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Of all the gifts,
the most precious is health

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Aryavaidyan is intended to encourage scientific writing and intellectual interactions among scholars, academicians, practitioners and students of ayurveda and allied subjects like Siddha, Unani, modern medicine, etc.

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PHARMACOGNOSTIC STUDIES OF *STRYCHNOS POTATORUM*

P. B. Mallikharjuna and Y. N. Seetharam

Abstract: *Strychnos potatorum* (clearing nut) is an important medicinal plant used in the traditional and folk medicine for treating several aliments including microbial infections, diarrhoea and diabetes. Some of its pharmacognostic studies such as fluorescent, organoleptic, ash and mineral contents of root, stem bark and seed (both collected sample and market sample) and GC- alkaloid profiles of seed are investigated here.

Introduction

Strychnos potatorum L.f. (family: Loganiaceae) is a medium sized (about 15 m) and much branched glabrous tree. It generally occurs as a pan tropic species in the peninsular India, Burma, Sri Lanka and also in the North and South-east parts of Africa (Fig. Ia-d). It is known as the clearing nut in English, kataka or ambuprasada in Sanskrit, nirmali in Hindi, cillibījagida in Kannada, illam or tettan-kottai in Tamil, cillacettu or indupacettu in Telugu, tēṭṭāmparal in Malayalam, kotaku in Oriya and nirmali in Bengali.

It is an important medicinal plant widely used for treating various ailments including microbial infections, anaemia and diabetes. The plant is acrid, bitter, alexiteric, increases appetite and improves taste. The seeds are the chief source of therapeutic importance and are used for treating eye and urinary tract infections and acute diarrhoea (Āyurveda), gonorrhoea (Unani) and venereal diseases including leucorrhoea and piles (Siddha) (Kirtikar & Basu, 1996; Annalakshmi, 2003). The root cures all kinds of leucoderma and the stem bark powder mixed with lime juice is said to be effective against cholera (Annalakshmi, 2003) (Fig. II a-d). The present paper deals with some of its pharmacognostic parameters that help to standardise its drugs in terms of fluorescent and organoleptic attributes and so also to know about the presence or absence of some metals and other secondary metabolites.

Materials and methods

Plant material: - The plant material viz., root, stem bark and seed of *Strychnos potatorum* were collected from Karpakpalli forest, Bidar district in December 2002. The material was identified and a voucher specimen of the same is deposited in the Herbarium of Botany

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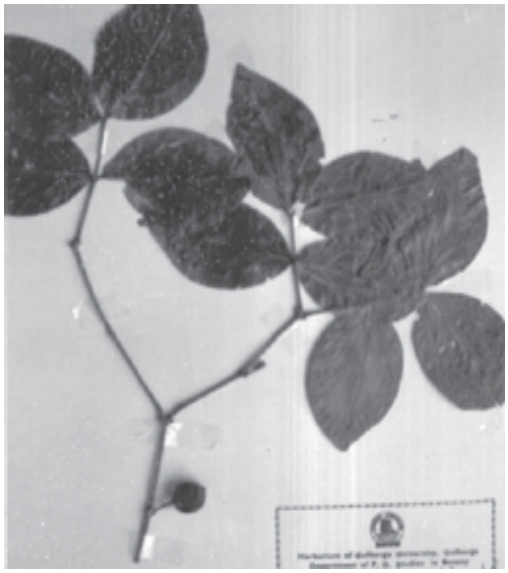
a



b

c

d

Fig. I a-d: *Strychnos potatorum*
a The habit; **b** A herbarium; **c** A twig with inflorescence; **d** A twig with a fruit

department, Gulbarga University (HGUG-214), Gulbarga. Further, another seed sample was procured from M/s. Jajee Ayurvedic stores, Gulbarga as the market sample, processed and stored for comparison. The plant material thus collected was shade-dried at room temperature. The powdered material was preserved in sterilized polythene bags.

Fluorescent studies: - Finely particulate (~300 µ) plant drugs were tested for their characteristic

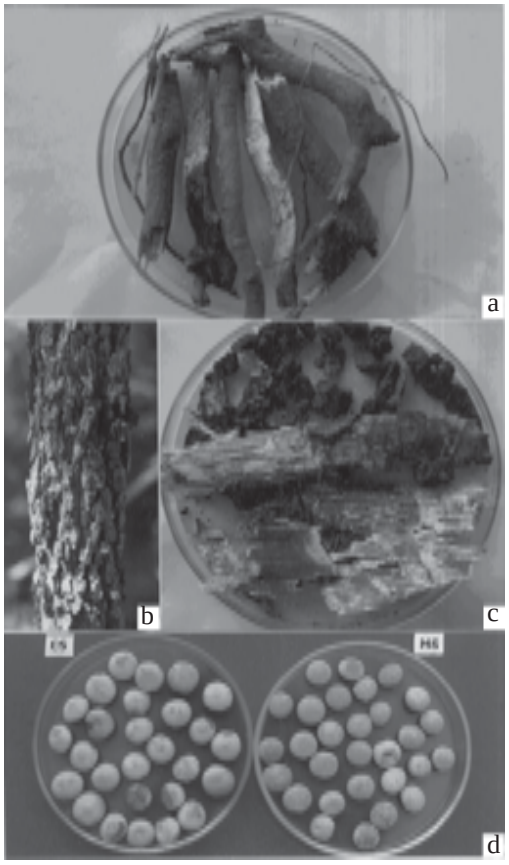


Fig. II a-d: *Strychnos potatorum*
a Root sample; **b** A portion of stem with bark; **c** Stem bark sample; **d** Collected seeds (CS) and market seeds (MS)

colours fluoresced both under visible and ultraviolet (UV_{365nm}) lights after treating with chemical solvents including alkalis and acids (Chase & Prat, 1949).

Organoleptic studies: - 100g powdered plant drugs of root, stem bark and seed of *S. potatorum* were successfully extracted in soxhlet extractor using petroleum ether, chloroform, 95% ehtnol and distilled water in the increasing order of polarity for 18 hours. The obtained extracts were condensed in *dry vacuo* (40°C) and the organoleptic properties such as colour, taste and yield (Chakraborti *et al*, 1988) were determined .

Determination of acid and mineral contents: - 2g of powdered plant material was taken in a sintered crucible and incinerated by keeping in muffle-furnace by gradually increasing its temperature (400 – 500 ^{0}C) for 6 hours. The ash value was calculated and further subjected to acid digestion using 25 % (v/v) HCl at 100 ^{0}C. The retained residue along with Whatman filter paper No.44 (ashless) was ignited in order to determine the acid insoluble ash content (Raghunathan, 1976). Further, four metals viz. copper, lead, iron and nickel were quantitatively estimated by atomic absorption spectrophotometric method (Smith-Hieftze 1000, Franklyn MA, USA) (Sawhney & Singh, 2002). Stock solutions were prepared from ash in double distilled water and the amount of metals were determined in percent of five replicates ± SD using standard graph.

GC Alkaloid profile of seed samples: - The total alkaloid extracts of both seed samples viz. collected and market seed, were dissolved in 5 ml of methanol of analytical grade and filtered through Whatman No.1 filter paper. 20 µL

samples were injected into the gas chromatogram column (Shimadzu QP-2000, column ULBON HR - with fused silica capillary tube of 0.25 mm X50 m). The initial temperature was 100^0C for 6 min and was increased up to 250 ^{0}C with 10^0C/min. Helium was the carrier gas pumped at 21 ml/min flow rate. All the reagents used were of analytical grade. The resulted number of peaks and their percent area were plotted on the software version 6.2.0.0:b27 and recorded.

Comparative Thin Layer Chromatography:- 20 µL of total alkaloid extracts of the seed of *S. potatorum* and *S. nux-vomica* (procured from M/s Jajee Ayurvedic stores, Gulbarga), the standard alkaloids, brucine and strychnine (Hi-media), were loaded on a precoated Alugram Sil G/UV_{254nm} (20x20 cm) and developed using EtOAc: *iso*PrOH: NH_4OH (80:15:5) mobile phase. The developed chromatogram was observed for the presence of strychnine and brucine in *S. potatorum* seed samples (Wagner & Bladt, 1996).

Results and discussion

The study of pharmacognostic aspects of medicinal plants in general and their therapeutic drugs in particular is an essential step in order to know their therapeutic value, efficacy, quality of the drug, to check for the adulterants and substitutes, and also for its toxicity.

The powdered drugs of *S. potatorum* viz. root, stem bark and seed (collected and market) have exhibited a wide range of characteristic fluorescent colour reactions when treated with various solvents ranging from acids to alkali both under visible and ultraviolet lights (UV_{365nm}). These were from ivory to dark black in colour. Further, the market seed samples exhibited broad range of colours (Table 1).

TABLE 2
The extractive and organoleptic characters of *S. potatorum* extracts

Characters	Extracts	Root	Stem bark	Collected seed	Market seed
Extractive values (%, w/v)	Pet. ether	1.30	0.50	2.74	2.16
	$CHCl_3$	0.12	0.56	0.34	0.76
	Et-OH	8.90	4.58	6.81	4.79
	Aqueous	5.90	6.97	15.68	10.23
Colour	Pet. ether	Old gold yellow	Mimosa yellow	Cinnamon	Cinnamon
	$CHCl_3$	Dark green	Fern green	Meadow green	Nile green
	Et-OH	Mint leaf green	Apricot	Inca gold yellow	Butter cup yellow
	Aqueous	Dark brown	Dark brown	Ivory	Ivory
Taste	Pet. ether	Pungent bitter	Pungent bitter	Pungent bitter	Pungent bitter
	$CHCl_3$	Pungent bitter	Pungent bitter	Pungent bitter	Pungent bitter
	Et-OH	Bitter	Bitter	Bitter	Bitter
	Aqueous	Bitter	Bitter	Bitter	Bitter
Nature	Pet. ether	Sticky	Sticky	Sticky	Sticky
	$CHCl_3$	Waxy	Waxy	Waxy	Waxy
	Et-OH	Resin	Resin	Resin	Resin
	Aqueous	Amorphous powder	Amorphous powder	Powder	Powder

TABLE 1
Fluorescent studies of *S. potatorum* powdered drugs

Treatment		Root		Stem bark		Collected seed		Market seed	
		VIS	UV_{365nm}	VIS	UV_{365nm}	VIS	UV_{365nm}	VIS	UV_{365nm}
01	Powder	Ivory	Ivory	Light black	Black	Ivory	Seafoam	Ivory	Ivory
02	+H_2O	Ivory	Dark black	Black	Black	Orange	Spring green	Nile green	Ivory
03	+HCl	Old gold	Brown	Chocolate	Black	Ivory	Mauve	Copper	Mimosa
04	+H_2SO_4	Cardinal red	Dark black	Brown	Black	Chocolate	Maroon	Chocolate	Black
05	+NaOH	Carrot red	Cream	Apricot	Black	Sea foam	Moss green	Burnt orange	Old gold
06	+$FeCl_3$	Citron	Sea foam	Butter cup yellow	Old gold	Cinnamon	Chocolate	Butter cup yellow	Moss green
07	+MeOH	Ivory	Sea foam	Black	Black	Ivory	Lettuce green	Chartreuse	Citron
08	+EtOH	Ivory	Sea foam	Black	Black	Ivory	Lettuce green	Chartreuse	Nile green
09	+BuOH	Ivory	Spring green	Black	Black	Ivory	Nile green	Chartreuse	Citron
10	+ Acetic acid	Ivory	Buff	Brickson brown	Sea foam	Ivory	Nile green	Cream	Butter cup yellow
11	+n-Hexane	Ivory	Seafoam	Cinnamon	Black	Ivory	Spring green	Buff	Spring green
12	+Lactic acid	Ivory	Light brown	Black	Rust red	Ivory	Old gold	Ivory	Old gold
13	+C_6H_6	Ivory	Light brown	Black	Black	Sea foam	Sea foam	Butter cup yellow	Moss green
14	+$CHCl_3$	Buff	Citron	Black	Black	Ivory	Spring green	Cream	Sea foam
15	+Pet. ether	Cream	Citron	Black	Black	Ivory	Nile green	Sea foam	Ivory

The organoleptic studies of *S. potatorum* have revealed variation in the colour, yield and taste of extracts. It is evident from the results, as expected the aqueous extracts are having the highest percent yield owing to the universal soluble nature of water followed by ethanol extracts (Table 2). However, the least percent of yields were recorded for chloroform extracts. Further, the yields of collected seed have greater value. The colours of these extracts were ranging from ivory to dark brown and bitter to pungent bitter in taste. They were amorphous powder to waxy in nature.

The highest percent of total ash and acid contents were obtained for stem bark followed by root and seed samples which may be due to the presence of lignin and tannins. The seed is rich in carbohydrates especially manno-galactans. But on digestion, lower yields of ash contents (Table 3) were obtained. Further, uneven distribution of metals in the plant was observed. Higher concentrations of iron were found in the root (2.2 %) and stem bark (1.161%). But the copper concentration is however low though it is uniformly distributed in all parts. Considerable amount of lead, a non-essential but toxic element was reported in the stem bark (0.0283%) and market seed (0.061%). However, the collected seed was completely devoid of lead element. Further, gradual increase of nickel from root to seed was observed. The presence of iron and copper is not only essential for plant growth but also is essential in treating anaemia. On the contrary, lead is a toxic element at higher concentration to the biota. The presence of lead more significantly in the market seed may be due to the method of processing and/or soil contamination (Cai *et al*, 1993). The lead poisoning is associated with carcinogenesis, reduced fertility, miscarriages and spermato-toxicity (Tandon & Suri, 1993).

Although, twenty-one alkaloid peaks were reported from the seed samples of *S. potatorum*, considerable variation in their percent areas were observed (Table 4, Fig. III). The most prominent alkaloid peak in the collected seed was reported with percent area 62.71% at 43.97 retention time (R*t*, min) whereas, in the market seed two

TABLE 3
Ash and mineral contents of *S. potatorum*

Plant part	Ash content (%, w/w)		Mineral content (%)			
	Total ash	Acid insol. ash	Iron	Copper	Lead	Nickel
1. Root	14.50	8.35	2.2000* (± 0.125)	0.1972 (± 0.005)	0.0005 (± 0.135)	0.2657 (± 0.091)
2. Stem bark	20.45	16.52	1.1610 (± 0.145)	0.2155 (± 0.015)	0.0283 (± 0.137)	0.3318 (± 0.042)
3. Collected seed	3.15	1.25	0.0643 (± 0.161)	0.2065 (± 0.015)	0.0000 (± 0.277)	0.4535 (± 0.088)
4. Market seed	3.00	1.39	0.0631 (± 0.199)	0.2013 (± 0.014)	0.0607 (± 0.104)	0.4297 (± 0.103)

*Values are percent of five replicates (± S. D.)

prominent peaks were observed at 43.850 and 61.422 (R*t*, min) with percent area 45.91% and 28.32%, respectively. The longevity of storage of plant drugs generally decreases its bioactive constituents. This is observed in TLC studies of secondary metabolites including alkaloids (Mallikharjuna, 2005). However, the method of processing aimed at increasing the potency of the drug and minimizing its toxicity is a very essential aspect. For instance, the seed of *Strychnos nux-vomica* are often used in clinical practices after processing in hot sand (220°C for 30min) which is intended not only to reduce toxicity but also to clean the fine hairs, which cause throat infection. Thus a better acceptance (Cai *et al*, 1993; Wu *et al*, 1994). Furthermore, the comparative thin layer chromatogram (co-TLC) has revealed the absence of strychnine and brucine, the known alkaloids from *S. potatorum*. However, further studies reveal that it is rich in diaboline and its derivatives (Mallikharjuna *et al*, 2007). As per the literature available strychnine and brucine were reported from the other Asian *Strychnos* species like *S. nux-vomica*, *S. wallichiana* and *S. ignatti* (Chakraborti *et al*, 1988).

Conclusion

The above results of pharmacognostic studies of *S. potatorum* have laid emphasis on the standardisation of its crude drugs, check of

TABLE 4
Alkaloid profile of *S. potatorum* seed by gas chromatography

Sl. No. of the peak	Collected seed		Market seed	
	Retention time (min)	Percent area (%)	Retention time (min)	Percent area (%)
1	28.276	0.32	28.148	0.47
2	36.926	1.08*	36.795	0.89*
3	38.455	0.22	40.275	0.48
4	42.860	2.54*	42.735	1.83*
5	43.987	62.71*	43.252	1.86*
6	45.456	0.74*	43.850	45.91*
7	46.129	0.10	45.989	1.91*
8	46.653	0.70*	46.532	0.77*
9	46.881	1.33*	48.428	1.91*
10	48.885	2.27*	48.762	0.69*
11	49.717	0.59*	49.638	0.07
12	50.482	2.07*	50.000	2.47*
13	51.569	0.88*	51.452	0.07
14	52.125	9.18*	52.011	7.15*
15	53.660	0.68*	53.533	0.53*
16	55.159	0.05	55.065	1.53*
17	55.992	3.89*	55.825	1.50*
18	57.051	9.42*	56.785	1.61*
19	59.623	0.16	59.460	0.17
20	61.729	0.47	61.424	28.32*
21	83.183	0.61*	82.667	0.40

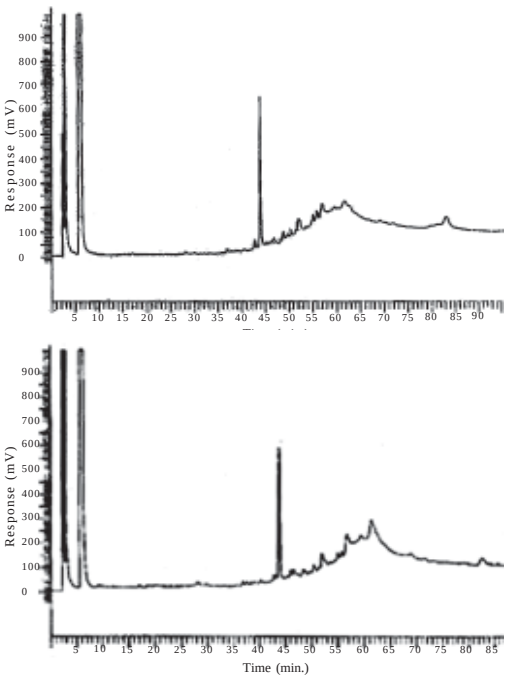


Fig. III
The gas chromatography alkaloid profile of *S. potatorum* seed

mineral contents, variation in the alkaloid profile and the absence of strychnine and brucine.

