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Quality control measure of *Jeeva Rasa Avaleha*: a male sexual stimulant

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In spite of numerous *Vajeekarana* formulations being available in the market, the prevalence erectile dysfunction is increasing irrespective of race or economic status. The aim of the present study was to measure the quality control parameters of *Jeeva Rasa Avaleha* used as male sexual stimulant. It was prepared by using seven drugs, namely *Shuddha Hingula, Shuddha Shilajatu,* with powders of roots of five herbs viz., *Aakarakarabha, Ashwagandha, Shweta Mushali, Shatavari* and tuber of *Vidarikanda.* Qualitative and quantitative analyses of JRA such as reducing sugar, non-reducing sugar, fat content, water soluble extractive, pH, thin layer chromatography (TLC) and energy dispersive x-ray spectroscopy (EDX) were performed. It was observed that 9.8% moisture content and 15.12% water-soluble extractive were in JRA, respectively. EDX and AAS study showed that formulation had heavy metals within permissible limit except mercury which is an ingredient of JRA. Both qualitative and quantitative analysis of JRA indicates safety of formulation and therefore it may be prescribed for the treatment of erectile dysfunction.

Keywords: Erectile dysfunction, Herbo-mineral, *Jeeva Rasa Avaleha, Vajeekarana*

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Erectile dysfunction (ED) is imperative to a man's prosperity and wellbeing and influences the person as well as purposes strain on a couple's way of life and relationship[1]. Its prevalence was probably 152 million in 1995 and will be 322 million in 2025[2]. Furthermore, its prevalence is 8% in 20–29 years age group and 11% among 30–39 years age group[3]. Food and Drug Administration (USFDA) suggested creating plant inferred medicate as magnificent option in contrast to engineered drugs because of faster rate of development and cheaper prices[4]. As per World Health Organization (WHO), about 80% of the total populace utilises spices and other customary prescriptions for their essential wellbeing care[5] indicating the widespread acceptability of herbal formulations as therapeutic agents for various disorders[6]. Therefore, appraisal of the wellbeing, adequacy and nature of home grown medicines are essential for worldwide harmonization of home grown drugs, so the time appears to be ready for botanicals of better quality.

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Plant inferred items are progressively being searched out as restorative items, nutraceuticals and cosmetics[7]. For that standardisation of herbal product is need of time at the level of collection, processing and after formulation[8]. JRA has been prepared as proprietary medicine containing *Hingula, Shilajatu, Aakarakarabha (Anacyclus pyrethrum)*, *Ashwagandha (Withania somnifera)*, *Mushali*, *Shatavari* (A. racemosus) *Vidarikanda (Pueraria tuberosa)* and prepared with the aim for management of erectile dysfunction on the basis of pharmacological properties of its ingredients such as quoted in classical as well as contemporary research. *Pueraria tuberosa* DC (Fabaceae) is used as aphrodisiac agent[9] and *A. racemosus* restores the sexual dysfunctions[10]. Furthermore, *Withania somnifera*, have potent aphrodisiac activity, Shilajit is employed for the management of male reproductive disorders[11] and Musli (*Chlorophytum borivilianum*) is aphrodisiac and sexual stimulant[12]. The formulations contain mixtures of different plant samples, which are multi component in nature and expected to act synergistically, therefore beneficial for treatment of

erectile dysfunction.The formulations developed in the present investigation require further evaluation of its quality control measure which includes qualitative and quantitative estimation to establish their utility beyond reasonable doubt.

Materials and Methods

Procurement & Identification

Hingula, Shilajatu, Aakarakarabha, *Ashwagandha*, *Mushali*, *Shatavari, Vidarikanda, Ghee* and sugar were procured from Gola Dinanath market, Varanasi. *Hingula* and *Shilajatu* were authenticated by concern experts of Faculty of Ayurveda of BHU with vouchers no. RSBK/17-18/03, RSBK/17-18/04, *Anacyclus*/DG/2017/01, *Withania*/DG/2017/02, *Mushali*/DG/2017/03, *Asparagus*/DG/2017/04 and *Pureria*/DG/2017/05.

