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Amla, Ashwagandha and Shatavari Formulations as Herbal Medicines and Nutraceuticals

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Abstract

Three herbal formulations namely, Amla, Ashwagandha and Shatavari Capsules, were examined for general test parameters, at different production stages, and a few basic nutritive test parameters. The general test parameters and assays were found to be satisfactory and the nutritive values were found to be quite significant. This proves that the three formulations can be used as herbal medicines, and the significant nutritive values prove that they can be used as dietary supplements.

Keywords: Amla, Ashwagandha, Shatavari, Herbal medicine, Dietary supplements.

Introduction

Herbs are staging a comeback and "herbal renaissance" is happening all over the globe. The herbal products, today, symbolise safety, in contrast to the synthetics that are regarded as unsafe to humans and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities, for centuries, the synthetic products of the modern age, surpassed their importance for a while. However, the blind dependence on synthetics is over and people are returning to the naturals, with the hope of safety and security[1].

Herbal medicine is still the mainstay of about 75 – 80% of the world population, mainly in the developing countries, for primary healthcare, due to better cultural acceptability, better compatibility with the human body and lesser side-effects[2]. The same, has been supported by Ogbe et al[3], in their study. The chemical constituents of herbal drugs, are a part of physiological functions of living flora, and hence, they are believed to have better compatibility with the human body. Nowadays, different types of plants are used in herbal medicine. Mahesh et al[4] have already proved the importance of *Boerrhavia diffusa* to treat large number of human ailments. The movement of certain minerals and plant-made compounds, into the body of humans, is called "Nutrition" [5]. The cells of the body must have a ready supply of carbohydrates and fats, for tissue integrity to be maintained and as a fuel for metabolism, a supply of proteins for building materials, and various miscellaneous components like vitamins and minerals as catalysts in biochemical reactions. These raw materials that the cells need from outside are called "nutrients", and their primary source is the plant cells, where all these nutrients are combined in ideal proportions and are neatly packaged for our use[6]. Herbal drugs are derived either from the whole plant or from their different parts like leaves, bark, roots, flowers, seeds, etc., and also from plant excretory products like gums, resins and latex.

The Chinese have maintained a centuries-old tradition of using various herbs and nutritional supplements, to treat many maladies of the body and spirit. Some herbs have been used only medicinally, others purely as food ingredients, but many more serve as both, food and drug. Today, their use is expanding, and therefore, it is important to evaluate and document the safety of those herbs, in common use, as food supplements[7].

The general analysis and nutritive study, thus evaluates, whether the drugs can be used as food supplements. A dietary supplement is a product taken by mouth that contains a dietary ingredient, intended to supplement or enhance the diet. They can be consumed in the form of tablets, capsules, soft-gels, gel-caps, liquids or powders. Dietary supplements are alternatives to conventional foods, for physical and mental well-being[8].

Nutritive value is defined as an indication of the contribution of a food to the nutrient content of the diet. Ranganathan S. et al[9] studied the nutritive value of Indian foodstuffs.

The three formulations chosen for nutritive value analysis were, single ingredient capsules of Amla, Ashwagandha and Shatavari. Amla consists of the fresh or dried fruit of *Emblica officinalis*. Amla is rich in tannins, gallic acid, ellagic acid and glucose, pectins and vitamin C. Amla is a very good liver tonic, a gentle purgative, an astringent and diuretic, and the richest known source of vitamin C, thus showing anti-scorbutic activity[10] and Amla has a very high nutritive value. Since vitamin C is heat and light sensitive, its content may vary during various processing methods.

Ashwagandha consists of dried roots and stem bases of *Withania somnifera.* It is rich in proteins, free amino acids like aspartic acid, glycine, tyrosine, alanine, praline, tryptophan, glutamic acid, cysteine, etc., starch, reducing sugars, alkaloids, steroidal lactones. Thus, it acts as a nutritive tonic, stimulant and offers powerful nutritional support, energy and rejuvenation. It is a good source of folic acid, thus is vital for foetal neuronal growth and blood formation[11]. The use of ashwagandha as a popular herbal medicine has been supported by Subhas I. et al[12].

