Research Article

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STANDARDIZATION OF SWASARI VATI WITH REFERENCE TO GAS CHROMATOGRAPHY, SCANNING ELECTRON MICROSCOPY AND EDAX

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ABSTRACT

Swasari Vati is a Herbo-mineral Kharaliya Rasayana which contains Parada (Mercury), Gandhaka (Sulphur), Vanga (Tin), Shunthi (*Zingiber officinale*), Pippali (*Piper longum*), Maricha (*Piper nigrum*) and Gomutra (Cow's urine) as bhavana dravya. It is indicated for the treatment of Swasa (Bronchial Asthma). This drug was pharmaceutically prepared and studied with modern analytical techniques like Gas chromatography-mass spectrometry (GC-MS), Scanning Electron Microscope (SEM) and Energy Dispersive X-ray Analysis (EDAX) with a view to standardize and to find out the chemical constituents and structural characterization of the drug. Under GC-MS study, the Swasari Vati found to have α-curcumene; α-zingiberene; shagol, Cyclohexane carboxylic acid; 1-piperidine carboxaldehyde; Benzoic acid, 3-hydroxy-, methyl ester; Gingerol etc. 2-(3, 4-methylenedioxyphenyl) cyclohexanone etc. 36 components were observed. The SEM photomicrograph of Swasari Vati sample shows bulk particles with a wide distribution of shapes and sizes. Individual, non-aggregated, microcrystalline particles, with irregular boundaries of various size and shapes were seen. The EDAX shows the presence of 11 different elements. The result shows that present as percentage of EDAX shows absence of heavy metals Cadmium, Lead

Keywords: Kharaliya Rasayana, Swasari Vati, Standardization, GC-MS, SEM, EDAX.

INTRODUCTION

In Ayurveda the quality and standardization of drugs was described elaborately, but it was based on the physical aspect of the drug such as colour, odor, taste, size, texture etc. In the modern era due to commercialization and bulk production of Ayurvedic drugs there is a chance of adulteration and incomplete processing of the drugs. Hence there is a need for standardization of all Ayurvedic drugs to maintain their quality. Therefore it is highly desirable that these drugs should be characterized with modern instruments, based on which the specifications of such drugs can be well standardized on a scientific basis. Among many sophisticated instrumentation GC-MS, SEM and EDAX are the important tools to analyze the Herbo-mineral compounds. In this study pharmaceutically prepared Swasari Vati[1] a Herbo-mineral Kharaliya Rasayana[2] commonly used for the treatment of Swasa was studied with these techniques to find out its chemical composition and also to standardize the drug. The analytical methods reveal out the chemical composition of formulation as well as their concentration. By this it helps to ensure safety limits and accuracy of the drug.

MATERIAL AND METHODS

The drug Swasari Vati was prepared in the P.G. Department of Rasa Shastra, N.R S. Govt. Ayurvedic College, Vijayawada, India. The analysis was conducted at Sastra University, Thanjavur and DMRL, Hyderabad, India.

Preparation of Swasari vati

The homogenous mixture of Samaguna Kajjali of purified Parada[3], Gandhaka[4], fine powders of Shunti[5], Pippali[6], Maricha[7] and Vanga Bhasma[8] were taken in equal quantity in a khalva yantra. Later triturated by adding Gomutra for 3 days, then vatis (tablets) were prepared in the dose of 500 mg and dried. These prepared vatis were subjected for analysis through GC-MS, SEM and EDAX.

Gas Chromatography- Mass Spectrometry (GC-MS)

Mass Spectrometry in conjunction with Gas chromatography has been reported to be a powerful tool in biological and chemical studies. It is a chromatographic separation technique based on the difference in the distribution of species between two non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives, which are volatilized under the temperatures employed. It is based on mechanisms of adsorption, mass distribution or size exclusion.[9]

Instrument Details

Make: PerkinElmer Clarus 500, Column type: Capillary Column Elite-5MS (5% phenyl 95% dimethylpolysiloxane), Column length: 30 m, Column id: 250 μm

GC Condition
Oven Program: 500C@80C/min to 150°C (5mi)@8°C/min to 250°C (10 min), Injector temp: 270°C, Carrier gas: He@ flow rate 1 ml/min, Split ratio 1:10

MS Condition
Mass Range: 40-600 amu, Type of Ionization: Electron Ionization (EI), Electron energy: 70 ev, Library: NIST 2005, Sample injected: 1 microlitre

Scanning Electron Microscope (SEM)
SEM can give images having three dimensional realistic effects. The inflorescence microscope is useful in studying labeled regions of a cell and monitoring of molecules that perform and for the technique known as photo leaching recovery.[10]

Analysis Procedure
SEM of the sample was analyzed by S-2400 Hitachi Scanning Electron Microscope. To produce the SEM image, the electron beam is swept across the area being inspected, producing many such signals. These signals are then amplified, analyzed and translated into images of the topography being inspected. Finally, the image is shown on a CRT.

