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Original Research Article

Physico-chemical study of *Panchvalkal* mentioned in *Himvan Agada*: An anti — Poisonous formulation in *Ayurveda*

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ABSTRACT

Agadatantra is one among the *Ashtangas* of Ayurveda, which deals with all cases of poisoning. In all these situations, *Sarpvisha* is of paramount importance because it is a condition that requires emergency management. *Himavanagada* is one among them. *Ayurveda* has grouped the stem bark of five plants viz. *Nyagrodha* (*Ficus bengalensis* Linn.), *Shirish* (*Albizzia lebbeck* Benth.), *Ashwatha* (*Ficus religiosa* Linn) *Vetasa* (*Salix caprea Linn.*) and *Plaksha* (*Ficus infectoria* Roxb.) as ingredients in *Himavan agada* (the stem bark of five trees). Itcontains 14 ingredients and indicated for *mandalivisha, visarpa, shwayathu, visphota, jwara* and *daha.*

Objectives: To analyze the Physico-chemical, phyto- chemical properties.

Materials and Methods: Procurement of Raw drugs was later subjected to Physico-chemical analysis and preliminary phytochemical analysis.

Conclusion: All the parameters, assays are done, upon which the standards of Pharmacopoeia depend.

1. Introduction

Ayurveda has grouped the stem bark of five plants viz. *Nyagrodha (Ficus bengalensis* Linn.), *Shirish (Albizzia lebbeck* Benth.), *Ashwatha (Ficus religiosa Linn) Vetasa (Salix caprea Linn.)* and Plaksha (Ficus infectoria *Roxb.)* as ingredients in *Himavan agada* (the stem bark of five trees).[1]

The *Himavan agada* are indicated as *Varnya (improve complexion), Stanyashodhana* (purifies breast milk), *Vranaghna* (wound healing), *Visarpa* (for treating Herpes zooster) and *Shophaghna* (anti-inflammatory) identified by *Arundatta* the commentator of *Ashtanga Hrudaya.*[2] Also *Himavan agada* (anti-poisonous formulation) used to treat the *Mandali Sarpa Visha.*

Therefore the present study has been planned to explore and analyze the poly herbal combination were subjected to Physico-Phytochemical analysis of Panchvalkal mentioned in Himvan agada.

2. Aims and Objective

To analyze the Physico-chemical, phyto- chemical properties of Himvan Agada by using HPTLC fingerprinting.

3. Materials and Methods

1. Preparation of *Himavan agada*

Raw drugs required for preparation were procured form authenticated market dealer. Procured drugs were authenticated in Department of Dravyaguna, Gaur

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Brahman Ayurvedic College, Rohtak. Drugs were pounded individualy in *Khalwa Yantra* to get *Yawakuta* form. After pounding drugs are mixed (I part each) to prepare *Himavan agada.* Ingredient of *Himavan agada* is mentioned in table.

Table 1: Ingredients of panchvalkl mentioned in *Himvan agada*

Sl no	Name of Ingredients	Quantity
1.	Nyagrodha (Ficus bengalensis Linn.)	1 part
2.	Shirish (Albizzia lebbeck Benth.)	1 part
3.	Asvattha (Ficus religiosa Linn.)	1 part
4.	Vetasa (Salix caprea Linn.)	1 part
5.	Plaksha (Ficus infectoria)	1 part

The sample of Panchvalkal was later subjected to Physico-chemical analysis and preliminary phytochemical analysis.

3.1. Determination of ash value/total ash[3]

3.1.1. Description

The residue remaining after incineration is the ash content of the drug, which represents inorganic salts. Ash value is a criterion to judge the identity or purity of crude drug. This test is applicable for Solid, Semi-solid dosage form.

3.2. Equipment and glassware used

Muffle-furnace, Silica Crucible, Desiccator, Ash less filter paper, Beaker.

3.3. Reagent/ chemicals used

NA

3.3.1. Procedure

Incinerate about 2 to 3 gm of drug (W_1) accurately weighed and ground in a tarred silica crucible (W_2). Keeps the crucible in a muffle-furnace at a temperature not exceeding 450°C- 600°C (According to the physical property of product) for 4 hrs. Cool in desicator and weigh (W_3). If a carbon free ash cannot be obtained in this way. After cooling reignite the crucible at same temp. for 01 hrs. and calculated the weight difference. Collect the residue on an ash less filter paper. Incinerate the residue and filter paper. Add the filtrate, evaporate to dryness. Ignite at a temperature not exceeding 450°C - 600°C. Calculate the percentage of ash with reference to the air-dried drug.