Acknowledgements

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LEECH THERAPY IN THE MANAGEMENT OF KNEE OSTEOARTHRITIS - A PILOT STUDY

Azad Hussain Lone[1], Tanzeel Ahmad[1], A.H Naiyar[2] and Rafiuddin[1]

Abstract: Osteoarthritis by far is the most common form of arthritis and is a major cause of pain and disability in the old. The reported prevalence of osteoarthritis from a study in rural India is 5.78%. Various renowned Unani physicians like Rhazes and Avicenna have described it as the inflammation or pain in joints caused by the accumulation of morbid humours or vitiated matter in the joint or its surrounding periarticular tissues. Leech therapy has been suggested and successfully practised by Unani physicians in the management of musculoskeletal and chronic skin disease since thousands of years ago. The study was conducted at National Institute of Unani Medicine Hospital, Bangalore with the aim and objective of assessing the efficacy and safety of Leech therapy in the management of knee osteoarthritis on modern scientific parameters.

Introduction

Osteoarthritis is a major health problem in terms of its prevalence, associated disability and effect on the quality of life. It is the commonest form of arthritis with a worldwide distribution, and leading cause of mobility related disability in the elderly people[1]. Patients usually complain of joint pain, stiffness and swelling which are worse in the morning[2]. It is one of the commonest musculoskeletal disorders affecting human beings thus making it an important cause of disability. According to World Health Organization, osteoarthritis is the second commonest musculoskeletal problem in the world population (30%) after back pain (50%).The reported prevalence of OA from a study in rural India is 5.78%[3]. In India osteoarthritis of knee joint is more common than hip joint, and is more common in women[4]. Osteoarthritis, also erroneously called degenerative joint disease, represents failure of the diarthrodial (movable, synovial lined) joint, that is characterised clinically by pain and functional limitations, radiographically by osteophytes and joint space narrowing, and histopathologically by alterations in cartilage and subchondral bone integrity. There is stiffness of the involved joints after a period of inactivity but not for more than twenty minutes[5]. In Unani system of medicine, the term wajual muffasil (arthritis) is collectively used for all joint diseases but the features of wajual muffasil

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balghami (phlegmatic arthritis) are same as that of osteoarthritis. Various renowned Unani physicians like Zakaria Razi, Ibne Abbas Majoosi, and Ibne Sina have described it as the inflammation or pain of joints and considered it as a maddi marz i.e. a disease caused by the accumulation of morbid humours (phlegm) or vitiated matter in the joint or its surrounding periarticular tissues. Ibne Sina in his famous treatise, "The Canon of Medicine" has mentioned that psychological factors and emotional states play an important role in the causation of this disease along with the weakness of joint[6].

Analgesics and non steroidal anti-inflammatory drugs (NSAIDs) relieve pain and improve function but the prolonged use of these drugs produce side effects[7], so there is a dire need to develop a long term control of symptoms by non pharmacological measures such as leech therapy, thermal modalities, tidal irrigation, patient education, exercise, massage and cupping. In Unani system of medicine leech therapy is one of the effective regimen and has been suggested and successfully practised by Unani physicians for long but the safety and efficacy of this therapy has not been scientifically evaluated till date. Hence, it was decided to conduct a clinical trial to find out the efficacy and safety of leech therapy in the management of knee osteoarthritis on modern scientific parameters. In classical Unani literature, two types of leeches are described - useful and poisonous leeches - along with their characteristics, indications and contra-indications. The features of therapeutically useful or nonpoisonous leeches are with thin tiny heads, emerald green colour, tiny and rounded like a rat's tail and found in moist rich places where frogs are in abundance. Leeches with long head, black, grey or green colour should be avoided as these are poisonous[8].

Materials and methods

The study was conducted in the Regimental Therapy Unit, National Institute of Unani Medicine Hospital, Bangalore from March 2009 to April 2010 and it was an open randomized uncontrolled trial. The patients were randomly selected from OPD and IPD of NIUM Hospital. A total of 30 diagnosed patients of knee osteo-arthritis qualifying the inclusion criteria were enrolled. The patients were clinically assessed and diagnosed on the basis of history, clinical and radiological examination. The duration of trial was 30 days and the assessment of subjective and objective parameters were done fortnightly.

Assessment criteria

Subjective parameters:- i) Pain in joints, ii) tenderness on the joint area, iii) morning stiffness, iv) swelling over the affected joint, v) restriction of movement[9].

Objective parameters:- Radiological (X- ray knee joint, AP and Lat. view) changes[13.]

Inclusion criteria

1. A clinically and radiologically diagnosed patient with knee joint osteoarthritis
2. Patients of age group 30-70 years of either sex
3. Patients who have agreed to sign the informed consent form and follow up the protocol.

Exclusion criteria

1. Patients with any systemic illness (hepatic failure, renal failure, ischaemic heart disease and malignancy).
2. Patients with anemia and or diabetes mellitus.
3. Patient suffering from rheumatoid arthritis,

tubercular arthritis, infective arthritis, gout, syphilitic arthritis, traumatic arthritis and gonorrheal arthritis.

4. Pregnant and lactating mothers.
5. Mentally retarded person.

Procedure

Leeches, procured from a local supplier and identified as medicinal leeches[11] (*Hirudinuria granulosa*) were used for the study, at Dept. of Zoology, Bangalore University. Prior to the procedure, the following investigations were done. Random Blood Sugar, Hb%, TLC, DLC, ESR, BT, CT, Serum Uric acid, CRP, RA factor, LFT, RFT, ECG, X- ray knee joint, HbsAg, Elisa test for HIV.

The method of leech therapy was explained to the patients and the part preparation was done thoroughly. Then 4 leeches were applied on the affected joints for a period of approximately 30 minutes according to the procedure as described by Ibne Sina[8]. The schedule of therapy was 0, 3rd, 9th, 15th, 21st and 27th day and the assessment of the subjective and objective parameters were done on 0, 15th and 30th day. The assessment was done with the help of Western Ontario and McMaster University (WOMAC) OA Scale[12] and Visual Analogue Scale (VAS) score[13] in which, decrease in scores suggests improvement. The severity of various clinical parameters were graded on 4 points as (0= nil, 1= mild, 2= moderate and 3= severe). The pre and post treatment data (scores) were tabulated and statistically analyzed by applying Paired Students 't' test to evaluate the efficacy of leech therapy. After the completion of trial, patients were again subjected to laboratory tests (BT, CT, LFT, RFT, etc) to explore the side effects or toxicity.

Results and discussion

Of 30 patients, the highest incidence was observed in the age group of 51-60 years while the least in 30-40 years. Distribution of patients according to age, sex, dietary habits, symptoms, etc. is shown in Table 1.

Effect of therapy on WOMAC score:- WOMAC OA Scale is an internationally accepted scale which consists of questions based on 3 symptoms viz; pain, stiffness and difficulty in performing physical activities. WOMAC scores were calculated in all patients before and after treatment according to the method described

TABLE 1
Distribution of patients according to age, sex dietary habits, affected joints and symptoms

Parameters	No. of Patients	Percentage
1. Age		
- 30-40	3	10
- 41-50	8	26.6
- 51-60	15	50
- 61-70	4	13.3
2. Sex		
- Male	11	36.6
- Female	19	63.3
3. Dietary habits		
- Vegetarian	10	33.3
- Non veg.	20	66.6
4. Affected Joint		
- Right knee	5	16.67
- Left knee	7	23.33
- Both	18	60
5. Symptoms		
- Pain	30	100
- Tenderness	18	60
- Stiffness	22	73.3
- Restricted movements	20	66.6
- Swelling	24	80

by S. Sontakke[14]. The mean WOMAC scores for pain, stiffness and difficulty in performing physical activities were reduced after the treatment (Table 2). The scores of both pre-treatment and post-treatment groups were compared and statistically analyzed by applying Paired students 't' test. The mean WOMAC scores of post-treatment groups was found significantly lowered ($p<0.001$) when compared to mean WOMAC scores of pre-treatment group. Thus, statistically leech therapy is an effective regimen in the management of osteoarthritis.

Effect of therapy on VAS score: - VAS scores were calculated in all the patients before and after the treatment. The mean VAS scores for pain, stiffness, tenderness, swelling and restriction of movements were reduced at the end of study (Table 2). The scores of both pre-treatment and post-treatment groups were statistically analyzed by applying "Paired Students 't' test." The mean VAS scores of post-treatment groups was found to be significantly lowered ($p<0.001$) when compared to mean VAS scores of pre-treatment group. Thus, statistically leech therapy is an effective regimen in the management of osteoarthritis.

TABLE 2
Effect of therapy on WOMAC and VAS scores

Symptoms	Mean score	
	BT	AT
1. WOMAC score		
- Pain	14.24	4.22
- Stiffness	8.68	3.34
- Daily activity	36.4	10.4
2. VAS score		
- Pain	4	1
- Stiffness	2	0
- Tenderness	3	0
- Swelling	1	0
- Restricted movements	2	0

The overall improvement in various clinical parameters might be due to elimination or evacuation of morbid humors present locally around the joints by leech therapy. This is in consonance with properties of leeches described by Raban Tabri, Razi, Majoosi and Ibn Sina[15]. The effectiveness may also be attributed to the analgesic and resolvent activities of leech therapy[16]. More over the saliva of a leech contains about 100 pharmacologically active biological substances like hirudin, hyaluronidase, vasodilators, inhibitors of kallikerine, anesthetics, antibacterial, fibrinases, collagenase, etc. This has been proved by various modern scientific researches. These substances are injected into human body when sucks the blood and are responsible for the analgesic, anti inflammatory and anesthetic effects of leech therapy[17].

Conclusion

On the basis of the above mentioned results, it is clear that leech therapy produced significant improvement on various symptoms and signs including pain, tenderness, stiffness, swelling, WOMAC score. But there was no effect in the degenerative changes of joints as observed in the X-rays of pre and post-treatment phases. Besides, the therapy was found safe and well tolerated as the safety parameters (Haemogram, TLC, DLC, KFT and LFT) remained within normal limits after the treatment. No obnoxious side effects were observed except for a mild local itching in some patients (30%). The overall

compliance to the therapy was good. Thus it may be concluded that the leech therapy is an effective and safe regimen in the symptomatic management of knee osteoarthritis.

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EFFICACY OF ĀMALAKYĀDI GHṚTA ALONG WITH VACĀDI GHṚTA NASYA (PRATIMARŚA) ON SENILE DEMENTIA - A CLINICAL EVALUATION

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Abstract: Progressive deterioration of memory, intellect, attention, thinking, comprehension and personality are encountered in senile dementia with intact normal consciousness. Āmalakyādi ghṛta is a newly formulated ghee-based medicine which contains āmalaki, haridra and rasona, whereas Vacādi ghṛta nasya contains vaca, kuṣṭha and pippali which have śirovirecana as well as rasāyana properties. Vacādi ghṛta was administered in the mode of pratimarśa nasya along with Āmalakyādi ghṛta orally. Āmalakyādi ghṛta along with Vacādi ghṛta nasya showed moderate improvement in 2.38% and mild improvement in 92.85% patients which reflect that the test drug is potentialy an alternative remedy to improve mental faculties in senile dementia.

Introduction

Dementia is a chronic confusional state associated with loss of higher mental function such as judgmental capacity, memory and language; but it is not associated with altered consciousness. Though senile dementia is not described as an independent disease entity in āyurveda, some terms can be correlated with dementia. Mādhava has mentioned smṛtināśa as a prodromal symptom of jara (ageing)[1]. Caraka too has described the symptom of smṛtibhramśa where smṛti is vitiated by rajas and tamas[2]. Hence, senile dementia can rightly be correlated with jarājanya smṛtibhramśa.

Dementia may be progressive or static, permanent or reversible in nature. An underlying cause is always assumed, although in rare cases it is impossible to determine a specific cause. The potential reversibility of dementia depends on underlying pathological condition and the availability and application of effective treatment. If treatment is given before irreversible damage sets in, approximately 15% of the cases can be reversed. Women are more susceptible to Alzheimer's disease than men which may be attributed to women's longer life span.

In āyurveda, there are many effective medicines described for the management of various psychiatric disorders. Medhya rasāyanas such as maṇḍūkaparṇī, guḍūcī, yaṣṭīmadhu and śaṅkhapuṣpī are well known psychotropic drugs which are being used successfully in the treatment of various psychiatric illnesses for

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long. As dementia is an ūrdhvajatrugata vikāra, nasya especially pratimarśa nasya is beneficial. The 9th and 10th chapters in Cikitsāsthāna of Caraka-samhita deal with neuropsychiatric disorders viz. unmāda (psychiatric diseases) and apasmāra (convulsions) where nasya (nasal insufflations) is mentioned as a suitable modality.

Aims and objectives: - The present study is aimed at evaluating the efficacy of Āmalakyādi ghṛta along with Vacādi ghṛta nasya (pratimarśa) in senile dementia.

Materials and methods

56 patients with signs and symptoms suggestive of senile dementia were registered, out of which 42 patients completed the 3 months treatment course.

Inclusion criteria:- Patients aged between 60-90 years attended the OPD of Kāyacikitsa department of I.P.G.T. & R.A, Jamnagar. They were screened and those fulfilling the clinical features of senile dementia both āyurvedic as well as modern descriptions were selected.

Exclusion criteria:- Patients suffering from psychiatric and neuropsychiatric conditions like Schizophrenia, Parkinsonism, Huntington's disease, Pick's disease, etc., persistent endocrine disorders, any other chronic systemic disorders and persons taking psychotropic drugs including alcohol were not chosen.

Method of study

Before registering the patients, written consent was taken and all were assessed by proposed parameters along with laboratory investigations. The study was conducted as a randomized single blind clinical one and was cleared by Institutional Ethics Committee. The dementia patients were prescribed Āmalakyādi ghṛta (10g) twice daily - in the morning and evening - for 3 months in empty stomach followed by drinking lukewarm water. Vacādi ghṛta pratimarśa nasya two drops in each nostril twice a day in the morning and evening were also administered for 3 months. Before taking the medicine, patients were advised to take harītakī cūrṇa in a dose of 3-4 g at bed time for 3-7 days for purgation according to the type of koṣṭha. All the patients were advised to follow appropriate diet (sāttvika āhāra) as per their prakṛti to correct their dietary habits.

Follow up: - After completion of treatment, patients were observed for 1 month and all parameters were re-assessed.

Assessment criteria

Subjective criteria:- Mini-mental state examination (questionnaires), Bender Gestalt Motor Visual Test, Hamilton anxiety rating scale (HARS), Hamilton depression rating scale (HDRS), brief psychiatry rating scale (BPRS), improvement in clinical features and assessment of doṣa, dūṣya and mānasa bhāva were the subjective criteria. Chief complaints of senile dementia and others symptoms related to specific rating scales such as Hamilton anxiety rating scale, etc. were assessed by 0-4 gradations according to the severity. Visual memory was assessed by Bender Gestalt Motor Visual Test by grading from 0-3 on the basis of severity.

Objective criteria: - Serum Cholinesterase, routine hematological and bio-chemical investigations, urine and stool examinations were the objective criteria.

Observations and results

Effect of the therapy on chief complaints: -

Irritability was relieved by 52.63%. Other symptoms like intentional tremor, assistance in personal care, disturbed sleep, mislaying of objects, etc. were improved. The therapy showed mild improvement in most patients (Table 1). These findings are suggestive of psycho activity of the test drug. The nourishment of mind provided by Āmalakyādi ghṛta may potentially enhance physical and mental faculties and decrease weakness and fatigue and in turn increase self confidence.

As kaphadoṣa is vitiated in most of the cases (50%), all the above signs and symptoms may be due to the obstruction of manovahasrotas by increased kapha doṣa. Āmalakyādi ghṛta contains drugs having memory enhancing, kapha śāmaka and rasāyana properties with uṣṇavīrya and kaṭuvipāka. Cow's ghee, the base of the drug, synergizes (yogavahi property) the cumulative effect of other drugs in the combination. Hence, by these properties, the drug may act over manovaha srotas, pacifies increased kapha and stabilises vātadoṣa to increase dhī, dhṛti and smṛti. Moreover, Vacādi ghṛta administered as nasya conains vaca, pippali and kuṣṭha which have śirovirecana property that removes vitiated kapha from śirosthāna and thus, absorption of nutrients at tissue level may be increased. These drugs have rasāyana and medhya prabhāva which can also improve the above mental qualities. Recent clinical studies have shown that, individual drugs of Āmalakyādi ghṛta (āmalaki, haridra, rasona) are established to have antioxidant

TABLE 1
Effect of Āmalakyādi ghṛta orally along with Vacadi ghṛta nasya on chief complaints

Chief complaints	Āmalaykāydi ghṛta + Vacādi ghṛta nasya		Paired t-test 'p'	÷2 (Chi-square)	p
	Mean ± SEM	% relief			
Forgetfulness	0.785 ± 0.09	43.42	<0.001	0.09	>0.5
Impaired attention	0.658 ± 0.10	39.70	<0.001	1.58	>0.01
Objects mislaid	1.690 ± 0.08	58.67	<0.001	0.08	>0.50
Forgetting names	0.939 ± 0.12	46.96	<0.001	0.89	>0.1
Forgetting numbers	1.690 ± 0.12	55.03	<0.001	0.008	>0.5
Decreased efficiency in household tasks	0.193 ± 0.08	16.21	<0.05	16.56	<0.001
Family faces, surrounding not recognised	0.833 ± 0.13	46.51	<0.001	0.33	>0.50
Making mistake in accounts	0.75 ± 0.12	42.85	<0.001	1.28	>0.10
Irritability	1.081 ± 0.11	52.63	<0.001	0.77	>0.10
Disturbed sleep	1.952 ± 0.20	63.07	<0.001	0.28	>0.50
Suicidal thoughts	0.5 ± 0.34	33.33	>0.05	5.12	<0.05
Intentional tremor	0.571 ± 0.17	38.09	<0.05	2.24	>0.10
Losing valuables	1.292 ± 0.07	53	<0.001	0.0006	>0.50
Forgetting food cooking on stove	0.666 ± 0.33	33.33	>0.05	1.01	>0.10
Gait difficulty	0.72 ± 0.12	41.86	<0.001	3.92	<0.05
Difficulty in preparing food	0.4 ± 0.24	28.57	>0.05	0.73	>0.10
Assistance in personal care	1 ± 0.32	50	<0.05	0.18	>0.50

property which can neutralize free radicals and reduce beta-amyloid burden in cortical area of brain and can thus improve brain functions viz memory, intellect, concentration, etc. Goghṛta (cow's ghee) by its lipophilic action can transport all properties of the drug into target organs of brain like hippocampus, hypothalamus and amygdala by crossing blood-brain barrier.

