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A DETAILED PHYSICOCHEMICAL AND PHYTOCHEMICAL STUDY OF *AMLAKYADI CHURNA* (AN AYURVEDIC MEDICINAL PREPARATION)

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ABSTRACT

Ayurveda, meaning the "Science of life" is said to be the oldest and most complete medical system in the world. Its roots are in ancient Indian civilization and Hindu philosophy. Food is the basis of life and it acts as a fuel for various metabolic functions taking place inside our body. *'Anna'* (Food) is considered as *'Prana'* (The Vital Life Force) in Ayurveda. A good and healthy appetite is an important factor which affects our nutrition, immunity & overall health. In the present era of stress coupled with irregular eating habits, *Aruchi* (loss of appetite or *Anorexia*) is quite prevalent. *Amlakyadi churna* is a traditional medicinal preparation, mentioned in *Sharangadhara Samhita* (a classical treatise on Ayurveda) & consists of *Amlaki* (*Phyllanthus emblica Linn., syn. Emblica officinalis Gaertn.*), *Citraka* (*Plumbago zeylanica Linn.*), *Haritaki* (*Terminalia chebula Retz.*), *Pippali* (*Piper longum Linn.*) and *Saindhav lavan* (Rock salt). It is a *Rucikara-Pachan dravya* that enhances appetite and aids in proper digestion. This study highlights the results of standardization (identity, purity & strength) tests, preliminary phytochemical screening and TLC findings. All the said tests are conducted in *State Drug Testing Laboratory, AYUSH, Guwahati, Assam*. These studies are important in way of establishing quality-control, efficacy & acceptability of herbal drugs.

Keywords: *Anna, Prana, Amlakyadi Churna, Saindhav lavan, Rucikara-Pachan Dravya*

INTRODUCTION

"PRAANINAAM MULAM AHARA''- Acharya Sushruta.

'Let Food be Thy Medicine & Medicine be Thy Food' - Hippocrates

In Ayurveda, *Ahara* (food) is considered as the basis of the ecosystem of life which includes our immune power. Appetite is the in-built mechanism of human body for regulation of food intake to meet our daily energy and micronutrients needs. *Aruchi* (loss of appetite or Anorexia) is quite prevalent now-a-days, causes of which varies from improper dietary habits to psychological causes like stress, depression, etc. Having a poor appetite can lead to improper nutrition, deficiency disorders, fatigue, malaise, etc.

Amlakyadi churna (a *Rucikara Pachan Dravya*) is a classical Ayurvedic medicinal preparation, which finds its mention in *Sharangadhara Samhita*. Even though many modern research works are available in respect to its individual ingredients, but a comprehensive profile in respect to the crude drug is lacking. Amlakyadi churna was subjected to pharmaceutical evaluation (evaluation of different physicochemical and phytochemical parameters) in order to prepare a profile of the formulation.

Amlakyadi churna consists of *Amlaki (Phyllanthus emblica Linn., syn. Emblica officinalis Gaertn.), Citraka (Plumbago zeylanica Linn.*), *Haritaki* (*Terminalia chebula Retz.*), *Pippali (Piper longum Linn.*) & *Saindhav lavan* (Rock-salt). The present study establishes its chemical profile and ascertains its purity as a crude drug. The air-dried plant material was subjected to preliminary phytochemical screening for detection of various plant constituents.

MATERIALS AND METHODS

Collection & Identification of raw materials – The dried parts used of every individual herb was collected from the state of Karnataka, India and Saindhav lavan was procured locally from Guwahati, Assam, India.

The identification was done in the Department Of Dravyaguna, GACH, Guwahati-14.

Preparation of medicine – The medicine was prepared as per guidelines mentioned in Ayurvedic Pharmacopoeia of India at State Ayurvedic Pharmacy, GACH, Guwahati-14.

Place of work - Preliminary phytochemical screening including standardization tests and TLC studies were carried out in State Drug Testing Laboratory, AYUSH, Govt. Ayurvedic College & Hospital, Guwahati-14.