Place of Study - SEM Dept. D.M.R.L. Hyderabad, India

Energy Dispersive X-ray Analysis (EDAX)
A Scanning Electron Microscopy (SEM) may be equipped with an EDAX analysis system to enable it to perform compositional analysis on specimens. EDAX analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen.

Place of Study: D.M.R.L, Hyderabad, India

RESULTS
GC-MS Study
The GC-MS of Swasari Vati has been carried out to get an idea of the separation and identification of degradation products of organic and organometallics making the elucidation of their structures and analysis of substance present in minute quantities. Herbs are composed of hundreds of active principles. Under GC-MS it was observed that, the Swasari Vati has α-curcumene; α-zingiberene; shagol Cyclohexane carboxylic acid; 1-piperidine carboxaldehyde; Benzoic acid, 3-hydroxy-, methyl ester; Gingerol, 2-(3, 4-methylenedioxyphenyl) cyclohexanone etc. This medicine has got majority of the ingredients which are having bronchodilator properties and also have Analgesic, Antipyretic, Anti-inflammatory, Anti-allergic, Anti-oxidant, Expectorant, antiseptic, carminative, Antispasmodic and Anti-rhino viral activities. Other compounds were Cyclohexane carboxylic acid; 1-piperdine carboxaldehyde; β-elemene; 1-H-Cycloprop (e) azulene, decahydro; 1, 1, 7-trimethyl-4-methylene and 2-(3, 4-methylenedioxy phenyl) cyclohexanone. (Table 1) (Graph 1)

EDAX Study
EDAX shows both the Elemental and Atomic percentage of the elements which are present in Swasari vati. As per EDAX Study elemental percentage in Swasari Vati-I (Table 2) were: Carbon - 29.95, Oxygen – 23.63, Na-0.70, Mg-0.48, Si- 0.54, P-0.57, S - 10.53, Cl-2.74, K-5.43, Ca-3.18, Hg-22.25. Sample-II (Table 3) elements are C-24.56, O-22.60, Na-0.77, Mg-0.46, Si-0.32, Mo-10.82, S-5.71, Cl-3.36, K-5.60, Ca-3.00, Hg-22.79 (Graph 2 and 3)

SEM Study
Individual, non-aggregated, microcrystalline particles, with irregular boundaries of various size and shapes were seen. Irregularly clumped amorphous particles of various sizes and shapes were present. A coating of some material was seen on most particles expecting the cellular structures. (Figure 1, 2, 3, 4, 5)

DISCUSSION
In recent days standardization and quality control of Ayurvedic drugs has become vital issue, since this system is gaining popularity and acceptance at global level. In this study a new pharmaceutical preparation Swasari Vati was subjected for analysis by commonly using techniques GC-MS, SEM and EDAX. This study shown Zingiberene, Curcumene, Gingerol and Piperidine as main constituents as the drug contains Sunthi, Pippali and Maricha. EDAX study reveals mercury, oxygen carbon and sulphur as the major elements. The final compound in SEM study is in the form of microcrystalline particles with various size and shapes.