The effect of therapy on doṣavṛddhi lakṣaṇa was statistically highly significant because of the vāta and kapha śamaka properties, kaṭu, tikta rasa predominance and uṣṇa vīrya (hot in potency) of the drugs. Moreover, cow's ghee has specially vāta and pittasamaka properties (Table 2). The effect of therapy on srotoduṣṭi was also statistically highly significant. The drugs of the formulation have rasāyana, balya (strengthening) properties. Hence, it can provide proper nutrition to all dhātus, and as a result majja and asthi-dhātu-duṣṭilakṣaṇas were relieved. Moreover, most of the drugs have uṣṇavīrya and kaṭu, tikta rasa. Therefore, it can enhance digestive power and increase appetite, and thus annavaha srotoduṣṭi lakṣaṇas are decreased. Again, most of the drugs possess pañcarasa and kaṭuvipāka. By these properties, it can relieve the obstruction of purīṣavaha-srotas. Thus, duṣṭilakṣaṇa of purīṣavahasrotas are subsided (Table 2).

Various parameters for mental health (Mānasa parīkṣabhāva) are utilised as subjective criteria to assess the mental state according to āyurveda. Improvement was observed in parameters like bhaya, krodha, etc. (Table 3).

Discussion

Āmalakī (*Emblica officinalis*), haridrā (*Curcuma longa*) and rosana (*Allium sativum*) are well known for their antioxidant and memory enhancing properties. Most of the ingredients of Āmalakyādi ghṛta are uṣṇa in vīrya and kaṭu in vipāka. Hence, by its uṣṇavīrya nature, these drugs pacify increased kapha and vāta doṣas

TABLE 2
Effect of therapy on doṣavṛddhilakṣaṇas and srotoduṣṭi

Description	No.of patients	Paired 't' test						
		Mean score		% relief	SD	SE	't'	p
		BT	AT					
1. Doṣavṛdhilakṣaṇa								
- Vāta	17	14.94	6.00	59.84	2.461	0.59	14.97	<0.001
- Pitta	2	11.00	7.00	36.36	0.00	0.00	1.000	>0.05
- Kapha	23	11.74	5.48	53.33	1.84	0.38	16.32	<0.001
2. Srotoduṣṭilakṣaṇa								
- Annavaha	23	3.74	1.91	48.84	0.58	0.12	15.19	<0.001
- Rasavaha	37	5.47	2.76	49.50	1.43	0.23	11.48	<0.001
- Asthivaha	39	3.85	2.00	48.00	0.84	0.13	13.65	<0.001
- Majjavaha	35	4.11	2.40	41.67	0.99	0.17	10.27	<0.001
- Purīṣavaha	29	3.24	1.03	68.08	1.23	0.23	9.62	<0.001

BT - before treatment, AT - after treatment, SD - standard deviation, SE - standard error

and clear the obstruction of manovahasrotas and by memory enhancing property, it improves smṛti. Cow's ghee has yogavāhi property and is medhya. The ingredients of Vacādi ghṛta have śirovirecana property which remove vitiated kapha doṣa from śirosthāna and thus increase absorption of nutrients at tissue level.

The assessment based on Hamilton Anxiety Rating Scales (HARS) on various parameters like fear, anxiety, depressed mood, etc. showed statistically highly significant improvement (Table 4). The drugs of the formulation have antioxidant, memory enhancing and rasāyana properties which may increase acetylcholine and acetylcholine esterase in brain and thus help in synaptic transmission of impulse through neurons to enhance the mental faculties. The assessment based on Hamilton Depression Rating Scale (HDRS) indicated that improvement was found in depressed mood, insomnia late, anxiety somatic and hypochondriasis (Table 4). The medhya and rasāyana properties of the drugs may have tranquilizing effect and could exert soothing effect on abnormal thoughts by scavenging free radicals and thus, effect in relieving depression.

Improvement was noticed in somatic concern, anxiety, emotional withdrawal, tension and depressed mood on Brief Psychiatry Rating Scale (BPRS)**,** which was highly significant (Table 5) statistically. The above symptoms may occur due to vāta and kapha vṛdhi. Hence, due to uṣṇa-vīrya and kaphaśāmaka properties of the drugs in Āmalakyādi ghṛta in conjunction with vātaśāmaka property of Vacādi ghṛta, the above symptoms may have improved. Vacā, kuṣṭha and pippalī have antidepressant properties. Based on these parameters, mild improvement was

TABLE 3
Effect of Āmalakyādi ghṛta orally along with Vacadi ghṛta nasya on disturbed mānasikabhāvas

Mānasikabhāvas (emotions)	Mean score			% relief	SD	SE	't'	p
	BT	AT	D					
Bhaya	1.314	0.714	0.6	45.65	0.603	0.102	5.87	<0.001
Krodha	1.342	0.842	0.5	37.25	0.506	0.082	6.08	<0.001
Śoka	1.111	0.814	0.2962	26.66	0.465	0.0895	3.30	<0.05
Manasa	1	0.818	0.181	18.18	0.404	0.121	1.49	>0.05
Dhairya	1.4	0.975	0.425	30.35	0.500	0.079	5.36	<0.001
Harṣa	1.048	0.853	0.195	18.60	0.4012	0.062	3.11	<0.05
Prīti	1	0.853	0.146	14.63	0.357	0.055	2.61	<0.05
Vīrya	1.028	1	0.028	2.77	0.169	0.028	1	>0.05
Śraddha	1	0.952	0.047	4.76	0.218	0.047	1	>0.05
Medha	1.761	1	0.761	43.24	0.4310	0.066	11.45	<0.001
Avasthān	1.04	1	0.04	3.84	0.2	0.04	1	>0.05
Upādhi	1.166	1	0.166	14.28	0.377	0.058	2.86	<0.05
Smṛti	1.690	1	0.6904	40.84	0.467	0.072	9.56	<0.001
Vijñāna	1.406	1	0.406	28.88	0.4989	0.088	4.60	<0.001
Sanjnya	1.048	1	0.048	4.65	0.2180	0.034	1.43	>0.05

TABLE 4
Effect of the treatment on HARS and HDRS*

Description	No.	%	SD	SE	Diff. M	't'	p
1. Symptoms of HARS:							
- Anxious mood	42	47.50	0.3	0.04	0.904	19.73	<0.001
- Tension	42	44.73	0.4	0.06	0.809	13.20	<0.001
- Fears	35	40.22	0.50	0.08	0.54	6.35	<0.001
- Insomnia	21	51.21	0.44	0.09	1	10.24	<0.001
- Difficulty in concentration and memory	41	20.37	0.44	0.06	0.26	3.81	<0.001
- Depressed mood	40	46.73	0.30	0.05	0.9	18.73	<0.001
- General Somatic (muscular)	28	6.89	0.26	0.05	0.071	1.44	>0.05
- Somatic (sensory)	13	47.05	0.50	0.14	0.61	4.38	<0.001
- Cardiovascular symptoms	1	0	-	-	0	-	-
- Respiratory symptoms	11	40	0.52	0.15	0.54	3.46	<0.05
- Gastrointestinal symptoms	25	45	0.46	0.09	0.72	7.85	<0.001
- Genitourinary symptoms	42	0	-	-	0	-	-
- Autonomic symptoms	5	28.57	0.54	0.24	0.4	1.63	>0.05
- Behavior at interview	34	48.07	0.45	0.07	0.73	9.57	<0.001
2. Symptoms of HDRS:							
- Depressed mood	40	44.44	0.40	0.06	0.8	12.49	<0.001
- Guilt	2	33.33	0.70	0.5	0.5	1	>0.05
- Suicidal attitude	6	33.33	0.83	0.34	0.5	1.46	>0.05
- Insomnia initial	19	22.72	0.45	0.10	0.26	2.53	>0.05
- Insomnia middle	2	0	0	0	0	-	-
- Insomnia late	14	71.42	0.46	0.12	0.71	5.70	<.001
- Work and activities	39	7.31	0.26	0.04	0.07	1.77	>0.05
- Retardation	11	8.33	0.30	0.09	0.09	1	>0.05
- Agitation	1	100	-	-	1	-	-
- Anxiety (psyche)	31	13.88	0.45	0.08	0.16	1.97	>0.05
- Anxiety (somatic)	11	63.63	0.50	0.15	0.63	4.18	<0.05
- Somatic general	25	24	0.43	0.08	0.24	2.75	<0.05
- Somatic (GIT)	24	16	0.38	0.08	0.17	2.14	<0.05
- Genital	1	0	-	-	0	-	-
- Hypochondriasis	40	41.79	0.46	0.07	0.7	9.53	<0.001
- Insight	8	0	0	0	0	-	-
- Loss of weight	0	0	-	-	-	-	-
- Diurnal variation	1	0	-	-	0	-	-
- Depersonalization	1	0	-	-	0	-	-
- Paranoid symptoms	2	40	1.41	1	1	1	>0.05
- Obsessional and compulsive symptoms	1	50	-	-	1	-	-
- Helplessness	16	5.55	0.25	0.06	0.06	1	>0.05
- Hopelessness	24	11.11	0.33	0.07	0.12	1.81	>0.05
- Worthlessness	5	0	0	0	0	-	-

*HARS - Hamilton Anxiety Rating Scale; HDRS - Hamilton Depression Rating Scale

observed in 57.14% patients and moderate improvement in 9.52% patients. Results also showed 0.644% increase in body weight, 0.245% decrease in ponderal index and 0.423% increase in body surface area which is statistically insignificant.

On Mini Mental State Examination (MMSE), 6% increase was found, which was statistically highly significant ($p<0.001$). All the drugs have memory enhancing, antioxidant and rasayana properties. So the improvement may be due to their antioxidant and rasayana effects on the brain. On Bender Gestalt Motor Visual test, 82.81% increase in scores was found which is highly significant statistically ($p<0.001$). On hematological and biochemical parameters, this therapy showed improvement in hemoglobin (0.37%), total leucocyte count (1.38%), which were statistically insignificant ($p>0.05$). Improvement in neutrophil count (5.03%) is statistically significant. 9.29% decrease and 7.53% increase were found in lymphocyte and eosinophil respectively which were statistically insignificant ($p>0.05$). 8.15% decrease was found in monocyte which was statistically insignificant ($p>0.05$). 7.308% decrease was found in ESR which was statistically insignificant ($p>0.05$). On biochemical parameters, 5.529% increase was found on fasting blood sugar and 0.569% increase in Serum Cholesterol which were statistically insignificant. Decrease in Serum Creatinine (3.95%) and increase in Serum Urea (0.28%) were found to be statistically insignificant ($p>0.05$) whereas Serum Triglyceride was decreased (11.04%) which is highly significant statistically

TABLE 5
Effect of the treatment on Brief Psychiatry Rating Scale (BPRS)

Symptoms of BPRS	No.	%	SD	SE	Diff. M	't'	p
Somatic concern	42	48.14	0.26	0.04	0.93	23.08	<0.001
Anxiety	42	47.50	0.297	0.045	0.90	19.73	<0.001
Emotional withdrawal	7	66.66	0.38	0.14	0.86	6	<0.001
Conceptual disorganization	26	32.25	0.496	0.097	0.38	3.95	<0.001
Disorientation	27	25.80	0.46	0.089	0.296	3.308	<0.05
Guilty feelings	2	33.33	0.707	0.5	0.5	1	>0.05
Tension	42	43.75	0.58	0.08	0.83	9.29	<0.001
Mannerism and posturing	4	25	0.5	0.25	0.25	1	>0.05
Suspiciousness	4	33.33	0.58	0.29	0.5	1.73	>0.05
Grandiosity	2	33.33	0.707	0.5	0.5	1	>0.05
Hostility	0	0	-	-	-	-	-
Hallucinatory behavior	1	0	-	-	0	-	-
Motor retardation	9	0	0	0	0	-	-
Uncooperativeness	1	0	-	-	0	-	-
Depressed mood	40	46.66	0.33	0.05	0.876	16.52	<0.001
Unusual thought content	32	21.05	0.44	0.08	0.25	3.21	<0.05
Blunted affect	32	18.42	0.42	0.074	0.218	2.946	<0.05
Excitement	25	31.03	0.489	0.097	0.36	3.674	<0.05

($p<0.001$). Mild increase was found in hematological and biochemical parameters after treatment but within the normal range. This change seems to be due to fluctuations which are observed normally but they are not statistically significant.

3.05% decrease in serum cholinesterase was found. But it is statistically insignificant. Serum Cholinesterase level is below normal in Senile Dementia. Recent studies show that in Alzheimer's disease[4], the ratio of Butyryl-cholinesterase to Acetylcholinesterase changes dramatically in cortical regions from 0.2 to as much as 11 and it is found that Acetyl-cholinesterase[5] is lost up to 85% in specific brain regions, whereas Butyrylcholinesterase[6] levels rise with disease progression. Hence, in total Acetylcholinesterase is decreased in Alzheimer's disease. The memory enhancing and antioxidant drugs of the formulation may enhance acetylcholine as well as serum cholinesterase concentrations in brain and may help in synaptic transmission of impulse through neurons and retard disease process. Therapy showed 1.807% decrease in pus cells in urine examinations ($p>0.05$) and 30.77% decrease in cyst in stool examination ($p>0.05$).

The Āmalakyādi ghṛta with Vacādi ghṛta showed overall mild improvement in 92.85% patients and moderate improvement in 2.38% patients. It may be due to the memory enhancing, rasāyana and antioxidant property of the drugs of Āmalakyādi ghṛta. Vacā, kuṣṭha and pippalī have antidepressant property. Vacā and goghṛta (cow's ghee) have medhya property which can directly act through hypothalamo-pituitary-adrenal axis to improve memory and other cognitive impairment such as attention, concentration, judgment, intellect, etc. Moreover, Vacādi ghṛta can clear the srotas of śirosthāna and can eliminate the morbid doṣas from siropradeśa and can help Āmalakyādi ghṛta to act directly through the above axis to achieve the needed objective.

Conclusion

Mind and body are interlinked. Depression, anxiety, negative thoughts and abstinence from sadvṛtta and svasthavṛtta disturb one's psychological health and play an important role in dementia in old age by vitiating mānasika doṣa; rajas and tamas, prāṇa, udāna, vyāna vāyus; rasavaha, majjāvaha and manovaha srotas and ojas. Patients, who indulge in defective life style and resort to undisciplined dietetic pattern (vātakaphaprokopa āhāra vihāra) are constantly exposed to factors that disturb mental faculties (manovighātakara bhāva) like bhaya, cinta, śoka, dveṣa, krodha and moha. Dementia is a progressive degenerative disease. In this context, rasāyana drugs may undoubtedly be efficacious, safe and cost effective in the management of senile dementia.

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ELEMENTAL ANALYSIS OF SOME INDIAN MEDICINAL PLANTS BY PARTICLE INDUCED X-RAY EMISSION (PIXE) TECHNIQUE

Rajeshwari. B. M[1], B. R. Kerur[1] and. T.R. Rautary[2].

Abstract: The trace element analysis was carried out in some medicinal plants viz. *Ocimum tenuiiflorum, Catharanthus roseus, Trigonella foenum-graecum, Azadirachta indica, Aegle marmelos, Zingiber officinale, Emblica officinalis, Anacardium occidentale, Momordica charantia* and *Syzygium cumini* using Particle Induced X-ray Emission (PIXE) technique. A 2 MeV proton beam was employed to excite the samples. The experiments were carried out using 3MV pelletron accelerator facility at IOP, Bhubaneswar. The elements K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Sr and Pb were identified to be prominently present in all the above medicinal plants.

Introduction

The therapeutic effect of a medicinal plant is based on the chemical compounds in it. Some medicinal plants are well known for their potential medicinal values and are also used in various formulations. Such plants were taken for elemental analysis using Particle Induced X-ray Emission (PIXE). The major components are organic compounds, some of which have biological activity, but none act independently and they cannot replace the functions of the medicinal plants as a whole.

Herbal drugs are being used as remedies for various diseases across the world from ancient time[1]. In recent years increasing interest has been focused on phytomedicines or āyurvedic medicines as safer and more congenial to the human body. Medicinal plants are used for preparation of various drugs single or in combinations[2]. Chemical constituents of medicinal plants exhibit biological activity. It has been reported that trace elements play a pivotal role in formation of the active constituents in medicinal plants[3]. However, most studies on such medicinal plants pertain to constituents such as essential oils, vitamins, glycosides and other organic components, while little has been reported about the elemental composition of the plants[4].

A literature survey revealed a significant

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modulatory role of trace elements in various diseases[5]. It has been documented that alteration of trace elemental homeostasis in an organism has direct correlation with different pathological conditions[6]. Thus, screening of the actual bioactive elements of plant origin and assessment of elemental composition of the widely used medicinal plants is essential[7]. In this perspective probing into the specific biological significance of trace elemental composition of plants is most crucial for developing new strategies of drug design based on natural resources.

The present investigation is an attempt to trace elemental composition of some commonly and widely used plants of the North Karnataka region of India. For this study we employed Proton Induced X-ray Emission (PIXE) a fast, nondestructive, multi-elemental analysis technique[8]. PIXE is a powerful technique to estimate trace elemental concretions. The main advantages of PIXE technique are 1) good detection limits. 2) multi elemental capacity and 3) small sample quantities required for analysis.

Materials and methods

Medicinal plants were collected from different areas of North Karnataka and identified with the help of experts. The medicinal plants were cleaned with distilled water and dried. The dried plants were powdered in an agate mortar. A known quantity of this powder is taken and mixed with a known quantity of high pure graphite powder to monitor beam current. All the targets were in the form of pellets made by mixing the powder with graphite in the ratio 1:1 and pressing by a hydraulic press under the pressure of 10 ton. These mixtures were compressed under a pressure of 10 ton, into pellets of 13 mm diameter and about 2mm thickness. The targets were fixed on a frame one by one outside the chamber for the irradiation. To assure that the targets were 45^0 to the beam, the targets were adjusted until a laser beam reflected from the target, was seen on the detector window.

Experimental details

The PIXE experiments were carried out at the Institute of physics, Bhubaneswar with a 3MV pelletron accelerator facility available there. The samples were mounted on the target ladder and were kept in the scattering chamber. The samples were positioned at an angle of 45^0 to the beam direction. These samples were excited with 2 MeV proton beam. The proton beam was collimated to a diameter of 4mm and made to fall onto the target. The beam current was kept at about 20nA. The position of the proton beam on the sample was adjusted properly by viewing through a window provided to the scattering chamber. The x-ray spectrum was recorded with a high resolution Si (Li) detector. The resolution of the detector is 160 eV at 5.9 KeV energy. The detector was kept at an angle of 45^0 to the target position and at an angle of 90^0 to the proton beam direction. Each sample was irradiated for about 1 hour. For each sample, the total charge collected and the average beam current were noted. The X-ray spectra thus recorded with some medicinal plants are shown in Fig, I&II.

