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Original Article

# Pharmaceutico-Analytical Investigation of Murvadi Agada with HPLC Marker Analysis

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## ABSTRACT

**Background:** *Agada yogas* are traditional *Ayurvedic* remedies that are recommended for the treatment of both acute and chronic poisoning as well as a number of illnesses brought on by exposure to toxins. *Murvadi agada* a unique polyherbal preparation used to treat *Gara visha* and *Agni vikaras* (digestive dysfunction) is discussed in relation to '*Garopahat Paavaka*" a disorder marked by disturbed Agni due to cumulative or artificial toxins. Scientific standardization is necessary to guarantee its quality, safety, and reproducibility despite its therapeutic significance. To standardize *Murvadi Agada* through comprehensive pharmaceutico-analytical evaluation including chromatographic profiling, physicochemical analysis, organoleptic assessment, and phytochemical screening. **Materials and Methods:** Authentic raw drugs yielded fine *churna* of *Murvdai agada*. Pharmacopeial assays quantified organoleptic traits, moisture, total alkaloids and flavonoids etc. Preliminary phytochemicals were screened for secondary metabolites. TLC furnished characteristic $R_f$ metrics, HPLC enabled piperine and quercetin quantification as biomarkers. **Results:** Parameters adhered to compendial thresholds, affirming purity/stability. Phytoconstituents encompassed flavonoids, alkaloids, glycosides and reductants. TLC exhibited reproducible $R_f$ bands. Flavanoid yield: 8.01 +- 0.78 mg QE/g. HPLC revealed piperine (0.978%) and quercetin (0.044%). **Conclusion:** This integrative method ensures therapeutic consistency and improved scientific credibility by bridging traditional *Ayurvedic* knowledge with contemporary analytical validation.

**Keywords:** *Gara visha*, HPLC, *Murvadi agada*, Phytochemical analysis, Standardization, TLC.

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## INTRODUCTION

Agada yogas constitute a distinct and specialized group of formulations for the management of *Gara visha*. These compositions are intended to fight inflammation, tissue damage and metabolic disruptions brought on by toxic exposure in addition to neutralizing toxins and restoring systemic equilibrium. *Gara visha* is any synthetic or artificial material that has an impact on the body either directly or indirectly through harmful metabolites. *Acharya Charaka* states that Gara *Visha* is the substance which is not instantly lethal as it has delayed digesting attribute (Agnivesha *et al*., 2015), while *Acharya Sushruta* and *Acharya Vagbhata* says that it is a poison that is made from animal waste, a mixture of medicines or *Bhasmas* that have opposing qualities, or a poison with reduced potency (Sushruta, 2016).

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Whether intentionally or inadvertently, people nowadays are exposed to a wide range of poisons, both natural and man-made. Nowadays *Gara visha* can be understood as the poisonous effect on the human body caused by the frequent and unmoderated consumption of junk food, preservatives, food additives, coloring agents, soft drinks, and processed meals in daily life.

*Gara Visha* physical and psychological symptoms described in classics are *Pandu, Krisha, Alpagni, Kasa, Jwara, Shwasa, Deena Vaak, Durbala, Alasa, Shopha, Suska Pada-Kara* etc., and a person may also see dried trees and barren reservoirs or have vivid nightmares about creatures like wild jackals, cats, mongooses, snakes, and monkeys (Vagbhata, 2014).

*Murvadi Agada* of the *Ashtanga Hridaya Uttarasthana Vishapratisheda* chapter is recommended in the context of "*Garopahat Paavaka"*: the symptoms brought on by Agni dysfunction carried on by the use of *Gara Visha* (Tripathi, 2013). The qualities of *Pitta Saraka, Anulomaka, Grahi, Shula Prashamaka, Pachaka, and Deepaka* are all present in *Murvadi agada* and four distinct *Anupanas* are listed specifically, *Amla Rasa, Masthu, Takram, and Koshnambu* (Murthy, 2009).

