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Research Article

PHARMACOGNOSTIC AND PHARMACEUTICO-ANALYTICAL STUDY OF *MURVADI AGADA*

Sachin Mangattu[1], Varsha R. Solanki[2]*

[1]PhD Scholar, *[2]Professor and Head, Department of Agad Tantra, Institute of Teaching and Research in Ayurveda, INI, GoI, MoA, Jamnagar, Gujarat, India.

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ABSTRACT

In the current era, the quality assessment of herbal formulations is of great importance in order to confirm the authenticity before its release into the market. Comprehensive guidelines have been set up by the World Health Organisation to ensure rigorous standards in their production and use. *Murvadi Agada* is a formulation which is composed of 10 herbal drugs and it is indicated in *Gara Visha* (artificial poison). **Aim:** To prepare *Murvadi Agada* and to establish its preliminary standard profile through pharmacognostic evaluation, physicochemical analysis, phytochemical screening and High-performance thin layer chromatography fingerprinting. **Materials and Methods**: The pharmacognostic evaluation was performed at the Pharmacognosy Department, ITRA, Jamnagar, while the pharmaceutical study was conducted at the Pharmaceutical Chemistry Department, ITRA, Jamnagar. High performance thin layer chromatography analysis was carried out at Vasu Research Centre, Vadodara. **Results:** Pharmacognostical findings confirmed the ingredients of *Murvadi Agada.* Values of physico-chemical parameters were as follows: loss on drying 1.038%w/w, pH 6, total ash value 8.053%w/w, water soluble extractive value 11.3645%w/w and alcohol soluble extractive value 11.3839%w/w. Qualitative tests revealed the presence of carbohydrates, steroids, glycosides, saponins, tannins, flavonoids in aqueous extract and carbohydrates, steroids, alkaloids, flavonoids in methanolic extract of the sample. High performance thin layer chromatography showed 6 major spots at 254nm, 5 major spots at 366nm and 9 major spots at 540nm. **Conclusion:** The trial drug is free from any impurities or adulterants and data generated can be used to develop a preliminary standard profile for *Murvadi Agada*.

INTRODUCTION

Agada Tantra (Ayurveda toxicology) deals with the symptoms and treatment related to the poisonous effect of both plant and animal poisons. The anti-poisonous drugs used to counter the action of the poisons are known as *Agada* (antidote). Diseases are caused due to exposure to cumulative, concocted poisons and incompatible dietary habits which signifies the need of detoxification at all levels. The *Agada Yogas* (anti-poisonous formulations) mentioned in *Samhitas* (treatises) possess anti-toxic, anti-oxidant and radical scavenging activities.

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The judicial use of *Agada Yogas* (anti-poisonous formulations) in certain diseases is found to be effective as per research.

Visha (poison) is basically classified into two, *Akritrima* (natural) and *Kritrima* (artificial). *Akritrima Visha* (natural poison) is further classified into *Sthavara Visha* (plant-based poison) and *Jangama Visha* (animal-based poison). *Kritrima Visha* (artificial poison) is also known as *Gara Visha* (artificial poison).[1] *Gara Visha* (artificial poison) is said to be prepared with parts of poisonous creatures, waste products, incompatible drugs etc. It is chiefly administered with food with the intention to kill enemies or by women to gain control over their husbands.[2] The poisoning acts on both physical and psychic levels which results in symptoms *Pandu* (anemia), *Jwara* (fever), *Alpagni* (depleted metabolism), *Kasa* (cough), *Swasa* (dyspnea), *Udara* (ascites), *Yakrut Roga* (hepatomegaly), *Pleeha Roga*

(splenomegaly) etc. [3] *Murvadi Agada* mentioned in *Ashtanga Hridaya, Visha Pratisheda* contains *Murva* (*Marsdenia tenacissima* (Roxb.) Moon), *Guduchi* (*Tinospora cordifolia* (Thunb.) Miers), *Tagara* (*Valeriana wallichii* DC.), *Pippali* (*Piper longum* L.), *Patola* (*Trichosanthes cucumerina* L.), *Chavya* (*Piper retrofractum* Vahl), *Chitraka* (*Plumbago zeylanica* L.), *Vacha* (*Acorus calamus* L.), *Musta* (*Cyperus rotundus* L.), *Vidanga* (*Embelia ribes Burm*. f.) plants.[4] *Dhatvagni Mandhya* (depleted tissue specific metabolism) is the primary symptom of *Gara Visha* (artificial poison) and the main action of *Murvadi Agada* is to boost the hampered digestive fire.