3.3.2. Calculation

$$Percentage\ of\ Ash := \frac{W3 - W2\ X\ 100}{W1}$$

Where, W_3= Weight of crucible with ash, W_2= Weight of crucible and W_1= Weight of drug taken.

3.4. Determination of acid insoluble ash[4]

3.4.1. Description

The residue remaining after incineration is the ash content of the drug, which represents inorganic salts. Ash value is a criterion to judge the identity or purity of crude drug. This test is applicable for Solid, Semi-solid dosage form.

3.5. Equipment and glassware used

Muffle-furnace, Silica Crucible, Desiccator, Ash less filter paper, Beaker.

3.6. Reagent/ chemicals used

Dil. hydrochloric acid

3.6.1. Procedure

Prepare ash. Transfer the ash in a 250 ml beaker without loss of ash and add 100 ml of dil. hydrochloric acid. Wash the crucible with 10 ml of acid and transfer the washings to the beaker. Heat the beaker till the liquid boils. Filter the solution and collect the insoluble matter on ash less filter paper (Whatman). Wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible. Dry on a hot plate and ignite at 600°C in a muffle furnace (until become white ash). Allow the residue to cool in suitable desiccators for 30 minutes and weigh without delay. Repeat the process until constant weight (W_3) is obtained. Calculate the acid insoluble ash with reference to the air dried drug.

3.6.2. Calculation

$$Percentage\ of\ Acid\ insoluble\ Ash : \frac{W3 - W2\ X\ 100}{W1}$$

Where, W_3= Weight of crucible with ash, W_2= Weight of crucible, W_1= Weight of drug taken.

3.7. Determination of water soluble ash[5]

3.7.1. Description

The residue remaining after incineration is the ash content of the drug, which represents inorganic salts. Ash value is a criterion to judge the identity or purity of crude drug. This test is applicable for Solid, Semi-solid dosage form.

3.8. Equipment and glassware used

Muffle-furnace, Silica dish, Desiccators, Ash less filter paper, Beaker.

3.9. Reagent/ chemicals used

Purified Water

3.9.1. Procedure

Prepare ash. Boil the total ash for 5 minutes with 25 ml of water. Collect insoluble matter in an ash less filter paper. Wash with hot water and ignite for 15 minutes at a temperature not exceeding 450°-600°C. Subtract the weight (W_3) of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water- soluble ash with reference to the air-dried drug.

3.9.2. Calculation

$$Percentage\ of\ Water\ insoluble\ Ash: \frac{(W3-W2)\ X\ 100}{W1}$$

Where, W_1= Weight of drug taken, W_2= Weight of empty crucible and W_3= Weight of crucible+ water insoluble ash.

Water soluble ash = Total ash — Water Insoluble Ash

3.10. Determination of alcohol soluble extractive[6]

3.10.1. Description

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired.

3.11. Equipment and glassware used

Hot air oven, Flask, Flat bottom shallow dish

3.12. Reagent/ chemicals used

Alcohol

3.12.1. Procedure

Macerate 5 gm (W_1) of the coarsely powdered drug with 100 ml of Alcohol of specified strength in a closed flask for 24 hours. Shake frequently during six hours. Allow to stand for eighteen hours. Filter rapidly taking precautions against loss of solvent. Evaporate 25 ml of the filtrate to dryness in a tarred flat bottom shallow dish (W_2). Dry at 105^0C to constant weight and weigh (W_3). Calculate the percentage of Alcohol– soluble extractive with reference to the air-dried drug.

3.12.2. Calculation

$$Percentage\ of\ Alcohol\ soluble\ extractive: \frac{(W3-W2)\ X100X100}{W1\ X\ 25}$$

Where, W_1= Weight of drug taken, W2= Weight of empty dish and W_3= Weight of dish+extractive residue.

3.13. Determination of water soluble extractive[7]

3.13.1. Description

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired.

3.14. Equipment and glassware used

Hot air oven, Flask, Flat bottom shallow dish

3.15. Reagent/ chemicals used

DM. Water

3.15.1. Procedure

Macerate 5 gm (W_1) of the coarsely powdered drug with 100 ml of distilled water in a closed flask for 24 hours. Shake frequently during six hours. Allow to stand for eighteen hours. Filter rapidly taking precautions against loss of water. Evaporate 25 ml of the filtrate to dryness in a tarred flat bottom shallow dish (W_2). Dry at 105^0C to constant weight and weigh (W_3). Calculate the percentage of water – soluble extractive with reference to the air-dried drug.

