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COMPARATIVE SCREENING BY HPTLC FOR ACTIVE COMPONENT IN FERMENTED BIOMEDICINE: VASARISHTAM

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ABSTRACT Ayurveda ,the alternative system includes .Arishtas as one of the popular formulation and have been used as appetizer and stimulant. They are unique to their indefinite shelf life. The proper standardisation for ensuring quality control draws its popularity and quality. To standardise Vasarishta, a well-known herbal formulation containing the herb vasaka (Malabar nut) by WHO guidelines and to perform the comparative quantification of vasicine in various formulation. Standardization parameters as per WHO guide lines are adopted and quantification of vasicine a major ingredient of vasaka has carried out by HPTLC method. The quantity of Vasicine present in various formulations are subjected to comparative evaluation. It has revealed that vasicine is the major constituent of these formulations by HPTLC Analysis. Standardization of these formulations confirms its identity, quality and purity of the Ayurvedic formulation.

KEYWORDS : Arishta, Vasarishta, Ayurvedic formulation, Vasicine, Standardization

INTRODUCTION

Arishtas are medicinal preparations of Ayurveda and are prepared by soaking the drugs, in powder form in a solution of sugar or Jaggery, for a specified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of the active principles contained in the drugs. The alcohol, so generated, also serves as a preservative.

Vasarishta is a well-known herbal formulation. Its main ingredient is vasa also known Malabar nut. It is a strong mucolytic and anti-asthmatic. Hence vasarishta is used to treat many respiratory disorders. It calms pitta and kapha. Vasarishtam has coagulative properties, therefore, it is also used to cure bleeding disorders like nose bleeding/ epistaxis, haemoptysis. It is also called vasakasavam, vasakadyarishtam, vasakarishtam. 8 Adhatoda vasica (L.) Nees (Acanthaceae), known commonly as Malabar nut tree, is a shrub growing throughout the Indian peninsula. The plant is used in the indigenous system of medicine in India and is a well-known expectorant in both Ayurvedic and Unani Systems of Medicine. The leaves are used to treat malarial fever, chronic fever, intrinsic hemorrhage, cough, asthma, leprosy, skin diseases, and piles. The plant is reported to show abortifacient, antimicrobial, and antitussive activities. The crude extract of A. vasica leaves was found to have conspicuous antifeedant and toxic effects on the larvae of S. littoralis. The plant contains alkaloids such as Vasicine, vasicinone, deoxyvasicine, vasicol, adhatodinine, and vasicinol. Vasicine is reported to have bronchodilatory, respiratory stimulant, and uterine stimulant effects. Vasicine acetate showed antimycobacterial activity. Essential oils of the leaves of A. vasica are also known to contain ketone, terpene, and phenolic ether which have antitumor, antioxidant, antiaging, antimutation, and sedative effects; the high phenolic content of essential oils contributes to their antimicrobial properties. In the present communication we report the antimicrobial, antioxidant, and anticancer effects of Vasicine acetate acetylated from Vasicine obtained from A. vasica leaves[11].

Need Of Standardization

Herbal medicines are natural products and their phytoconstituents depending on time and region, processing and storage, variations in the collection, processing or storage of an herb could impact its efficacy profile.The parameters of testing the quality of materials (dravya) in traditional medicines, such as rasa (taste), guna (properties), vipaka (post digestion effects) and karma (action) are very different from the western methods. These traditional parameters reflect not only the quality but also efficacy. [1]

The increased use of herbal medicines in developed countries is mainly due to the failure of modern medicine in providing effective treatment for chronic diseases and emergence of multi-drug resistant bacteria and parasites[2].

The Ayurvedic Pharmacopoeia of India and Pharmacopoeial standards for Ayurvedic formulations mention only the study of physico-chemical parameters and thin-layer chromatography of raw materials and formulations, which are not sufficient for proper standardization in present era [7,8]

Therefore, modern analytical method high performance thin layer chromatography (HPTLC) is applied to ascertain the quality of herbal products. Fingerprints obtained from HPTLC, used as important tools for identification of marker compounds in the phyto-constituents and for quality control development of herbal formulations. Development and application of analytical techniques[9].

MATERIALS AND METHODS

I. The three marketed formulations of Vasarishtam were procured from market.: Sample A0, Sample A1, and Sample A2 respectively
II. The standard drug Vasicine procured from YUCCA ENTERPRISES,

Asavas and aristas are medicinal preparations made by soaking the drugs, either in coarse powder or in the form of decoction (kasaya), in a solution of sugar or jaggery, as the case may be, for aspecified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of the active principles contained in the drugs. Alcohol, so generated, also serves as a preservative. Standardized ayurvedic formulations of uniform quality are essential for beneficial therapeutic use. Due to lack of standards and quality control methods, there are batch-to-batch variations in the formulation. As part of standardization procedure, the formulations were tested for physicochemical parameters like pH, total solids, loss on drying, acid value, boiling range, specific gravity, ethanol content, viscosity and weight per ml. The variation in ethanol content in asava and arista preparations stands a major problem in fixing up their limits as a quality control parameter. Thus to establish its limit over a period of time the present study focused on determining the ethanol content of the formulations

The pH value of vasarishta preparation was determined by using standard buffer as a primary standard and then pH of vasarishta was determined by using pH meter.