Even though *Murvadi Agada* has been used therapeutically for millennia, strong scientific validation and quality assurance

are necessary for its translation into modern clinical practice and broader acceptability. Due to the presence of several phytochemicals, changes in raw drug sources, regional variances, seasonal fluctuations and a variety of processing techniques, polyherbal formulations are complicated. Therefore, modern analytical tools such as TLC and HPLC are essential to establish identity, purity and consistency of *Murvadi agada*.

## MATERIALS AND METHODS

### Method of preparation

Instruments and Equipment's-Weighing machines, pulveriser, clean cotton swabs or cloth, analytical balance, steel vessels, masks, caps, aprons, sieves 85 and 120 mesh, gas and burners are some of the supplies and instruments.

### Preparation of Churna

We devised the formulation by procuring the raw drugs from the reliable sources and prepared the formulation in the Khasbhag pharmacy.

Each of the ten premium ingredients are chosen in their unprocessed state and metered in the same amounts. A pulveriser was used to grind each ingredient into a fine powder. These churnas are first passes through an 85# mesh and a 120# sieve independently to produce a homogenous mixture that is uniformly blended (Hiremath and Digra, 2022).

They are then all mixed together in equal portions. The evenly mixed churna is then placed in a UV chamber and exposed to UV light for more than 2 hr in order to maintain its purity and prevent microbiological contamination. The produced churna is then sealed in pouch or airtight containers to preserve its efficacy and prevent moisture penetration.

### Analytical Study

The analytical investigation was conducted at Shri B.M.K. Ayurveda Mahavidyalaya Belgaum Central Research Laboratory, which has been approved by AYUSH. The HPLC test was done at Drug Testing Laboratory in Rajiv Gandhi Education Society's Ayurvedic Medical College and Hospital, Ron.

The sample was analysed for:

1. Organoleptic Characters.
2. Physiochemical parameters.
3. Phytochemical Properties.
4. Thin Layer Chromatography.
5. Total Alkaloids and Total Flavonoids.
6. High Performance Liquid Chromatography.

## RESULTS

### Organoleptic Parameters

The following are examples of a substance's macroscopic qualities that may be seen without a microscope: colour, texture, taste, odour, and formulation state or form. The *Murvadi Agada's* macroscopic characteristics are characteristic odour in churna form with light-brownish colour and astringent taste.

The Indian Pharmacopeia served as the basis for the techniques employed. Water- soluble extract, acid insoluble ash, moisture content, and total ash value were all measured. We employed the methods specified in the API (Ministry of Health and Family Welfare, Department of AYUSH, 2008).

### Total ash and Acid Insoluble Ash (AIA)

#### *Method*

A precisely weighed, air-dried powdered sample of Murvadi Agada was incinerated in a silica crucible at a temperature not exceeding 600°C until carbon free ash was produced. After cooling in desiccator, the residue was weighed to compute total ash.

For AIA the total ash was boiled with 2 M HCl, filtered, washed, ignited, cooled, and weighed to calculate acid-insoluble ash representing siliceous matter.

The physicochemical properties of *Murvadi Agada* are moisture content 8.710%, total ash value 7.338%, acid insoluble ash 1.823%, water soluble extractive 14.812% and alcohol soluble extractive 11.266%.

### Phytochemical Screening

The *Murvadi Agada* aqueous extract and alcohol extract was subjected to phytochemical screening for major secondary metabolites by standardized internationally accepted protocols as described by Harborne (1967) Trease and Evans' and other pharmacopeial standards (Harborne, 1998; Trease and Evans, 2009) (Table 1).

The results of phytochemical screening of *Murvadi Agada* aqueous extract and alcohol extract is shown in Table 1.

