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Research Article

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PHARMACOGNOSTICAL AND PHYTO-CHEMICAL STANDARDIZATION OF *ASHTAANGAAVALEHA*: A POLYHERBAL FORMULATION

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ABSTRACT

The present work was carried out to standardize the finished product *Ashtaangaavaleha* to confirm its identity, quality and purity. *Ashtaangaavaleha* is indicated for the management of *Jwara* (Fever), *Kaasa* (Cough), *Swaasa* (Dyspnoea/Asthma), *Aruci* (Tastelessness) and *Chardi* (Emesis). There has been an increase in demand for the Phyto-pharmaceutical products of *Ayurveda* so a new pharmaceutical preparation in the form of *Ashtaangaavaleha* was tried to standardize which is economical in terms of time and machinery usage. Pharmacognostical and phyto-chemical observations revealed the specific characters of all active constituents used in the preparation. The phytochemical analysis and High Performance Thin Layer Chromatography has also been performed. The drug combination was also characterized for its physico-chemical properties. The presence of Endosperm cells, Vittae cells, Prismatic crystals, Schleroids, Trichome, Epicarp-beaker shaped were the characteristic features observed in the microscopy of drug combination. Phyto-chemical analysis indicated presence of alkaloids, tannins, saponins, flavonoids, glycosides and Steroid. On the basis of observations and experimental results, the study may be used as reference standard in the further quality control researches. Further studies may be carried out on *Ashtaangaavaleha* based on identification and separation of active ingredients with the help of various Biomarkers.

Keywords: *Ashtaangaavaleha*, Pharmacognosy, phytochemistry.

INTRODUCTION

Ashtaangaavaleha is a poly herbal formulation.[1] The poly herbal formulations described in Ayurveda have been the basis of treatment of various human diseases. *Ashtaangaavaleha* is indicated for the management of *Vaata-Kapha Jwara* (Fever due to *Vaata* and *Kapha* vitiation), *Kaasa* (Cough), *Swaasa* (Dyspnoea/Asthma), *Aruci* (Tastelessness), *Chardi* (Emesis).[2] Traditional Medicines[3] are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs.[4] It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine.[5] The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards.[6] Selection of scientific and systematic approach for the biological evaluation of herbal formulations based on their use in the traditional systems of medicine forms the basis for an ideal approach in the development of new drugs from plants.[7] But the most important challenges faced by these formulations arise because of their lack of complete standardization. Detailed research on the chemistry and pharmacology of products of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases. In the light of above background, the present study aimed to standardize the finished product of *Ashtaangaavaleha* using pharmacognostical and phytochemical parameters. The authenticity, quality and purity of herbal drugs are established by references given in pharmacopoeia.[8]

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MATERIALS AND METHODS

Collection, Identification and authentication of raw drugs

The raw drugs for the study were procured form the Pharmacy of Institute of Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar. The ingredients were identified and authenticated in the Pharmacognosy Laboratory, Institute of Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar. The ingredients and parts used are listed in the table **1**.

Table 1. Ingredients of *Ashtaangaavaleha*

S no	Name	Botanical Name	Part Used	Form	Part
1	Katphala	*Myrica negi* Buch-Ham.	Bark	Churna	1
2	Pushkaramoola	*Inula racemosa* Hook. F.	Root	Churna	1
3	Shringee	*Pistacia integerrima* Stewart ex Brandis	Gall	Churna	1
4	Yamaanee	*Ptychotis ajawan* Linn.	Fruit	Churna	1
5	Kaaravee	*Carum carvi* Linn.	Seed	Churna	1
6	Shunthee	*Zinziber officinale* Roxb.	Rhizome	Churna	1
7	Marica	*Piper nigrum* Linn.	Fruit	Churna	1
8	Pippalee	*Piper longum* Linn.	Fruit	Churna	1
9	Madhu	Honey	.....	.....	2
10	Aardraka	*Zinziber officinale* Roxb.	Rhizome	Swarasa	2
11	Guda	*Saccharum officinarum* Linn	Swarasa	Sugar	16

Preparation of drug

The drugs enlisted from 1 to 8 (Table **1**) were washed, dried and made into fine powder and then sieved in mesh no. 85 separately. The ingredients are mixed well in equal quantity in mass mixing machine till a homogenous mixture was obtained. Washed and peeled rhizome of Aardraka (*Zinziber officinale Roxb*.) was grinded and squeezed. Then the juice was filtered through a muslin cloth. To make the drug combination palatable, process of drug preparation was modified.[9] The juice of Aardraka was added with Guda (concentrated juice of *Saccharum officinarum Linn)* and heated in low flame. As it attains *Avaleha Siddha Lakshanas*[10] the homogenous powder of ingredients 1 to 8 (table **1**) were added after removing the vessel from the fire and stirred continuously to form a homogenous mixture. After cooling the prepared material for 24 hours, honey was added and stirred and then the drug was packed in airtight plastic containers.