Similarly, Shatavari consists of tuberous roots of *Asparagus racemosus*. It is rich in saponin glycosides. Shatavari is a galactogogue and a powerful nutritive tonic and rejuvenative, which can be given to a person with any type, constitution, sex, age. Charaka has categorized it as balya, promoting strength[13].

Thus, to prove that these three single ingredient formulations can be used as nutritive supplements, their nutritive value analysis was carried out.

Material and Methods

Methods for Extracts:, Description: The sample was spread in a clean, previously dried Petri dish and visually observed with the naked eyes. The colour and nature (form) of the sample was recorded.

Loss On Drying at 105°C[14]: About 1g sample was weighed in an LOD bottle. The sample was dried at 105°C for about 6 to 7 hours, cooled to room temperature in a desiccator and weighed till constant weight.

Total Ash[14]: About 1g sample was accurately weighed in a crucible. The sample was ignited at about 550°C to 600°C for 4 to 5 hours, then allowed to attain room temperature in a desiccator and weighed till constant weight.

Acid Insoluble Ash[14]: The ash obtained above was dissolved in about 25ml dil. HCl, boiled for about 15minutes and filtered through ashless filter paper No.41. Washings with hot water were given to the residue till the filtrate was free from acid. The filter paper was dried in an oven and incinerated at 550°C to 620°C for about 3 to 4 hours. Then the residue was cooled to room temperature in a desiccator and weighed till constant weight.

Water Soluble Extractive[14]: About 1g of sample was soaked overnight, in 100 ml water and slightly warmed. Next day, this solution was filtered and 25 ml of filtrate was dried at 105°C, to constant weight, in a petri dish.

Alcohol Soluble Extractive[14]: About 1g of sample was soaked overnight, in 50 ml alcohol. Next day, this solution was filtered and 25 ml of filtrate was dried at 105°C, to constant weight, in a petri dish.

pH of 1.00% w/v solution[14]: About 1 g of sample was dissolved in 100 ml water, slightly warmed, cooled and the pH was recorded on a suitable, previously calibrated pH meter.

Content of Total Tannins[15]: Appropriate dilutions of sample and tannic acid standard were prepared, and color development was carried out, as given under the colorimetric procedure by S. Ranganna. Optical densities of sample and standard, were recorded at 760nm and content of tannin was calculated.

Content of Total Alkaloids: About 3g of Ashwagandha extract was accurately weighed and 5ml of ammonia solution was added to it. The contents were transferred to a suitable Soxhlet apparatus and extracted for 18 hours with 60ml of 95% ethyl alcohol. The alcohol was removed by distillation. The remaining soft extract was extracted with 5 X 25ml of 1N HCl. The contents were then transferred to a separating funnel and washed with 10ml chloroform. Then, the pH of acid solution was adjusted to alkaline, to litmus paper, with ammonia solution and this solution was then extracted with 5 x 25ml chloroform. The chloroform layer was washed with 10ml water and evaporated to dryness on a water bath. Then, 10ml alcohol was added to the dried residue and evaporated to dryness, to constant weight. The content of total alkaloids was calculated.

Observations:
(i) Weight of the sample (A) = _____ g
(ii) Weight of empty conical flask (B) = _____ g
(iii) Weight of conical flask + Residue (C) = _____ g

Calculation:
Total Alkaloids (%w/w) = (C – B) X (100 / A)

Content of Total Saponins: About 3g of shatavari extract was accurately weighed and extracted with 90%v/v methanol (25ml) by refluxing for half an hour. The residue was extracted two more times by taking 25ml methanol. The methanolic extracts were combined and the solvent was distilled off. After distillation of alcohol, the soft extract obtained, was treated with with petroleum ether 60-80°C, 25ml by half an hour. After cooling, the solvent was removed by decantation and discarded. Now, the same soft extract was treated successfully with chloroform 25ml and ethyl acetate 25ml, under reflux, for half an hour and after cooling, the solvents were removed by decantation and discarded, keeping the soft extract in the same flask. After the three extractions cited above, the soft extract was dissolved in 25ml of 90%v/v methanol, and transferred into a beaker. Then, with constant stirring, acetone was added into the solution in the beaker, for complete precipitation of saponins. Then, the precipitate was filtered and dried to constant weight at 105ºC. The content of total saponins was calculated.