Presently in the market, the need of herbal material is increasing on daily basis. Hence, there is the need to maintain the quality of the material. Unfortunately, the regulation norms to maintain the quality of the herbal medicines are not stringent when compared to synthetic drugs. The quality can be affected by various physical, chemical and geographical aspects. Moreover, deceptive practices such as adulteration or substitution compromise the quality of the herbal material. All of this is leading to hazardous effects on the health of the consumers. Hence it is important to validate the herbal materials before bringing it to the market. Standardization and the phytochemicals investigation are carried out; apart from these, there are various quality control tools which are used to assure the quality aspects of the herbals. The present study aims to authenticate and develop an analytical profile of *Murvadi Agada*.

MATERIALS AND METHODS

Collection of raw drugs

Most of the raw drugs of *Murvadi Agada* were collected from the Pharmacy, ITRA, Jamnagar, Gujarat. *Murva, Patola* and *Chitraka* samples were collected from the Narayan Aushadha Bhandar, Jamnagar, Gujarat.

Preparation

All the ten ingredients (mentioned in Table 1) were taken in equal quantity, dried and made into *Sookshma Choorna* (very fine powder) separately. These individual fine powders obtained were sieved through 80-number mesh. All the drugs were mixed homogenously for more than seven times. The homogenously mixed *Choorna* (fine powder) was packed in airtight containers.

Table 1: Contents of *Murvadi Agada*

No.	Drug name	Botanical nomenclature	Family	Partsused [5]	Composition
1	*Murva*	*Marsdenia tenacissima* (Roxb.) Moon	Apocynaceae	Root	1 part
2	*Guduchi*	*Tinospora cordifolia* (Thunb.) Miers	Menispermaceae	Stem	1 part
3	*Tagara*	*Valeriana wallichii* DC.	Caprifoliaceae	Rhizome	1 part
4	*Pippali*	*Piper longum* L.	Piperaceae	Fruit	1 part
5	*Patola*	*Trichosanthes cucumerina* L.	Cucurbitaceae	Whole plant	1 part
6	*Chavya*	*Piper retrofractum* Vahl	Piperaceae	Stem	1 part
7	*Chitraka*	*Plumbago zeylanica* L.	Plumbaginaceae	Root	1 part
8	*Vacha*	*Acorus calamus* L.	Acoraceae	Rhizome	1 part
9	*Musta*	*Cyperus rotundus* L.	Cyperaceae	Rhizome	1 part
10	*Vidanga*	*Embelia ribes* Burm. f.	Primulaceae	Fruit	1 part

Pharmacognostical evaluation

As per API, drugs which are used in the finished product of *Murvadi Agada* were identified and authenticated by the Pharmacognosy Laboratory, ITRA, Jamnagar. The identification was carried out based on the organoleptic features and microscopy of the prepared drug. [6,7,8,9]

Powder Microscopy

Powdered drug was studied microscopically and microscopic characters of individual drugs were noted. The powder of the drug was dissolved with water followed by microscopy of the sample without stain and after staining with Phloroglucinol + HCl. Microphotographs of the sample were also taken under Carl Zeiss trinocular microscope.

Physico-chemical parameters

Loss on drying, total ash, water soluble extract, alcohol soluble extract and pH value were assessed.[10]

Determination of loss on drying at 110°C

To determine the loss on drying of the sample, 2gm of precisely weighed sample was taken in dried and previously weighed petri-dish; spread evenly and dried in the oven at 110°C till constant weight. The weight after drying was noted and the loss on drying was calculated. The percentage of loss on drying was considered based on the weight of air-dried sample and expressed as % w/w.

