Basic standardization and development of standard operating procedure for the same were achieved by doing pH, percentage of alcohol and total reducing sugar, specific gravity on both MS and Vasarishta, during and after completion of process. Finally, MS and Vasarishta (built upon MS) made in laboratory were compared with marketed samples of MS and Vasarishta using gas chromatography. Results: The types of alcohols and volatile acids in MS and Vasarishta, prepared in laboratory, are similar but the proportions differ, which is taken as an indicator of process standardization. Values of furfural, ethyl acetate, and I-butanol in lab samples are within permissible limits as against the values of the market samples. **Conclusions:** The textual process for the production of *Vasarishta* proved to produce organoleptically acceptable product which is virtually free of toxic compounds such as furfural. Key words: Distillation, fermentation, furfural, gas chromatography, Mritasanjeevani Sura, Mayurakhyayantra, Sandhana

Introduction

Out of 25 different dosage forms described in Ayurveda, "Asava-Arishta" are commonly used owing to greater shelf life,^[1] faster absorption followed by quick and targeted results. These are polyherbal hydro-alcoholic formulations prepared by fermentation process, known as Sandhana Kalpana. Apart from the management of chronic disease conditions, some of the formulations from this class are advocated in acute conditions.

Vasarishta is one of the prominent formulations used to treat various respiratory conditions.^[2] It is the advanced category of *Arishta*, which is built upon another "Asava-Arishta"

Address for correspondence: Dr. Asmita A. Wele, D 1104, Green Valley Society, Kool Homes, Bavdhan, Pune - 411 021, Maharashtra, India. E-mail: asmita.wele@gmail.com formulation, *Mritasanjeevani Sura* (MS).^[3] Due to its unique processing, *Vasarishta* became a very potent medicine and therefore administered at very low dose of 1 *Masha* (corresponds to 1 ml as per metric conversion), as compared to the standard dose^[4] of 1 *Pala* (corresponds to 40 ml) of *Asava-Arishta*.

Standard manufacturing process of Asava involves three steps. First step is of juice extraction or soaking of cereals or dry fine powders of plant mixtures or boiling of the mixture at

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low temperature for a short period as the case maybe. Next is to add sugars, natural plant-based yeasts such as flowers of *Dhataki* (Woodfordia fruticosa, Kurz.) or Madhuka (Bassia latifolia Roxb.) and aromatic agents to the mixture, which is sealed and stored in temperature-controlled area for about 3 weeks. Third step is decanting and filtration of the formulation and bottling. Arishta formulations differ from Asava where standard decoction (Kwatha) is made and used instead of juice or moderately boiled plant infusion, while all further steps are similar. Sura is that type of Asava where cereals or plant powders are cooked or boiled for some time and set aside to ferment. It is then separated and used to extract phytochemicals from herbal juices by mixing and setting for a particular time depending on the plants or formulations where no distillation is involved.^[5]

In case of *Vasarishta* which is built upon MS, it was observed that pharmacies do not practice a specific method of distillation, mentioned in text, perhaps due to the complexity involved in the preparation and unavailability of optimized procedure or standard operating procedure (SOP).

Although standardization and efficacy-related research works have been reported on various Asava-Arishta formulations including Vasarishta earlier, authors did not come across any publication on manufacturing methods and standardization of this particular Vasarishta. Hence, the present study was carried out to prepare MS and further Vasarishta. The process was standardized and the products were compared with the marketed sample that quoted same reference to find out the difference in standards if any, owing to the change in manufacturing procedure.

Phytochemical profiles of almost all drugs of MS are available, and Vasa (Adhatoda vasica [L.] Nees.) is extensively studied for its components and pharmacology. Vasa leaves contain phenols, tannins, alkaloids, anthraquinones, saponins, flavonoids, amino acids, and reducing sugars.^[6] Ethanol extracts of the leaves show antimicrobial activity against many bacterial strains.^[7] The alkaloids vasicine and vasicinone have been thoroughly studied for structures; also bronchodilator, anti-inflammatory, and hepatoprotective activities.^[8]

It is well accepted clinically that the response to drug is dose-specific. Since the *Vasarishta* built upon MS is used in very small dose, the authors felt that it is important to decipher the rationale behind the peculiar manufacturing technique described only for this "*Vasarishta*" in the light of available information.

