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Research Article

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Nutritional Profiling and Heavy Metal Analysis of *Withania Somnifera* Based Polyherbal Formulation- Aswagandhadi Lehyam

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Abstract: Aswagandhadi lehyam, is one of the most popular formulations prescribed as Rasayana (tonic) in the traditional medical system of India, Ayurveda. As like other lehyam formulations, Aswagandhadi lehyam prepared as per the avaleha Kalpana in ayurveda. The formulation received its name from its major ingredient, Ashwagandha (*Withania somnifera*). Even though the Aswagandhadi lehyam used generally as rasayana, its major ingredient Ashwagandha has various medical benefits. Ashwagandha is compared well with *Eleutherococcus senticosus* (Siberian Ginseng) and *Panax Ginseng* (Chinese / Korean Ginseng) in its adaptogenic properties, and hence it is popularly known as Indian Ginseng. Since it is used as majorly as rasayana, in addition to its pharmacological studies and chemical standardisation its nutritional profiling also needs to be studied. In the present study the nutritional profiling of the Aswagandhadi lehyam is studied, which will provide a better idea regarding the nutritional values while consumed generally for increasing physical strength, endurance, stamina, vitality and vigor. It is also important to have an understanding regarding the heavy metal contamination of the formulation. Heavy metal analysis of the formulation is done in Inductively coupled plasma mass spectrometry (ICP-MS). The *aswagandhadi lehyam* prepared as per avaleha kalpana and Sahasrayogam, is regarded as the industrial working standard, and is considered for the study.. The organoleptic analysis reported - as smooth, dark brown sweet product, pleasant aroma and creamy texture. Nutritional profiling results evidenced the health benefits of the formulation including ~389 kCal of energy value, 2.1% fibre content etc. Heavy metal contamination study done with ICPMS reported the presence of trace elements like Mercury (Hg), Arsenic (As), Lead (Pb), and Cadmium (Cd) in a range of 0.02-0.39 ppm.

Keywords: *Withania somnifera*, ayurveda formulation, rasayana, nutritional profiling, heavy metal analysis

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1. INTRODUCTION

Avaleha Kalpana is an important pharmaceutical preparation in Ayurveda. Avaleha Kalpana ensures, absorption of active therapeutic properties of ingredients. The literary review of Avaleha suggests that these are preparations prepared from aqueous extracts of the plants used as a base and powders, pulp, sugar and Prakshepa are incorporated to the base Kashaya or prescribed liquid media. There are chief ingredients, Kwatha Dravyas, sweetening agent and Prakshepa Dravyas etc. Avaleha is a semisolid preparation of herbal drugs prepared in decoction or extracts of different herbs by adding sweetening agents like jaggery, sugar or sugar candy[1]. The word Avaleha has been derived from the root word "lihaswadane", in which 'lih' means substance which is licked and 'aswadane' means that which has good taste[2]. It is claimed that the therapeutic activity of the Avaleha preparation depends on the drugs which are thus used. Ashwagandhadi lehyam is one among the most popular avaleha preparations with ashwagandha as a major ingredient (Sahasrayogam). The formulation received its name from its major ingredient Aswagadha (*Withania somnifera*). Which is also called Indian Ginseng, and is compared well with *Eleutherococcus senticosus* (Siberian Ginseng) and *Panax Ginseng* (Chinese / Korean Ginseng) in its adaptogenic properties[3]. In Ayurveda ashwagandha is majorly used in Rasayana formulations and prepared in the form of Lehya[4,5]. Ashwagandha is an important ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity [6,7]. It is widely used in Ayurvedic medicine, the traditional medical system of India. Critical analysis of the published literature suggests an anti-cancer potential of WS with a key role in cancer prevention[8]. The study on CHO cells has scientifically evidenced the antitumor efficacy of Ashwagandha, which showed an inhibitory rate of 49%[9]. The anxiolytic study result of ashwagandha proved its antistress property[10]. The chemical and pharmacological standardisation studies have been done already on Ashwagandhadi lehyam, with FTIR and HPLC fingerprinting. The heavy metal analysis of the product is done on Atomic absorption spectrometry (AAS), which reported the basic physico-chemical analysis of the formulation[11]. The HPTLC fingerprinting is also done in a much broader way by comparing various market samples available along with withanolides, the active ingredients of ashwagandha[12]. The basic chemical analysis like the pH, ash etc has been done already to provide a basic understanding in previous studies[13]. Antimicrobial potential is also an important parameter regarding generic medicine like tonics, which have been established against E coli like microbes[11]. Ashwagandhadi Lehya helps as a health tonic in reducing complaints related to aging like dizziness, aching muscles, urge incontinence, gener- alised weakness etc. This indicates that regular consumption of Ashwagandhadi Lehya can reduce the deleterious effects of aging and improve their general health by facilitating an active, independent and stress-free life in elderly population[14]. In addition to the pharmacological aspect, it is also important to understand the benefits of general consumption of the ashwagandhadi lehyam as it is generally prescribed as rasayana (tonic). Nutritional profile analysis of the product will exactly provide the nutritional values per quality of the formulation. Since the formulation includes herbal ingredients, fat and jaggery, the energy value and fibre content of the product need to be studied, and will give a scientific support to its claim as tonic. Previous studies of heavy metal analysis have been done with Atomic Absorption Spectophotometer and this study aimed to conduct the heavy metal contamination analysis with the help of more accurate ICP-MS instrumentation system.