Data analysis

The PIXE spectra were analyzed using the GUPIX 2000 software package. GUPIX is a versatile software package for fitting PIXE spectra for thin, thick, intermediate and layered specimens. The pecks are described by Gaussian or voigtian with low energy tailing. Escape peaks are included. The background is removed by top-hat filtering and no model is needed. This

package has provision to convert the X-ray peak intensities into elemental concentrations using a standardisation technique involving fundamental parameters and predetermined instrumental contents. Spectrum fitting provides tables of peak areas, two error recipes, detection limits, etc. The spectra corresponding to different medicinal plants were analyzed using GUPIX 2000 software package. The elements were identified and their relative concentrations were estimated. The low Z elements were identified according to their K X-ray energies while the high Z elements were identified according to their L X-ray energies. Internal standard "graphite" with known concentration was added to the experimental sample. Using GUPIX software program, for the given input parameters such as solid angle and charge collected, the concentration of the graphite powder was reproduced within 5%. This confirms the reliability of the input parameters in estimating the concentration of different elements from their respective X-ray intensities. The elements were identified in different medicinal plants and their concentrations of different elements thus obtained in different medicinal plants are given in Table 1. For some of the elements, the $K_{\hat{a}}$ X-ray component of low Z element overlaps with the $K_{\acute{a}}$ X-ray component of high Z element. In GUPIX software package, there is a provision to correct for this overlap thus giving the correct intensities of the elements.

Results and discussion

The trace element analysis was carried out in the medicinal plants *Ocimum tenuiflorum, Catharanthus roseus, Trigonella foenum-graecum, Azadirachta indica, Aegle marmelos, Zingiber officinale, Emblica officinalis, Anacardium occidentale, Momordica charantia*

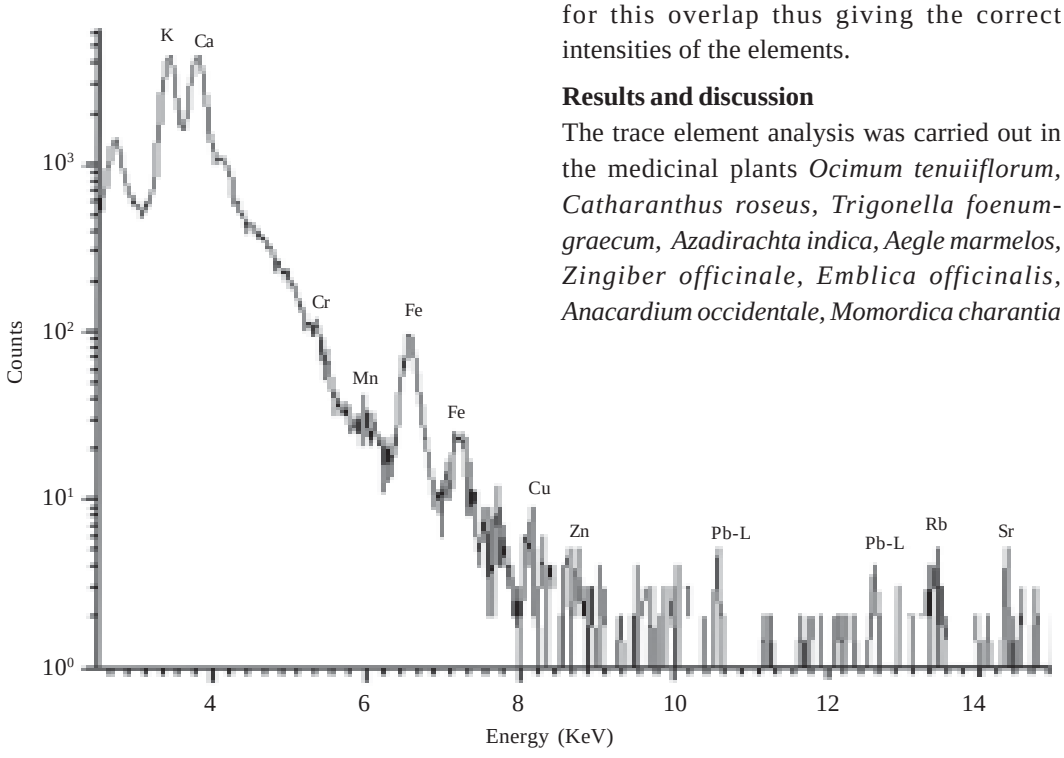

Fig. I. *Ocimum tenuiflorum*

and *Syzygium cumini.* The elements K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Sr and Pb appear prominently in all the above medicinal plants. The variation of the concentrations of different elements in different medicinal plants is shown as bar diagrams (Fig. I&II).

Analysis of the present investigation documents a wide range of variation in the elemental constitution of different plant parts taken as study samples. From the observed data, some of the traditional use of plant parts can well be correlated with their elemental composition. Concentrations of Cr and Sr were observed to be in the range of 3.2 to 15.5 ppm in the different plant parts (Table 1). The relatively high concentration of Cr *Momordica charantia* and *Aegle marmelos* may account for their traditional use in the treatment of anemia[9] since Cr is an effective intrinsic factor which has indirect modulory role in diabetes[10]. From the analysis of data it was found that in a particular part of a specific plant, in all the samples chosen, the concentration of Copper was much higher than that of Cr or Sr in the same plant parts and these were also found to contain remarkable concentrations of Fe (Table1). In short, the samples under study rafted a high level of Cu in the presence of still higher Fe concentration with lesser levels of Cr and Sr. This data is similar to the observation of Nielsen *et al*.,[11] where it was documented that in an environment with significant level of Fe, Rb and Cu might exhibit antagonistic interactions. This actually reflects differential interaction between essential trace and ultra trace elements in a particular biological system or environment. The traditional use of *Catharanthus roseus* leaf, *Trigonella foenum-graecum* seed and neem leaf in the treatment of diabetes can be well correlated with significant Zinc concentration (>100ppm) in each of these

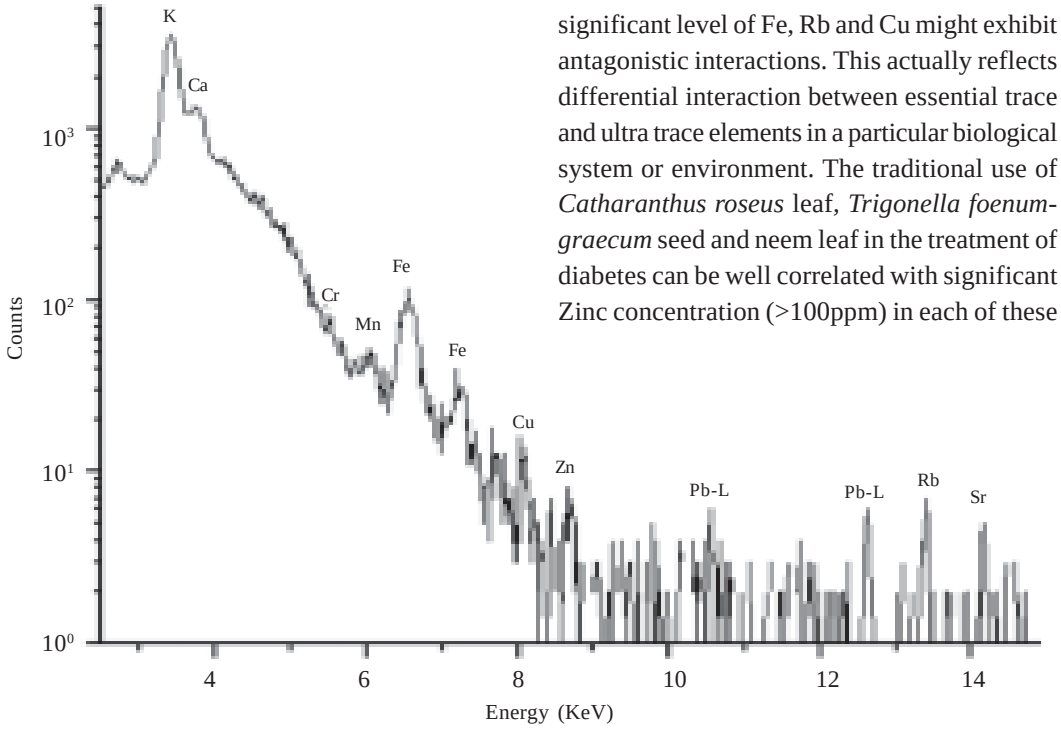

Fig. II. *Syzygium cumini*

TABLE 1
Concentrations of different elements obtained in different medicinal plants

Sample name	Coding	K (%)	Ca (%)	Cr	Mn	Fe	Cu	Zn	Rb	Sr	Pb
Ocimum tenuiiflorum	G01	0.61	1.83	31.6	14.2	93.8	15.6	57.9	6.4	13.7	2.1
	S01	0.59	1.74	23.2	10.8	75.6	11.8	61.2	6.8	12.9	1.8
	K01	0.64	1.77	35.6	16.1	108.1	18.5	65.7	5.4	10.7	1.9
Catharanthus roseus	G02	0.54	1.04	3.7	19.6	96.4	5.7	21.4	4.9	12.4	1.9
	S02	0.43	0.93	4.5	15.8	83.2	5.4	16.7	5.3	11.1	2.1
	K02	0.49	0.91	4.1	12.7	75.4	5.4	12.8	4.2	10.8	1.9
Trigonella foenum-graecum	G03	3.91	4.92	9.6	53.7	154.1	26.5	29.5	14.2	19.4	8.4
	S03	3.75	4.78	9.8	39.7	134.6	19.4	21.4	15.4	18.7	8.5
	K03	3.79	4.85	9.9	47.1	149.1	18.4	25.2	13.7	17.6	8.1
Azadirachta indica	G04	0.29	1.31	3.1	5.4	50.7	4.3	14.6	2.7	4.8	1.6
	S04	0.27	1.28	3.0	5.8	47.8	5.2	12.1	3.3	5.2	2.1
	K04	0.32	1.31	3.4	5.1	56.4	4.8	16.3	2.8	5.3	2.2
Aegle marmelos	G05	0.84	0.96	2.7	30.7	85.4	9.3	5.1	9.2	18.4	4.7
	S05	0.76	0.89	3.2	28.4	73.6	10.7	5.3	8.4	16.5	4.2
	K05	0.81	1.01	3.4	25.8	78.3	12.1	5.9	7.4	15.5	4.9
Zingiber Officinale	G06	1.37	0.49	8.8	76.4	389.6	7.6	32.9	6.7	7.2	3.2
	S06	1.29	0.42	8.1	66.8	371.1	7.9	35.6	5.4	7.3	2.8
	K06	1.33	0.45	7.6	72.5	372.5	7.6	25.4	5.1	7.6	2.5
Emblica officinalis	G07	0.51	0.78	6.3	37.2	82.4	14.5	24.6	14.1	8.4	6.4
	S07	0.47	0.65	6.1	30.8	69.1	10.6	19.3	12.7	7.1	5.2
	K07	0.46	0.70	6	41.5	68.4	10.8	16.7	10.5	5.9	4.5
Anacardium occidentale	G08	0.39	0.86	7.3	32.9	325.1	16.4	19.6	3.5	4.1	2.1
	S08	0.42	0.71	6.7	28.1	291.5	12.7	15.5	3.2	4.3	2.2
	K08	0.48	0.78	5.1	45.8	302.4	15.1	12.7	3.2	4.9	2.1
Momordica charantia	G09	0.72	0.53	26.8	25.4	745.9	16.1	26.7	6.8	10.4	7.5
	S09	0.67	0.51	23.5	19.4	734.6	12.5	18.4	6.2	8.9	6.4
	K09	0.71	0.51	21.1	19.1	720.4	11.6	14.8	6.5	9.3	6.7
Syzyguimi cumini	G10	0.34	0.72	7.9	20.1	271.6	8.4	12.8	2.8	5.7	3.4
	S10	0.26	0.61	5.4	14.6	260.8	8.6	10.4	3.1	3.4	2.6
	K10	0.26	0.61	5.4	14.6	260.8	8.6	10.4	3.1	3.4	2.6

plant parts since Zn is known to have beneficial effects on vision[12]. It may be interesting to note that in the present study elemental analysis of these samples highlighted a significant correlation of Zn and Pb. Out of 10 samples, Pb was detected only in 8 samples all of which were found to contain a high concentration of Zn (viz. *Zingiber officinale* rhizome, *Syzygium cumini* seed and *Emblica officinalis* fruit). The average Zn concentrations in the Pb-containing samples were found to be more than twofold higher than that of the samples where Pb was not detected. This might have same implications on the potential ameliorating role of Zn an Pb metabolis[13]. The species' specific compositional variation as observed in cases of the concentration and distribution patterns of Mn and Fe, in plants like neem and *E. officinalis* (Table 1) may reflect a definite correlation between the functional property of the element and physiology of the specific plants and its parts. This view gets support in the present finding with regard to Rb and Ca also. Fe level of K was found to be as low as 0.26 ppm in *Ocimum tenuiiflorum* leaf while it exhibited a very high concentration of 389 ppm in *Zingiber officinale* (Table1). Use of the decoction of *Zingiber officinale* as traditional remedies for patients suffering from oladdes stones 2 might probably be correlated with the high concentration of Fe in *Zingiber officinale* and its potential to replace Ca, thereby helping Ca in *Emblica* fruit; *Anacardium occidentale* and neem leaf may be attributed to the use of these plant parts in the treatment of leprosy[14].

Conclusion

The trace element analysis was carried out in the medicinal plants commonly used in India using PIXE technique. The elements K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Sr and Pb appear prominently in the spectra of the above medicinal plants. The elements K and Ca are present in very small quantities. A total of 10 elements have been determined in 30 medicinal plants commonly used in India by using PIXE. The data of this study documents variations in elemental composition and concentration, thereby reflecting difference in physiological functioning of the specific plant depending upon the elemental interaction within it. This preliminary study having baseline information about mineral constituents of medicinal plants in North Karnataka, will be helpful in developing an approach towards direct link between elemental content and its curative probability, having coherence with traditional use.

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THE REDISCOVERY OF AYURVEDA
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IMPORTANCE OF GUGGULUKALPANA IN ĀYURVEDIC THERAPEUTICS

Ramesh Kumar Gupta[1], Sumer Singh[2], Akhilesh Kumar Singh[3]

Abstract: Guggulukalpana is well known for its vāta and medohara properties. Apart from an important therapeutic agent, guggulu (*Commiphora wightii*) is used as a fumigation material in yāgakarma and as a rakṣoghna drug to protect unwanted forces i.e. grahabādha, etc. Various herbo-mineral formulations containing guggulu have been indicated in diseases like dhamani praticaya (hyperlipidaemia), hṛdroga (heart disease), sandhivāta (osteoarthritis), āmavata (rheumatoid arthritis), medoroga (obesity), vātavyādhi (mainly nervous system disorders), etc. By virtue of its lekhana property guggulu formulations have become one of the most popular medicines for the treatment of atisthaulya (overweight/obesity) which is the most hazardous pathological condition of the human body.

Introduction

Carakasamhita is one of the richest source of knowledge about the use of herbal drugs indicated for many incurable diseases. Ācārya Caraka has enumerated four qualities for a drug viz. applicability, richness of quality, abundance and high potent therapeutic agent. Cūrṇa, kvātha, vaṭi, sneha, guggulu, āsava-ariṣṭa and avaleha kalpana are the main āyurvedic dosage forms predominantly used. These kalpanas are chiefly herbal drug formulations prepared by different āyurvedic pharmaceutical procedures.

Palaṅkaṣa, mahiṣākṣa, kauśika, pura, kumbha and marudeśya are the synonyms of guggulu. It is of two types i.e. mahiṣākṣa and kanaka. Guggulu is amorphous, translucent, solid adhesive, oleo-gum-resinous oily latex (niryāsa) of a small tree *Commiphora wightii* which is produced by the process of gummosis from its plant. In āyurveda, guggulu is used either alone or with combination of other herbal, animal or mineral drugs in so many āyurvedic dosage forms i.e. vaṭi, rasa yogas, rasakriya, lepa, dhūpa, etc. for various treatments like obesity, rheumatoid arthritis, osteoarthritis, gouty arthritis, heart diseases etc. (Table 1) Here an attempt is made to compile some important guggulu preparations which are very much popular due to their therapeutic effectiveness.

Purification procedure

Crude guggulu is collected and broken into small pieces. It is bundled in a piece of cloth and immersed into one of the following liquids i.e. triphala kvātha, vaśā patra kvātha, vaśā patra svarasa, godugdha, gomūtra or nirguṇḍī patra svarasa with haridrā cūrṇa, and boiled. Boiling is done in constant slow heat until it is mashed so that it passes through the cloth. Thus the

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TABLE 1

Guggulukalpanas and their therapeutic uses

	Name	Main ingredients:	Indications	Reference
1.	Abhayādi guggulu	Śudha guggulu (purified *Commiphora wightii*), harītaki (*Terminalia chebula*), āmalaki (*Emblica officinalis*), drākṣa (*Vitis vinifera*), miśreya (*Foeniculum vulgare*), bhṛṅgarāja (*Eclipta alba*), śveta and kṛṣṇa śāriba (white and black variety of *Hemidesmus indicus*), mañjiṣta (*Rubia cordifolia*), haridra (*Curcuma longa*), dāruharidra (*Barberis aristata*), vaca (*Acorus calamus*), musali (*Asparagus adscendens*), mulethi (*Glycyrrhiza glabra*), trivṛta (*Operculina turpethum*), muramānsi (*Nordostachys jatamansi*), caturjātaka (*Elettaria cardamomum*, bark of *Cinnamomum zeylanicum, C. tamala, Mesua ferrea*), trikaṭu (*Zingiber officinale, Piper nigrum, Piper longum*), viḍaṅga (*Embelia ribes*) and durālabha (*Fagonia cretica*).	Snāyu and mastiṣka vikara (disorders of tendon and brain)	BR 101/18-22
2.	Ābhā guggulu	Śudha guggulu, babul tvak (*Acacia nilotica* ssp. *indica*), triphala (*Emblica officinalis, Terminalia chebula, Terminalia bellirica*) and trikaṭu.	Sandhi bhaṅga (joint dislocation)	BR 49/15
3.	Amṛta guggulu	Śudha guggulu, guḍūci (*Tinospora cordifolia*), pāṭha (*Cissampelos pareira*), mūrva (*Marsdenia tenacissima*), bala (*Sida alnifolia*), kuṭaki (*Picrorhiza kurroa*), dāruharidra, triphala, guḍūcisatva (extract of *Tinospora cordifolia*), śuṇṭhi (*Zingiber officinale*), pippali (*Piper longum*).	Kuṣṭha (leprosy), vātarakta (gouty arthritis), āmavāta (rheumatoid arthritis), kāmala (jaundice), pratiśyāya (rhinitis).	BP 29/177-182
4.	Amṛtādya guggulu	Śudha guggulu, guḍūci, ela (*Elettaria cardamomum*), viḍaṅga (*Embelia ribes*), vatsaka (*Holorrhena pubescens*), harītaki and āmalaki.	Medoroga (obesity), and prameha-pīḍika (carbuncle)	CD 222/17
5.	Ādityapāka guggulu	Śudha guggulu, triphala, pippali and dālcini (*Cinnamomum verum*).	Sandhi, asthi and majjagatavāta	CD 139/66-67
6.	Ekavimśati guggulu	Śudha guggulu, citraka (*Plumbago zeylanica*), triphala, trikaṭu, śveta and kṛṣṇa jīraka (white and black variety of *Cuminum cyminum*), vaca, saindhava lavaṇa (rock salt), ativiṣa (*Acotinum heterophyllum*), kuṣṭha (*Saussurea lappa*), cavya (*Piper retrofractum*), ela, yavāsa (*Alhagi camelorum*), viḍaṇga, ajamoda (*Carum roxburghianum*), nāgarmotha (*Cyperus rotundus*), devadāru (*Cedrus deodara*).	Kuṣṭha (leprosy), krimi (worm infestation), duṣṭavraṇa (infected wound), grahaṇi (sprue), arśa (piles), gṛdhrasi (sciatica) and bhaṅga (fracture).	BP 54/67-72