Preparation of *Jeeva Rasa Avaleha*

Before preparation of *Jeeva Rasa Avaleha, shodhana* of *hingula* and *shilajitu* were carried out as per reference of *Rasa Tarangini*[13, 14]and powder of *Aakarakarabha*, *Ashwagandha, Shweta, Mushali, Shatavari* and *Vidarikandawere* were prepared separately and strained through 80 mesh of sieve to get homogenous powders as per the reference of API[15]. After that by using these drugs JRA was prepared as per reference of *Sharangdhara Samhita* and AFI reference[15,16] in the departmental laboratory.

Method of preparation

All the ingredients were taken in the ratio mentioned in Table 1. *Chasani* was prepared by boiling of sugar in potable water up to desired concentration and then powder of all ingredients [1-6 of Table 1] were added as *prakshepa* and homogeneously mixed, and *ghrita* was added. After appearance of desired characteristics of *Avaleha, Jeeva Rasa Avaleha* was packed.

Table 1 — Composition of *Jeeva Rasa Avaleha*

S.N.	Ingredients	Ratio	Percentage
1	*Shuddha Hingula*	20 g	0.57
2	*Shuddha Shilajatu*	80 g	2.29
3	*Aakarakarabha*	80 g	2.29
4	*Ashwagandha*	80 g	2.29
5	*ShwetaMushali*	80 g	2.29
6	*Shatavari*	80 g	2.29
7	*Vidarikanda*	80 g	2.29
8	*Ghrita*	400 mL	11.42
9	Sugar	2.00 g	57.14
10	Water	600 mL	17.13
	Total parts	3.500 kg	100 %

Analytical standardisation of *Jeeva Rasa Avaleha*[17]

The physicochemical parameters of *Jeeva Rasa Avaleha* were performed to validate pharmaceutical processes. Analysis of JRA was carried out in term of qualitative and quantitative analysis for standardisation of JRA.

Qualitative analysis

Substances are recognized or ordered based on their concoction or physical properties, which looks for data about the character or type of substance present

Reducing sugar

Two mg of JRA was dissolved in 2ml of purified water in a test tube to prepare the test solution. 2 mL of Benedict's solution was added in to the test solution and heated in a water bath at 60°C for 10 min. The color of solution turned yellowish to orange, which is indicative of reducing sugar.

Non reducing sugar

Two mg of JRA sample was dissolved in 2 mL of distilled water to prepare the test solution, in a test tube. 1 mL of dilute Hydrochloric Acid was mixed and boiled for 1 minute. When the solution cooled, 1 mL of Sodium bicarbonate was mixed. Then the combination was heated in a water bath at 60°C for 10 min. Yellowish brick red precipitate appeared.

Determination of fat

1 mL of Liebermann B solution was added in 2 mL of ethanolic extract of JRA . Mixture was heated on a water bath for 10 min at 60°C. The color of solution turned dark purple green which is indicative of fat in JRA.

Quantitative analysis

Amount or concentration of an analyte may be determined (estimated) and expressed as a numerical value in appropriate units

Determination of loss on drying

Two g of sample was taken in a perfect, dried and tarred silica pot. The sample was kept in a stove at 105°C for 5 h. After 5 h crucible was picked out from oven and weighed it.

Determination of total ash value

Two g precisely gauged test was taken in a tarred silica pot and burned at temperature not surpassing

450°C, until liberated from carbon. The example was cooled and gauged. At that point the level of debris regarding air dried example was determined.

Determination of acid insoluble ash

The obtained ash was transferred in to flask containing 25 mL of 6 N HCl and boiled for 5 min now filtered, washed and ignited to constant weight. After that percentage of insoluble ash was calculated respect to the air-dried drug.

Determination of water soluble ash

The debris were heated up for 5 min with 25 mL of water, insoluble matter was gathered in a Gooch pot, or on a debris less channel paper, washed with boiling water and touched off to consistent load at a low temperature.

Determination of alcohol soluble extractive value

Two g of medication was taken in tapered cup and 50 mL of ethanol was added and shacked for 6 h, persistently then permitted to represent 18 h. Following day, the concentrate was sifted. The filtrate was dissipated to dryness in tarred vanishing dish on water shower and dried at 105°C to a consistent weight.

Determination of water soluble extractive

2 g of JRA was taken in a cone shaped carafe, 50 mL of refined water was added and shacked persistently for 6 h. Then allowed to stands for 18 hrs then filtered. The filtrate was dissipated to dryness in tarred vanishing dish on water shower and dried at 105°C to a steady weight.