Determination of Ash value

To determine the ash value of the sample, about 2 g of powdered and accurately weighed sample drug was taken in previously weighed dry silica crucible. It was then scattered in a fine even layer at the bottom of the crucible and incinerated in a muffle furnace by gradually increasing the heat, not exceeding 450°C until freed from all carbon. It was then allowed to cool slowly and weighed to a constant weight in an accurate digital weighing machine. The percentage (%w/w) of ash was calculated with reference to the air-dried sample.

Determination of Water-soluble extractive value

5 g of the coarsely powdered drug was weighed and transferred into a round bottomed flask. To this 100ml of water was added and kept for 24 hours. The content was shaken frequently for 6 hours and allowed to stand for 18 hours. After that it was filtered through ordinary filter paper. 25ml of filtrate was evaporated to dryness in a previously weighed porcelain evaporating dish on hot water bath and then at 110°C hot air oven till it attains constant weight. The evaporating dish was then allowed to cool and weighing was continued till a constant weight was obtained. The percentage of cold water soluble extract was calculated from the weight of the residue with respect to the sample drug taken.

Determination of Alcohol soluble extractive value

5gm of the coarsely powdered drug was weighed and transferred into a round bottomed flask. To this 100ml of alcohol of specified strength (95%) was added and kept for 24 hours. The content was shaken frequently for 6 hours and allowed to stand for 18 hours. After that it was filtered through ordinary filter paper. 25ml of filtrate was evaporated to dryness in a previously weighed porcelain evaporating dish on hot water bath and then at 110°C hot air oven till it attains constant weight. The evaporating dish was then allowed to cool and weighing was continued till a constant weight was obtained. The percentage of alcohol soluble extract was calculated from the weight of the residue with respect to the sample drug taken.

Determination of pH

5% w/v aqueous solution of the sample drug was prepared by putting 2.5gm sample in 50ml water, and was kept overnight. It was then filtered and the pH of the filtrate was noted.

Preliminary qualitative chemical tests

The aqueous and methanolic extractives were subjected to qualitative analysis for identification of various plant constituents. Tests were done to assess the presence of various phyto-constituents in the sample such as carbohydrates, proteins, steroids, glycosides, saponins, alkaloids, tannin and flavonoids.[11]

Test for Carbohydrates

Molisch's test: 2 drops of fresh 10% a-naphthol reagent was mixed with 2ml of test solution. 2ml of concentrated H_2SO_4 was poured to the mixture. Formation of a red-violet ring indicates the presence of carbohydrates.

Test for Proteins

Biuret test: 2ml of 4% NaOH and 2 drops of 1% copper sulphate solution was added to 3ml test solution. Appearance of violet or pink colour indicates the presence of two or more peptide bond of proteins.

Test for Steroids

Salkowski test: 2ml chloroform was added to 2ml of test solution then 2ml concentrated H_2SO_4 was added gently from the side of the test tube and is shaken. The chloroform layer changes from red to purple and the acid layer shows greenish-yellow fluorescence indicating the presence of steroids.

Test for Glycosides

Keller-Kiliani test: Few drops of glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 was added to 2ml of test solution. The appearance of reddish brown colour at junction of the two liquid layers and bluish-green colour in the upper layer indicates the presence of glycosides.

Test for Saponins

Foam test: The test solution is shaken vigorously with distilled water in a test tube. The appearance of honeycomb like foam, which persists for few minutes confirms the presence of saponins.

Test for Alkaloids

Dragendorff's test: Few drops of Dragendorff's reagent to 2-3 mL of test solution. Appearance of orange-brown precipitate indicates the presence of alkaloids.

Test for Tannin

Ferric chloride test: Few drops of 5% Ferric chloride solution ($FeCl_3$) were added to 1-2 mL of test solution. A brown-coloured precipitate indicates the presence of tannins.

Test for Flavonoids

Lead acetate test: The appearance of white precipitate when the test solution was treated with lead acetate indicates the presence of flavonoids.

HPTLC

High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of herbal chemical constituents. Here an attempt has been made to develop HPTLC pattern of the drug under study *Murvadi Agada.* [12]

Sample Preparation for HPTLC

1gm of sample was weighed accurately in a conical flask. To it 10ml methanol was added and the mixture was refluxed for 30 minutes on a water bath. It was then allowed to cool and filtered using Whatman

filter paper No. 1. The filtrate thus obtained was used for HPTLC fingerprinting.