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	Name	Main ingredients:	Indications	Reference
7.	Kāñcanāra guggulu	Śudha guggulu, kāñcanara tvak (*Bauhinia variegata*), trikaṭu, triphala, varuṇa tvak (*Crataeva nurvala*), trijātaka, (*Elettaria cardamomum*, bark of *Cinnamomum verum* and leaves of *C. tamala*).	Gaḷagaṇḍa (cervical lymphodenitis), apaci (scrofula), arbuda (tumour) and granthiroga (cysts).	BR 44/64-69
8.	Kaiśora guggulu	Śudha guggulu, triphala, guḍūci, trikaṭu, viḍaṅga, danti (*Baliospermum montanum*) and trivṛta mula.	Vātarakta, vraṇa (abscess), kuṣṭha and prameha piḍika.	BR 27/98-108
9.	Triphalā guggulu	Śudha guggulu, triphala and pippali	Bhagandara (anal fissure), gulma (abdominal distention), arśa and śotha (edema).	SSM 7/82-83
10.	Trayodaśāṅga guggulu	Śudha guggulu, babul tvak, aśvagandha (*Withania somnifera*), hauber (*Juniperus communis*), guḍūcisatva, śatāvari (*Asparagus racemosus*), gokṣura (*Tribulus terrestris*), rāsna (*Pluchea lanceolata*), śudha vidhara bīja (purified seed of *Argyreia speciosa*), miśreya (*Foenieculum vulgare*), kaccūra (*Curcuma zedoaria*), ajmoda and śuṇṭhi.	Gṛdhrasi (sciatica), kaṭīgraha (plumago), hanugraha (lock jaw), sandhi, asthi, majja, snāyu and koṣṭha gata vāta.	BR 26/98-101
11.	Navaka guggulu	Śudha guggulu, trikaṭu, triphala, trivaṅga (calcined nāga, vaṅga and yaśada)	Medoroga, āmavāta and kaphavikāra	BR 39/43
12.	Navakaṣāya guggulu	Śudha guggulu, guḍūci, vasa (*Adhatoda vasica*), paṭolapatra (leaves of *Trichosanthes dioica*), nimbatvak (bark of *Azadirachta indica*), triphala, khadirasāra (*Acacia catachu*), amḷavetasa (*Garcinia pedunculata*).	Visarpa (erysipelas) and kuṣṭha	CD 302/19
13.	Navakārṣika guggulu	Śudha guggulu, triphala and pippali.	Śotha, gulma (abdominal distention), arśa and bhagandara (anal fissure)	BR 51/28
14.	Pañcāmṛtaloha guggulu	Śudha guggulu, kajjali (black sulphide of mercury), abhraka, rajata, lauha and svarṇamākṣika bhasma.	Mastiṣka and snāyu gata roga (diseases of tendon and brain), all vātavyādhis (nervous system disorders i.e. hemiplagia, paralysis, etc.)	BR 101/14-17
15.	Pañcatikta-ghṛta guggulu	Śudha guggulu, nimba tvak, guḍūci, vasa pañcāṅga (whole plant of *Adhatoda vasica*), kaṇṭakārīmūla (*Solanum surattense*), pāṭha (*Cissampelos pareira*), viḍaṅga. devdāru, gajapippali (*Piper chaba*), svarjika and yavakṣara (sodium and potasium corbonate), śuṇṭhi,	Kuṣṭha, vātarakta, arśa, arbuda, nāḍīvraṇa (sinus), duṣṭavraṇa, gudaroga (ano-rectal disease), gulma and vidradhi.	BR 54/233-236

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Name	Main ingredients:	Indications	Reference
	haridra, miśreya, cavya, kuṣṭha, jīra (*Cuminum cyminum*), tejovati (*Zanthoxylun armatum*), marica (*Piper nigrum*), kuṭaja (*Holarrhena pubescens*), citraka, kuṭaki, sudha bhallātaka (purified *Semicarpus anacardium*), vacā, pippalīmūla, mañjiṣṭhā (*Rubia cordifolia*), ativiṣa (*Aconitum heterophyllum*), triphala, ajamoda.		
16. Pathyādi guggulu	Śudha guggulu, triphala, viḍaṅga, danti (*Baliospermum montanum*), guḍūci, pippali, trivṛt, śuṇṭhi, marica.	Gṛdhrasi, nav khanjata (acute mono plegia cruralis)	BP 24/145-150
17. Punarnavā guggulu	Śudha guggulu, punarnava (*Boerhavia diffusa*), eraṇḍamūla and taila (root and oil of *Ricinus communis*), śuṇṭhi, trivṛt (*Operculina turpethum*), danti, guḍūci, triphala, trikaṭu, citraka, saindhavalavaṇa, viḍaṅga, śudha bhallātaka, punarnavamūla and svarṇamākṣika bhasma (calcined copper pyrite)	Vātarakta, vṛdhi (hydrocoel), gṛdhrasi and āmavāta.	BR 27/109-113
18. Punarnavādi guggulu	Śudha guggulu, punarnava, devadāru, harītaki and guḍūcī	Tvakroga (skin disorders), śotha, udararoga (abdominal disorders) and pāṇḍuroga (anemia).	BR 42/135
19. Rasa guggulu	Śudha guggulu, hiṅguḷotha pārada (mercury extracted from cinnabar)	Kuṣṭha and vraṇa	BR 52/55-63
20. Rasābhra guggulu	Śudha guggulu, kajjali, lauha and abhraka bhasma (calcined iron and mica), trikaṭu, triphala, dantīmūla, guḍūcī, kuṭaja, trivṛt (*Operculina turpethum*), viḍaṅga and nāgarmotha (*Cyperus rotundus*).	Vātarakta, kuṣṭha, krimi, gaḷagaṇḍa (inflammation of cervical gland) and apaci (scrofula).	BR 27/91-97
21. Saptavimśatika guggulu	Śudha guggulu, trikaṭu, triphala, nāgarmotha, viḍaṅga, guḍūcī, citraka, kacūra (*Curcuma zedoaria*), ela, hauber (*Juniperus communis*), devadāru, tumbaru (*Zanthoxylun armatam*), puṣkaramūla (*Inula racemosa*), cavya, indrayana (*Cirullus colocynthis*), haridra, dāruharidra, viḍa (black salt), saindhava (rock salt) and sauvarcala lavaṇa (sodium sulphate mixed with sodium chloride), yava and svarjikakṣāra (potasium and sodium carbonate), gajapippali (*Scindapsus officinalis*)	Kāsa, śvāsa, śotha, arśa, bhagandara, hṛdaya, pārśva and udara śūla (angina pectoris, chest and abdominal pain).	CD 271/13-19
22. Svayambhuva guggulu	Śudha guggulu, bakuci (*Cullen corylifolium*), śilājatu (asphaltum), svarṇamākṣika and lauha bhasma, muṇḍi (*Spaeranthus indicus*),	Kuṣṭha (leprosy)	BP 54/64-67

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Name	Main ingredients:	Indications	Reference
	harītakī, āmalakī, karañjapatra (*Pongamia pinnata*), khadirasāra, guḍūcī, trivṛta, jaipāla (*Croton tiglium*), nāgarmotha, viḍaṅga, haridra and kuṭaja.		
23. Lākṣa guggulu	Śudha guggulu, lākṣa (*Lacifer lacca*), asthisamghāra (*Cissus quadrangularis*), arjuna tvak (*Terminalia arjuna*), aśvagandha (*Withania sominifera*), nāgabala (*Sida cordata*).	Asthi and sandhi bhaṅga (bone fracture and joint dislocation)	BR 49/13-14
24. Varādi guggulu	Śudha guggulu, triphala, nimba tvak, aśvatha (*Ficus religiosa*), khadirasāra, asana tvak and vāśā.	Upadamśa (soft chancer)	BR 52/65-66
25. Vātāri guggulu	Śudha guggulu, eraṇḍatailam (oil of *Ricinus communis*), śudha gandhaka and triphala.	Āmavāta, kaṭīśūla (pain in lumber region), śotha, ghṛdhrasi, khañjata (mono plegia cruralis), paṅguta (diplegia) and vātarakta.	BR 29/152-154
26. Viḍaṅgādi guggulu	Śudha guggulu, viḍaṅga, triphala and trikaṭu.	Duṣṭavraṇa, nāḍīvraṇa, apaci and kuṣṭha.	CD 263/69
27. Vraṇāri guggulu	Śudha guggulu, pippalī, triphala, rasasindūra (red sulphide of mercury).	Vraṇa	BR 58/18
28. Vāśā guggulu	Śudha guggulu, vāśā, nimba tvak, paṭolapatra (*Trichosanthes dioica*), asana (*Pterocarpus marsupium*), yavāsa (*Alhagi camelorum*) and triphala.	Amḷapitta (acid peptic disorder)	CD 296/14
29. Śivā guggulu	Śudha guggulu, triphala, eraṇḍatailam, śudha gandhaka, rāsna, viḍaṅga, marica, dantimūla, jaṭamānsi (*Nordostachys jatamansi*), śuṇṭhi and devadāru.	Āmavāta, kaṭīśūla, gṛdhrasi, koṣṭrukaśirṣa (inflammed knee), sandhivāta and vātarakta.	RSS 3/17-20
30. Ṣaḍaṅgakvātha guggulu	Śudha guggulu, triphala, paṭola, nimba and vāśā patra.	Netra roga (eye disorders)	BR 64/45
31. Saptāṅga guggulu	Śudha guggulu, trikaṭu and triphala	Duṣṭavraṇa	BP 49/30-31
32. Simhanāda guggulu	Śudha guggulu, sarṣapa taila (oil of *Brassica compestris*), triphala, trikaṭu, nāgarmotha, viḍaṇga, devadāru, guḍūcī, citraka, dantī, trivṛta, cavya, marica, kajjaḷi, sūraṇa (*Amorphophallus campanulatus*), manakanda (*Alocasia indica*), jaipālabīja.	Āmavāta, śiro, sandhi, janu and jaṅgha gata vāta, kaṭīgraha, aśmari (stone), kāsa, mūtrakṛcchra.	BR 29/181-188

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	Name	Main ingredients:	Indications	Reference
33.	Samaśarkara guggulu	Śudha guggulu, yavakṣāra (Potassium carbonate), devadāru, saindhava, ela, vaca, ajamoda, trikaṭu, yavāni (*Hyoscyamus niger*), śveta and kṛṣṇa jīraka, viḍaṅga and citraka.	Vātarakta, udararoga, yakṣma (tuberculosis), viṣamajvara (infectious fever), gṛdhrasi and vraṇa.	BR 29/170-176
34.	Daśāṅga guggulu	Śudha guggulu, trikaṭu, triphala, nāgarmotha and viḍaṅga.	Sthūlata (obesity), āmavāta, sandhivāta, and pakṣāghāta (paralysis)	BP 39/30
35.	Yogarāja guggulu	Śudha guggulu, citraka, pippalimūla, yavāni, śveta and kṛṣṇa jīraka, viḍaṅga, ajmoda, devadāru, cavya, saindhava lavaṇa, kuṣṭha, rāsna, gokṣura, dhaniya (*Coriandrum sativum*), triphala, trikaṭu, caturjātaka, khas (*Vetiveria zizanioidis*), yavakṣāra and tālīspatra (leaves of *Abies webbiana*).	Āmavāta, āḍhyavāta (gouty arthritis), duṣṭavraṇa, arśa, krimi, plīhāvṛdhi (spleenomegaly), udararoga and anāha (flatulence).	BR 29/156-161
36.	Mahāyogarāja guggulu	Śudha guggulu, trikaṭu, triphala, pāṭha, miśreya, haridra, dāruharidra, yavāni, vaca, hiṅgu (*Ferula assa-foetida*), hapuṣa (*Juniperus communis*), gajapippali, kṛṣṇajīraka, kacūra (*Curcuma zedoaria*), tulasi (*Ocimum tenuiiflorum*), dhaniya, vida (black salt), saindhava and sauvarcala lavaṇa (sodium sulphate mixed with sodium chloride), trijātaka, pippalimūla, rāla (secretion of *Shorea robusta*), rāsna, gokṣura, ativiṣa (*Acotinum heterophyllum*), śuṇṭhi, yavakṣāra, cavya, amḷavetasa, citraka, puṣkaramūla, vṛkṣāmḷa (*Garcinia indica*), dāḍima (*Punica granatum*), eraṇḍa, trivṛta, dantīmūla, aśvagandha, vilvaphala (fruit of *Aegle marmelos*), devadāru, haridrā, kuṭaki, mūrvamūla, trāyamāṇa (*Gentiana kurroo*), durālabha (*Fagonia cretica*), viḍaṅga, vāśāmūla tvak, abhrakabhasma (calcined mica) and vaṅgabhasma (calcined tin).	Kuṣṭha, vātarakta, āmavāta, kāmala (jaundice) and pratisyāya (rhinitis).	BR 29/162-171
37.	Nirguṇḍi guggulu	Śudha guggulu, trikaṭu, triphala, trivaṅga (calcined nāga, vaṅga and yasada)	Āmavāta, sandhivāta, vātarakta and all types of vāta disorders	RSS 3/29-30
38.	Vyādhi śārdūla guggulu	Śudha guggulu, sarṣapa taila, triphala, trikaṭu, nāgarmotha, viḍaṅga, guḍūcī, citrakamūla, trivṛt, dantīmūla, cavya, sūraṇa, mānakanda, jaipālabīja, kajjali, lauha and abhraka bhasma.	Āmavāta, arśa, aśmari, mūtrakṛcchra, kāsa, śvāsa, śḷīpāda (elephantiasis) and jvara (fever).	BR 29/172-179
39.	Gokṣurādi guggulu	Śudha guggulu, gokṣura, trikaṭu, triphala and nāgarmotha	Prameha, mūtrakṛcchra, mūtrāghāta (retention and suppresion of urine), pradara (leucorrhea), vātarakta, aśmari and śukra vikāra.	BR 51/28

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Name	Main ingredients:	Indications	Reference
40. Harītakyādi guggulu	Śudha guggulu, harītaki, śuṇṭhi and vṛdhadāruka (*Argyreia speciosa*)	Āmavāta, adhyamana (flatulence), anāha (gaseous distention in abdomen), sandhivāta, vātaja udara roga (abdominal disorders), vātarakta, and all types of vāta disorders.	NR, Part-2, P 474
41. Varādya guggulu	Śudha guggulu, śatāvari (*Asparagus racemosus*), eraṇḍamūla (root of *Ricinus communis*), śuṇṭhi (*Zingiber officinale*), devdāru (*Cedrus deodara*), kuṣṭha (*Saussurea lappa*), saindhava lavaṇa (rock salt), rāsna (*Pluchea lanceolata*).	Āmavāta, sandhivāta, vātarakta and all types of vāta disorders	NR, Part-2, P 429
42. Dvātrimśaka guggulu	Śudha guggulu, trikaṭu, triphala, nāgarmotha, viḍaṅga, cavya, citraka, vaca, ela, pippalīmūla, hapuṣa, devadāru, tumbaru (*Zanthoxylum armatum*), puṣkarmūla, kuṣṭha, ativiṣa (*Aconitum heterophyllum*), haridra, dāruharidra, jīraka, śuṇṭhi, sauvarcala and saindhava lavaṇa, yavakṣāra, svarjikakṣāra (sodium carbonate) and gajapippali.	Āmavāta, āntravṛdhi (hernia), unmāda (insanity), jīrṇajvara (chronic fever), pārśvaśūla (pain in ribs), gṛdhrasi, hanustambha (lock jaw), pakṣāghāta and apatānaka (tetany).	NR, Part-2, P 427
43. Nimbādi guggulu	Śudha guggulu, nimba tvak (bark of *Azadiracta indica*), triphala (*Emblica officinalis, Terminalia chebula, Terminalia bellirica*), vasa (*Adhatoda vasica*), paṭolapatra (leaves of *Trichosanthes dioica*).	Vāta-śleṣmaja śūla (pain due to vitiation of vāta and kapha), śira:śūla (headache).	NR, Part-2, P 821
44. Trikaṭvādi guggulu	Śudha guggulu (purified *Commiphora wightii*), trikaṭu (*Zingiber officinale, Piper nigrum, Piper longum*), triphala (*Emblica officinalis, Terminalia chebula, Terminalia bellirica*), nāgarmotha (*Cyperus rotundus*), gokṣura (*Tribulus terrestris*).	Adhyamana (flatulence), prameha (diabetes), vātarakta, mūtradoṣa (urinary problem).	NR, Part-2, P 576
45. Lohādi guggulu	Śudha guggulu (purified *Commiphora wightii*), loha bhasma (Calcined iron), mulethi (*Glacyrrhiza glabra*), triphala (*Emblica officinalis, Terminalia chebula, Terminalia bellirica*), pippali (*Piper longum*).	Śuklagata netra roga (conjuctival disorders of eye).	NR, Part-2, P 576

*BR - Bhaiṣajyaratnāvali; RSS - Rasendrasārasaṅgraham; BP - Bhāvaprakāśam; SSM - Śārṅgadharasamhita - Madhya khaṇḍa; CD - Cakradattam; NR - Nighaṇṭuratnākar

obtained liquid is filtered through a cloth. Allow the heavy sand particles to settle. Then the supernatant part of teh fluid is decanted. This fluid is again boiled to evaporate its water content till it becomes a semisolid mass. This mass is further taken in an enamel tray and kept in the sun for drying. This dried guggulu is poured in a stone mortar and ground with ghee till it becomes waxy. The end product is called śodhita guggulu.

Pharmacological properties:- i) rasa - madhura, kaṭu, tikta, kaṣāya; ii) guṇa - laghu, tīkṣṇa, snigdha, pichila, sūkṣma, sara; iii) vīrya - uṣṇa; iv) vipāka - kaṭu; v) doṣaprabhāva - tridoṣahara; vi) karmaprabhāva - medohara, vedanāhara, vraṇaśodhana and vraṇaropaka.