Calculations :
% of Saponin = (Weight of the dried precipitate) X 100/wt of the spl

Methods for Capsule Formulations: Description: The capsule size was observed. Then, the capsule was opened and the colour and form (appearance) of the contents was recorded.

Disintegration Time[16]: 6 capsules were put in a suitable disintegration time machine along with sliding discs. The temperature of water was maintained at 37°C. The disintegration machine was switched on and the time required to disintegrate all the 6 capsules was recorded.

Average filled weight of capsule[16]: The contents of 10 capsules were removed and this powder was accurately weighed on a suitable balance and the weight was recorded as the net filled weight.

The tests for Loss on Drying at 105°C, total ash, acid insoluble ash, alcohol soluble extractive, pH of 1.00%w/v solution, content of total tannins, content of total alkaloids and content of total saponins are similar to those mentioned in methods for extracts.

In water soluble extractive 1g of capsule blend was soaked overnight, in 50ml water, instead of 100ml and the rest procedure is same as mentioned in methods for extracts.

HPTLC Fingerprinting:
Concentration of sample→ Blend of 1 capsule in 10ml methanol and 300mg of corresponding extract in 10ml methanol
Stationary phase→HPTLC Aluminium sheets silica gel 60 F 254.
Solvent system→ chloroform : methanol (9:1).
Sample volume→ 10 microlitre
Saturation time→ 30 minutes
Spotting level→ 1cm
Time of run→ 20 minutes
Length of run→ 8cm
Evaluation→ peak height and area
Detection→after spraying with vanillin sulphuric acid reagent.

Heavy Metals: As Per AYUSH Guidelines. The limits for lead (Pb)→10ppm, cadmium (Cd)→0.3ppm, arsenic (As)→3ppm and mercury (Hg)→1ppm, respectively.

Microbiology: As per USP/BP Guidelines.

Methods for Nutritive Study: Proteins: Accurately weighed, 0.5g of the blend of capsule was digested by heating the same with a mixture of 10g potassium sulphate, 1.5 g of copper sulphate and 20ml concentrated sulphuric acid till it turned green. After the contents were cooled, they were transferred to ammonia distillation flask. Cooled 40% NaOH solution was added into it to make it alkaline. These contents were then distilled immediately, and liberated gas was gently collected in 50ml of 0.1M HCl. After completion of distillation, the solution was titrated against 0.1M NaOH using methyl red as indicator, the end point being red to yellow. Similarly, blank was carried out by titrating 50ml 0.1M HCl against 0.1M NaOH, using methyl red as indicator.

Observations:
Weight of sample (A) = ----------g
B.R. of Blank (B) = --------ml
B.R. of Sample (C) = -------ml
Calculations:
Proteins (%w/w) = [(C – B) X 0.0014 X 6.25 X 100 X NF] / A,
Where, NF = Normality Factor of 0.1M NaOH, 6.25= conversion factor of nitrogen to protein

Fats: Accurately weighed 1g of the capsule blend was properly dissolved in 10 to 20ml of distilled water, with continuous stirring and heating, if necessary. Then the same was hydrolysed by adding concentrated HCl and boiled for 4 to 5 minutes This solution was then cooled to room temperature and was extracted 4-5 times with solvent ether. The ether layer was collected and washed with water, and then collected in a pre weighed flask. The washed ether layer was then evaporated to dryness on a water bath and then dried at 105ºC to constant weight. The respective weights were recorded and the % fat content in the sample was calculated by the formula mentioned.