Pharmacognostical evaluation

One gram of finished product *Ashtaangaavaleha* dissolved in distilled water then filtered through filter paper, the filtrate used study under the corl zeiss microscope with stain (Phloroglucine and concentrated HCl) and without stain to study the characters of the product. The microphotographs were taken attached with the microscope.

Phyto-chemical assay of drug

Ashtaangaavaleha was analyzed by using qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory, Institute of Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar. All Physico-chemical parameters such as Loss on Drying, Ash value, Water soluble extract, Methanol soluble extract, P^H, Reducing sugar, Non- reducing sugar and Total sugar were determined. The water and methanol extract of the sample was analyzed qualitatively for different functional groups.[11]

High performance thin layer chromatography (HPTLC)

Methanol extract of *Ashtaangaavaleha* was used for High performance thin layer chromatography (HPTLC) study. Methanol extract of *Ashtaangaavaleha* was spotted on pre-coated silica gel GL60254 aluminum plate as 10mm bands by means of a Camag Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene (9ml) and ethyl acetate (1ml) was used for *Ashtaangaavaleha* as a mobile phase. The development time was 30 minutes. After development, Densitometry scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 254nm and 366 nm under control of Win CATS software (V1.2.1. Camag).[12,13] Then the plate was sprayed with Anisaldehyde sulphuric acid followed by heating and then visualized in day light.

RESULTS AND DISCUSSION

Organoleptic parameters

The organoleptic characters of the formulation are tabulated in the table **2**.

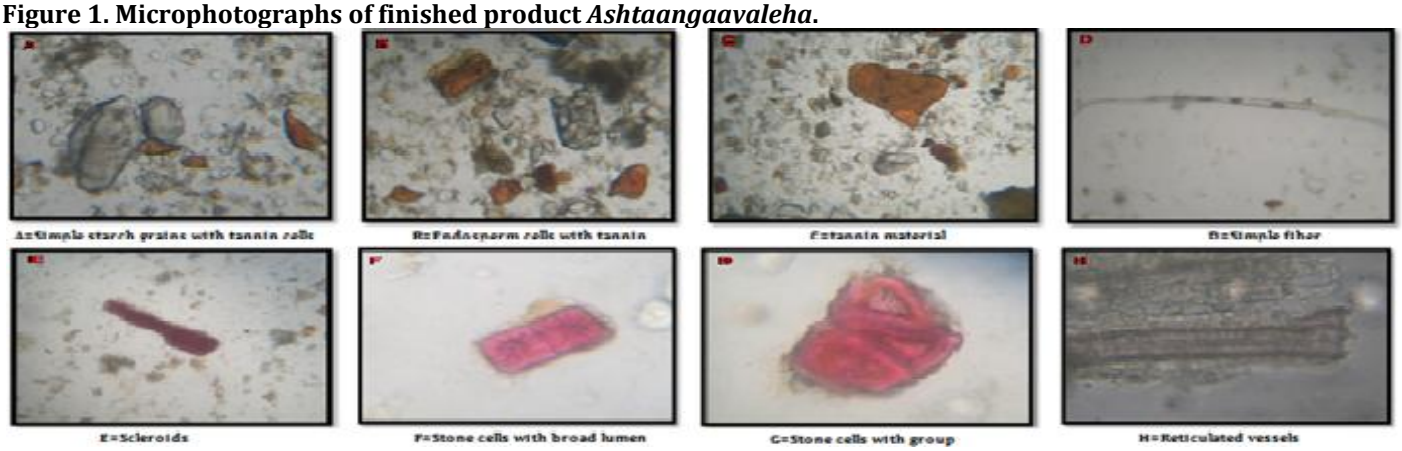
Table 2. Organoleptic parameters of *Ashtaangaavaleha*.