Determination of pH

Ten g of medication was broken up in to 100 mL refined water by macerating 2 h and afterward sifted it and filtrate was utilized for deciding of pH by utilizing pH meter (Eutech Instrument Company, Model no. EU780480).

Thin layer chromatography (TLC)

TLC was carried out by using TLC plate silica gel 60 F_{254} (Merck) 10×10 cm with mobile phase Benzene: Ethyl acetate (8:2) and spraying with Liebermann Buchard Reagent (Anisaldehyde : glacial Acetic acid : Sulphuric acid 97%; 0.5: 50:1) for examination of spots in the chromatogram.

Energy dispersive X-ray spectroscopy (EDX)

The percentage weight of elemental analysis of *Jeeva Rasa Avaleha*, was done by Energy-dispersive X-ray spectroscopy study[18,19] (Instrument ZEISS EVO 18 model).

Atomic Absorption Spectroscopy (AAS)

The quantitative assessment of Zinc, Cadmium and Lead were estimated as per the method described by Gomez *et al*., 2007[20] by atomic absorption spectrophotometer (Shimadzu–AA6300).

Results

Water soluble extractive & loss on drying was 15.12% and 9.80% respectively and pH & ash value was 6.71 & 1.04% respectively (Table 2). In TLC study, three spots were observed at R_f 0.105, 0.245 and 0.672 in mobile phase of Ethyl acetate: Methanol (8:2) and 0.618, 0.872, 0.90 in mobile phase of Benzene: Ethyl acetate (8:2). EDX study showed that JRA have maximum 50.6% of oxygen and minimum 0.08% of silica (Table 3) and AAS study showed that heavy metals were within normal limit (Table 4).

Discussion

Ash value represents the presence of inorganic salts in drug. Therefore the determination of ash value plays an important role to justify the identity and purity of

Table 2 — Qualitative and quantitative study of *Jeeva Rasa Avaleha*

Sr. No.	Parameters	Findings
1	Loss on drying	9.80%
2	Total ash value	1.40%
3	Acid insoluble ash	0.13%
4	Water insoluble ash	0.93%
5	Water soluble extractive	15.12%
6	Alcohol soluble extractive	0.40%
7	pH value	6.71
8	Reducing sugar (qualitative)	+
9	Non reducing sugar (qualitative)	+
10	Determination of fat (qualitative)	+

Table 3 — EDX analysis of *Jeeva Rasa Avaleha*

Element	Weight%
C	48.45
O	50.06
Mg	0.14
Si	0.08
Ca	0.88
Hg	0.39
Totals	100.0

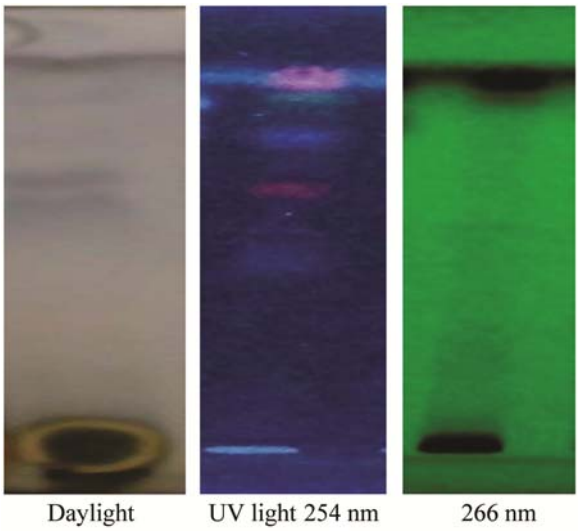
Table 4 — AAS study of *Jeeva Rasa Avaleha*

Zn	0.6530 ppm
Cd	0.0021 ppm
Pb	0.8757 ppm

the sample. The water solvent debris is the piece of the complete debris content, which is dissolvable in water, is acceptable pointer of either past extraction of water dissolvable salts in the medication or inaccurate detachment. The water dissolvable extractive worth demonstrated the nearness of sugar and acids in the compound. The liquor dissolvable extractive worth demonstrated the nearness of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and optional metabolites present in the plant test.