Table 1: Result of GC-MS Study of Swasari Vati

S. NO	Peak Name	Retention Time	Peak area	%Peak Area
1	Name: Cyclohexane Carboxylic acid Formula:$C_7H_{12}O_2$, Mw:128	10.51	164973	0.0882
2	Name: 1Piperidinecarbozaldehyde Formula:$C_6H_{11}NO$, MW: 113	10.77	324832	0.1737
3	Name: 3-Phenyl-propionic acid(4-Methoxy-benzylidene)hydrazide Formula:$C_{17}H_{18}N_2O_2$, MW:282	15.05	533138	0.2850
4	Name: n-Decanoic acid Formula:$C_{10}H_{20}O_2$, MW:172	15.30	828701	0.4430
5	Name : Bicyclo[3.1.1]hept-2-ene,2,6-Dimethyl-6- (4-methyl-3-pentenyl) Formula:$C_{15}H_{22}$, MW: 204	18.49	98465	0.0526
6	α-Curcumene, Formula:$C_{15}H_{22}$, MW:202	18.63	238666	0.1276
7	α-Zingiberene, Formula:$C_{15}H_{24}$, MW:204	19.01	239132	0.1278
8	α-Farnesene, Formula:$C_{15}H_{24}$, MW:204	19.18	129759	0.0694
9	Name: Benzoic acid,3-hydroxy-, methyl Ester, Formula: $C_8H_8O_3$, MW: 152	19.38	3131405	1.6741
10	Name:1-Hydroxy-6-(3-isopropenyl-Cycloprop-1-enyl)-6-methyl-heptan-2-one Formula:$C_{14}H_{22}O_2$, MW: 222	19.89	142769	0.0763
11	Name: β-Elemene, Formula: $C_{15}H_{24}$ MW:204	20.75	688220	0.3679
12	Name: 1,6,10-Dodecatrien-3-ol,3,7,11-Trimethyl-,(E) Formula: $C_{15}H_{26}O$, MW:22	20.83	22687	0.0121
13	Name: Dodecanoic acid, Formula:$C_{15}H_{24}O_2$, MW:200	21.15	1834124	0.9806
14	Name: Benzoic acid,2-(1-oxopropyl) $C_{10}H_{10}O_3$, MW:178	21.63	740451	0.3959
15	Name:1H-Cycloprop[e]azulene,Decahydro-1,1,7-trimethyl-4-methylene-, Formula:$C_{15}H_{24}$, MW: 162	22.56	55813	0.0298
16	Name:Cyclohexene,1,5,5-trimethyl-6-(2-Propenylidene)- , Formula: $C_{12}H_{18}$, MW:162	22.65	268506	0.1435
17	Name: 1-Nathalenol, 1,2,3, 4,4a, 7,8,,8a-Octahydro-1,6-dimethyl- 4-(1-methylethyl):[1S (1α,4α, 4aβ,8aβ)] Formula: $C_{12}H_{18}$, Mw: 162	23.01	384190	0.2054
18	Name:2-Butatone,4-(4-hydroxy-3-Methoxyphenyl)-, Formula: $C_{10}H_{12}O_3$, Mw:194 Zingiberone, Gingerone	23.06	76847	0.0411
19	Name:Isopropyl-4-hydroxybenzoate Formula:$C_{10}H_{12}O_3$, MW:180	23.25	2801459	1.4977
20	Name: α-Bisabolol, Formula: $C_{15}H_{26}O$ MW:222, Tetradecatriene, Formula:$C_{15}H_{26}$, MW:206	23.72	503903	0.2694
21	Name: 1,E-8,z-10-Tetradecatriene, Formula:$C_{14}H_{24}$, Mw:192	24.62	160034	0.0856
22	Name: tetradecanoic acid,Formula:$C_{14}H_{28}O_2$, Mw:228	25.09	4821819	2.5779
23	Name:4-phenoxybenzaldyde, Fomula:$C_{13}H_{10}O_2$, Mw:198	25.25	2211608	1.1824
24	Name:Tricyclo[4.3.0.0(7,9)]nonane,2,2,5,5,8,8-hexamethyl-(1α,,6β,7α ,9α)- Formula:$C_{15}H_{26}$, MW:206	25.98	552982	0.2956
25	Name: Butyl(2-nonyn-1-yl)amine Formula:$C_{13}H_{25}N$, MW: 195	26.31	1170242	0.6256
26	Name: Pentadecanoic acid,Formula:$C_{15}H_3O_2$, MW:242	26.65	304135	0.1626
27	Name:Naphthalene,decahydro-1,1-Dimethyl-, Formula:$C_{12}H_{22}$, MW: 166	27.83	60408512	32.2957
28	Name: n-Hexadecanoic acid, Formula:$C_{16}H_{32}O_2$, MW: 256	28.11	20079920	10.7352
29	Name: 2-Propenamide,N-octyl-3-phenyl- Formula:$C_{17}H_{25}NO$, MW: 259	30.27	140216	0.0750
30	Name: 9-Hexadecanoic acid Formula:$C_{16}H_{32}O_2$, MW: 254	30.42	2336103	1.2489
31	Name: 3-cyclohexene-1 methanol ,α,4-Dimethyl- α -(4-methyl-3-penteny l)- [R-(R*,R*)]- Formula:$C_{15}H_2$6$0$MW: 22,(+)-α-Bisabolol	31.62	72914	0.0390
32	Name:4-Vinylbenzoic acid Formula : $C_9H_8O_2$, MW:148	31.76	1950076	1.0426
33	Name: Gingerol, Formula: $C_{17}H_{26}O_4$, MW:294	32.85	1106867	0.5918
34	Name:2-(3,4 Methylene dioxyphenyl)cyclohexanone, Formula: $C_{13}H_{14}O_3$	33.54	75437704	40.3307
35	Name: o-Anisic acid, 4-nitrophenyl ester Formula:$C_{14}H_{11}NO_5$, MW:273	35.36	1704404	0.9112
36	Name:2-Propenamide,N-dodecyl-3-Phenyl-, Formula: $C_{21}H_{33}NO$, MW:315	38.48	1382375	0.7390
				100.000