Observation:
Weight of Sample (A) = ----------g
Weight of empty conical flask (B) = ----------g
Weight of conical flask after drying at 105ºC (C) = ----------g
Calculations: Fat Content (%w/w) = [(C – B) X 100] / A

Fibre: A weighed quantity of the test sample, representing about 2g of the blend of capsule, was exhausted with ether. 200ml of boiling dilute sulphuric acid was added to the ether-exhausted marc, in a 500ml flask, and the flask was connected to reflux condenser. The mixture was refluxed for 30 mins, accurately timed, and then, it was filtered through a hardened filter paper, and the residue on the filter paper was washed with boiling water until the effluent washing was no longer acid. The residue was rinsed back into the flask with 200ml of boiling NaOH solution. Again, the mixture was refluxed for 30mins, accurately timed, and then rapidly filtered through a tared filter paper. The residue on the filter paper was washed with boiling water, until the last washing was neutral, and dried at 110ºC, to constant weight. The dried residue was incinerated, ignited to constant weight, cooled in a dessicator and the ash was weighed. The difference between the weight obtained by drying at 110°C and that of the ash represented the weight of the crude fibers.

Observations:
Weight of sample (A) = ----------g
Weight of filter paper (B) = ----------g
Weight of filter paper + residue after drying at 110ºC (C) = ---g
Weight of empty crucible (D) = ----------g
Weight of crucible + residue after ignition (E) = ----------g

Calculations:
Fibre Content (%w/w) = [(C – B) – (E – D) X 100] / A

Calorific Value: 4(% carbohydrates) + 4(% proteins) + 9(% Fats) = calorific value in kilocalories

Carbohydrates: Carbohydrate content (%w/w) = 100-(%moisture + %ash + %protein + %fats + %fibre)

Table-1
General physico-chemical parameters of Amla, Ashwagandha and Shatavari Extracts

Sr. No.	Test Parameters	Amla Extract findings	Ashwagandha Extract findings	Shatavari Extract findings
1	Description	Grayish brown colored powder having astringent taste	Brown colored powder	Brown colored powder with slight bitter taste
2	Loss On Drying at 105°C	8.220 %w/w	7.251% w/w	2.717% w/w
3	Total Ash	3.930 %w/w	10.021% w/w	1.339% w/w
4	Acid Insoluble Ash	0.950 %w/w	6.069% w/w	0.235% w/w
5	Water Soluble Extractive	97.450%w/w	98.384% w/w	97.500% w/w
6	Alcohol Soluble Extractive	32.310%w/w	59.050% w/w	6.675% w/w
7	pH of 1.00% w/v solution	3.01	4.68	4.16
8	Content of Total Tannins	32.300%w/w	NA	NA
9	Content of Total Alkaloids	NA	5.810% w/w	NA
10	Content of Total Saponins	NA	NA	28.300% w/w

Table-2
General physico-chemical parameters of Amla, Ashwagandha and Shatavari Capsule formulations