Total ash represents inorganic contents in any formulation whereas acid-insoluble ash indicates acid insoluble inorganic content. Higher inorganic content indicates adulteration of raw ingredients by substances such as silica etc. Total ash value and acid insoluble ash of JRA is 1.40% and 0.13% respectively (Table 1). This indicate that formulation have very less percentage of inorganic content which further indicate that although adulterant is present but their presence is in negligible percentage. During determination of acid insoluble ash, drug was passed through acidic solution as the same course follows the drug after intake. So the acid insoluble ash test is therapeutically very important. Less the acid insoluble ash, it should be physiologically more available in human body. The less acid-insoluble ash may affect absorption in gastrointestinal canal. Moisture content reduces the chance of microbial contamination (bacterial and fungal growth) and decomposition due to unwanted chemical transformations and also helps to determine the stability of the drug. Lower moisture contents indicate more stability of the drug. JRA formulation have very less percentage of moisture so chance of microbial contamination is very less and formulation is stable for longer time.

EDX study showed that 0.39% of cinnabar present in formulation, the reduction in cinnabar (*Hingula)* percentage may be due to *Shodhana* process which was done with *Bhavana* of ginger juice. Ginger is a rich source of micro elements and probably acts as binding agent and has good antioxidant property. EDX is a diagnostic procedure utilized for the natural examination or substance portrayal of an example. In *Jeeva Rasa Avaleha* sample, the elemental concentrations were Carbon (48.45%), Oxygen (50.06%), Magnesium (0.14%), Silica (0.08%), Calcium (0.88%) and Mercury (0.39%) (Fig. 2). The concentration of Mercury was more due to the presence of *Hingula* in the formulation (Fig. 1). The AAS study of JRA revealed that heavy



Daylight UV light 254 nm 266 nm
Solvents — Benzene: Ethyl Acetate (8:2) Benzene: Ethyl Acetate (8:2)
Fig. 1 — TLC pictures showing spot in daylight and UV light at wavelength 254 and 266 nm

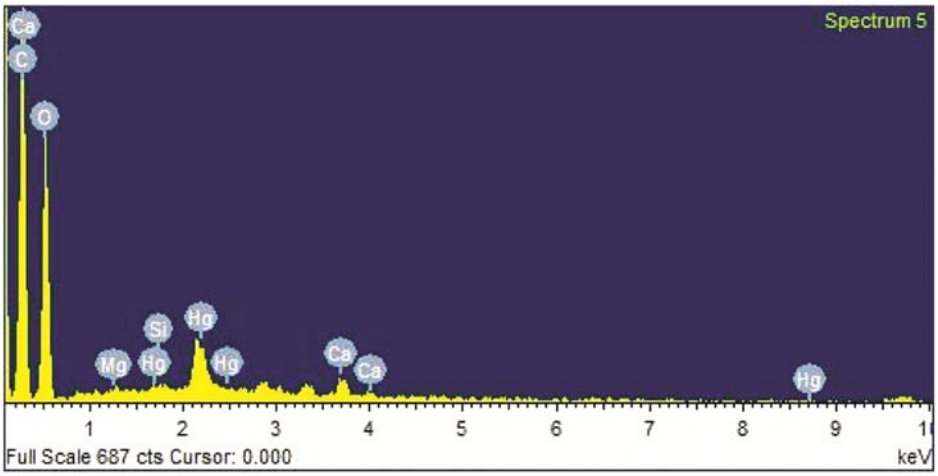


Fig. 2 — Elemental analysis graph of Jeeva Rasa Avaleha

metals are present in the sample but in permissible limit. *Shodhana* of *Shilajatu* was done through *Triphala Kwatha* as *Triphala* has various qualities like antioxidant property, hepato-protective property etc.[21]and acts as binding agent[22] therefore it might reduce harmful elements of *Shilajatu* and adds beneficial elements. The percentage of *Shilajatu* in JRA is approx. 2.7% and the sample of JRA taken for EDAX or AAS was in very small amount furthermore the instrument analyzes only few point not as whole sample hence it may be the reason to not detect Iron in JRA.

Conclusion:

The herbal drugs used in the formulation are easily available as they grow widely in India and hence rendering the formulations is economical to produce. Qualitative and quantitative analysis of JRA revealed that all the parameters were within normal limit which indicate formulation is safe and may be prescribed for the management of Erectile Dysfunction.

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Conflict of Interests

Nil

Author Contributions

Concepts and design of research has been carried out by V K, T S and A K C. The article was written and critically reviewed and K D Y. All authors read and approved the final manuscripts

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