Materials and Methods

Any ayurvedic-fermented formulation is prepared using three basic components such as medicinal drugs, sugars and fermentation agents, or natural yeasts. MS contains 27 plant drugs, water as the liquid medium, jaggery as a source of sugar, and no specific yeast. Two of these 27 ingredients, such as *Granthiparni (Polygonum aviculare* Linn.) and *Elvaluka (Prunus cerasus* Linn.) are on the verge of extinction and hence were not used in the formulation. Table 1 shows the details of herbal ingredients of MS (*Vasarishta* contains only two ingredients, MS and *Vasa* leaf juice). Identification and selection of all ingredients [Table 1] were done using consensus method by ayurvedic experts and botanists. Further, these drugs were authenticated using macroscopic, microscopic, and physicochemical parameters and compared with standards given in Ayurvedic Pharmacopeia of India (API).

The raw materials of MS were screened and cleaned to remove foreign matter and powdered to homogeneity by rearing 80 mesh size. Only two ingredients had foreign matter such as *Jatamanasi* (*Nardostachys jatamansi* DC.) 3.0% and *Methika* (*Trigonella foenum graecum* L.) 2.2%.

Five of the powdered drugs, namely Babbula (Acacia arabica Willd.), Badara (Ziziphus jujuba Lam.), Puga (Areca catechu L.), Lodhra (Symplocos racemosa Roxb.), and Shunthi (Zingiber officinale Roscoe.) were mixed with 2160 ml distilled water in quantities given in Table 1. The mixture was then poured in equal parts into three sterilized conical flasks. The flasks were sealed with cotton plugs and were autoclaved for 1 h at the pressure of 15 kg/cm². After cooling, 168 g of jaggery was added to each flask. The mixture was stirred till the jaggery was completely dissolved. Then, the flasks were corked and stored in a dark room for 20 days. Temperature of the room was maintained in between 25°C and 31°C and humidity was maintained between 60% and 70%. During the fermentation process, samples for physicochemical analysis were withdrawn daily in laminar air flow.

On the 21st day, fermented wort from each flask was decanted. Mixture of powders of the remaining twenty drugs were divided into three equal parts and added to the three flasks. The wort mixture was then distilled between 70°C and 75°C with the help of *Mochikayantra* [Figure 1].^[2] The distillatory titled *Mochikayantra* looks like upper portion of head of an elephant (*Gajakumbha*). To assemble it, a round bottom flask having single inlet and two outlets was taken. Both outlets were attached to two condensers which are further attached to a bottle and a conical flask. After filling the wort through inlet of the flask, it is sealed with a cap. The round bottom flask is heated using heating mantle.

The distillate obtained is MS. The yield was in the range of 580–590 ml per flask. The MS samples from all three flasks were subjected to organoleptic tests and analyzed for pH (ref: IS 3865:2001), specific gravity (ref: IS 3752:2005 [4.2]), alcohol (ref: IS 37529 [4.3]), and total reducing sugar (TRS) (ref: 1162:1958, reaffirmed 2009) percentages.

This MS was further used to prepare *Vasarishta*. Fresh *Vasa* leaves were autoclaved for 15 min at a pressure of 5 kg/cm², as a modification of classical *Putapaka* technique standardized earlier in the in-house laboratory. This process softened the leaves and optimum juice was expressed with the help of industrial mixer. To get 1000 ml juice, 1.5 kg leaves were used.

MS and fresh juice of *Vasa* leaves were mixed thoroughly in equal quantity, that is, 300 ml MS and 300 ml *Vasa* leaf juice in one flask. All the three flasks thus prepared were allowed to stand for 7 days. On 8th day, the mixture was filtered and stored in amber-colored bottles. The fermentation was monitored by daily sampling and assessing for changes in pH, specific gravity, alcohol%, and TRS%. The finished products were subjected to organoleptic and physicochemical tests.