Amlakyadi churna was subjected to the following tests as specified below-

i. PHARMACOGNOSTICAL EVALUATION (Organoleptic Analysis) [2] **:**

Microscopic and macroscopic studies of the *Churna* were done at State Drug Testing

Laboratory, AYUSH, Govt. Ayurvedic College and Hospital, Guwahati. 0.5mg of the powdered sample was analyzed in dry form with the help of Trinocular Research microscope (MAGNUS). The powder was analyzed in 10X and 40X magnification lens and observations were made regarding changes in appearance. The evaluation of colour, odour, texture and taste includes the macroscopic appearance and were done as per standard procedures.

ii. PHYSICOCHEMICAL EVALUATION/ QUANTITATIVE ESTIMATION [3]**:**

Physicochemical parameters (Total ash value, pH value, LOD, Alcohol soluble extractive and Water soluble extractive values) were determined as per the guidelines of WHO.

iii. PRELIMINARY PHYTOCHEMICAL SCREENING [4][5]***:***

An amount of 50 mg of air-dried powdered plant material was extracted successively with following solvents in a Soxhlet apparatus-

a. Petroleum ether (60 ˚C- 80 ˚C)
b. Benzene
c. Chloroform
d. Acetone
e. Ethanol (95 %)
f. Methanol

Each time before extracting with the next solvent, the powdered material was washed with chloroform water and dried in an air oven below 50˚C. Each individual extract was concentrated by distilling off the solvent and then evaporating to dryness on the water bath.

Fluorescence analysis of different extracts obtained thus was done under UV radiation at 254 nm.

QUALITATIVE CHEMICAL EXAMINATION**:**

The extracts obtained as above are then subjected to qualitative tests for the identification of various plant constituents. In addition, 50 gm of air-dried or fresh plant material is also subjected to hydro-distillation to detect the presence of volatile oil.

a. ***Detection of alkaloids***:
A small portion of the solvent free chloroform, alcoholic extract is stirred separately with a few drops of dil.HCL acid and filtered. The filtrate was tested carefully with alkaloidal reagents such as Dragendorff's reagent (orange brown precipitate).

b. ***Detection of carbohydrates and glycosides*** :
A small quantity (300 mg) of alcoholic extract is dissolved in 4 ml of distilled water and filtered. The filtrate was treated with Molisch's test to detect the presence of carbohydrates.
A small portion of the extract was hydrolysed with dil. HCL acid for a few hours in water bath. The hydrolysate is subjected to Leibermann-Burchard's test to detect the presence of glycosides.

c. ***Detection of phytosterol*** :
The petroleum ether extract is refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The saponification mixture with distilled water is diluted with distilled water and the extract is diluted with ether. The residue is treated with Leibermann-Burchard's test.

d. ***Detection of fixed oils and fats*** :
Small quantities of petroleum ether and benzene extracts are pressed separately between two filter papers. Oil stains on the paper indicate the presence of fixed oils.

e. ***Detection of saponins*** :
1 ml of alcoholic extract is diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A one cm layer of foam indicates the presence of saponins.

f. ***Detection of proteins and free amino acids:***
A small quantity of alcoholic extract is dissolved in a few milliliters of water and the solution is subjected to Minhydrin test.

iv. THIN LAYER CHROMATOGRAPHY [5]:
The chromatogram of *Amlakyadi churna* was carried out with methanolic extract and maximum spots had been separated on precoated silica gel TLC plate with trial and error methods. The pigments were separated from their spots at UV 254 nm wavelength. A solvent system of n-hexane: ethyl ether: glacial acetic acid (80:20:1) was selected after trial and error. The Rf value of each spot was calculated and then compared with literature.

RESULTS AND OBSERVATION-
The air-dried plant material was subjected to preliminary phytochemical screening for the detection of various plant constituents on the following lines to rationalize its use as a drug of therapeutic importance-

i. Successive solvent extraction
ii. Qualitative chemical examination
iii. Fluorescence analysis

Table 1: Organoleptic characteristics of *Amlakyadi churna*

SL. NO.	PARAMETERS	RESULT
1	Colour	Brown
2	Odour	Characteristic(aromatic)
3	Taste	Sour, pungent & a sweet after-taste
4	Texture	Fine powder
5	Foreign matter	0.8 -1 %
6	Microscopic analysis	No any significant change

Table 2: Results of Successive Solvent Extraction of Air-Dried Powder

SL . NO.	SOLVENT	COLOUR CONSISTENCY OF EXTRACT
1.	Petroleum Ether	Dark Brown
2.	Benzene	Blackish Brown
3.	Acetone	Blackish Brown
4.	Ethanol	Blackish Brown