### Thin Layer Chromatography

Thin Layer Chromatography (TLC) was performed for qualitative phytochemical analysis. 50 gms of Murvadi agada powder was extracted with 100 mL ethanol, filtered and shaken for 6 hours. The extract was applied on silica gel 60F254 plates and developed in toluene:ethyl acetate (7:3) after chamber saturation. Spots were visualized under UV light (254 and 366 nm) and $R_f$ values were calculated (Khandelwal, 2008) (Table 2).

## Total flavonoids and Alkaloids

Using the Aluminium Chloride ($Alcl_3$) colorimetric method, the total flavonoid content of Murvadi agada was calculated and represented as mg Quercetin Equivalents (QE)/g of dry material. 80% ethanol was used to extract 1 g of powdered formulation which was then centrifuged and combined to create 50 mL. A reagent blank was utilized to quantify absorbance, and a quercetin standard (10-100 µg/mL) was used for calibration (Table 3).

**Table 1:** **Phyto-chemical screening of *Murvadi Agada*.**

| Tests | Water | Alcohol |
|---|---|---|
| Test for Carbohydrates | Positive | Negative |
| Test for Reducing sugar | Positive | Positive |
| Test for Monosaccharides | Negative | Positive |
| Test for Pentose sugar | Negative | Negative |
| Test for Non reducing sugar | Negative | Negative |
| Test for Hexose sugar | Negative | Positive |
| Test for Proteins | Negative | Negative |
| Test for Amino acids | Negative | Negative |
| Test for Steroids | Negative | Negative |
| Test for Flavonoids | Positive | Positive |
| Test for Alkaloids | Negative | Positive |
| Test for Tannins | Negative | Negative |
| Cardiac glycosides | Positive | Positive |
| Anthraquinone glycosides | Negative | Negative |
| Saponin glycosides | Positive | Negative |

While acid base extraction was used to assess total alkaloids. Diluted acid was used to extract the sample, which was then alkalinized to release free alkaloids, separated using an organic solvent, and measured using standard acid titration.

## HPLC

High Performance Liquid Chromatography (HPLC) was used to quantify piperine in Murvadi Agada. The sample was extracted with methanol by sonication (International Conference on Harmonisation, 2005). Analysis was performed on a Waters UPLC system with PDA detector using a Symmetry C18 column (4.6 × 250 mm, 5 µm). HPLC-grade solvents and reference piperine standard were used (Table 4).

A standard stock solution of piperine (10 mg) was produced in methanol to obtain 1000 µg/mL. To generate a calibration curve, aliquots were diluted to concentrations ranging from 100 to 500 µg/ml. To conduct sample analysis, 10 mg of Murvadi Agada (192/MA) was extracted with methanol, sonicated for 30 minutes, and filtered through a 0.2 µm membrane filter (Figures 1 and 2).

To estimate quercetin Waters UPLC system was used with a Symmetry C18 column (4.6 × 250 mm, 5 µm), a PDA detector, and Empower software. HPLC-grade solvents were employed, and the quercetin reference standard was obtained from Sigma-Aldrich. To calibrate, a standard stock solution (1000 µg/