Sanskrit name	Botanical name	Part used	Amount	Total ash (%)	Acid- insoluble ash (%)	Alcohol- soluble extractive (%)	Water- soluble extractive (%)	Volatile oil (%)
Badar	Ziziphus jujuba Lam.	Bark	80 g	11	5.2	7.5	6.5	Nil
Babbul	Acacia Arabica Willd.	Bark	80 g	14	1.2	7.4	6.4	Nil
Puga	Areca catechu Linn.	Fruit	80 g+2.5 g	2.1	0.2	21.2	12.4	Nil
Lodhra	Symplocos racemosa Roxb.	Bark	20 g	10.2	1	13	16.5	Nil
Ardraka	Zingiber officinale Roscoe.	Rhizomes (fresh)	10 g	4.2	1	6.4	12.2	Nil
Devdaru	Cedrus deodara (Roxb.) Loud.	Bark	2.5 g	1.5	0.07	10.4	2.4	Nil
Lavanga	<i>Syzygium aromaticum</i> (Linn.) Merr. and L.M. Perry	Fruit	2.5 g	4	1	5.4	10	20
Padma	Nelumbium speciosum Willd.	Whole plant	2.5 g	12	2.9	2.0	7.2	Nil
Ushira	Vetiveria zizanioides (Linn.) Nash.	Roots	2.5 g	7.6	4.8	5.2	6.8	1
Gandharaj	Santalum album (Linn.)	Inner bark	2.5 g	0.2	0.1	8	1.5	1.8
Shatapushpa	Anethum sowa Kurz.	Seeds	2.5 g	7.2	0.2	4.4	15.1	4.3
Yavani	Trachyspermum ammi Linn.	Fruit	2.5 g	8.0	0.2	10.4	14	3.0
Maricha	Piper nigrum Linn.	Fruit	2.5 g	3.2	0.3	8	7.3	Nil
Jeeraka (Shweta)	<i>Cuminum cyminum</i> Linn.	Seeds	2.5 g	6.4	1	12	18.3	Nil
Jeeraka (Krishna)	<i>Carum carvi</i> Linn.	Seeds	2.5 g	8.7	1.5	10	15	4
Shathi	Hedychium spicatum Ham. ex Smith.	Rhizomes	2.5 g	5.2	1	6.4	9.4	Nil
Jatamansi	Nardostachys jatamansi DC.	Roots	2.5 g	6.2	5.3	3.6	5.4	0.22
Twaka	Cinnamomum zeylanicum Blume	Bark	2.5 g	2.1	1	3.4	7	2.6
Ela	Elettaria cardamomum Maton	Fruit	2.5 g	5.4	4	3.2	10.2	5.4
Jatifala	Myristica fragrans Houtt.	Fruit	2.5 g	2.1	0.32	12	8.4	0.1
Mustaka	Cyperus scariosus R. Br.	Rhizomes	2.5 g	8	3.5	5.4	12	1.4
<i>Shunthi</i> (dry)	Zingiber officinale Roscoe	Rhizomes	2.5 g	4.2	1	6.4	12.2	Nil
Mishreya	Foeniculum vulgare Mill	Seeds	2.5 g	9	11	4.5	10.2	5
Methika	<i>Trigonella foenum graecum</i> Linn.	Seeds	2.5 g	2.2	0.2	5	Nil	Nil
Raktachandan	Pterocarpus santalinus Linn. f.	Inner bark	2.5 g	2	0.2	4.2	4	Nil
Vasa	Adhatoda Vasica Nees	leaves	1.5 kg	14	1	4.6	28	Nil

Table 1: Ingredients and their physico-chemical values used to prepare *Mritasanjeevani Sura* (base) and *Vasarishta* (final product)



Figure 1: Mochikayantra (a distillery described in Sanskrit verse for the preparation of Vasarishta built upon Mritasanjeevani Sura)

Further, MS and *Vasarishta* prepared in the present study were qualitatively compared with the market samples of MS and *Vasarishta* for the presence of different alcohols, volatile oils,

and acids using gas chromatography [GC] (Agilent Model [6890 plus] RTX [Stabilwax DA column 60 m \times 0.25 mm ID \times 0.25 micron thickness]). The concentration of individual component was measured by flame ionization detector.

Results

Analysis of Mritasanjeevani Sura

Raw materials were subjected to physico-chemical analysis, and the results of the analysis are shown in Table 1. All the ingredients were complied with the API standards.

The in-process samples and finished product were subjected to different analyses. The values of each parameter, namely pH, TRS percentage, etc., for three batches were compared and the mean is taken. All values and per batch variation between them are within expected limits. The gradual drop in pH was observed from the 1st day up to 17th day. Hereafter, the pH stabilized at 3.7. An increase in alcohol content was seen up to 17th day, and then it stabilized to 9.8%. A change in specific gravity was observed from the 4th day that remained stable further on. A significant drop in the percentage of TRS was observed from the 3rd day of experiment which is the result of microbial growth. After 20th day toward the end of the experiment, TRS percentage was reduced to nondetectable limits [Figure 2].