Therapeutic properties: - Sthaulyata (obesity), sandhivāta (osteoarthritis), āmavāta (rheumatoid arthritis), vātarakta (gouty arthritis), snāyu-śotha (tendenitis), mūtrakṛcchra (urinary tract disorders) and vātavyādhi (upper and lower motor neuron disorders). Some important guggulukalpanas and their indications described in various ayurvedic classics are detailed in Table 1.

Conclusion

Guggulu kalpana is a unique dosage form mainly used in the management of sthaulyata (obesity) and vātavyādhi (mainly joint disorders) in āyurvedic therapeutics. Apart from its effectiveness in obesity and joint disorders, different compound formulations of guggulu is effective in kuṣṭha, duṣṭa vraṇa, nāḍīvraṇa, arśa, bhagandara, aśmari, prameha, krimiroga and many other vātaja, pittaja and kaphaja disorders.

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FUNDAMENTALS OF RASAŚĀSTRA AND ITS IMPORTANCE IN THE HERBOMINERAL FORMULATIONS OF ĀYURVEDA

(Part - II)

Neetu Singh and Anand K. Chaudhary*

Abstract: Continued from the previous issue. This part contains descriptions regarding shelf-life, safety and therapeutic profiles of rasa-auṣadies. Mercurial products (pāradīya yoga) are also dealt with.

4. **Validation and shelf life**

Validation literally means to render the process valid after substantiating the known process with a scientific rationality in order to deliver a particular product. A safe and effective quality product is the combination of only two features - i) the defined quality of raw material and ii) accuracy in the process. These two are the most essential elements to deliver a genuine product on every parameter. This may be achieved by preparing a Validation Master Protocol (VMP) for each product. The recent advances made in the processes of purification, isolation and structure elucidation of naturally occurring substances have made it possible to adopt appropriate strategies for the analysis of quality and the process of standardisation/validation of a herbomineral preparation in order to maintain the homogeneity of the final product

a. Quality control: - The pursuit of quality is being approached through the concept of Total Quality Management (TQM) and continuous improvement. Quality control of a product includes analytical testing of raw material, process tests, physical inspection of the products and operations at critical intermediate stages, tests on finished dosages form, packaging and labelling, environmental monitoring and inspection of the entire operations.

b. Quality assurance: - Quality assurance of a product means to assure the consumer about the quality accountable for safety, purity and effectiveness of a product. This specific phenomenon helps in identification and preparation of necessary standard operating procedures (SOPs) for all the objectives of a product.

c. Standardisation:- Standardisation means application of suitable methods and processes by which optimum conditions are ensured to obtain predictable results and products which confirm to certain set of standards in quality, purity, stability, safety, etc. (To cope up with all these requirements for herbomineral formulations, Deptt. of AYUSH, Ministry of Health and

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Family welfare, GOI has taken many appreciable measures since last five years and the outcome of all that is very fascinating and encouraging.)

d. Stability/shelf-life: - Stability of a pharmaceutical product may be defined as the capability of a particular formulation to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications in a specific container/closure system. In other words, shelf-life of a product may be defined as the time from the date of manufacture and packaging of the formulations until its chemical or biological activity is not less than a predetermined level of labelled potency, and its physical characteristics have not changed appreciably or deleteriously.

There are ancient and contemporary concepts for stability period (savīryata avadhi) of herbo-mineral formulations: According to Śāraṅgadharasamhita, the shelf-life period of herbo-mineral formulations is indefinite with the concept that the older the better its efficacy. Ayurvedic Drug Manufacture Associations (ADMA) in its petition to the Deptt. of AYUSH has suggested indefinite expiry period for all herbo-mineral formulations. The Government of India in its official gazette has notified the shelf-life period of different dosages forms of herbomineral formulations as under:

- Guṭika - Rasauṣadhies 5 years
- Bhasma No expiry
- Nāga,vaṅga, tāmra bhasmas 5 year
- Maṇḍūr loha 10 year
- Parpaṭi No expiry
- Kupīpakvarasa No expiry

(But due to protest from many stakeholders this matter has been referred to ASUDTAB and from there it has been referred to Ayurvedic Pharmacopoeia committee which is considering this issue critically. Meanwhile implementation of this notification is under abeyance.)

5. **Safety profile**

These herbomineral formulations were practised in India thousands of years ago with a safety and therapeutic efficacy. But in the last decades questions over safety of these preparations were aroused. This got a high level audience when these reports were published in JAMA twice (December 2004 and August 2008). Exclusively mercurial and minerallic/metallic products of āyurveda are under scan for its safety profile. Many organizations reacted sharply on these reports including national governments in protest or support of these. After a series of hue and cry with genuine or doctored reasons many steps have been initiated by all concerns to address this issue with rationality.

a. Toxicity study:- Schedule Y of Drugs and Cosmetics Rule 1945 consists of parameters required to establish safety profile of a drug which includes provisions of acute toxicity studies, sub chronic toxicities studies, chronic toxicity studies, mutagenic studies, reproductive toxicity studies and teratogenic toxicities studies along with guidelines of different phases of clinical studies. Many forums have raised queries on admissibility and applicability of provisions of schedule Y for Ayurvedic drugs on account of safe and efficacious practice of these drugs. After reviewing these facts, Deptt. of AYUSH has notified new guidelines for toxicities studies as well as for clinical studies of Ayurvedic drugs under Rule 170 of Drugs and Cosmetics Rule 1945 on Dec 24, 2008.

b. Adverse effects: - Adverse drug reactions (ADRs) have been defined by the WHO as “any

response to a drug which is noxious and unintended, and which occurs at doses used in man for prophylaxis, diagnosis, or therapy." A vigilant eye on the deleterious effects of a drug and to create awareness of the nature and scale of the drug-induced diseases has to be the primary focus. It is also important to understand the cause and make the patients aware of these problems and to analyze the possibilities of adverse effects so that precaution to prevent their occurrence or at least minimize their impacts is possible.

c. Pharmacovigilance:- On focusing the problems of ADRs, the department of AYUSH launched a program in September, 2008 namely National Pharmacovigilance Programme for Ayurvedic Siddha and Unani Drugs to support more comprehensive drug monitoring programs. This drug monitoring includes studies of drugs used and ADRs in a variety of in patients and ambulatory settings. Detection of unsuspected side effects and drug interactions, quantification of known effects and evaluation of the role of influencing factors are the primary objectives of this programme. To achieve these objectives for herbomineral formulations, information may be collected on patient characteristics, drug exposures, events, adverse reactions, efficacy of the drug therapy and manufacturing detail of the suspected drug. This may be termed as drug-surveillance program too.

6. **Therapeutic profile**

a. Indications: - The therapeutic profiles of herbo-mineral formulations of āyurveda are spread over various indications. It has been perceived since ancient times that these drugs are quite efficacious for the treatment of chronic disorders. The therapeutic profile of a single bhasma and its combinatory formulations are indicated to treat various ailments so also as rasāyana to enhance immunity and as vajīkaraṇa in textual classics of Rasaśāstra (except ancient texts like Rasendramaṅgal, Rasaratnākar, Rasahṛdaya tantra, where the subject matter is solely on alchemy), especially from 11th century onwards, viz. Rasendracūḍāmaṇi, Rasendra-cintāmaṇi, Rasaratnasamuccayam, Āyurveda-prakāśa and Rasataraṅgiṇi.

b. Contraindications: - Contraindications of these drugs are also notified in all ancient classics in specific cases of disease, status of patient, seasons, habitat, etc.

c. Dose: - Dose of these drugs is also important; it is completely depending upon the chemical constituencies of the individual drug. The dose of arsenical drugs are always in the range of 16-32 mg/day, whereas the rest of the metallic/ minerallic bhasma may be administered in the dose of 125-250 mg/day; and examples of śudhavarga have a dose pattern up to 1g/day. However, in accordance with the circumstances prevailing at the moment with various anupāna (adjuvant), a physician can reconsider or re-schedule the dose pattern according to the condition of the patient and the disease.

d. Duration: - There are queries and confusions on the duration of administration of these drugs which need to be settled. Interestingly what we have noticed in many classics is that duration of administration of these drugs are very particularised; it is varying from single dose to year long administration increasing or decreasing orders with intermittent intervals.

e. Antidote: - It is worthy to note here that in books of Rasaśāstra adverse reactions of these drugs are narrated with caution if they are not manufactured properly. And, of course, treatments, antidote of those undesired effects are also drawn there systematically. Few

examples in support of the above facts are quoted in Table 7.

7. **Regulatory obligation**

Regulations are highly required to ascertain uniformity in pharmaceutical products. The Office of Drug Controller General of India is responsible to take care of proper implementation of these regulations. The functionaries of this office are empowered for inspections of drug manufacturing units and they have every control on these units except price regulations in some cases. These officers pay attention to manufacturing, marketing and post marketing surveillance of drugs to promote and protect all interest of health of common public. There are a set of laws which cover rules and regulations related to drugs [from Drug Act 1930 to amendments (in 2008) of Rule 122 of Drugs and Cosmetics Rule 1945].

a. Drugs & Cosmetics Act, 1940:- The chapter IV-A (instituted in 1964) of this act containts all provisions expanding from sections 33 to section 33 N regarding Ayurveda Siddha and Unani drugs. This includes definitions of misbranded, adulterated and spurious drugs and all other issues such as inspection of unit, duty of analyst, penalty and confiscation desired for regulation of drugs. Definitions of classical āyurvedic drugs and ayurvedic patent and proprietary drugs are quoted in section 3 (a) and (h) of chapter I of D&C Rule 1940 respectively. Herbo-mineral formulations are represented in definitions as mentioned in section 3. Schedule I of the said act has listed 55 books which are termed as authoritative text books of Ayurveda. Formulations mentioned in these books are covered under different sections of chapter IV-A of D&C Act 1940.

b. Drugs & Cosmetics Rule, 1945:- Part XVI to XIX, Rule 151 (instituted in 1970) to Rule 170 (instituted in 2008) are conventionally dealing with rules made for licensing, standardisation,

TABLE 7
Characteristics of therapeutic uses of Materia Medica in Rasaśāstra

Materia Medica	Indications	Dose / Duration	Anupāna	ADR/antidote
Svarṇa (gold)	Kṣaya, agnimāndya, śvāsa, kāsa, aruci, pāṇḍu, ojovivardhanam, balakara	2 guñja (ratti)	Trikaṭu + ghṛta	Improperly prepared bhasma leads to loss of bala and vīrya and causes death
Lauha (iron)	Pāṇḍu, yakṣma, arśa, grahaṇi, jvara, śopha, prameha, gulma, plīha, kuṣṭha	¼ - 2 guñja	Ghṛta + madhu	Improperly prepared bhasma is jīvakāri, madakāri, dehaśūlakṛt and generating hṛtpīḍa. Treatment: viḍaṅga along with Agastya svarasa.
Abhraka (mica)	Sarvarogahara	½ guñja	According to disease	Candrikayukta bhasma leads to prameha and mandāgni. Treatment: āmalaki for 3 days
Haratāḷa (orpiment)	Kuṣṭha, phiraṅga, vātarakta, visarpa, arśa, apasmāra	¼ - ½ ratti	According to disease	In any kind of doṣas due to haratāḷ - Kūśmāṇḍa svarasa along with jīra and sugar
Śankh (conch shell)	Agnimāndya, grahaṇi, . pariṇāmaśūla, tāruṇyapiḍika	2 guñja	According to disease	-

packaging, labelling (for export too), shelf-life period of āyurvedic formulations. Form 24D-27D, 49 and 50 of schedule 'A' are concerned with Ayurvedic medicaments. Schedule 'T' of the said rule is regarding GMP of Ayurvedic Formulations. Separate notification as GMP of Herbo-mineral formulations are under active process for approval of the concerned authority i.e. Ayurvedic Pharmacopoeia Committee.

c. Schedule E of Drugs & Cosmetics Rule, 1945:- Schedule E (1) is depicting list of poisonous substances under the Ayurveda (including Siddha) and Unani system of medicines of all three origin i.e. drugs of vegetable origin - 15 substances such as ahiphena, vatsanābha, etc., drugs of animal origin - only one substance sarpaviṣa and drugs of mineral origin - 9 substances such as manaśśila, tutha, sindūr, etc. As per the directions of the concerned rule, if such ingredients are used in a formulation, it must be shown on the label.

d. Permissible limits of heavy metals: - Considering many issues on the toxicity of herbo-mineral formulations, Government of India, through Deptt. of AYUSH, has responded the queries and a press release were notified on 3 Aug 2008 to assure that these drugs are safe for practice. However, presence of contaminated metals in herbal formulations may not be appreciated by any means. Therefore, department of AYUSH on 14 October 2005, had notified maximum permissible limit of heavy limits in herbal formulations: i) mercury - 1 ppm, ii) lead - 10 ppm, iii) cadmium - 0.3 ppm and iv) arsenic -10 ppm. However, these permissible limits may not be applicable for formulations, which consist of mercury, lead and arsenic as ingredients. In such cases concept and theme of limits are different.

Mercurial products (pāradīya yoga)

a. Application of pārada: - Pārada (mercury) is a key element in the studies of Rasaśāstra. Every development, whether it is lohavāda (alchemical processes - conversion of lower element to higher element) or dehavāda (metabolic processes - potentiation /strengthening of body tissues to enhance immunity and longevity with proper physiological functioning), has its root with the properties of pārada. After researches of thousands of years the Indian sages invented therapeutic uses of pārada in many dosage forms which vary in its pharmacological functions despite the fact that ingredients of these different dosage forms are the same. Almost in all cases, pārada is treated with many other elements viz. gandhaka, svarṇa, haratāḷ, etc. to convert it into the form of a medicine. Credit of conversion in the properties of these substances including pārada can be attributed to the pharmaceutical processes viz. śodhana, samskāra, jāraṇa, kupīpakva methodology, etc. developed for the same.

b. Doṣa of pārada:- All the parameters of pārada have been addressed in the literature of Rasaśāstra which include its synonyms, occurrence, blemishes, physico-chemical, pharmacological and therapeutic properties. 12 doṣas (blemishes) of pārada are described in the literature of Rasaśāstra, which may be due to its position and surroundings in mines or adulteration after its procurement. The side effects of all these doṣas are also mentioned in the literature. These doṣas may be reduced or completely eliminated on virtue of some pharmaceutical processes rendered to pārada.These processes are known as śodhana and samskāra.

c. Śodhana and samskāra of pārada:- This is a unique phenomenon applied over pārada for the purpose of purification/detoxification and

change in properties by infusing good qualities. In this process, pārada is treated with many herbal media of solid or liquid nature in a specific arrangement of instrumentations called doḷā yantra, pātanayantra, etc. Some specified characteristic features have also been indicated to know the completion of these processes. These processes of śodhana and samskāra are the basic measures of conversion of pārada from a metal to medicine. The knowledge of this process is of utmost importance as a fundamental of Rasaśāstra.

d. Products range: - This wide range of mercurial preparation used in āyurveda has already been discussed in the preceding portion of this article under the heading of Dosage forms.

e. Dose and duration: - The dose of mercurial product is just varying from 35 mg to 250 mg / day as per the drug and disease. The duration of administration of these drugs is also variable as long as one year or a single dose as per the indications and contraindications.

Discussion and conclusion

Medical mineralogy is an up coming branch in medicine to look into the matters related to deficiency or excess syndrome of particular elements like copper, iron, calcium, manganese, etc. This is also a matter of concern in the present researches as to how these trace elements are significant in structural development and physiological functions of enzymes, hormones, vitamins, various specialized proteins. Many post graduate dissertations, doctoral theses and independent research papers by various laboratories have established these facts. Recently data was published in Indian Drug in support of the claim of essential and indispensable changes taking place due to unique pharmaceutical processes of Rasaśāstra by applying sophisticated analytical tests like ICP, AAS, TEM and SEM, etc.

Meanwhile attention has been paid to the safety of these rasauṣadhis and to validate data on this account at the department of Rasaśātra of Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University. Many bhasma like mākṣika, abhraka, lauha, vaṅga, etc. are tested for its acute and chronic toxicity studies in collaboration with the Deptt. of Pathology and Pharmacology of IMS. BHU. Recently (in 2008), teratogenic studies of Lauhabhasma were also done in our Institute by an author of this paper and results were in favour of fearless administration of these drugs. Same pursuits have been adopted by the Institute of Post Graduate Teaching in Research in Ayurveda, Gujarat Ayurveda University in the Deptt. of Rasaśāstra with the collaboration of Pharmacology unit of Institute. All these have been published in different journals and their findings were varying from case to case from mild toxicity to completely free from toxicity even in higher doses. On the basis of these studies, it can be assumed that if the pharmaceutical processes are adopted classically, rasauṣadhis will be free from toxicity. There are many hypotheses which are under the scan of researchers to establish absorption, distribution, metabolism and excretion (ADME) pattern of these bhasma.

One has to propose real fundamentals needed for understanding of these herbo-mineral formulations which may address all issues raised in this review write up. We tried for the same and the matter is open to the evaluation and suggestions of all academicians involved in the process.

A holistic and interpretative approach to every piece of information put down in this review write up may be agreeable to every stakeholder

of Rasaśāstra as fundamentals of Rasaśāstra which will ultimately lead to the manufacture of a safe and efficacious herbo-mineral formulations of āyurveda. Any suggestions or queries to provide more perfection to thrust areas of Rasaśāstra will be cordially accepted.

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(Concluded)

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STANDARDISATION OF KAṆḌŪGHNA TAILA (MEDICATED OIL FOR FILARIASIS)

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Abstract: Kaṇḍūghna taila, an āyurvedic herbal oil prepared from kaṇḍūghna daśaimāni (a group of 10 drugs indicated in itching), is referred to in Carakasamhita. Though these drugs are mainly indicated in itching, their actions like dāhapraśamana (reduces burning sensation), vedanāsthāpana (pain killer), śothahara (anti inflammatory) and vraṇaropaṇa (wound healing) have been described in various āyurvedic literatures. Based on these properties the oil was prepared with this group of drugs using sesame oil as the base. The oil was subjected to certain chemical studies to find out the iodine value, saponification value, acid value, peroxide value, total fat, weight for ml and HPTLC finger printing for standardisation.

Introduction

Chronic filariasis patients often suffer from acute periodic episodes of local inflammation involving skin and lymphatic vessels. Blisters and wounds with secondary bacterial infections and cellulites conditions worsen the patient's condition. Many of those chronic patients develop chronic skin problems with inflammation and itching. Keeping all these views and based on the classical āyurvedic and contemporary scientific reviews we selected the drugs of kaṇḍūghna daśaimāni for the preparation of external herbal oil. The prepared oil was studied to find out the iodine value, saponification value, acid value, peroxide value, total fat, weight for ml and HPTLC finger printing.