2. MATERIALS AND METHODS

Table 1: Ingredients of Aswagandhadi Lehyam

Sl.No	Ingredients	Scientific name	Quantity
1	Ashwagandha	Withania somnifera Pauquy	100g
2	Ellu	Sesamum indicum L.	100g
3	Uzhunnu	Vigna mungo (L.) Hepper	100g
4	Thippali	Piper longum L.	100g
5	Ghee		As needed
6	Jaggery		As needed

Formulation prepared as per the Sahasrayogam reference. Table 1- Showing the ingredients of ashwagandha rasayana

2.1. Physico chemical study

Once the formulation is prepared as per references and avaleha kalpana, the sample is subjected to organoleptic analysis, nutritional profiling and heavy metal analysis.

2.2. Organoleptic study

Sensory analysis done to study the organoleptic characters like Appearance, Taste, Smell and consistency of the product.

2.3. Nutritional profiling

Nutritional profiling of the product is done as per AOAC methods [5] and method designed in similar study for *Brahma Rasayana* is also considered for this study[7]

2.3.1. Total Fat

20g of sample is weighed in thimble and extracted the same with petroleum ether at 60- 80 °C for 6 hours in soxhlet. After six hours evaporated the petroleum ether by keeping it in a water bath. The extract is weighed.

Fat % =weight of the extract/weight of the sample *100

2.3.2. Total sugar

2.3.2.1. Standardization of Fehling's solution

5 ml of solution A and solution B were pipetted out accurately into a conical flask and added 20 ml distilled water to it. Boil ed the mixture and standard dextrose from the burette was added. 1ml Methylene blue indicator added towards the end of reaction while keeping the solution heating. The end point of titration being indicated by change of colour from blue to red. Strength of $CuSO_4$ can be calculated from the sugar solution used and will give the quantity of sugar required to reduce the copper in 5 ml $CuSO_4$ Solution.

2.3.2.2. Estimation of Total Sugars

1 g of the sample is weighed accurately in a beaker and dissolve it in 50 ml water. 1ml HCl is added to it and heat to boil. The solution kept overnight for inversion. After inversion neutralise the solution using sodium carbonate solution. The solution is transferred to 100 ml volumetric flask and made up to with water later transferred to burette. 5 ml each of solution A and solution B were accurately pipetted to a conical flask and added 20ml water. A few ml of solution from burette is added and heated to boil. 1ml of methylene blue indicator is added towards the end of the reaction. Titration completed within three minutes, the end point being indicated by the change of colour from blue to red. Volume in ml of the solution required for titration is noted.

$$\text{Strength of fehling's solution} = \frac{\text{Titre volume of std dextrose (ml)} * \text{weight of dextrose(g)}}{\text{Volume of fehling's solution}}$$

$$\text{Total sugar percentage (\%)} = \frac{100 * \text{Strength of fehling's solution} * 100}{\text{Weight of sample (g)} * \text{titre volume (ml)}}$$