Density is determined by using pycnometer.

The viscosity of vasarishta was determined by using Ostwald viscometer. A stopwatch was used to determine the time[21].

Fig.: Ostwald Viscometer

Total Solid Content : 25 ml of formulation was taken in evaporating dish which was previously weighed and allowed to evaporate, so that only solid content remains in the dish and rest of the fluid gets evaporated, weighed and the solid content of formulation was calculated[22]

Acid value was determined by dissolving 10 g of formulation in 50 ml equal volume of ethanol previously neutralized with 0.1M KOH to phenolphthalein solution. titrated with 0.1M KOH until solution remains faint pink after shaking for 30 s[3]. A distillation unit fitted with a thermometer was employed to determine the boiling range of the Vasarishta. Then the temperature was increased in such a way that the receiver could collect 4-5 ml/min. The process was continued until 25% (25 ml) of the distillate reached the receiver and the temperature of the last drop of the distillate to the receiver was also noted

Alcohol Content : clevenger apparatus is used for distillation process. Take 150ml of vasarishta in round bottom flask. Temperature is maintain in 60^0C,. A separator is used to separate and the alcoholic content is collected by the receiver,

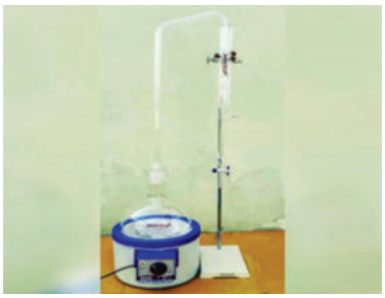


Fig. 3: Clevenger Apparatus

Specific gravity:The specific gravity bottle was used and the procedure has followed. [12]

For the determination of Ash value.Ash is produced by ignition and Weigh the Ash and calculate the percentage of total Ash[13].

Figure 4: Ash Test

The refractive index of formulation was found out by using Abbe's refractometer.

High Performance Thin Layer Chromatography (HPTLC)
HPTLC fingerprinting analysis was done using the CAMAG linomat 4 sample applicator using micro syringe (100 µl, Hamilton)

1µl, 5µl, and 10µl of standard Vasicine in water and 5µl of samples of vasarishtam is dissolved in corresponding solvents are tracked in precoated silica gel plate 60 F 254 plate from Merck (10×10 cm). The chromatograms of samples were developed in ascending technique in twin trough chamber. The solvent system optimized was Ethyl acetate: Methanol: Ammonia (7:3:0.3).

The detection was done under UV 254, UV 366 nm. Rf values and peak areas of the corresponding peak for different samples were then recorded

RESULTS AND DISCUSSION

Table1: Organoleptic Characteristics

Evaluation Parameters	Sample A0	Sample A1	Sample A2
Colour	Dull brown	Dark brown	Dark brown
Odour	Alcoholic	Alcoholic	Alcoholic
Taste	Sweet bitter taste	Sweet bitter taste	Sweet bitter taste

Table.2: Physico Chemical Evaluation

parameters	sample	Trial 1	Trial 2	Trial 3	Mean, standard deviation
pH	A0	5.41	5.41	5.42	5.4133 ± 0.0058
	A1	5.18	5.19	5.19	5.1867 ± 0.0058
	A2	5.4	5.41	5.4	5.4033 ± 0.0058
Viscosity(cp)	A0	4.119	4.123	4.12	4.1207 ± 0.0021
	A1	3.133	3.134	3.131	3.1327 ± 0.0015
	A2	1.6781	1.6756	1.6773	1.6770 ± 0.0013
Density(g/ml)	A0	1.171	1.7	1.73	1.5337 ± 0.3144
	A1	1.109	1.11	1.107	1.1087 ± 0.0015
	A2	0.972	0.974	0.973	0.9730 ± 0.0010
Specific Gravity (g/ml)	A0	1.1133	1.1133	1.1135	1.1134 ± 0.0001
	A1	1.1133	1.1136	1.1134	1.1134 ± 0.0002
	A2	0.9758	0.976	0.98	0.9773 ± 0.0024
Total Solid Content (g)	A0	11.59	10.47	11.24	11.1000 ± 0.5730
	A1	8	8.5	8.8	8.4333 ± 0.4041
	A2	4.18	3.56	4.01	3.9167 ± 0.3204
Alcohol Content (%v/v)	A0	28.666	28.67	28.75	28.6953 ± 0.0474
	A1	27.333	27.34	27.36	27.3443 ± 0.0140
	A2	21.333	21.36	21.35	21.3477 ± 0.0137
Ash Value (%)	A0	5.5	5.4	5.5	5.4667 ± 0.0577
	A1	3.5	3.6	3.4	3.5000 ± 0.1000
	A2	2.5	2.8	2.5	2.6000 ± 0.1732
Refractive Index	A0	0.406	0.407	0.409	0.4073 ± 0.0015
	A1	0.366	0.364	0.358	0.362 7 ± 0.0042
	A2	0.368	0.402	0.396	0.3887 ± 0.0181