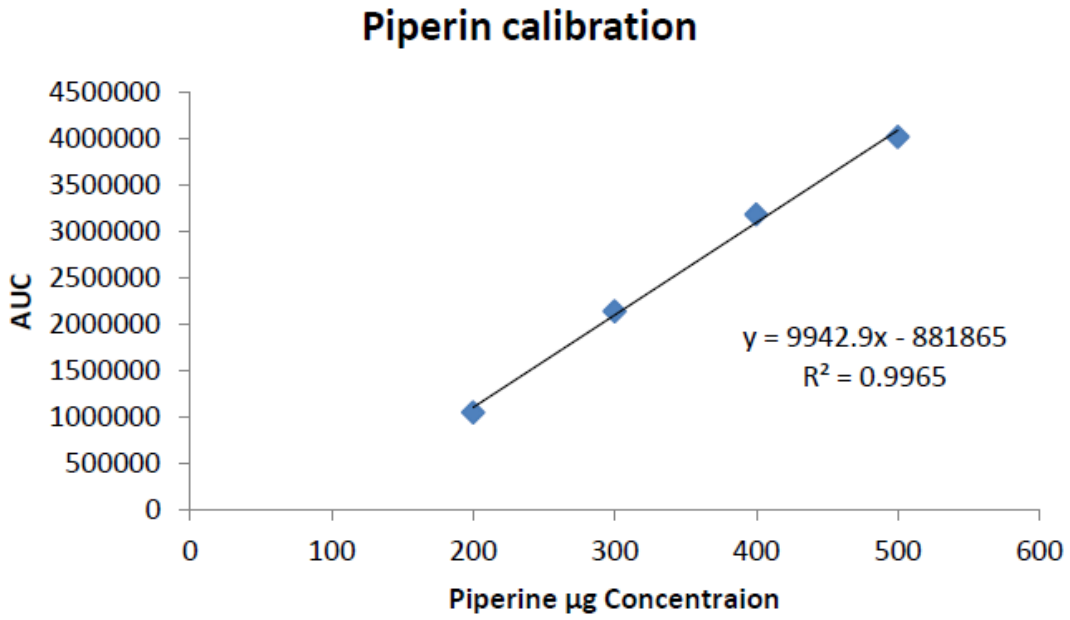


**Figure 1:** HPLC Piperin Curve.

**Table 2:** **TLC (Alcohol Extract) with the $R_f$ Values.**

| Phase | Ratio | Short wave | Long wave | Day wave |
|---|---|---|---|---|
| Mobile Toluene: Ethyl Acetate | 7:3 | 0.25,0.45,0.57,0.65,0.72,0.82, 0.92 | 0.18,0.22,0.31,0.42,0.48,0.52,0.62,0.65,0.71,0.78, 0.85,0.88,0.92 | 0.04,0.22,0.42,0.94 |

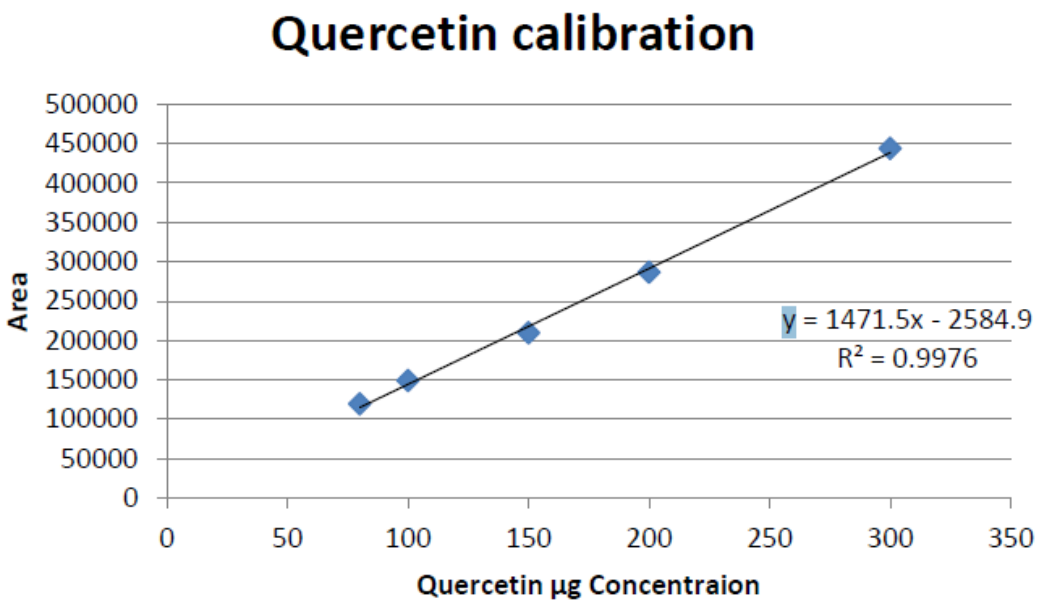


**Figure 2:** HPLC Quercetin Curve.

**Table 3:** **Report of Total Flavonoids and Alkaloids.**

| Extract | Mean concentration result |
|---|---|
| Alcoholic extract (cold) Flavonoid | (8.01 +- 0.78) mg QE/gram of Extract |
| Soxhlet Methanolic extract Alkaloids | (414.86 mg AE/100 g of sample) |