Sr. No.	Test Parameters	Amla Capsule findings	Ashwagandha Capsule findings	Shatavari Capsule findings
1	Description	CT/CT "0" size HPMC capsule filled with grey colored powder	CT/CT "0" size HPMC capsule filled with light brown colored free flowing powder.	CT/CT "0" size HPMC capsule filled with greyish - brown colored powder.
2	Disintegration Time	13 minutes	5 minutes	8 minutes
3	Average filled weight of capsule	446.00 mg	464.30 mg	472.90 mg
4	Loss On Drying at 105°C	4.854% w/w	5.270% w/w	7.410% w/w
5	Total Ash	4.486 % w/w	6.565 % w/w	5.299 % w/w
6	Acid Insoluble Ash	0.626 % w/w	3.803 % w/w	3.728 % w/w
7	Water Soluble Extractive	53.525% w/w	59.080% w/w	69.009% w/w
8	Alcohol Soluble Extractive	12.600% w/w	9.706% w/w	30.809 % w/w
9	pH of 1.00%w/v solution	3.03	4.90	4.02
10	Content of Total Tannins	83.961 mg/capsule	NA	NA
11	Content of Total Alkaloids	NA	3.858 mg/capsule	NA
12	Content of Total Saponins	NA	NA	83.523 mg/capsule
13	HPTLC Fingerprinting	Complies	Complies	Complies
14	Heavy Metals			
a	Lead (Pb)	0.995 ppm	0.586 ppm	0.398 ppm
b	Cadmium (Cd)	0.057 ppm	0.015 ppm	0.014 ppm
c	Arsenic (As)	0.134 ppm	0.211 ppm	0.089 ppm
d	Mercury (Hg)	0.274 ppm	0.547 ppm	0.442 ppm
15	Microbiological Testing			
i)	Total Aerobic Microbial Count	220 cfu/g	170 cfu/g	280 cfu/g
ii)	Total Combined Yeast/Moulds Count	<10 cfu/g	<10 cfu/g	<10 cfu/g
iii)	Bile-Tolerant Gram Negative Bacteria	<10 cfu/g	<10 cfu/g	<10 cfu/g
iv)	*Escherichia coli*	Absent	Absent	Absent
v)	*Salmonellae spp.*	Absent	Absent	Absent
vi)	*Staphylococcus aureus*	Absent	Absent	Absent
vii)	*Pseudomonas aeruginosa*	Absent	Absent	Absent
viii)	Clostridia spp.	Absent	Absent	Absent

Table-3
Nutritive value study of Amla, Ashwagandha and Shatavari capsule formulations

Parameters	Amla Capsule Formulation	Ashwagandha Capsule Formulation	Shatavari Capsule Formulation
Loss On Drying at 105°C	4.854% w/w	5.270% w/w	7.410% w/w
Total Ash	4.486 % w/w	6.565 % w/w	5.299 % w/w
Proteins	7.784 mg/capsule	18.587 mg/capsule	22.797 mg/capsule
Fats	8.518 mg/capsule	15.921 mg/capsule	12.967 mg/capsule
Fibre	Negligible	Negligible	Negligible
Carbohydrates	388.042 mg/capsule	374.840 mg/capsule	377.038 mg/capsule
Calorific Value	1.659 Kcal/capsule	1.717 Kcal/capsule	1.716 Kcal/capsule

Results and Discussions

Table-1 shows results of the general physico-chemical parameters of amla, ashwagandha and shatavari extracts. Table-2 shows results of the general physico-chemical parameters of amla, ashwagandha and shatavari capsule formulations. As mentioned in table-1 and 2, amla extract gives 32.30% w/w of tannins, whereas in capsule, it was found to be 83.96mg/capsule. Similarly, the functional group, alkaloids, present in Ashwagandha, was found to be 5.81% w/w in extract, whereas in capsule, it was found to be 3.85mg/capsule. Also, shatavari extract was showing 28.30% w/w of saponins, and capsule was showing 83.52mg/capsule. With respect to quality aspects, these results were found to be satisfactory. Table 3 emphasizes on the nutritive value study of the three capsule formulations, including proteins, fats, fibre, carbohydrates and calorific value. Considerable amount of proteins, fats and carbohydrates, in all the three capsule formulations, imparts dietary value to them.

Presence of corresponding extracts in final formulations, are confirmed by using the well-versed HPTLC technique. The comparison of bands at similar Rf's and overlapping of spectra, as shown in Figure-1, 2, 3 and 4, indicates the same.