3.15.2. Calculation

$$Percentage\ of\ Water\ soluble\ extractive: \frac{W3-W2X100X\ 100}{W1\ X\ 25}$$

Where, W_1= Weight of drug taken, W_2= W2ght of empty dish and W_3= Weight of dish+extractive residue.

3.16. Loss on drying (Oven Method)[8]

3.16.1. Description

Moisture content is the amount of moisture present in a crude drug sample. It should be minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination.

3.17. Equipment and glassware used

Hot air oven, LOD Bottle, Petri Dish

3.18. Reagent/ chemicals used

NA

3.18.1. Procedure

(Oven Method)

Sample is prepared by cutting shredding so that the parts are about 3 mm in thickness. Place about 10 gm

Table 2: Qualitative Phyto-chemical investigation in himavan agada formulations[9]

Sl. no	Parameter	Reagent preparation	Observation
1.		**Tests for Alkaloids**	
A.	Dragendroff's test	Bismuth sub nitrate- 1.7 gm Glacial Acetic acid- 20 ml Pott. Iodide (50%in water)- 100 ml Water- 80 ml	Alcoholic extract acidified with 2 M HCl + Reagent= Reddish brown precipitate
B.	Mayer's test	Mercuric Chloride- 1.36 gm Pott. Iodide- 5 gm Water- 100 ml	Alcoholic extract+Reagent= cream precipitate
C.	Wagner's test	Iodine- 2 gm Pott. iodide- 6 gm Water- 100 ml	Alcoholic extract acidified with 1.5% HCl + Reagent = Yellow or brown precipitate
D.	Hager's test	Picric Acid- 1.0 gm Water- 100 ml	Alcoholic extract + Reagent = yellow precipitate
2.		**Tests for Glycosides**	
A.	Keller-killiani Test (for deoxy sugar):	1 ml of glacial acetic acid containing traces of FeCl3 and 1 ml of conc. H2SO4 were added.	Aqueous extract + Reagent=A reddish-brown colour formed at the junction of two layers and the upper layer turned bluish green.
B.	Legal test (for cardinolides)	Conc. Ethanolic extract was made alkaline with few drops of 10% NaOH and then add freshly prepared Sod. Nitroprusside solution.	Conc ethanolic extract + Reagent= Blue coloration observed.
C.	Baljet test	Picric acid is added to the extract and made alkaline.	Aqueous extract + Reagent= Stable orange color.
D.	Borntrager's test	Dil. HCl is added to powdered drug and heated for 5 mins then filtered and add equal volume of CHCl3, shake well and collect the lower org. layer of CHCl3, add NH3 half of its volume, shake well.	Aqueous extract or powder drug + Reagent= Lower Ammonical layer turned rose pink colour.
3.		**Tests for Saponins**	
A.	Foam Test:	Powdered drug residue was taken in a test tube and shaken vigorously with a small amount of NaHCO3 and water.	Drug + Reagent= Characteristic honeycomb like froth obtained.
4.		**Tests for Steroids**	
A.	Salkowaski reaction	Residue of extract taken in 2 ml of CHCl3 and 2 ml of conc. H2SO4 added from the side of the test-tube and then shaken for few minutes.	Alcoholic extract + Reagent= Red colour developed in the CHCl3 layer.
B.	Libberman Burchard's Test	Acetic Anhydride Sulphuric acid	2mg of extract was dissolved in acetic anhydride, boil and cool then add 1 m conc H2SO4. Formation of pink colour.
5.		**Tests for Tannins and Phenolic Compounds**	
A.	Ferric chloride reagent	5% FeCl3 sol. added to test sol.	Aq. or alcoholic ext + Reagent= Dark green or deep blue colour is obtained.
B.	Lead acetate test	A 10% w/v solution of basic lead acetate in distilled water was added to test sol.	Aq. extract +Reagent= Precipitation occurred.
C.	Gelatin solution tes.	1% w/v solution of gelatin in water, with 10% sodium chloride and then added to test sol.	Aq. extract + Reagent= White precipitate is obtained
6.		**Tests for Flavonoids**	
A.	Shinoda Test:	Test residue dissolved in 5 ml ethanol (95% v/v) and reacted with few drops of conc. HCl and 0.5 g of Mg metal.	Hydro-alcoholic or alcoholic extract + Reagent= The pink, crimson or magenta colour is developed.
B.	Ammonia Test:	Filter paper strips were dipped in the alcoholic extract and ammoniated.	Alcoholic extract + Reagent= Strips turned yellow

(W_1) accurately weighed up to third decimal place of drug (Without preliminary drying) in a tarred evaporating dish + drug weight (W_2). Dry at 105°C for 5 hours and weigh (W_3). Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 percent. (Constant weight is reached when two consecutive weighing after drying for 30 minutes). Cool for 30 minutes in desiccator, show not more than 0.001 g difference.