Method for developing HPTLC

Chromatographic separation of *Murvadi Agada* was carried out using HPTLC on pre-coated silica gel 60 F254 aluminium plates (Merck). Sample application was performed using a CAMAG Linomat 5 automatic sample applicator (Camag, Muttenz, Switzerland) equipped with a 100µL Hamilton syringe. The volume of sample application was 8.0µL. The plates were developed in a CAMAG glass twin-trough chamber, pre-saturated with the mobile phase for 30 minutes. The mobile phase consisted of Toluene : Ethyl Acetate : Acetic Acid in a ratio of 7:2:1 (v/v). After development, the plates were scanned using a CAMAG TLC Scanner II with CATS 3 software. Post-derivatization was done using anisaldehyde–sulphuric acid reagent, and plates were dried on a pre-heated TLC plate heater at 100 ± 5°C for 3 minutes prior to analysis at 254 nm, 366 nm, and 540 nm.

RESULTS AND OBSERVATION

The results and observation of the present study are tabulated below:

Pharmacognostical study

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Murvadi Agada*. The data reported from microscopic and macroscopic studies helps in authentication of genuine herbs. Results were matched with the API and thus confirmed the genuineness of all the drugs used in the finished product.

Organoleptic findings

Table 2: Organoleptic characters of *Murvadi Agada*

No.	Characters	Results
1	*Rupa* (colour)	Brown
2	*Rasa* (taste)	Astringent
3	*Gandha* (odour)	Aromatic
4	*Sparsha* (consistency)	Smooth

Finished product microscopy

Microscopic evaluation of the finished product of *Murvadi Agada* was conducted under 10X magnification, characters were noted down and microphotographs were taken (Figure 1).