The finished product MS was watery and transparent, having bitter and sweet tastes as described in ayurvedic texts and with mild alcoholic aroma.

Analysis of Vasarishta

It was observed that pH gradually decreased from the initial 5.5 to 5.1. Specific gravity, alcohol, and TRS percentages remained constant for all 7 days [Figure 3]. It can be inferred that Vasa juice may not contain enough reducing sugars or yeast as there was no sign of fermentation and subsequently no increase was seen in the percentage of alcohol in all the three batches. There may not be any microbial activity at this stage since the alcohol percentage is around 4.58% after double dilution of MS with Vasa juice. It could be envisaged that extraction of phytochemicals from Vasa leaf juice in the hydro-alcoholic environment is the important factor.

The finished product, Vasarishta, was sticky, faint brown, having bitter and sweet tastes, and with mild alcoholic aroma.

Gas chromatography analysis

The various acids in table are the fermentation end products. Methanol in quantity ranging up to 11 is nontoxic. Other volatile acids and alcohols contribute to the fragrance of the product and affect the organoleptic properties. In addition, they also constitute toward the stability of the product.

Marketed sample showed marked difference in the values of acetaldehyde, 1-propanol, and furfural in case of MS, while in case of *Vasarishta*, market sample showed marked difference in the values of ethyl acetate, acetic acid, and furfural. These substances may also have been added externally as preservatives in the marketed preparations.

Furfural content was above toxicity levels^[9] in case of *Vasarishta* market sample, whereas in the sample prepared in the present study, the value of furfural was well below permissible limit. The results of GC analysis are shown in Table 2.

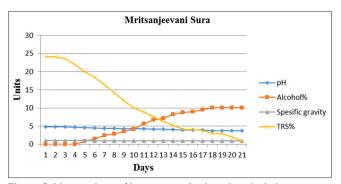


Figure 2: Mean values of in-process physico-chemical changes during development of Mritasanjeevani Sura

Discussion

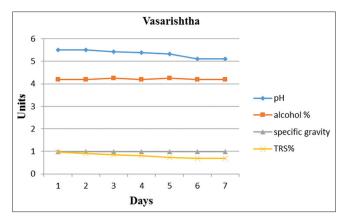
The study was undertaken intrigued by a line of the Sanskrit verse that dose of the particular formulation titled *Vasarishta* is 1 ml (1 *Masha*) as against the standard dose of 40 ml (1 *Pala*) for ayurvedic *Asava–Arishtas*. The verse clearly mentions a formula and process of preparation of MS by distillation and further using the distillate to extract, perhaps, specific phyto-ingredients from the juice of *Vasa* leaves. As the rationale behind the exceptionally low dose of the formulation and its peculiar process is not explained in classical texts, author thought to manufacture and standardize the process and analyze the product. It was essential to prepare three batches of MS, which were subsequently used to make *Vasarishta*. The means of physico-chemical values of MS and *Vasarishta* helped to establish the SOP for this particular method in the absence of standard comparators.

As stated earlier, MS and *Vasarishta* are two different products in the market. The samples of *Vasarishta* available in the market quote the same reference^[1] author used, but do not

Table 2: Gas chromatography analysis of								
Mritasanjeevani Sura and Vasarishta in comparison								
with market samples (units)								

Components	MS prepared	MS market sample	<i>Vasarishta</i> prepared	Vasarishta market sample
Alcohols				
Acetaldehyde	1.46	13.84	6.90	11.37
Acetone	0	0.4	0	5.19
Ethyl acetate	0.52	2.34	1.14	134.20
Methanol	6.22	8.6	10.39	49.68
1-propanol	3.16	38.23	40.30	21.87
Iso-butanol	4.50	32.55	6.61	8.58
lso-amyl alcohol	87.28	92.26	39.23	13.5
Volatile acids				
Acetic acid	0.4	1.24	0	11.14
Furfural	1.6	29.58	0	14,924.74

MS: Mritasanjeevani Sura





use the processing method mentioned in the formulation of *Shloka* (verse). They use the classical method of mixing and fermentation, which maybe the reason that the dose on label is mentioned as 10-20 ml twice a day.