Mean ± standard deviation, n=3

Organoleptic characteristics of different marketed formulations of Vasarishtas (Table 1) revealed, this is palatable to use because of sweet taste combined with fine aroma which masks the unpleasant taste and odour of added herbal ingredients.

Measured pH indicates that vasarishta formulation have weak acidic properties (Table 2).

Alcohol percentage in each of the tested brands of Vasarishtam was different. The maximum was 28.6953 ± 0.0474 and the minimum was 21.3477 ± 0.0137. The results highlighted that the levels of alcohol in Vasarishtam were higher than those prescribed in Ayurvedic pharmacopoeias.

HPTLC
We had done HPTLC using the stationary phase, silicagel 60 F254 plate optimized Solvent system Ethyl acetate: Methanol: Ammonia (7:3:0.3)

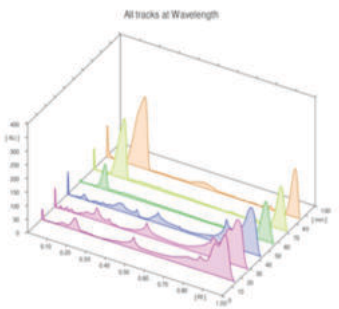
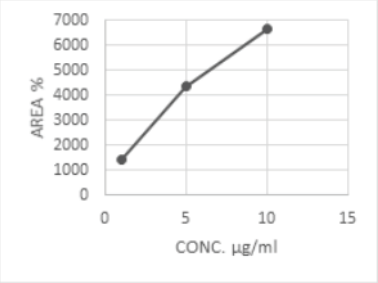


Table : 3

Sl. No	Sample	Concentration ()	Max . Rf	Area
1	A0	5	0.81	1358.2
2	A1	5	0.81	1598.9
3	A2	5	0.81	1811.4
4	Vasicine	1	0.81	1430.2
5	Vasicine	5	0.81	4351.6
6	Vasicine	10	0.81	6645.5

Calibration Curve For Vasicine



The Rf value of Vasicine by HPTLC analysis was found to be 0.81 .The peak coincide with all the marketed formulations, so we can say that the marketed formulations contain vasicine as one of the major component .

The HPTLC profiling of formulations and vasicine at 254nm, 366nm and visible region are given below.

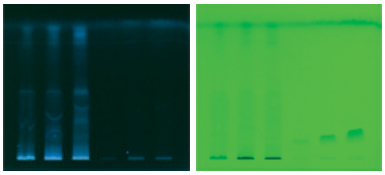
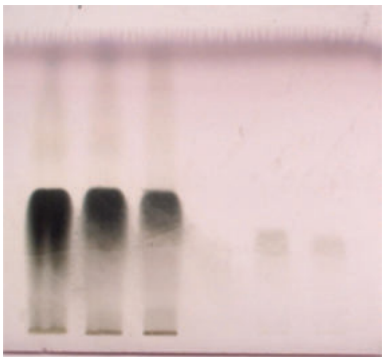


Image visualization: 254nm 366nm



Visible

CONCLUSION

Vasarishtam is a commonly used Ayurvedic formulation.Although it has so many medicinal properties, proper standardization is not done so far. Vasarishtam. It was revealed that the formulation is a weakly acidic liquid formulation and the major constituent was revealed as vasicine (Rf 0.81) from HPTLC and which can be attributed to the expectorant, anti-asthmatic and bronchodilatory actions of this particular Ayurvedic Formulation. Alcohol percentage in each of the tested brands of Vasarishtam was different and the levels of alcohol in Vasarishtam were higher than those prescribed in Ayurvedic Pharmacopoeias. The total as value of A0 was found to be 5.4667 ± 0.0577., and 3.5000 ± 0.1000, 2.6000 ± 0.1732 for A1 and A2 respectively. From the recorded levels of pH, Alcohol content, Total solid content, Viscosity, Refractive index, Specific gravity and Ash value it can be considered that the basic standardisation parameters for the quality control of arishtas have been performed and which will to improve the patient compliance and acceptance.

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