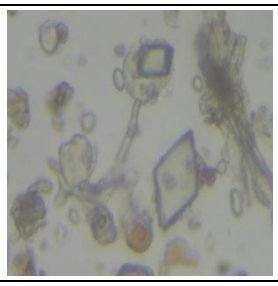


Fig 1(a): Prismatic crystals of *Murva*

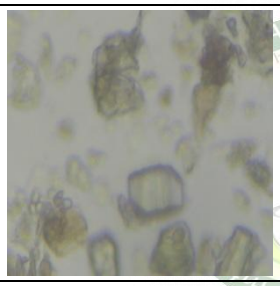


Fig 1(b): Rhomboidal crystals of *Murva*

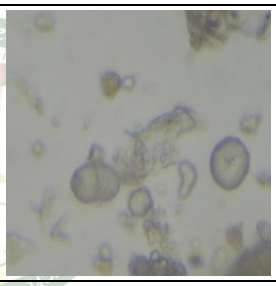


Fig 1(c): Starch grains of *Murva*

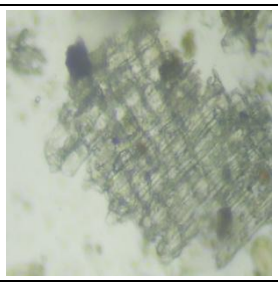


Fig 1(d): Cork cells of *Murva*

Fig 1(e): Pitted vessels of *Guduchi*

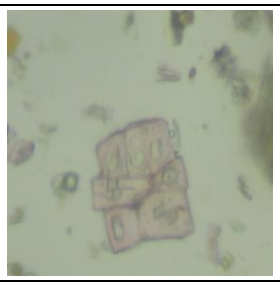


Fig 1(f): Lignified collenchyma cells of *Guduchi*

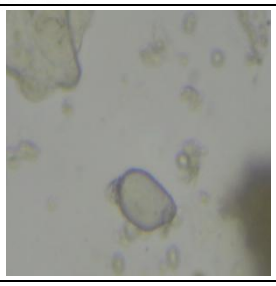


Fig 1(g): Starch grains of *Guduchi*

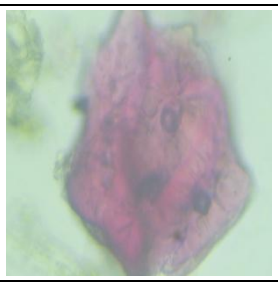


Fig 1(h): Stone cells of *Tagara*

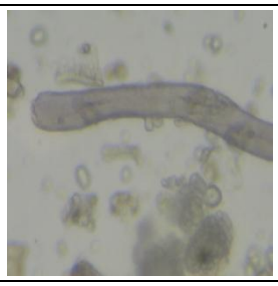


Fig 1(i): Fibres of *Tagara*

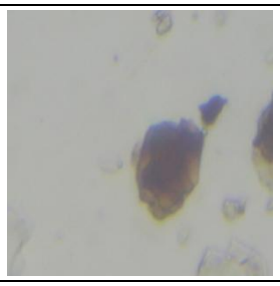


Fig 1(j): Brown content of *Tagara*

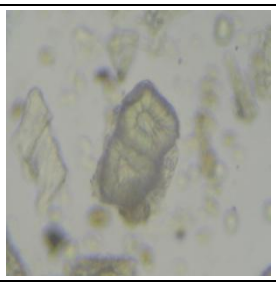


Fig 1(k): Stone cells of *Pippali*

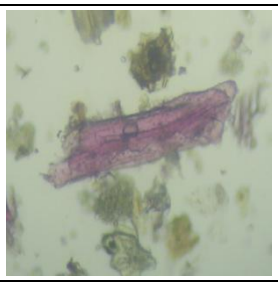


Fig 1(l): Lignified fibres of *Patola*

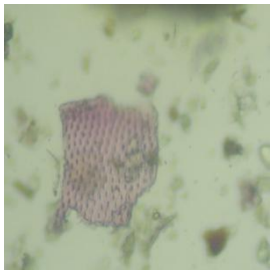
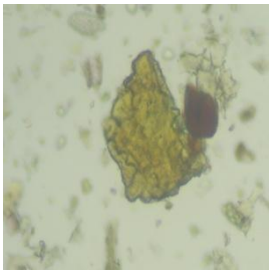
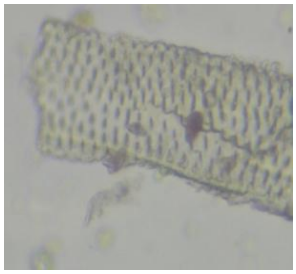
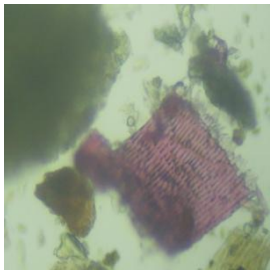
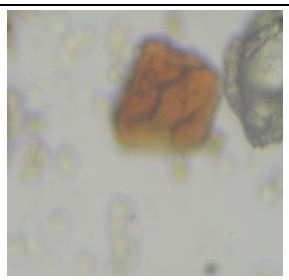
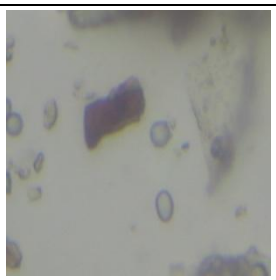
			

Fig 1(m): Lignified pitted vessels of *Patola*	**Fig 1(n): Brown content of *Patola***	**Fig 1(o): Pitted vessels of *Chavya***	**Fig 1(p): Lignified pitted vessels of *Chavya***
			
Fig 1(q): Lignified pitted vessels of *Chitraka*	**Fig 1(r): Brown content of *Chitraka***	**Fig 1(s): Starch grains of *Vacha***	**Fig 1(t): Annular vessels of *Vacha***

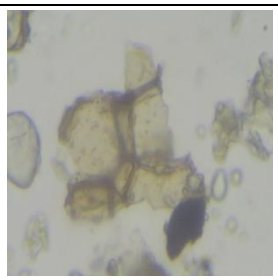
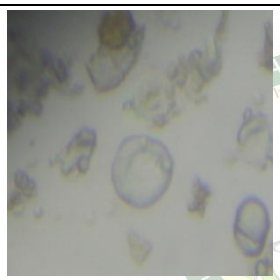
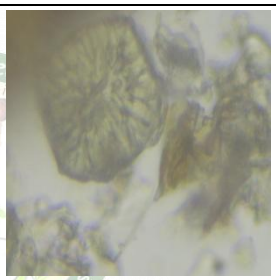
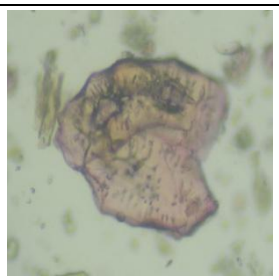