The Mochikayantra is a double arm distillation unit, seldom used in the modern methods of distillation and is put to use for a very few processes in ayurvedic domain too. Etymology of the name Mritasanjeevani indicates that its use is to revive a dying patient or individual. Furthermore, the MS is used as base to facilitate the extraction of specific components from Vasa fresh juice, which in turn is named as Vasarishta. It is indicated in Tamaka Shwasa (asthma - bronchial and cardiac) where cardinal sign is breathlessness. In acute attack of Tamaka Shwasa, bronchodilation, anti-inflammatory, antispasmodic, and antihistaminic activities are expected to be present in the formulation; whereas in acute attack of cardiac asthma, vasodilatation is expected. It can be put forth, therefore, that composition and manufacture of the Vasarishta built upon MS is perhaps targeted to extract specific phytochemicals imparting above-mentioned actions to the formulation.

It has been demonstrated earlier that larger, more complicated alcohols are often isolated from volatile oils of plants by the process of steam distillation.^[10] The plant material is boiled in water, and the volatile oils are carried over by the steam, condensed, and separated from the water.

Out of the total ingredients used here, 15 contain essential oils while another 8 contain volatile oils of complex nature. These are extracted and separated by soaking and distillation using double arm technique. Along with the oils, many alcohol soluble phytochemicals also enter into the distillate. Due to limitations of the resources, analysis of distillate other than MS could not be performed. However, it is known that *Vasa* leaf juice contains active ingredients such as vasicinone and vasicine. Further, the activity profiles including vasicine that have been elucidated in previous research papers of the plants used in MS are suggestive of presence of phytochemicals having anti-inflammatory, antispasmodic, bronchodilator, and antihistaminic activities. Some like piperine are known to enhance bioavailability of the formulation.^[11]

It could be noted here that Sura fermentation begins with 25% sugar which is significantly lower than Asava-Arishta fermentation (40-50%). This may allow several nonfermenting micro-organisms to grow. As stated by previous research work, nonfermenting yeast do occur in W. fruticosa flowers or in other ingredients that could be involved in mobilizing different phytochemicals.^[12] Similar to this observation, it is worth noticing here that the percentage of alcohol in MS stabilizes at 9.8% in spite of the absence of external natural yeasts such as flowers of W. fruticosa or B. latifolia and also after distillation. In general, self-generated alcohols are distilled several times to increase the percentage of alcohol to the tune of 30-42. One such product from Vitis vinifera is described in Ayurveda where a distillate is sequentially distilled 10 times using an assembly called Varuniyantra to get finished Drakshasava.[13] It is possible that the plants used to make MS contain such "nonfermentative" yeasts, which produce little amount of alcohol, which is enhanced by distillation process, yet restricted within tolerance limits. It explains the rationale behind specific procedure using Mochikayantra. Furthermore, excess alcohol may be responsible to extract or generate furfural from the MS mixture, above the permissible limit, hence there is a difference seen in the furfural values of market sample which is prepared by adding yeasts to the formulation.

It is seen that 7-day contact period of MS with the juice of *Vasa* leaves facilitates extraction of phytochemicals present in the leaves into MS. It is always possible that during maturation process, the mixture may develop a fungal layer. In ancient days when aseptic techniques such as laminar flow were absent, fungicide activity of alcohol may have been prohibiting fungal contamination, during maturation of MS with *Vasa* juice.

Furfural or furaldehyde (C5H4O2) is made from agricultural material rich in pentosan.^[14] In the present study, though initial mixture of plants did not analyzed, it may be said that the plant materials may be the source of pentosan. It is important to note that in the lab-made MS, percentage of furfural is 1.6, as against 29.58 in the market sample. Similarly, Vasarishta prepared in house reports percent of furfural 0 whereas in market sample, it is 149.24, which is well beyond the permissible limit. An unpublished study reports an Reference dose for chronic oral exposure (RfD) of 0.003 mg/kg/day or 0.2 mg/day for a 70 kg human.^[15] In addition, the oral toxic dose of furfural in rats as per a study was 65 mg/kg which calculates to 10.31 mg/kg in humans.^[7] This explains the deaths reported after consumption of MS a few years ago which led to the unfortunate ban on MS. It is also possible that the marketed formulation either adds furfural as preservative or it is generated during the process. Since these high levels of furfural are toxic, our current procedure may be followed for the preparation of MS or Vasarishta.

Volatile acids and volatile alcohols are responsible for the aroma of the finished product. They make the formulation sweet smelling and increase its organoleptic properties. This correlates with the sweet smell and milder test of the formulation generated in lab while the marketed formulation was much acrid and stronger in test. The acceptable organoleptic properties of the formulation automatically mean satisfactory patient compliance and translate into better clinical output.