3.18.2. Calculation

$$Percentage\ of\ loss\ on\ drying: \frac{W2 - W3\ X\ 100}{W1}$$

Where, W_1= Weight of drug taken, W_2= Weight of evaporating dish + drug before drying and W_3= Weight of evaporating dish + drug after drying.

3.19. Thin layer chromatography (TLC) test[10]

3.19.1. Description

TLC is the technique in which a solute undergoes distribution between two phases, stationary phase and a mobile phase. The stationary phase acts as an adsorbent in a relatively thin uniform layer of a dry finely powdered material applied to a glass, plastic or metal sheet.

Separation may be achieved on the basis of partition a or a combination of partition and adsorption, depending on the particular type of stationary phase, its preparation and uses of different solvents.

3.20. Equipment and glassware used

Oven, TLC developing chamber, Visualizing chamber, Pre-coated aluminium plates with silica gel 60 F_{254}, Beaker, measuring cylinder with stoppered and glass pipettes.

3.21. Reagent/ chemicals used

Different solvents and post-derivatise reagents.

3.21.1. Conditions

1. Stationary phase: Pre-coated silica gel 60 F_{254} aluminium plates
2. Mobile phase: Toluene: Ethyl acetate: Formic acid (8:2:0.1).
3. Chamber Saturation Time: 20 mins.
4. Test Solution: Dissolve 500 mg of sample in 10 ml of methanol.
5. Derivatising Agent- 5% Sulphuric Acid in Methanol

3.22. HIGH performace thin layer chromatography (TLC) test[11]

HPTLC is the technique in which a solute undergoes distribution between two phases, stationary phase and a mobile phase. The stationary phase acts as an adsorbent in a relatively thin uniform layer of a dry finely powdered material applied to a glass, plastic or metal sheet.

Separation may be achieved on the basis of partition a or a combination of partition and adsorption, depending on the particular type of stationary phase, its preparation and uses of different solvents.

Instrument contains different parts to applicate, scan and visualize the elution of different spots after development of TLC plate. So, we can easily develop a fingerprint profile for the same.

3.23. Equipment and glassware used

Linomats TLC applicator, Oven, TLC developing chamber, Visualizing chamber, Pre-coated aluminium plates with silica gel 60 F_{254}, Beaker, measuring cylinder with stoppered and glass pipettes, Camag TLC scanner and photographic chamber.

3.24. Reagent/ chemicals used

Different solvents and post-derivatise reagents.

4. Observation and Results

Table 3: Results of preliminary physicochemical and phytochemical analysis

Sl.No.	Parameters	Sample
1.	Alcohol Soluble Extractive	7.62%
2.	Water Soluble Extractive	15.26%
3.	LOD	10.29%
4.	Total Ash	8.16%
5.	Water Soluble Ash	2.55%
6.	Acid Insoluble Ash	0.91%

4.1. Organoleptic character

Table 4: Organoleptic characters ofPanchvalkal

Sr. No.	Parameters	Panchavalka
1	Appearance	Crude raw drug
2	Odour	Characteristic
3	Taste	-
4	Colour	Brownish

4.2. Qualitative parameters

4.2.1. Conditions

1. Stationary phase: Pre-coated silica gel 60 F_{254} aluminium plates
2. Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1).
3. Chamber Saturation Time: 20 mins.

Table 5: Results of phytochemical profile

Sl. no	Qualitative chemical Test	Results	Method
1.	Alkeloids	Negative	API 2008
2.	Glycosides	Positive	
3.	Tannins	Positive	
4.	Flavonides	Positive	
5.	Saponins	Negative	
6.	Steroids	Negative	

Table 6: Results of TLC analysis

Test Parameter	Limits	Result	Test Method
TLC Profile Sample	254nm	0.68	API 2008
	366nm	0.93,0.82,0.62,0.4,0.2	
	Derivatized	0.93, 0.82, 0.75, 0.70, 0.5, 0.31	

Table 7: Results of HPTLC fingerprinting analysis

Test Parameter	Limits	Result
HPTLC Fingerprinting Profile sample B	254 nm	0.18, 0.29, 0.54, 0.57, 0.64
	310 nm	0.18, 0.54, 0.58, 0.66, 0.68
	366 nm	0.18, 0.34, 0.54, 0.58, 0.65, 0.66, 0.7, 0.72, 0.79