**Table 4:** **Chromatographic Condition for Piperine and Quercetin.**

| Factors | Piperine | Quercetin |
|---|---|---|
| Mobile Phase | 0.5%Acetic acid in water:Acetonitrile (40:60) | 1% Acetic acid: Methanol (40 :60%v/v) |
| Stationary Phase | Symmetry C18 (4.6 x 250 mm, 5 µ particle size) | Symmetry C18 (4.6 x 250 mm, 5 µ particle size) |
| Wavelength | 334nm | 334 nm |
| Run time | 10 Mins | 6 Min |
| Flow Rate | 1 mL/Min | 1 mL/Min |
| Injection Volume | 100 µL | 10 µL |
| Temperature | Ambient | Ambient |
| Mode of Operation | Isocratic elution | Isocratic elution |

mL) was generated by dissolving 10 mg quercetin in methanol and diluting to concentrations ranging from 80-300 µg/mL.

The HPLC analysis revealed linearity across concentrations, with the sample containing 9.78 µg of piperine per 10 mg, equivalent to 0.978% w/w. The formulation contains 4.4 µg quercetin per 10 mg sample, which is equivalent to 0.044% w/w.

## DISCUSSION

Analytical measurements show Murvadi Agada's quality and purity. Moisture concentration below 10% provides stability and resistance to microbial infection. Ash values indicate low levels of inorganic contaminants. Higher water-soluble extractives indicate hydrophilic bioactive components crucial to digestive correction.

Flavonoids and alkaloids discovered are pharmacologically significant. Flavonoids have antioxidant and anti-inflammatory properties, which aid in detoxification and metabolic restoration. Alkaloids aid in gastric stimulation and have antibacterial effects. TLC profiling generates a repeatable chemical fingerprint, which is critical for polyherbal compositions. HPLC measurement of Piperine confirms the presence of Pippali and promotes bioavailability. The assessment of quercetin validates its antioxidant potential. The findings are consistent with the traditional Deepana-Pachana and Agni-restorative acts outlined in Ayurvedic texts. Scientifically established standardization improves translational potential in integrated toxicology and metabolic disease therapy.

## CONCLUSION

The current study successfully established pharmaceutical and analytical standards for Murvadi Agada. Physicochemical parameters were within acceptable ranges. Phytochemical analysis revealed bioactive components. TLC fingerprinting and HPLC marker measurement (piperine and quercetin) confirmed identity and quality.

This study establishes a framework for quality control, batch consistency, and future pharmacological and clinical research.

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## ABBREVIATIONS

**HPLC:** High Performance Liquid Chromatography; **API:** Ayurvedic Pharmacopoeia of India; **TLC:** Thin Layer Chromatography; **QE:** Quercetin Equivalent; **AE:** Alkaloid Equivalent; **AIA:** Acid Insoluble Ash; **KAHER:** KLE Academy of Higher Education and Research.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

Murvadi Agada, a traditional Ayurvedic polyherbal formulation used for Gara Visha and digestive disorders, was scientifically standardized using pharmaceutico-analytical methods including physicochemical tests, phytochemical screening, TLC, and HPLC. The study confirmed its purity and stability while identifying key phytoconstituents such as flavonoids and alkaloids, with quantified biomarkers piperine and quercetin, supporting its therapeutic consistency and scientific validation.

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