Vasa leaves contain 6-hydroxypeganine and vasicine as main alkaloids. Many researches have shown that vasicine acts as a stimulant in respiratory system. When used in lower doses, it produces bronchodilation and relaxation of tracheal muscles, while at higher dose, it protects from histamine-induced bronchospasm.^[6] The dose of the formulation is 1 ml. It is known that 1 ml alcohol is readily absorbed from the buccal mucosa and palate, bypassing the systemic circulation.^[16] Sublingual absorption of drug shows effect within minutes.^[17] Moreover, it has been proved that bioavailability of the phytochemicals increases due to piperine, which is an important component of MS. Considering the nature of bronchial and cardiac asthma, this particular formulation, prepared by employing the particular technique and administered over tongue may have been proven as emergency medicine in olden days. Further, it may have been administered over a considerable period of time to get consistent effect.

It can be summarized by the way of reverse pharmacology that while designing this particular formulation *Vasarishta*, ancient ayurvedic chemists might have thought of following logic. Plants containing specific phyto-constituents having specific activities on respiratory system are selected. Dosage form is self-generated alcohol which is a very good solvent for the extraction of phyto-constituents and it also has vasodilatation activity. Ayurvedic theoretical basis explains that alcohol (Asava-Arishta) dilates vessels (Vikasi) and has a fast reach to the site of action before getting metabolized (Vyavayee).[18] Hence, the extracted phytochemicals in Vasarishta reach the site of action fast to revive the patient in acute bronchial and or cardiac asthma. It may have been also thought that the amount of alcohol should not go beyond a specific percent and hence, routine fermentation yeasts are not added during the process and the two armed distillery is used to extract complex alcohols and chemicals from the plants. Small dose of 1 ml helps to increase its palatability also for a significant period of administration. The study opens up a possibility of exploratory clinical study on Vasarishta prepared using this particular method.

Conclusions

The optimized process for the production of MS and *Vasarishta* proved to produce a good product which is virtually free of toxic compounds such as furfural. The procedure should be adopted for the commercial manufacturing of *Vasarishta* wherever the reference is quoted.

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Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

मृतसंजीवनी सुरा से निर्मित वासारिष्ट का निर्माण एवं परीक्षण

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मृतसंजीवनी सुरासे निर्मित वासारिष्ट की मात्रा अन्य आसव तथा अरिष्ट की मात्रा से कई गुना कम दी गयी है। अन्य आसव ४० मिली मात्रा में दिए जाते हैं परंतु इस वासारिष्ट की मात्रा केवल १ मिली हैं। इसका कारण वासारिष्ट की विशेष निर्माणविधी हो सकता हैं यह मानकर हमने इसका अध्ययन किया। शास्त्रानुसार मृतसंजीवनी सुरा तथा वासारिष्ट उसी विशिष्ट पद्धती से निर्माण किए। विशिष्ट पद्धती का परिक्षण तीनों नमूनों के चार परीमाणों के आधार पर किया– आम्लता, एलकोहल प्रतिशत, तथा शर्करा प्रतिशत व विशिष्ट गुरुता मापी गई। इस प्रकार से तैयार हुये वासारिष्ट एवं मृतसंजीवनीसुरा की तुलना बाजार में उपलब्ध वासारिष्ट एवं मृतसंजीवनी सुरा के साथ की गई। इसके लिए गैस क्रोमॅटोग्राफी तकनीक का प्रयोग अपनाया। परीक्षण तथा तुलना के उपरांत यह सिद्ध हुआ की प्रयोगशाला में तथा फार्मेसी में तैयार किए हुये प्रॉडक्टस में एलकोहल के प्रकार व उड़नशील तैल समान है परंतु उसके प्रमाण भिन्न हैं। ग्रंथोक्त पद्धती से निर्मित प्रॉडक्ट में फुरफुराद, इथाईल ॲसीटर, १–ब्युटानॉल इनके प्रमाण निश्चित मानदंड के अनुसार आये हैं चूंकी बाजार से उपलब्ध प्रॉडक्ट में इनकें प्रमाण निश्चित मानदंड से अधिक हैं। इसलिए ग्रंथोक्त पद्धती का पालन करके ही औषधी पाठ निर्मिती करना जरुरी है।