2.3.3 Crude Fibre

50g of Sample is weighed and dried by keeping it in a water bath. 5g of the dried sample is weighed accurately in a thimble and extracted for about 1 hour with petroleum ether in a Soxhlet extractor. The material in the thimble is transferred to a 1 litre flask. 200 ml of dil. sulphuric acid taken in a beaker and brought it to boil. The boiling acid is transferred to the flask containing sample and immediately connected to the flask begins to boil within 1 minute. Flask is rotated frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid. Continued boiling for exactly 30 minutes. The flask and filter are removed through fine linen or through a coarse acid wash. Hardened filter paper held in a funnel and weeds with boiling water until the washing is no longer acid to litmus paper. Some quantity of sodium hydroxide solution is brought to boil under a reflux condenser and transferred the residue on the filter into the flask with 200 ml of boiling sodium hydroxide solution. Immediately connected the flask with the reflux condenser and boils for exactly 30 minutes. The flask is removed and immediately filtered through the linen or filter paper. The residue washed thoroughly with Hardened filter paper held in a funnel and wash with boiling water until the washing is no longer acid to litmus paper. Some quantity of sodium hydroxide solution is brought to boil under a reflux condenser and transferred the residue on the filter into the flask with 200ml of boiling sodium hydroxide solution. Immediately connected the flask with the reflux condenser and boils for exactly 30 minutes. The flask is removed and immediately filtered through the linen or filter paper. The residue washed thoroughly with hot water and transferred to a Gooch crucible prepared with a thin but compact layer of asbestos. The residue washed thoroughly first with hot water and then with about 15 ml of ethanol and with 3 successive washings of petroleum ether. Gooch crucible dried and contents kept in an air oven at 105 ± 1^0c for 3 hours. Cool and weigh. Repeat the process of drying for 30minutes, cooling and weighing need to be continued until the difference between two consecutive weighings reach below 1 mg. Incinerated the contents of the Gooch in a muffle furnace at 550 ± 20^0c until all carbonaceous matter is burnt. Gooch crucible is cooled in a desiccator and weighed.

Table 2: Organoleptic profile

Sl.No.	Parameters	Results
1	Appearance	Dark brown
2	Taste	Sweet, creamy
3	Smell	Characteristic
4	Consistency	Semisolid

Where,
M_1 = Mass in g of Gooch crucible and contents before ashing,
M_2 = Mass in g of Gooch crucible containing asbestos and ash,
M = Mass in g of the material taken for the test,
H = Moisture content of the sample as received in percent

2.3.4 Total protein

Total protein content in sample is analyzed by kjeldahl method with 2g of sample (AOAC International 1995)[15].

Table 3: Nutritional profile		
Sl.No.	Parameters	Results
1	Energy value	389.14 kcal/100 gms
2	Total fat	6.7%
3	Cholesterol	0
4	Carbohydrate	81.4%
5	Total sugar	52.4%
6	Protein	0.81%
7	Crude Fibre	2.1%

Table 4: Nutritional profile		
Sl.No.	Heavy metals	Results
1	Mercury (Hg)	0.02 ppm
2	Lead (Pb)	0.19 ppm
3	Cadmium (Cd)	0.15 ppm
4	Arsenic (As)	39. ppm

2.3.5 Cholesterol

Cholesterol in the sample is determined by using GC FID detector using standard sterol

2.3.6 Carbohydrate

Total carbohydrate in the simple is determined by calculation.

Total carbohydrate = 100 - weight in grams of protein + fat + Water + Ash +

3. RESULT AND DISCUSSION

3.1. Organoleptic profiling

Table 2 - Showing the organoleptic profile of Aswagandhadi lehyam. Like other formulations prepared based on Avaleha Kalpana, the sample is of semi solid creamy texture. Presence of jaggery has contributed towards the sweetness and thippali towards the slight pungent feel of the product. The thippali and ellu might be the reason behind its good characteristic aroma.

3.2. Nutritional profiling

Table 3 - Nutritional profile of the *Aswagandhadi lehyam* is analysed and charted. Since the main ingredient in the formulation is Aswagandha root, the obtained nutritional profile values is primarily compared with that of the Aswagandha root. Studies of nutritional values of roots of aswagadha collected from two different sources in India, namely Karthikere, Chickamanglore district and Sondekola, Chithradurga district done previously, reported a total protein of 0.621% ad 1.631% respectivly, where our formulation reported 0.81%[16]. The aswagandhadi lehyam was reported a Total fat content of 6.7%, where in previous study of Ashwagandha, it is reported 1.138% and 0.328%, which is clearly because of the ghee added in the formulation. The fibre content of the Ashwagandha root found in previous study is 5% and 4% respectively, where our formulation reported the same as 2.1% only, due to the addition of non fibrous ingredients also. The carbohydrate value of aswagandha showed a wide range of 23.34% to 63.37% in previous study might be because of the influence of condition of farming in two different districts, and the aswagandhadi lehyam has reported 81.4% indicates the prescience of carbohydrates from other ingredients especially jaggery. Coming to the previous studies, the study on Brahma rasayanam, similar Ayurvedic formulation, prepared as per avaleha Kalpana also satisfies similar results in nutritional values[16]. 2.1% of fibre content in the formulation is a very good sign of the products health benefits which is only 1.37% in previous study. The protein content the formulation is resulted 0.81% and fat content is 6.7% in current study and similarly 0.77% and 7.09% respectively for both parameter in brahma rasayanam. In aswagadhadi lehyam, the total carbohydrate content is 81.4% where the total sugar content is 52.4%, while in brahma rasayanam the carbohydrate and sugar content is 77.69% and 50.72% respectively.Same like the studies on brahma rasayana the cholesterol value is nil in the current formulation. The energy value in the aswagandhadi lehyam is resulted as 389.14kcal/100g and which satisfies the previous history of 375.65 kcal/100g of the brahma rasayanam. The non detection of cholesterol in the sample and high energy value gives a clear nutritional mark to the *Aswagandhadi lehyam.*