			

Fig 1(u): Cork cells of *Musta*	**Fig 1(v): Starch grains of *Musta***	**Fig 1(w): Stone cells of *Vidanga***	**Fig 1(x): Lignified stone cells of *Vidanga***

Figure 1: Microphotographs of Ingredients

Physico-chemical parameters

Physico-Chemical parameters of *Murvadi Agada* like loss on drying, total ash, water soluble extract, alcohol soluble extract and pH value were assessed (mentioned in Table 3).

Table 3: Physico-Chemical parameters of *Murvadi Agada*

S.No.	Parameters	*Murvadi Agada*
1	Loss on drying	1.038%w/w
2	Ash value	8.053%w/w
3	Water soluble extract	11.3645%w/w
4	Alcohol soluble extract	11.3839%w/w
5	pH	6

Preliminary qualitative chemical tests

Qualitative analysis was carried out by means of methanol and aqueous extracts of *Murvadi Agada*. The test samples were appraised for carbohydrates, proteins, steroids, glycosides, saponins, alkaloids, tannins, flavonoids etc (mentioned in Table 4).

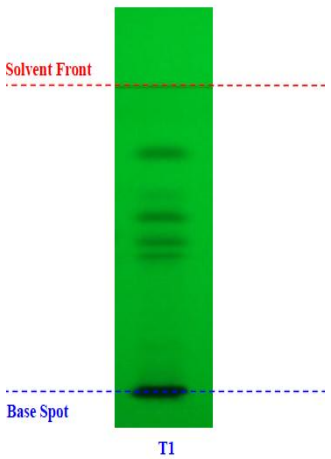
Table 4: Preliminary qualitative chemical tests

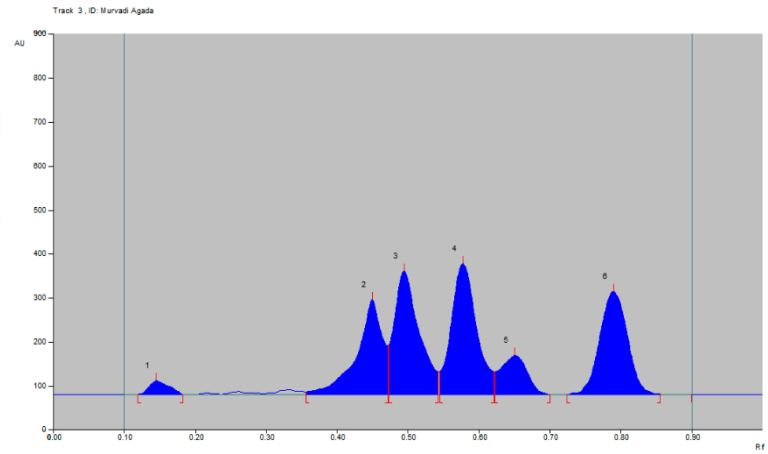
No.	Active constituent	Test	Aqueous extract	Methanolic extract
1	Carbohydrates	Molisch's test	+	+
2	Proteins	Biuret test	-	-
3	Steroids	Salkowski test	+	+
4	Glycosides	Keller-Kiliani test	+	-
5	Saponins	Foam test	+	-
6	Alkaloids	Dragendorff's test	-	+
7	Tannins	Ferric chloride test	+	-
8	Flavonoids	Lead acetate test	+	+

High Performance Thin Layer Chromatography (HPTLC) of *Murvadi Agada*

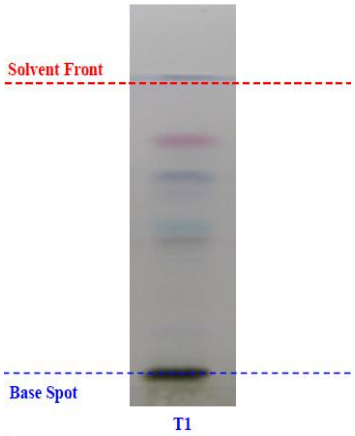
Table 5: High performance thin layer chromatography (HPTLC)