Materials and methods

The study was undertaken at Regional Research Institute (Ay), Vijayawada as a part of PhD study under Dr. NTR, University of health sciences, Vijayawada, A.P. The source and authentification of raw drugs was done by Plant Taxonomy Division, Laila Impex R&D Centre and CCRAS/RRI Research Centre, Vijayawada.

Mode of preparation

Coarse powder of candana (*Santalum album*), naḷada (*Vetiveria zizanioides*), kṛtamāla (*Cassia fistula*), naktamāla (*Pongamia pinnata*), nimba (*Azadirachta indica*), kuṭaja (*Holarrhena pubescens*), sarṣapa (*Brassica nigra*), madhuka (*Glycyrrhiza glabra*), dāruharidra (*Berberis aristata*) and mustā (*Cyperus rotundus*) were

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taken in equal quantities and mixed uniformly. 5kg of this mixture was boiled in 80 liters of water and reduced to 20 liters of decoction. The decoction along with 1250g kalka (paste prepared from the mixture of powder of all the above drug and water) and 5 liters sesame oil were boiled till the oil alone remained. The oil so prepared was studied to find out the iodine value, saponification value, acid value, peroxide value, total fat, weight for ml and HPTLC finger printing.

Observations and results

Organoleptic characters:- a) colour - dark-brown; b) odour - fragrant and pleasant; c) touch - oily.

Determination of weight per millilitre: - The weight per ml of a liquid is the weight in gram of 1 ml of a liquid when weighed in air at 25^0, unless otherwise specified. Thoroughly cleaned and dried pycnometer was calibrated by filling it with boiled and cooled water at 25^o and the contents weighed. It was assumed that the weight of 1 ml of water at 25^o when weighed in air of density 0.0012 g per ml is 0.99602 g. The capacity of the pycnometer was calculated. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). The temperature of the substance was adjusted to about 20^0 and the pycnometer was filled with it. The temperature of the filled pycnometer was adjusted to 25^0 and excess substance and weight were removed. The tare weight of the pycnometer was substracted from the filled weight. Finally the weight per millilitre dividing the weight in air, expressed in gram of the quantity (of liquid that filled the pycnometer at the specified temperature) by the capacity expressed in ml of the pycnometer at the same temperature was determined. The weight per milliliter of the oil is 0.92 g/ml.

Determination of saphonification value: - The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat. 35 to 40 g of potassium hydroxide was dissolved in 20 ml water, and added with sufficient alcohol to make 1,000 ml. It was left overnight, and the clear liquor collected. About 2 g of the oil was weighed accurately in a tared 250 ml flask, added with 25 ml of the alcoholic solution of potassium hydroxide, attached a reflux condenser and boiled on a water-bath for one hour by frequently rotating the contents of the flask to cool; and 1 ml of solution of phenolphthalein was added and finally titrated the excess of alkali with 0.5 N hydrochloric acid. The number of ml required (a) was noted. The experiment was repeated with the same quantities of the same reagents in this manner but omitting the oil. The number of ml required (b) was noted. Finally the saponification value was calculated by the formula: $\frac{(b\text{-}a) \times 0.02805 \times 1000}{W \text{ (oil weight in g)}}$. The saphonine value of the oil is 182.1

Determination of iodine value:- The iodine monochloride method is that the approximate weight (in g) of the oil to be taken may be calculated by dividing 20 by the highest expected iodine value. The oil was accurately weighed, in dry iodine flask; 10 ml of carbon tetrachloride was added and dissolved. This was added with 20 ml of iodine monochloride solution. The stopper which was previously moistened with the solution of potassium iodine was inserted and left in a dark place at a temperature of about 17^oC for thirty minutes. Later this was added with 15 ml of solution of potassium iodine and 100 ml water. Total

solution was shaken, and titrated with 0.1 N sodium thiosulphate using solution of starch as indicator. The number of ml required (a) was noted. The operation exactly in the same manner was carried out at the same time but without the oil being tested, and the number of ml of 0.1 N sodium thiosulphate required (b) was noted. The iodine value was calculated by the formula: $\frac{(b-a) \times 0.01269 \times 100}{W \text{ (oil weight in g)}}$. The iodine value of oil is 114.2.

Acid value: - The acid value is the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the substance. 10 g of the oil was weighed accurately and taken into a 250 ml flask with 50 ml of a mixture of equal volumes of alcohol and solvent ether. This solution was neutralized after the addition of 1 ml of solution of phenolphthalein. Later total solution was heated gently on a water-bath, until the substance had completely melted and titrated with 0.1 N potassium hydroxide by shaking constantly until a pink colour which persists for fifteen seconds is obtained. The number of ml required was noted and the acid value was calculated by the following formula:
$\frac{a \times 0.00561 \times 1000}{W \text{ (oil weight in g)}}$, where 'a' is the number of ml of 0.1 N potassium hydroxide required.The acid value of oil is 7.0

Peroxide value: - The peroxide value is the number of mill equivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance. 5 g of the oil was accurately weighed and taken into a 250-ml glass-stoppered conical flask. This oil was added with 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform; swirled until dissolved and added with 0.5ml volumes of saturated potassium iodide solution. The mixture was allowed to stay for exactly 1 minute, with occasional shaking and added with 30 ml of water and titrated gradually with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. 0.5 ml of starch solution was added and the titration was continued by shaking vigorously until the blue colour just disappears ('a' ml). The same operation was repeated omitting the oil being examined ('b' ml). The peroxide value was calculated by the formula:-$\frac{10 \text{ (a-b)}}{W}$. The peroxide value of oil is 5.9

Total fat: - 2 g of the oil was taken in a thimble made of coarse filter paper and knotted with thread. This sample was extracted with 25 ml anhydrous alcohol in a soxlet extraction apparatus. The solvent was evaporated and the extract was dried to a constant weight at 110^{0}C Finally the fat content percentage was calculated. The total fat in oil is 93.0%

TLC/HPTLC

Sample preparation: - 1 g of extract sample was refluxed with 10ml methanol and spotted using Linomativ (Camaq Switzerland)

Stationary phase (application):- The prepared sample was applied over the pre-coated silica gel 60 F_{254} plate, 0.2 mm thickness (Merk, Germany).

Development (mobile phase): The sample was developed with the help of mobile phase- Toluene: Ethyl acetate: Formic acid (50:15:05)

Visualization (scanning):- For visualization the plate was dried at 100^o C and scanned at 366 nm UV.

Observations:- The sample plate scanned under UV, wavelength 366 nm showed 4 peaks (spot) and the observed Rf. values were 0.34, 0.48, 0.55, and 0.62 (Fig. I)

Discussion and conclusion

Kaṇḍūghna taila was subjected to physico chemical analysis to standardise for further studies and utility. The oil was prepared from the drugs kaṇḍūghna daśaimāni (a group of 10 drugs indicated in itching) as referred to in Carakasamhita. The oil is not available in the market. Based on its actions and indications an effort was made to prepare and standardise the oil. Though the oil was basically selected for a clinical study on Filariasis, it can also be tried on relevant clinical conditions. Physico-chemical studies of oil viz. weight for ml, iodine value, saponification value, acid value, peroxide value and total fat are certain parameters selected to establish the standards of the oil. The developed HPTLC finger prints of the oil are also useful for varification and comparision in future studies.

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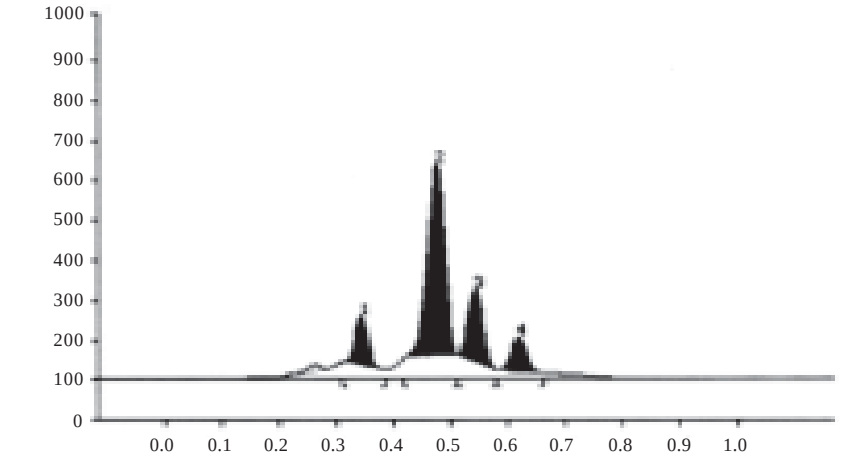

Wave length: 366 nm

Track: 3, noise level: 0.076AU, raw data file: BAL27_5A

U4.03 S/N: 0302A017 CAMAG SOFTWARE (c) 1996 SCANNER 3: 030106

Track: 3. Analysis c: KANDUGHNA TAILA OIL

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.31	1.5	0.34	120.8	14.05	0.39	0.0	1379.6	10.94
2	0.42	1.4	0.48	473.6	55.06	0.51	0.0	7552.2	59.89
3	0.51	0.0	0.62	86.8	10.09	0.67	0.0	1215.0	9.64
		Total height -		860.0			Total area -		12609.4

Fig. I

HPTLC Finger prints of Kaṇḍūghnataila(UV 366nm)

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ANTIBACTERIAL SCREENING OF MUGDHARASA

Surekha S Patil and R. S. Hiremath*

Abstract: Environmental and personal hazards are factors that invade micro-organisms on different systems of human body. These cause diseases like infectious diarrhoea. Mugdharasa is one of the āyurvedic formulation mentioned as very effective in treating diarrhoea of various types. In the present study, an effort is made to study the anti-bacterial activity of Mugdharasa on four different strains by MIC and MBC methods.

Introduction

Mugdharasa is one of the Kharaliya rasāyana that come under nirgandha anaghni mūrchana of pārada auṣadi yogas. It is mainly indicated in vamana, sahaja phiraṅga, bālātisara harita, pīta, mṛttikavaraṇa, durgandhayukta atisāra, kāmala, mandāgni and kaṇṭhaśālūka.

To detect, isolate, cultivate and culture sensitivity of the drugs there are the techniques adopted by the modern microbiologists. This helps to assess the probable effect of a drug on different micro-organisms. Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation, and minimum bactericidal concentrations (MBCs) the lowest concentration being that of antimicrobial. This will prevent the growth of an organism.

The antimicrobial activity of a drug is generally expressed as it is inhibiting effect towards the growth of bacterium in nutrient broth or nutrient agar. For this study the following conditions are required: i) the substance must be in contact with the test organism, ii) condition must be favourable for the growth of micro-organisms in the absence of antimicrobial substances, iii) there must be a means of estimating the amount of growth and thereby the percentage of growth of inhibition, iv) the activity of powder substance should be observed and determined by the growth response of micro-organisms.

Limitations of *in-vitro* study:- Antimicrobial susceptibility testing results cannot and should not be used as predictors of therapeutic outcome for the use of particular antimicrobial agents because of the lack of correlation between *in-vitro* test conditions and the *in-vivo* setting.

Advantages:- i) Broth dilution testing allows the option of providing both quantitative (MIC) and qualitative (category interpretation) results; they provide more quantitative information and may be applied to a wide range of isolates than

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the diffusion tests, ii) most susceptibility test batteries require testing several antibiotics at several different concentrations, the smaller volume used in micro dilution allows this to be accomplished in a single micro titre tray format. Use of test tubes as required by the macro dilution method becomes substantially cumbersome and labour intensive, especially because most laboratories must test several bacterial isolates daily. For this reason, macro dilution is rarely used in most clinical laboratories and subsequent comments regarding broth dilution focus on the micro dilution approach.

Aims and objectives

To study the *in-vitro* evaluation of its bacteriostatic and bactericidal activity against strains of gram positive and gram negative bacteria responsible for infectious diarrhea by the following methods: i) minimum inhibition concentration method and ii) minimum bactericidal method

Materials and methods

In the present study, broth dilution test (MIC) and MBC test were performed to determine the level of bacteriostatic and bactericidal effect on the Mugdharasa.

Broth dilution test method: - The broth dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for MBC tests also: The procedure was carried out mainly under three steps: step i) sterilization, preparation of inoculums, preparation of stock solution, preparation of broth media; step ii) serial dilution procedure, reading MIC results; step iii) minimum bactericidal concentration (MBC) procedure and reading of MBC results.

Materials:- i) BHI broth media, ii) overnight broth culture of test organisms (inoculated micro-organisms), iii) Mugdharasa powder (required antibiotic in powder form), iv) required solvent - sterile distilled water, iv) sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles, v) incubatorr, vi) cotton swabs, vii) sterile graduated micropipettes - 5,10, 50, 200, 500 ml size and viii) a suitable rack to hold 40 tubes in four rows i.e. 10 tubes in each row.

Organisms used:- Bacteria - a) gram positive - *Staphylococcus aureus* - ATCC No. - 700699; b) gram negative - *Escherichia coli* - ATCC No. - 25922, *Shigella flexneri* - ATCC No. - 29508 and *Vibrio cholerae* - ATCC No. - 17802.

Step 1

1. Sterilization: - Sterilization of the medium, tubes for slants, etc. was done by autoclaving at 15 lbs/square inch for 20 minutes. The glasswares like syringes, pipettes, empty test tubes, were sterilized by dry heat in an oven at a temperature of 160^{O}C for 1 hr.

2. Preparation of inoculums:- Mueller-Hinton agar medium of the following composition was used for preparation of slants: i) peptone - 5.0g, ii) beef extract - 1.5g, iii) sodium chloride - 5.0g, iv) agar - 15.0g, v) yeast extract - 15.0g and vi) distilled water - 1000 ml.

About 28g of prepared medium was taken in 1000 ml of distilled water and boiled to dissolve completely. After being streaked with micro-organisms and aseptic conditions the slant were incubated at 37^{O}C + 1^{O}C for 24 hrs.

3. Preparation of stock solution: - Stock solution is prepared by using saline water in the concentration of 1gm/ml.The volume of the solution used during the procedure is 0.2ml, so the concentration of the drug finally stands as 1 mg/ml.

d) Preparation of broth media: - Brain Heart Infusion media was used for the preparation of broth solution. The following compositions were used: i) Calf brain infusion from - 200.00, ii) beef heart infusion from - 250.00, iii) protease peptone - 10.00, iv) dextrose - 2.00, v) sodium chloride - 5.00, vi) disodium phosphate - 2.50.

Suspend 37.0 gm in 1000 ml distilled water dispense it into bottles or tubes as desired and sterilize by autoclaving at 15 lbs pressure (121^0C).

Step 2

1. Serial dilution procedure:- To start with, glass test tubes plugged with cotton are autoclaved. Ten borosilicate glass test tubes are taken in four rows each, one corresponding for the serial dilution with respect to one organism. Test tubes are serially numbered from 1-10. The first test tube is placed in the row in rack with the name of the organism used in the study. 200 ml of the (BHI) brain heart infusion broth is initially taken in all the test tubes of eight rows. To the first tube of all the four rows, 200ml of the stock solution is added. It is pipetted in and out to ensure uniform mixing. 200 ml of the first test tube is then pipetted to the second tube and the process is repeated till the 10^{th} tube. From the 10^{th} tube 200 ml is pipetted out and discarded. Every time the 200 ml solution is introduced in the test tube it is gently pipetted in and out within the test tube to ensure proper mixing. During every withdrawal of 200 ml of the solution and introduction to another tube, a new pipette is made use of.

In the above said method, serial dilutions are made similarly with 10 test tubes arranged in four rows corresponding to four micro-organisms of two drug samples. The concentration in the test tube now stands as 500mg (tube 1), 250mg (tube 2), 125mg (tube 3), 62.5mg (tube 4), 31.5mg (tube 5), 16mg (tube 6), 8mg (tube 7), 4mg (tube 8), 2mg (tube 9) and 1mg (tube 10). Now adjust the bacterial count $1.58*10^8$ organisms per ml by comparing it with McFarland's standards. The suspension of the organisms *Staphylococcus aureus*, *E. coli*, *Shigella flexneri* and *Vibrio cholerae* in the (BHI) brain heart infusion broth is then inoculated to all the test tubes in the corresponding four rows with the concentration of 200 ml. All tubes are inoculated at 35^0C for 24hrs without shaking or agitation.

After 24hrs of incubation the test tubes are ready for MIC. This is defined as the concentration of the first tube in the series to show visible traces of growth of bacteria, which is evident, by turbidity or cloudiness of the solution in the test tube. Turbidity indicates that bacterial growth has not been inhibited by the concentration of the preparation contained in the medium. The main advantage of the 'broth dilution' method for the MIC determination lies in the fact that it can readily be converted to determine the MBC as well.

2. Reading of MIC results: - MIC is expressed as the lowest dilution with inhibited growth and is judged by lack of turbidity in the tube; because very faint turbidity may be given by the inoculums itself. The inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

Step 3

1. MBC: - The MBC (Minimum Bactericidal Concentrations) values also known as MLC (Minimal Lethal Concentration) is the estimation

of bacterial activity and is defined as the lowest concentration of the preparation of Mugdharasa to kill 99.9% of the initial inoculums after incubation for 24hrs under a standardised set of conditions. Here, in the present study, MBC was obtained by platting out the contents on an agar medium to count the growth.

3. Procedure and reading result:- After incubation of serial dilution tubes (after 24 hrs), next day MIC readings are taken, after visually clear tubes 0.1ml from each of the last four tubes i.e. (7,8,9 &10) were taken and spread across the surface of dried agar plates with the help of sterile bent glass rods. Then next plates were inoculated overnight at 35^0C for 24hrs. Next day plates are observed for growth of colonies and readings are noted. The number of colonies that grew on each MBC subculture plates after overnight incubation was counted. The concentration in which petriplate showed either no growth or reduction in 99.9%, was considered the MBC of specific antimicrobial agent.

These subcultures may show similar number of colonies that indicate bacteriostasis only. A reduced number of colonies indicate a partial or slow bactericidal activity. If the whole inoculum has been killed, it indicates no growth. The highest dilution showing at least 99% inhibition is taken as MBC.

Result and discussion

The MIC values of Mugdharasa showed sensitivity towards all the four tested organisms in different concentrations i.e. from 500 to 1 mg/ml of the drug (Table 1). The MBC values of Mugdharasa on different organisms are shown in Table 2. The comparative study of MIC and MBC values of Mugdharasa are shown in Table 3

Antimicrobial study by broth dilution method: - MIC study of Mugdharasa was carried out on *S. aureus* gram +ve and *Vibrio cholerae*, *Shigella flexneri*, *E.coli* - gram -ve strains respectively by serial dilution. The stock solution of Mugdharasa was prepared by taking 1 gm of drug sample separately and mixing it in 1ml of saline water. From this stock solution, 10 concentrations were taken by serial dilution method ranging from 500 mg, 250 mg, 125mg, 62.5mg, 31.25mg, 16mg, 8mg, 4mg, 2mg and 1mg/ml for all organisms. Mugdharasa has shown maximum inhibitory concentration (MIC) against all four organisms, even up to 1mg/ml concentration.