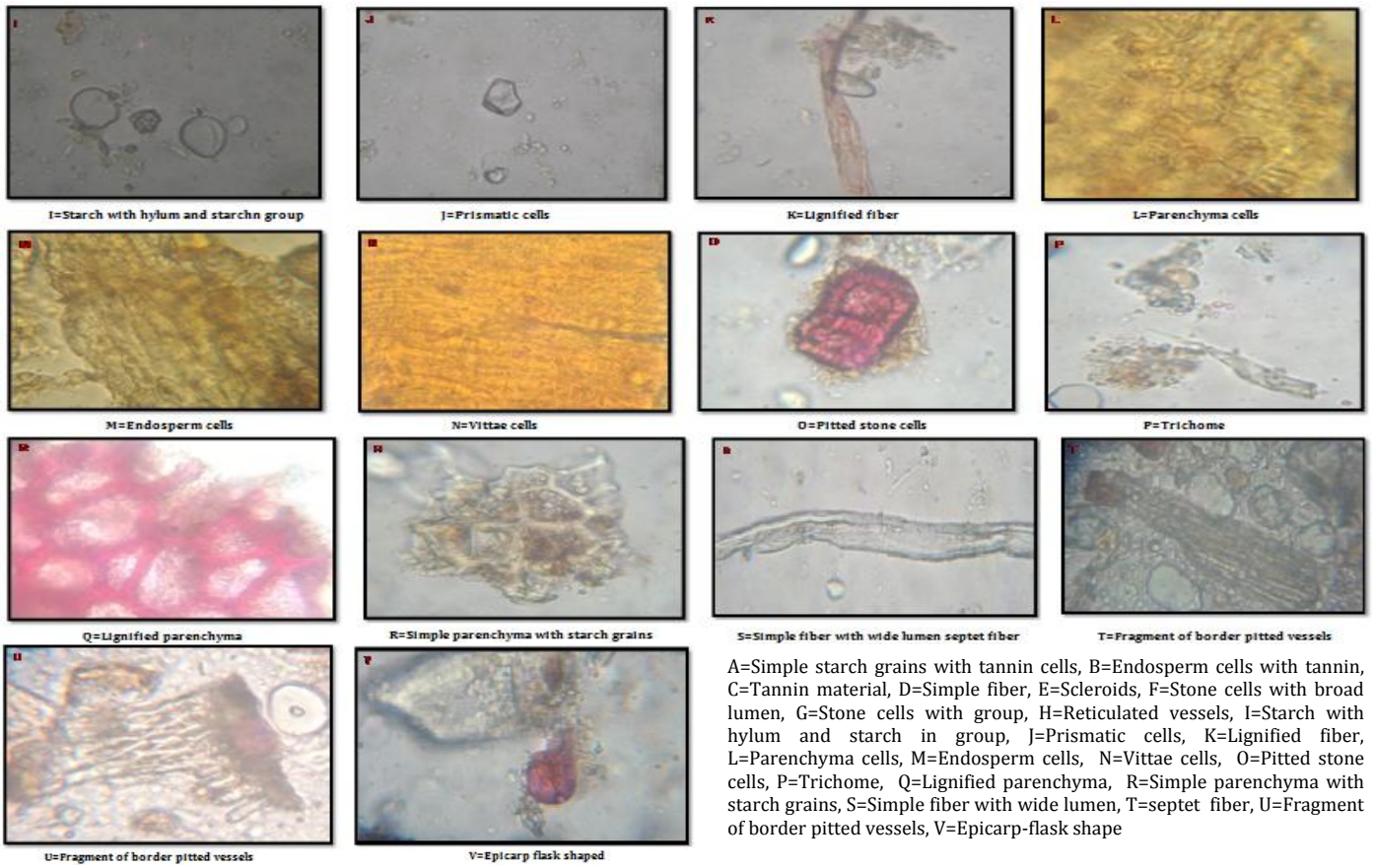
Organoleptic parameters	Sample
Appearance	Semisolid
Color	Dark brown
Taste	Sweet, Astringent
Odor	Characteristic

Pharmacognostical evaluation

Diagnostic characters of the finished product under the microscope were Simple starch grains with tannin cells (*Zinziber officinale* Roxb.), Endosperm cells with tannin, Simple fiber (*Inula racemosa* Hook. F.), Scleroids (*Pistacia integerrima* Stewart ex Brandis), Stone cells with broad lumen(*Piper longum* Linn.), Reticulated vessels(*Zinziber officinale* Roxb.), Starch with hylum (*Piper longum* Linn.) and starch in group, Prismatic crystal of calcium oxalate (*Myrica negi* Buch-Ham.), Lignified fiber, Parenchyma cells(*Piper nigrum* Linn.), Endosperm cells, Vittae cells(*Carum carvi* Linn.), Pitted stone cells, Trichome (*Ptychotis ajawan* Linn.), Lignified parenchyma, Simple parenchyma with starch grains, Simple fiber with wide lumen, septet fiber (*Zinziber officinale* Roxb.), Fragment of border pitted vessels, Epicorp-beaker shaped(*Piper longum* Linn. and *Piper nigrum* Linn.).[14] (Figure **1 A-V**)

Figure 1. Microphotographs of finished product *Ashtaangaavaleha*.