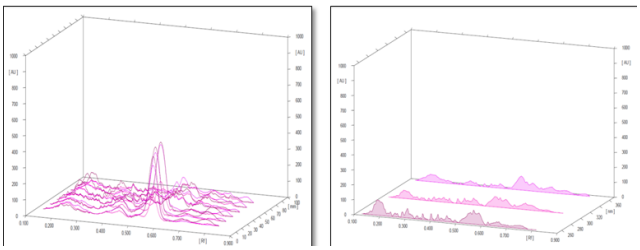

Fig. 1: Images of HPTLC fingerprinting at various nm

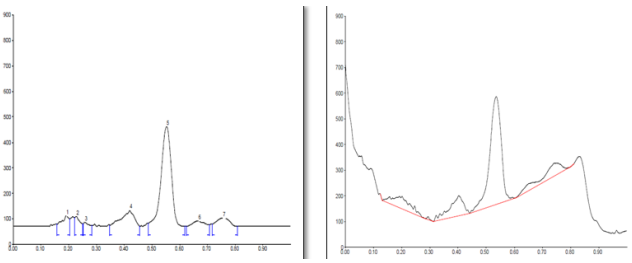

Fig. 2:

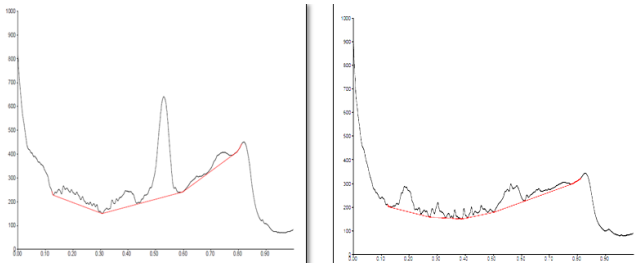

Fig. 3:

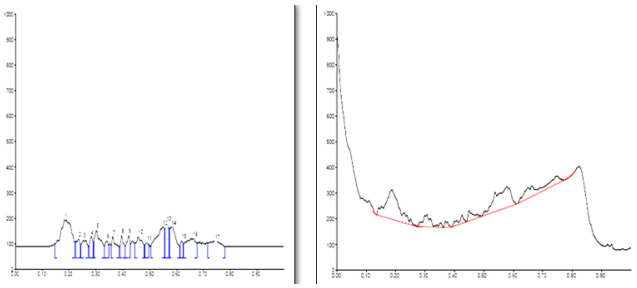

Fig. 4:

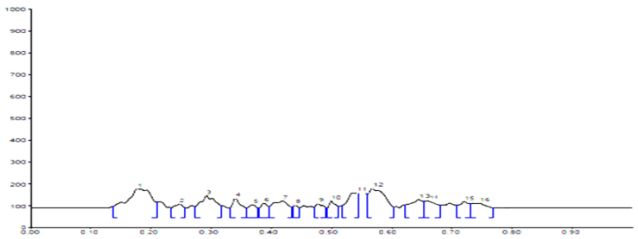

Fig. 5:

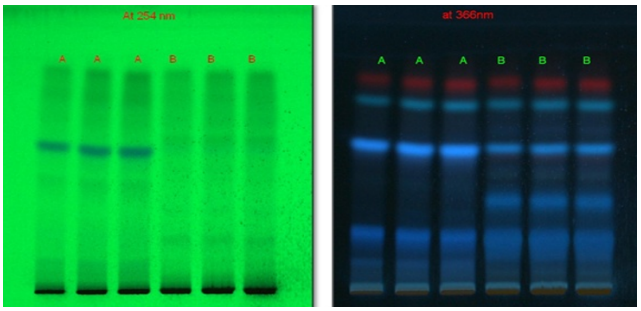

Fig. 6: HPTLC fingerprinting at various nm

4. Test Solution: Dissolve 100 mg of sample in 10 ml of methanol.
5. Derivatising Agent- Anisaldehyde Sulphuric Acid

5. Discussion & conclusion

5.1. HPTLC fingerprinting profile

The different concentration of Methanolic extract of samples was applied on HPTLC plates in 6, 8 and 10 μl. Plate was developed and elution of different spots visualized under 254, 366nm and white light after derivatization with Anisaldehyde sulphuric acid reagent.

5.2. Physico-phytochemical analysis

The quantitative tests e.g. total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, water- soluble extractive, ether-soluble extractive, moisture content, volatile oil content and assays are the methods upon which the standards of Pharmacopoeia depend. So it is necessary to analyze Ayurvedic formulation on these parameters.

6. Source of Funding

None.

7. Conflict of Interest

None.

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