Table 3: Qualitative Analysis of Air-Dried Powder Extract

COMPOUNDS FOR DETECTION	METHANOLIC EXTRACT
1. Proteins	+ve
2. Carbohydrate	**-ve**
3. Saponins	+ve
4. Phenols	+ve
5. Glycosides	+ve
6. Steroids	**-ve**
7. Tannins	+ve
8. Alkaloids	+ve
9. Fixed Oil	+ve

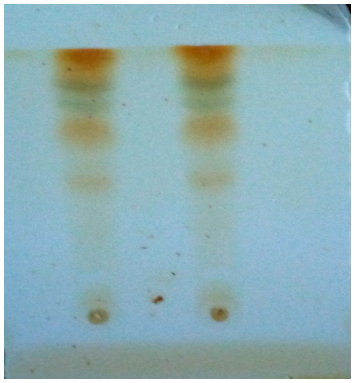
Table 4: Fluorescence Analysis of Different Extract under UV RADIATION

SL. NO.	EXTRACT	BEFORE UV	AFTER UV AT 254 nm
1.	Petroleum Ether	Dark Brown	Green
2.	Benzene	Blackish Brown	Greenish Red
3.	Acetone	Blackish Brown	Greenish Red
4.	Ethanol	Blackish Brown	Greenish red

Table 5: Determination of Quantitative Standards

SL. NO.	PARAMETERS	VALUE
1.	Total Ash Value	21.81%
2.	pH (10% aqueous sol.)	3.67
3.	LOD	5.8%
4.	Alcohol soluble extractive	37.41 %
5.	Water soluble extractive	17.89%

Fig 1: Chromatographic profile of Crude drug Extract (*TLC under UV chamber)*[*]:



* (The details of solvent system and the Rf values are mentioned in Table 6)

Table 6: Details of solvent system and Rf values

EXTRACT	SOLVENT SYSTEM	NO. OF SPOTS	RF VALUES
Methanol	n-hexane: ethyl ether: glacial acetic acid (80:20:1)	5	0.17 0.48 0.58 0.66 0.83

DISCUSSION[6]

Owing to the medicinal properties attributed to a herbal drug, it is necessary to maintain its quality and purity for its proper use. In the recent past, it has become possible to suggest a practicable quality assurance profile for a herbal drug or its bioactive constituent(s), given the advent of new analytical tools and sophisticated instrumental technology. The crude drugs are subjected to a suitable method of extraction and purification for the isolation of phytopharmaceuticals. Extractive values also help in estimation of specific constituents soluble in particular solvents. Microscopic evaluation helps in proper identification of source materials. Macroscopic characters, ash values and extractive values serve as diagnostic parameters and help in evaluation of purity of drugs.

The pharmacognostical evaluation showed that the sample drug was brown in colour; sour, pungent in taste along with a sweet after taste; aromatic in odour and fine powder in consistency. Sourness is due to *Amlaki* and a sweet after taste is due to *Amlaki* & *Haritaki* and pungent due to presence of *Pippali* and *Citraka*. The palatability of the drug is good. In microscopic evaluation, no significant changes or appearance of the powdered material was seen. Foreign matters were limited to (0.8%-1%).

The phytochemical analysis showed positive presence of proteins, saponins, phenols, glycosides, tannins, alkaloids and fixed oil. It was also noted that carbohydrates and steroids were absent in the sample drug. The fluorescence analysis of different extracts revealed the fluorescent property of the sample drug.

The quantitative standards were well within normal limits. The alcohol soluble extractive value is more than water soluble extractive value, which indicates the presence of more alcohol soluble contents in the drug. The pH value of the sample drug is 3.67, which indicates its acidic nature.

The TLC showed 5 spots and Rf value of each spot was calculated. The Rf values were then compared with literature and found to be same. The Rf values were found to be of 0.17, 0.48, 0.58, 0.66, & 0.83 values respectively.

CONCLUSION

The ancient science of Ayurveda is a heritage of Indian culture and boon to the world. The fundamental concepts of Ayurveda are very complicated and complete understanding of this science is rather difficult. Thus, extensive research work is necessary to establish its strong scientific footing along with understanding its basic concepts. A systematic study of a crude drug is essential in the present era for quality-control and analysis of phytopharmaceuticals derived from them.

From this study, we have been able to gather important information regarding *Amlakyadi Churna* which has ascertained its purity as a drug, and simultaneously establishes its basic chemical profile. The authors hope that the information provided by this present study can be useful for further studies on *Amlakyadi churna*.

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