Table 2: EDAX of Swasari Vati-I

| Element	Wt %	At %	K-Ratio	Z	A	F
EDAX ZAP Quantification (Standardless); Element Normalized; SEC Table : Default						
C K	29.95	52.02	0.0581	1.0771	0.1799	1.0002
O K	23.63	30.81	0.0415	1.0589	0.1660	1.0001
Na K	0.70	0.63	0.0025	0.9907	0.3621	1.0009
Mg K	0.48	0.41	0.0024	1.0155	0.4951	1.0018
Si K	0.54	0.40	0.0041	1.0141	0.7465	1.0065
P K	0.57	0.38	0.0047	0.9854	0.8317	1.0110
S K	10.53	6.85	0.0956	1.0121	0.8925	1.0045
C1K	2.74	1.61	0.0188	0.9659	0.7100	1.0038
K K	5.43	2.90	0.0418	0.9674	0.7936	1.0029
Ca K	3.18	1.66	0.0257	0.9891	0.8170	1.0000
Hg L	22.25	2.31	0.1526	0.6669	1.0288	1.0000
Total	100	100				

Element	Net Inte	Bkgd Inte	Inte. Error	P/B
C K	20.33	1.09	2.33	18.65
O K	38.85	0.81	1.64	47.96
Na k	3.35	2.67	8.80	1.25
Mg K	3.34	4.03	10.11	0.83
Si K	5.26	6.94	8.32	0.76
P K	5.43	7.68	8.40	0.71
S K	105.46	6.80	1.03	15.51
Cl K	19.48	5.86	2.87	3.32
K K	36.69	5.01	1.86	7.32
Ca K	20.47	4.62	2.66	4.43
HgL	6.80	1.26	4.49	5.40