3.3. Heavy Metal Analysis

Table 4 - The formulation is tested for the presence of heavy metals using ICPMS. As discussed in Nutritional profiling the results obtained from heavy metal study of formulation, is compared with that of the previously done Withania somifera, the major ingredient in the formulation. Since heavy metals are generally considered as poisonous, the reposrts are thus compared. Lead is regarded as highly hazardous for plants, animals and particularly for microorganisms. The main sources of lead pollution in agriculture and plants are lead mines, fuel combustion, sewage sludge applications and farmyard manure. The maximum acceptable concentration for food-stuffs is around

1 mg/kg. Long-term exposure to lead can result in a buildup of lead in the body and severe symptoms. These include anemia, pale skin, a decrease handgrip strength, abdominal pain, nausea, vomiting and paralysis of the wrist joint. Prolonged exposure may also result in kidney dam- age. If the nervous system is affected, usually due to very high exposure, the resulting effects include severe headache, coma, delirium and death. Continued exposure can lead to decreased fertility and/or increased chance of miscarriage or birth defects[17,18]. The Lead presence of the roots of aswagadha collected from various spots of India reported 0.16, 0.1 and 0.06ppm where the current study reported the same as 0.19ppm, which is satisfiable in the light that the formulation includes other herbal ingredients also and still maintains the level under 1ppm. Cadmium is a toxic metal and can cause serious health problems. Recently attention has been focused on its availability in water, soil, milk, dietary products, medicinal plants, herbal drugs, etc. The most common sources for cadmium in soil and plants are phosphate fertilizers, non-ferrous smelters, lead and zinc mines, sewage sludge application and combustion of fossil fuels[19,20]. Critical levels for cadmium in soil are between 3-5 mg/kg[21]. The cadmium is found not detected in the roots of Aswagandha in previous studies which was collected from various spots, but there is a trace of 0.15ppm presence in aswagadhadi lehyam. The detection is negligible since the critical level is between 3-5ppm, even in the ingredient, but the presence might be resulted from other herbal ingredients. Since the similar study on Aswagandha rasayanam for evaluating the presence of heavy metal by using AAS instrumentation, resulted in slightly high value of trace elements but satisfiable[11]. In the light of the previous study which reported the presence of 0.05ppm mercury, our study reported 0.02ppm. cadmium and arsenic have reported a presence of 0.15 and 0.39ppm in our current study while the previous study reported 0.18 and 0.48ppm. There is a significant deviation from the previous study in the value of lead, which is on a very high-level of 3.2ppm in previous study where the present study reported a presence of 0.19ppm. The high value reported in the previous study might be because of the area of sample collection, lack of proper preprocessing or instrumentation variation. The chances of such a high detection of heavy metals in the product is not satisfiable as similar study on the Brahmarasayana also reported zero detection of trace elements in previous study[22].

4. CONCLUSION

Aswagandhadi lehyam is one of the most popular Rasayana formulation used in India under Ayurvedic Medicine. The formulation is prepared based on Avaleha kalpana, which ensures the proper absorption of active ingredients in the raw materials used. Scientific study on the formula helped to create an in-house quality standard for the Aswagandhadi lehyam. The nutritional profiling and heavy metal analysis provided an introduction to the Rasayana effect of formulation by stating its nutritional values. The negligible values of heavy metal presence in the formulation providing a significant support to its health benefits as a rasayana. Vast scope is pending for researchers to explore on the Pre-clinical and clinical studies of the formulations.

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6. AUTHORS CONTRIBUTION STATEMENT

I have carried out the experiments. Madhan Shankar have guided, supervised and encouraged Ben Raj in the findings of this work. Both the authors discussed the results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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