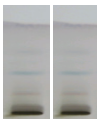
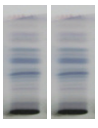
Amla	Ashwagandha	Shatavari
		
Extract Capsule	**Extract Capsule**	**Extract Capsule**
After spraying plate with vanillin sulphuric acid reagent and observing under white light		

Figure-1
Comparison of TLC Bands

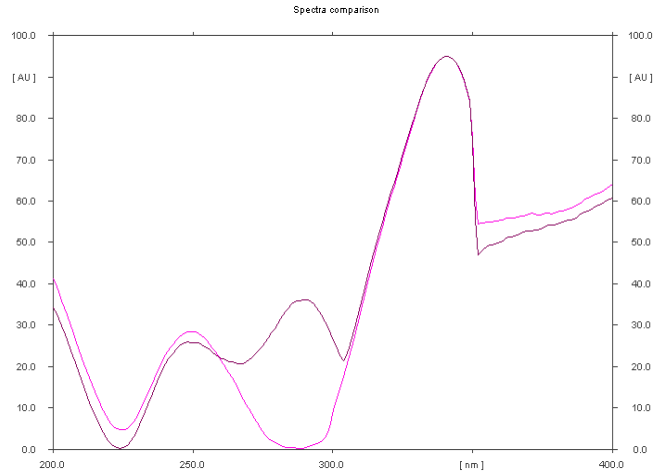


Figure-2
Overlapping spectrum of Amla extract and Capsule

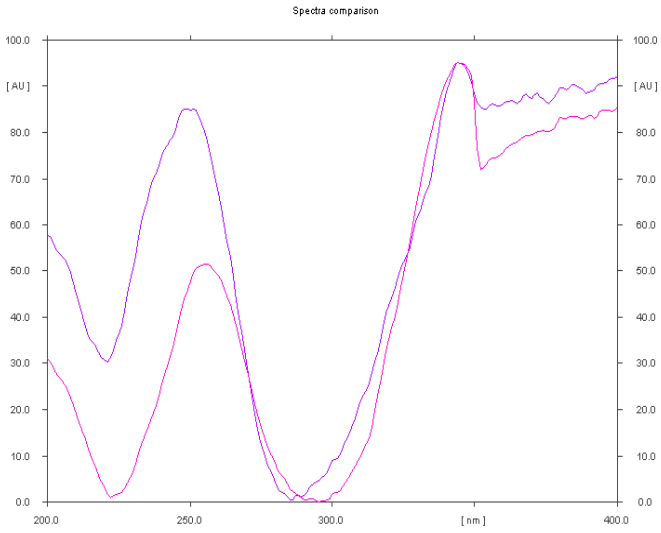


Figure-3
Overlapping spectrum of Ashwagandha extract and Capsule

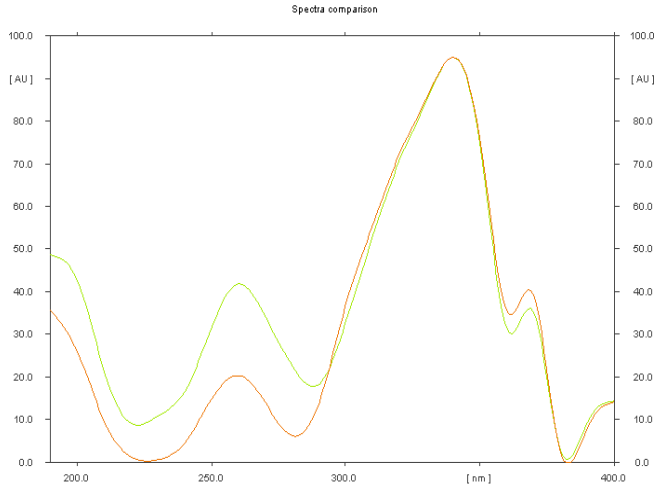


Figure-4
Overlapping spectrum of Shatavari extract and Capsule

Conclusion

From the above results and discussions, we can conclude that, although, the results of physico-chemical tests, heavy metals and microbiology, prove that amla, ashwagandha and shatavari formulations can be used as herbal medicines, the significant nutritive study results prove that, they can also be used as dietary supplements, to enhance our daily diet.

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