Table 3: Results of EDAX of Swasari Vati –II

Element	Wt. %	At %	K-Ratio	Z	A	F
EDAX ZAF Quantification (Standardless); Element Normalized; SEC Table : Default						
C K	24.56	48.24	0.0481	1.1025	0.1777	1.0002
O K	22.60	33.32	0.0383	1.0838	0.1566	1.0001
Na K	0.77	0.79	0.0027	1.0138	0.3414	1.0011
Mg K	0.46	0.45	0.0023	1.0390	0.4682	1.0021
Si K	0.32	0.27	0.0024	1.0374	0.7192	1.0077
Mo L	10.82	2.66	0.0966	0.8350	1.0658	1.0037
S K	5.71	4.20	0.0526	1.0409	0.8800	1.0044
Cl K	3.36	2.24	0.0228	0.9927	0.6810	1.0034
K K	5.60	3.38	0.0418	0.9922	0.7504	1.0024
Ca K	3.00	1.77	0.0238	1.0140	0.7818	1.0000
Hg L	22.79	2.68	0.1611	0.6886	1.0263	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte	Inte.Error	P/B
C K	18.18	1.33	2.76	13.64
O K	38.71	0.76	1.80	50.70
Na k	3.85	2.48	8.48	1.55
Mg K	3.35	3.87	10.95	0.87
Si K	3.29	7.38	14.21	0.45
Mo L	50.22	7.42	1.77	6.77
S K	62.59	7.39	1.55	8.47
Cl K	25.46	7.05	2.72	3.61
K K	39.62	6.58	2.02	6.02
Ca K	20.46	5.90	3.06	3.47
HgL	7.74	1.53	4.67	5.07
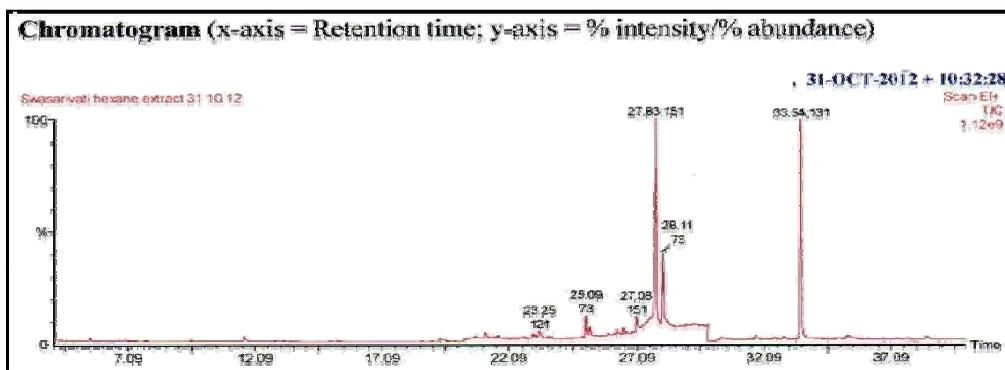
Graph 1: GC-MS of Swasari Vati
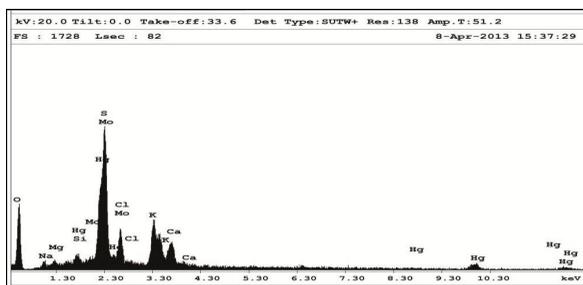
Graph 2:- EDAX result of Swasari Vati – I
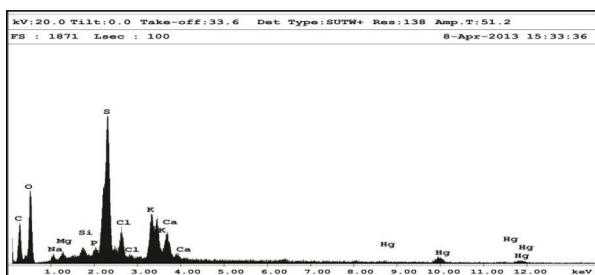
Graph 3: EDAX result of Swasari Vati- II
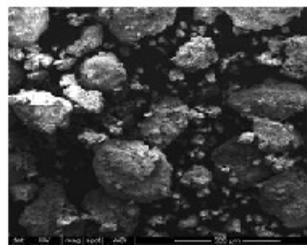
FIGURES:

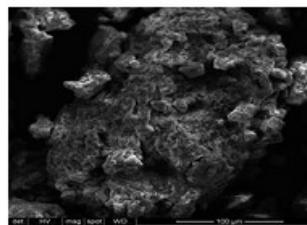Figure 1: SEM Picture of Swasari Vati- 100X

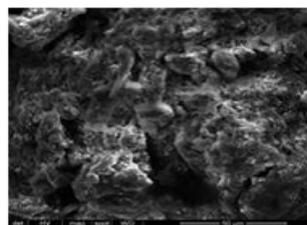Figure 2: SEM Picture of Swasari Vati-500X

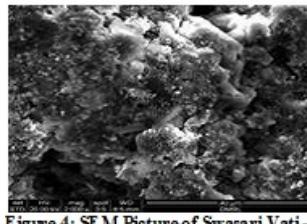

Figure 3: SEM Picture of Swasari Vati – 1000X
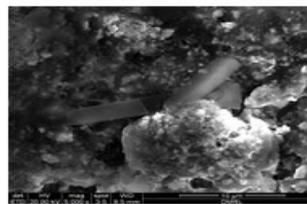

Figure 4: SEM Picture of Swasari Vati-2000X

Figure 5: SEM Picture of Swasari Vati-5000X

CONCLUSION

Under GC-MS study, the Swasari Vati has α-curcumene; α-zingiberene; shagol ,Cyclohexane carboxylic acid;1-piperidine carboxaldehyde; Benzoic acid, 3-hydroxy-, methyl ester; Gingerol etc. 2-(3, 4-methylenedioxyphenyl) cyclohexanone etc. 36 components were observed. The SEM photomicrograph of Swasari Vati sample shows bulk particles with a wide distribution of shapes and sizes. Individual, non-aggregated, microcrystalline particles, with irregular boundaries of various size and shapes were seen. The EDAX shows the presence of 11 different elements. The result shows that the present as percentage of EDAX shows absence of heavy metals Cadmium and Lead.

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