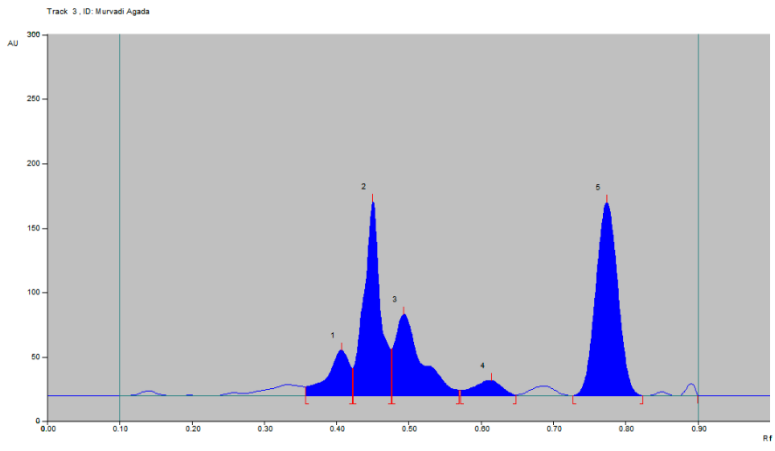
Name of Drug	Wave length	No. of Spots	Maximum Rf Value
Murvadi Agada	At 254 nm	6	0.14, 0.45, 0.49, 0.58, 0.66, 0.80
	At 366 nm	5	0.41, 0.45, 0.49, 0.61, 0.77
	At 540 nm	9	0.14, 0.28, 0.34, 0.38, 0.45, 0.49, 0.61, 0.66, 0.80



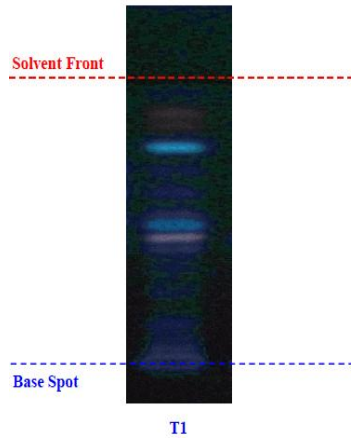


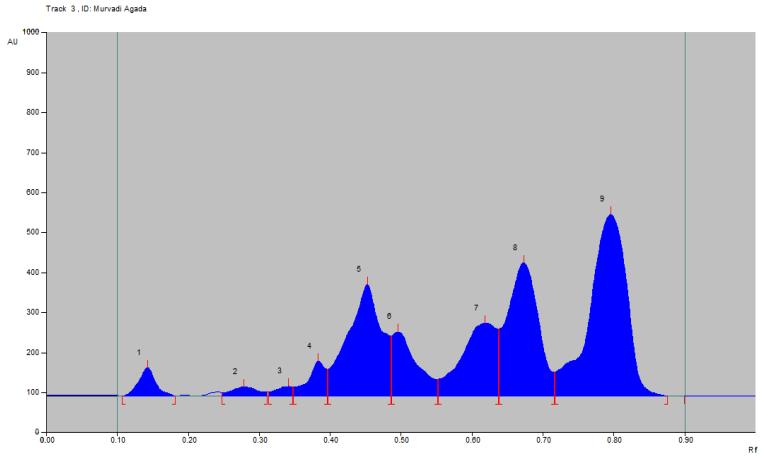
UV 254nm | **Figure 2(a) HPTLC Chromatogram 254nm**





UV 366nm | **Figure 2(b) HPTLC Chromatogram 366nm**





UV 540nm	Figure 2(c) HPTLC Chromatogram 540nm

Figure 2: HPTLC of *Murvadi Agada*

DISCUSSION

All systems of medicine consider quality assurance as an integral part to ensure quality medication. Even though WHO has set up specific guidelines to ensure quality, the number of studies related to their scientific assessment are less. For the present study, *Murvadi Agada* was prepared in the method explained in *Ashtanga Hridaya*. The formulation consisted of 10 herbal drugs which were properly collected and authenticated. All the drugs were powdered and mixed together.