TABLE 1
The MIC values of Mugdharasa on organisms

Micro Organism	Concentrations (in mg/ml)									
	500	250	125	62.5	31.25	16	8	4	2	1
S.aureus	S	S	S	S	S	S	S	S	S	S
E coli	S	S	S	S	S	S	S	S	S	S
S. flexneri	S	S	S	S	S	S	S	S	S	S
V.cholerae	S	S	S	S	S	S	S	S	S	S

TABLE 2
MBC of Mugdharasa on the four organisms

Organisms	7 (8mg)	8 (4mg)	9 (2mg)	10 (1mg)
S. aureus	Ng	Ng	Ng	100 Cl
E. coli	Ng	Ng	2 Cl	12 Cl
S. flexneri	12 Cl	5 Cl	4 Cl	150 Cl
V. cholerae	Ng	Ng	Ng	Ng

*Ng = No growth; Cl = Colonies

TABLE 3
MIC & MBC values of Mugdharasa

Micro Organisms	MIC	MBC
1. *S. aureus*	1mg/ml	2mg/ml
2. *E. coli*	1mg/ml	4mg/ml
3. *S. flexneri*	1mg/ml	16mg/ml
4. *V. cholerae*	1mg/ml	1mg/ml

MBC was expressed as the lowest concentration which was able to kill the bacteria. After getting MIC values, the 7th (8mg), 8th (4mg), 9th (2mg), and 10th (1mg) tubes were sub-cultured and inoculated for 24hrs and had the organisims observed the next day. The results showed Mugdharasa is able to kill all four organisms on its different concentrations. In other words, it showed 100% result on *V. cholerae* up to 1mg, *S. aureus* up to 2mg, *E. coli* up to 4mg and *S. flexneri* up to 32 mg.

The standard drug prepared and tested for the antimicrobial activity on selected bacterial species and the antimicrobial effect showed sensitivity towards tested micro-organisms by both methods in different concentrations of Mug-dharasa. The drug was found to have both bacteriostatic and bactericidal properties on both gram-positive and gram-negative bacteria.

Conclusion

Mugdharasa is having significant anti-bacterial activity on micro-organisms viz. *S. aureus, V. cholerae, S. flexneri and E. coli* tested by both agar disc diffusion method and MIC method. MBC of Mugdharasa shows 100% bactericidal effect on all the 4 microbes. Thus the *in-vitro* susceptibility testing detects resistance by both methods and it provides valuable data that are used in conjunction with other diagnostic information to optimize the therapy. *In-vitro* study has shown the effects of Mugdharasa on microbes. To prove its therapeutic efficacy *in-vivo* study on animals is to be carried out to rule out any toxicity of drug before clinical trials.

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Clinical observation

CARCINOMA PANCREAS

K.V. Rajagopalan*

A 26 year old mother of a 2 year old boy was brought to our Secunderabad Branch in a stretcher on 16.09.1999 for consultation of Dr. P.K. Warrier, Chief Physician, Arya Vaidya Sala, Kottakkal. The patient was a housewife. Her condition then was as follows:

- Severe weakness
- Anaemia
- Loss of weight
- Nausea
- Poor appetite
- Stomach pain
- Back pain
- Cough
- Lack of confidence

History

The symptoms started with pain and swelling in the epigastric region in May 1999, for which she consulted her family doctor and as per her advise, she underwent an ultrasonography (20.05.1999) of the abdomen, which revealed:

1) Pseudo pancreatic cyst?
2) Lymphoma?

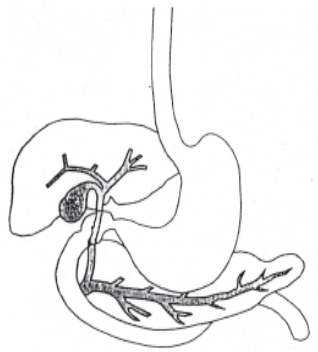
Further to this, ERCP (Endoscopic Retrograde Cholangio Pancreatography) was performed at the Deptt. of Gastro-enterology of Medenova hospital and confirmed Pancreatic ductal block mid body (Report 1). The patient was advised to take allopathic medicines for 3 months. As the pain and swelling did not reduce, the patient underwent a repeated USG of abdomen on 16.08.1999, which showed an increase in the size of the mass i.e. 94 x 102 mm became 114 x 98 x 88 mm (Report 2). For further investigation, the FNAC (Fine Needle Aspiration Cytology) was done on 30.08.1999 and the case was confirmed as papillary adeno carcinoma (Report 3).

Viewing the above condition, the doctor advised her to go in for an immediate surgery. It was arranged on 30.8.1999. Blood transfusion was done during the procedure because of severe anaemia. But on Laperotomy, surgeons deferred the surgery because of the extensive nature of the disease (Report 4)

**Arya Vaidya Sala, Kottakkal*

Report 1

Endoscopic Retrograde Cholangio Pancreatography - 22.05.99



INDICATION : PSUEDOCYST OF PANCREAS FOR EVALUATION

PROCEDURE : EVIS JF 130 SCOPE USED
PAPILLA NORMAL
PANCREATIC DUCT SELECTIVELY CANNULATED
PANCREATIC DUCT SHOWS BLOCK IN THE MID BODY

IMPRESSION : PANCREATIC DUCTAL BLOCK MID BODY

This was her condition when she reached our Secunderabad Branch on 16.09.1999.

Examination

B.P. : 100/70 mm of Hg
P.R. : 65/mt.
Weight : 41 kg.

On palpation a mass was felt on the epigastric and left hypochondrium region with severe tenderness.

Hemetological examination:
Hb% : 10.49%
SAP : 160 iu/l (normal range 38 – 117)
SCEA : 0.7 mg/dl
S.Ca 19.9 : 76 iu/l (0-30)

Vitals:
Bowel : Constipated
Appetite : Very poor
Sleep : Disturbed
Urine : Normal

No relevant family history

Medicines

After a thorough examination by the Chief Physician, the following medicines were prescribed:

1. *Sahadevyadi Leham* (3g) twice daily (6.00 A.M. and 5.00 P.M) followed by *Nimbamritadi Panchatiktam Kashayam* (15 ml) + 60 ml *Gomutram* (cow's urine)
2. *Sahadevi extract* (2g) with warm water before lunch and dinner
3. *Arushkaram extract* (1g) with honey after lunch and dinner

4. A mixture of *Dasamularasayanam* (10g) + *Hingulabhasmam* (400 mg) to be taken in a day in small doses at different intervals
5. *Sanjeevani tailam* for external application followed by veshtanam (bandage) with arkapatra (leaves of *Calotropis gigantea*) on the mass area.

Diet suggested: - at 8.00 AM - breakfast; at 12.00 noon - lunch; at 7.00 PM - fruits + milk

Special instruction was given to take vegetable juice once or twice a day instead of coffee/tea; to take more buttermilk whenever necessary (buttermilk is considered as nectar in ayurveda); and to avoid red chillies, tamarind, pickles, oily, fried and cold items.

She followed the advice very religiously. Her husband gave her all the moral support which boosted her will-power. He kept us informed of her condition. Sometimes both of them visited the branch office at Secunderabad. Gradually she showed a remarkable improvement. It was like the lush growth of good seeds under favorable conditions.

Report 2
USG Abdomen - 16.08.99

LIVER	:	SIZE & SHAPE ARE NORMAL PARENCHYMAL ECHO TEXTURE IS NORMAL NO FOCAL LESION NO EVIDENCE OF INTRAHEPATIC BILARY DILATATION CBD & PV ARE NORMAL
GALL BLADDER	:	WELL DISTENDED NORMAL WALL THICKNESS NO CALCULUS
SPLEEN	:	NORMAL IN SIZE & PARENCHYMAL, ECHOTEXTURE. NO SOL
PANCREAS	:	HEAD OF THE PANCREAS IS WELL VISUALISED & APPEARS NORMAL OUTLINE OF THE BODY & TAIL OF THE PANCREAS NOT CLEARLY VISUALISED & APPEARS TO BE COMPRESS BY A LARGE MIXED ECHOTEXTURE MASS MEASURING 114 x 98 x 88 MM IN SIZE, WHICH IS NOTED ALONG THE TAIL OF PANCREAS AND MEDIAL TO THE SPLEEN.
KIDNEYS	:	RIGHT KIDNEY MEASURES 99 x 37 MM LEFT KIDNEY MEASURES 93 x 42 MM BOTH RENAL SITE SIZE & SHAPE ARE NORMAL BOTH THE RENAL PARENCHYMAL ECHOTEXTURE IS NORMAL NO FOCAL LESION NOTED NO EVIDENCE OF CALCULUS OR HYDRONEPHROSIS THERE IS NO EVIDENCE OF ANY FREE FLUID IN THE ABDOMEN
URINARY BLADDER	:	EMPTY
IMPRESSION	:	EVIDENCE OF LARGED MIXED ECHOTEXTURE MASS IN THE EPIGASTRIC REGION OBLITERATING THE OUTLINE OF THE BODY AND TAIL OF THE PANCREAS - S/O? INFECTED PSEUDO-PANCREASTIC CYST.

Patient at Kottakkal

After 5 - 6 months' medication, the patient's husband wrote to us on 23.03.2000 expressing her willingness to come to Kottakkal and meet the Chief Physician. The following points were noted:

- Weight increased from 41 to 45.5 kg
- Normal blood pressure
- The lump is not felt
- Does all household works on her own
- Eats well and feels hungry
- No pain in the abdomen

The patient visited our AH&RC, Kottakkal on 23.5.2000 and the following improvements were noted:

- Pain in the abdomen, nausea, and back pain subsided
- Weight gained from 41 to 46 kg
- Normal bowel, appetite, urine and sleep

The patient was advised to continue the same medications except Arushakaram extract and Sahadevi extract, and also the diet was modified as follows:

- 07.30 AM - Fruit juice
- 09.30 AM - Breakfast
- 11.00 AM - Fruits
- 01.00 PM - Meals + vegetables + buttermilk
- 05.00 PM - Fruits / fruit juice
- 07.30 PM - Fruits / milk

After seven months, the patient visited the Chief Physician at Hyderabad on 22.12.2000. This time she did not have any specific complaints to report. She was advised to take *Sahadevi Extract* (3g)

Report 3
Fine Needle Aspiration Cytology - 30.08.99

GROSS: MICROSCOPIC EXAMINATION OF STRAINED SMEARS SHOWS – RBC, COLLECTIONS OF INFLAMMATORY CELLS WITH COLLECTING LARGE CELLS CONTAINING DEEP STAINING NUCLEI. THESE CELLS ARE ARRANGED IN A GLANDULAR PATTERN. WELL FORMED PAPILLARY PROCESSES ARE SEEN.

IMPRESSION: THE ARRANGEMENT OF CELLS IS IN FAVOUR OF PAPILLARY ADENO CARCINOMA

with cow's urine twice a day i.e. at 6.00 AM and at 5.00 PM in empty stomach and to stop all other medicines.

Patient back to family life

After a certain time, she bid goodbye to medicines and came to normal life. Meanwhile, in July 2002, she conceived for the second time. In the 4th month of pregnancy i.e. on 22.10.2002, the USG of the abdomen showed -

1) A large fibroid (9 x 10 cm) in the uterus
2) Pancreatic mass same in size, not increased

TABLE 1
Comparative statement of the pancreatic mass

Date	Size of pancreatic mass
16.08.1999	114 x 98 x 88 mm
28.09.2003	51 x 39 mm
13.10.2004	36 x 42 x 36 mm

The patient was advised to go ahead as per the advice of the then gynecologist. To be on the safer side, she was prescribed *Nimbamrithadi Panchathiktham Kashayam* 15 ml + *Gomutram* 60 ml at 6.00 AM and 6.00 PM.

The patient was asymptomatic till delivery and gave birth to a healthy boy on 16.3.2003 by caesarean.

Report 4
Medical Report - 09.09.99

DIAGNOSIS : ADVANCED INOPERABLE PANCREATIC MALIGNANCY
EVALUATED FOR UPPER ABDOMINAL PAIN WITH WEIGHT LOSS AND ANAEMIA.
USG/CT SCAN FEATURES SUGGESTIVE OF A MASS LESION INVOLVING THE PANCREAS
AFTER PREOPERATIVE EVALUATION WAS TAKEN UP FOR SURGERY BY DR. G.V. RAO
LAPAROTOMY PERFORMED THROUGH ROOF TOP INCISION
EVIDENCE OF A LARGE MASS LESION INVOLVING ALMOST THE ENTIRE PANCREAS. LESION SHOWING CYSTIC AREAS. THE MASS LESION WAS SEEN INFILTRATING SUPERIOR MESSENTERIC PEDICLE AND WAS INSEPARABLE. EVIDENCE OF EXTENSIVE COLLATERALS MULTIPLE NODES WERE FOUND AT THE PORTA. LIVER WAS FREE. IN VIEW OF THE EXTENSIVE ADVANCED NATURE OF THE DISEASE SURGERY WAS DEFERRED.
OPERATIVE AND POST OPERATIVE PERIODS WERE UNEVENTFUL

ADVISED : TAB. TAXIM O 200 MG 1 TAB TWICE A DAY FOR 5 DAYS
CAP. PROTOLOC 20 MG 1 CAP DAILY IN THE MORNING ON EMPTY STOMACH
CAP. VIZYLAC 1 CAP DAILY
REVIEW AFTER 3 DAYS FOR THE REMOVAL OF SKIN CLIPS
PLANNED FOR CHEMOTHERAPY

Report 5

B Mode 2-Dimensional Real time Ultrasound Scan of Pelvic organs - 28.09.03

U.B.D.	:	NORMAL		
UTERUS	:	SHAPE AND SIZE - ANTEVERTED 106 X 65 X 70 MM MYOMETRIUM - NORMAL. ENDOMETRIUM - 7.5 MM MASS LESION - A SINGLE LARGE 57 X 46 MM HYPOECHOIC AREA SEEN AT BODY REGION		
OVARIES	:		Right	Left
		Size	28 X 17 mm	30 X 17 mm
		Shape	Normal	Normal
		Follicles	Normal	Normal
		Mass lesions	No	No
		Free fluid in Gul de sac	Not seen	Not seen
		Adnexal mass	Not seen	Not seen
IMPRESSION	:	PANCREATIC MASS SIZE 51 X 39 MM		

Report 6

Ultrasound scan of abdomen and pelvis - 13.10.04

LIVER	:	SIZE 155 MM SHAPE AND ECHO PATTERN ARE NORMAL. NO MASS LESION ARE SEEN. PORTAL VEIN 12 MM. CHD 2MM NORMAL. INTRAHEPATIC BILIARY RADICALS AND INTRA HEPATIC VEINS ARE NORMAL.
G.B.D.	:	SIZE, SHAPE AND WALL THICKNESS ARE NORMAL. NO CALCULUS OR NO MASS LESIONS ARE SEEN
SPLEEN	:	SIZE 100, SHAPE AND ECHO PATTERN AND SPLENIC VEIN ARE NORMAL. NO ABNORMAL CALCIFICATIONS ARE SEEN
PANCREAS	:	SIZE, SHAPE AND ECHOPATTERN ARE NORMAL. NO ABNORMAL CALCIFICATIONS ARE SEEN. A SINGLE HYPOECHOIC WELL DEFINED AREA OF 36 x 42 x 36 MM SEEN AT TAIL OF PANCREAS
KIDNEYS	:	SHAPE, CONTOUR, RENAL CAPSULE, PERIRENAL AREAS AND CORTICAL ECHOPATTERN ARE NORMAL. CORTICOMEDULLARY DIFFERENTIATIONS ARE NORMAL. NO HYDRONEPHROSIS. NO CALCULUS OR MASS LESIONS ARE SEEN. RIGHT KIDNEY SIZE 97 x 48 MM. LEFT KIDNEY SIZE 88 x 48 MM
U.B.D.	:	NORMAL IN COLOUR. NO MASS LESIONS ARE SEEN. NO RESIDUAL URINE SEEN.
UTERUS	:	ANTEVERTED 103 x 64 x 86 MM MYOMETRIUM NORMAL. A SINGLE HYPOECHOIC AREA OF 62 x 54 MM SEEN FUNDAL AND BODY REGION. ENDOMETRIUM NORMAL.
OVARIES	:	RIGHT OVARY 22 x 12MM. LEFT OVARY 28 x 13 MM. SIZE, SHAPE AND ECHOPATTERN ARE NORMAL. NO CYSTIC OR NO SOLID MASS LESIONS ARE SEEN

NO ASCITES

AORTA AND PVC ARE NORMAL. NO PRE OR PARA AORTIC LYMPHADENOPATHY

BOWEL GAS PATTERN INCREASED

A comparative statement of the pancreatic mass at different period of the treatment is tabulated in Table 1. After two years of delivery she underwent hysterectomy and is now leading a good family life. (Report 5&6)

Discussion

In Suśrutasamhita, cancer (arbuda) is explained in the context of granthi and is considered to be more complicated than granthi due to its māmsa predominance.

गात्रप्रदेशे क्वचिदेव दोषाः संमूर्च्छिता मांसमभिप्रदूष्य
वृत्तं स्थिरं मन्दरुजं महान्तमनल्पमूलं चिरवृद्ध्यपाकम्।
कुर्वन्ति मांसोपचयं तु शोफं तमर्बुदं शास्त्रविदो वदन्ति ।। (सु. सं. नि. 11/13,14)

The treatment principle of arbuda according to the text is śophacikitsa, in other way śoṣaṇacikitsa. The treatment principle of rasāyana also should be adopted to keep up the standard of patient's strength.

All the ingredients of *Nimbamritadi panchatiktam kashayam* are anti-cancerous. The ingredients like nimba (*Azadirachta indica*), vṛṣa (*Justicia beddomei*) and amṛta (*Tinospora cordifolia*), functions as rasāyana also. Gomūtra (cow's urine), having the properties like tīkṣṇa, rūkṣa, dīpana, and pācana, acts as an anti-cancerous drug when therapeutically administered. So also is the case with aruṣkaram (*Semecarpus anacardium*); which acts as a herbal chemotherapy. Sahadevi (*Vernonia cinerea*) based medicines have been in use in cancer treatment with encouraging results.

As cancer is considered to be a life style disease, modification in lifestyle and diet helped to relieve the symptoms. Mind is the healing factor of any disease; the attention of physician, affectionate care from family and society helped to bring her back to life.

The general treatment principle here can be summarised as śoṣaṇa (on the excited dhātus), rasāyana, arbudaharatva, pathyāhāra, pathyavihāra and manobala.