A=Simple starch grains with tannin cells, B=Endosperm cells with tannin, C=Tannin material, D=Simple fiber, E=Scleroids, F=Stone cells with broad lumen, G=Stone cells with group, H=Reticulated vessels, I=Starch with hylum and starch in group, J=Prismatic cells, K=Lignified fiber, L=Parenchyma cells, M=Endosperm cells, N=Vittae cells, O=Pitted stone cells, P=Trichome, Q=Lignified parenchyma, R=Simple parenchyma with starch grains, S=Simple fiber with wide lumen, T=septet fiber, U=Fragment of border pitted vessels, V=Epicarp-flask shape

Physico-chemical parameters

Ashtaangaavaleha was evaluated for various physic-chemical parameters. The results are shown in the table **3**.

Table 3. Physico-chemical parameters of *ashtaangaavaleha*

S no	Test	Sample
1	Loss on Drying at 110 ˚C	12.44 %w/w
2	Ash value	5.54 %w/w
3	Water soluble extract	71.67 %w/w
4	Methanol soluble extract	71.77 %w/w
5	PH	5.35
6	a. Reducing sugar	31.99 %w/w
	b. Non- reducing sugar	30.87 %w/w
	c. Total sugar	62.86 %w/w

Qualitative tests

Qualitative tests indicated presence of alkaloids, tannins, glycosides, saponins, flavonoids, protein, carbohydrates and steroid. Details are shown in the table **4**.

High performance thin layer chromatography (HPTLC) study

In High performance thin layer chromatography (HPTLC) study, visual observation under UV light showed few spots, but on analyzing under densitometer much more was observed. Chromatogram shows 8 prominent spots at R_f 0.02, 0.13, 0.22, 0.32, 0.49, 0.56, 0.77, 0.94 in short wave UV254 nm and 5 prominent spots at R_f 0.02, 0.20, 0.49, 0.56, 0.65 in long wave UV 366nm. Details are noted in the table **5** and figure **2**. Then the plate was sprayed with Anisaldehyde sulphuric acid followed by heating and then visualized in day light shows 3 prominent spots at R_f 0.18, 0.36, 0.55. Details are noted in the table **6** and figure **2**.

Table 4. Qualitative tests of *Ashtaangaavaleha* for different functional groups

S no	Functional group	Test	Sample
1	Alkaloid	(Dragendroff's & Wagner's test)	+
2	Tannin and phenolics	(Lead acetate solution)	+
3	Glycosides	(Keller-Killiani test)	+
4	Saponin Glycosides	(Lead acetate solution)	+
5	Flavanoids	(Lead acetate solution)	+
6	Protein	(Lead acetate solution)	+
7	Steroid	(Liebermann's Burchard)	+
8	Carbohydrates	Molish's test	+

Figure 2. HPTLC of *Ashtaangaavaleha* (Methanol Extract)

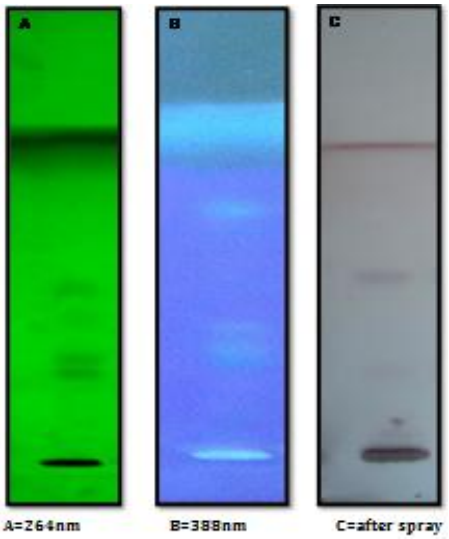


Table 5. HPTLC of *Ashtaangaavaleha* (Methanol Extract)

Extract	Solvent system	Wavelengths	Spots	R_f value	Observation under UV light
Methanol extract	Toluene(9ml): Ethyl acetate(1ml)	366nm UV	5	0.02, 0.20, 0.49, 0.56, 0.65	Light yellow, Light brown, Grayish brown, Light brown, Brown
		254 nm UV	8	0.02, 0.13, 0.22, 0.32, 0.49, 0.56, 0.77, 0.94	Light yellow, Light brown Gray, Brown, Grayish brown, Light brown, Brown, Brown

Table 6. HPTLC of *Ashtaangaavaleha* (Methanol Extract) after spraying anisaldehyde sulphuric acid

S No	spray	Spots	Rf value	Observation
1	Anisaldehyde sulpjuric acid	3	0.18, 0.36 0.55	Brown, Pink, Light brown

CONCLUSION

Pharmacognostical and Phyto-Chemical evaluation of *Ashtaangaavaleha* illustrated the specific characters of all ingradients which we used in the preparation. For the first time a new pharmaceutical preparation *Ashtaangaavaleha* was tried which is economical in terms of time and machinery usage. On the basis of our observations and experimental results, this study may be used as reference standard in the further quality control researches. Further studies may be carried out on *Ashtaangaavaleha* based on identification and separation of active ingredients with the help of various Biomarkers.

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