The finished product was analysed for organoleptic characters, pharmacognosy, and other quality checks of standardization as per Ayurveda Pharmacopeia of India. Pharmacognosy evaluation showed the presence of prismatic crystals, lignified stone cells, cork cells and starch grains of various ingredients.

To validate the pharmaceutical process various physico-chemical parameters were used. The pH showed the acidic nature of the final product. Reduced loss on drying indicates less chances of microbial contamination. It also indicates that stability of the drug is good. Ash value or ash content is the amount of inorganic residue that remains after oxidizing or incinerating organic matter. The ash value was found to be 8.053%w/w, which suggests that the inorganic substances present in the sample were within the standard limits. Water soluble extract value 11.3645%w/w means that it consists of a moderate number of water-soluble compounds like sugars, acids, and inorganic salts within its composition. The alcohol soluble extractive value measures the number of polar components in plant material that are soluble in alcohol. A moderate percentage of the phytochemical components of *Murvadi Agada* are soluble in alcohol media, according to its alcohol soluble extractive value of 11.3839%w/w. It is found that *Murvadi Agada* has a higher alcohol-soluble extractive value compared to its water-soluble extractive value.

Qualitative test revealed presence of carbohydrates, steroids, glycosides, saponins, tannins and flavonoids in aqueous extract of the sample. The methanolic extract of the sample showed presence of carbohydrates, steroids, alkaloids and flavonoids. Glycosides, saponins and tannins were found to be absent in methanolic extract. Alkaloids were absent in aqueous extract. Proteins were found to be absent in both aqueous and methanolic extract of the sample.

The HPTLC fingerprinting of *Murvadi Agada* was carried out using a silica gel 60 F254 plate and visualized at 254nm, 366nm, and 540nm after derivatization. Under each wavelength, the sample (Track T1) showed distinct chromatographic profiles, suggesting a rich phytochemical composition. At 254 nm, six prominent peaks were observed at Rf values 0.14, 0.45, 0.49, 0.58, 0.66, and 0.80, which denotes the presence of multiple UV-absorbing compounds [Figure 2(a)]. Under 366nm, five peaks were noted, including major spots at Rf 0.41, 0.45, 0.49, 0.61, and 0.77 indicating fluorescence characteristics of certain phytoconstituents [Figure 2(b)]. After derivatization and visualization at 540nm, nine peaks appeared at Rf 0.14, 0.28, 0.34, 0.38, 0.45, 0.49, 0.61, 0.66, and 0.80, which reflect colour-producing compounds such as terpenoids or phenolics [Figure 2(c)].

Moreover, the repeated appearance of Rf 0.14, 0.45, 0.49, 0.61, 0.66, and 0.80 across multiple wavelengths suggests the presence of stable bioactive markers in *Murvadi Agada.* These consistent bands can be taken as potential phytochemical markers for the formulation.

CONCLUSION

Murvadi Agada comprises of *Murva* (*Marsdenia tenacissima* (Roxb.) Moon), *Guduchi* (*Tinospora cordifolia* (Thunb.) Miers), *Tagara* (*Valeriana wallichii* DC.), *Pippali* (*Piper longum* L.), *Patola* (*Trichosanthes cucumerina* L.), *Chavya* (*Piper retrofractum* Vahl), *Chitraka* (*Plumbago zeylanica* L.), *Vacha* (*Acorus calamus* L.), *Musta* (*Cyperus rotundus* L.), and *Vidanga* (*Embelia ribes Burm*. f.). Pharmacognostical findings confirmed the ingredients of *Murvadi Agada*. Physico-chemical analysis helps to create a preliminary standard analytical profile for *Murvadi Agada* as no standard is available in the pharmacopoeia. Hence, data generated by this study can be used as a tool for the identity and purity of the formulation, *Murvadi Agada.*

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***Address for correspondence**
Dr. Varsha R. Solanki
Professor and Head,
Department of Agad Tantra,
Institute of Teaching and Research in Ayurveda, INI, GoI, MoA,
Jamnagar, Gujarat.
Email: vrs@itra.edu.in