RESEARCH PAPER

Pharmaceutico-Analytical Study Of Karanjadi Lepa: A Multi-Stage Phytochemical Assessment From Raw Drugs To Final Formulation

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Received: xxth Month, 2026 | **Revised:** xxth Month, 2026 | **Accepted:** xxth Month, 2026 | **Available Online:** xxth Month, 2026

Abstract:

Bahirparimarjana Cikitsa is an important modality for treating *twak vikaras*, and *Lepa* is one among them. *Karanjadi Lepa*, made from *Karanja beeja* (*Pongamia pinnata*), *Chakramarda beeja* (*Cassia tora*), *Kushta moola* (*Saussurea lappa*), and fresh *Gomutra*, is known for its *kushtaghna* and *krimighna* properties. In this study, raw materials from a GMP-certified pharmacy were authenticated, powdered into *Karanjadi Choorna*, and then mixed with *Gomutra* to prepare *Karanjadi Lepa*. Physicochemical and phytochemical analysis were carried out at each stage, with microbial testing for the intermediate *choorna*. Findings showed significant drug loss during *choorna* preparation and notable phytochemical changes across stages. The addition of *Gomutra* enhanced drug penetration and therapeutic effect. This work highlights the need for stage-wise analysis and evaluation of Ayurvedic formulations to ensure their quality, safety, and effectiveness.

Key words*: Karanjadi lepa, Gomutra*, Phytochemicals, *kushtaghna, choorna*

How to cite this article: Namratha U, Roopa Dodamani, Manjula Vagarali, Naveenraj A, Anil Kumar K M. Pharmaceutico-Analytical Study of Karanjadi Lepa: A Multi-Stage Phytochemical Assessment from Raw Drugs to Final Formulation. Int J Drug Deliv Technol. 2026;16(37s): 832-838. DOI: 10.25258/ijddt.16.37s.107

1.Introduction:

Bahirparimarjana chikitsa plays an important role in the management of *twak vikaras*. *Lepa* is one among that *bahirparimarjana chikitsa* modalities and more convenient than other types of topical applications like *upanaha, kalka* etc. To make the application process faster and more efficient, the topical formulations should be carefully rubbed over the skin in an upward or backward direction of the hairs. As a result, the medication enters the pores at the hair's root, where it is absorbed into the capillary network, travels to small veins, and eventually enters the systemic circulation. In the end, this leads to medication absorption and the intended outcomes. This rubbing technique makes the skin warm, which may hasten the drug's pilosebaceous absorption and skin penetration since heat dilates capillary endings[1].

Karanjadi lepa, one of the *kusthahara pradeha* mentioned in *Ayurveda* classics. It contains *Karanja beeja*(*Pongamia pinnata*), *Chakramarda beeja*(*Cassia tora*), *Kushta moola*(*Saussurea lappa*) and *Gomutra*[2]. As the drugs of *Karanjadi lepa* are *kushtahara, krimihara* in nature, it can be advised in *twak vikaras.*

Analytical studies will provide information regarding its purity, safety, potency and therapeutic effectiveness of medicines. Without these if the formulations are used for administration, there may be chances of ill effects and further complicate the things. So before administration, analytical studies are needed. The Physicochemical parameters, Phytochemical Screening Quality standards for *Karanaja, Kushta* and *Chakramarda* are available in API, whereas the quality standards are not explained for *Karanjadi lepa*. So there is a need to evaluate and assess quality parameters for *Karanjadi lepa*. So in this study quality analysis of *Karanjadi churna*(intermediate product) and *Karanjadi lepa*(final product) were done and compared.

2. Materials and methods

Raw material collection

The raw drugs *Karanja beeja, Kushta moola, Chakramarda beeja* were collected from GMP certified KLE *Ayurveda* Pharmacy, Khasbhag, Belagavi and fresh *Gomutra* was collected from natural habitat (Table I)

Authentication & Analysis

Authentication & Analysis of raw drug, intermediate product and finished product were done at AYUSH Approved ASU Drug Testing Laboratory Lic. No. TL-8/2011, KAHER's SRI BM Kankanawadi Ayurveda Mahavidyalaya, Shahapur, Belagavi.

Method of preparation of *karanjadi churna* & *lepa*

Preparation of *Karanjadi churna*:

Initially, the raw drugs were taken and kept in sunlight for one day and then authenticated and analysed for physicochemical and phytochemical screening. After passing the normal limits they were subjected for the preparation of *churna*.

The raw drugs *Karanja beeja, Kushta moola* and *Chakramarda beeja* were taken in the quantity of 300gm each as shown in fig. 1,2,3. Then they were separately made into powder form using pulveriser. Then each powder is passed under 120 mesh to collect fine powder as shown in fig. 5,6. The *Karanja beeja choorna* was somewhat sticky and was not easily passing through the mesh. So after passing it through the mesh for once, the remanent part was kept in sunlight for 2-3 hours and again sieved. This was repeated for 2-3 times. On contrary, *Chakramarda beeja* and *Kushtamoola churnas* were easily passed through the mesh. The final yield obtained from each drug are *karanja beeja choorna*-86 gm, *Kushta moola churna*-110gm, *Chakramarda choorna*-100gm which are mentioned in table no II. The prepared *churnas* of all the three drugs were taken in equal quantity and mixed homogenously and *karanjadi choorna* was made as shown in fig.7

Fig 1: *Karanja beeja*

Fig 2: *Chakramarda beeja*

Fig 3: *Kushta moola*

Fig 4: *Gomutra*

Fig 5: Powdering of raw drugs in pulveriser

Fig 6: Sieving the powder

Fig 7: *Karanjadi choorna*

Table II: Preparation of fine powder from raw drugs

Sr. no	Ingredient	Initial weight	Weight after preparing *churna*	Loss %
1	*Karanja*	300gm	86gm	71.33
2	*Kushta*	300gm	110gm	63.33
3	*Chakramarda*	300gm	100gm	66.66

Preparation of *Karanjadi lepa*:

Karanjadi churna was analysed for physicochemical, phytochemical and microbial limit test. Freshly collected *Gomutra* (fig.no 4) was also analysed for physicochemical and inorganic element analysis. Then the *churna* and *gomutra* were taken for the preparation of *lepa.*

Karanjadi churna was taken and sufficient quantity and freshly collected *gomutra* was added and mixed homogenously to get *lepa* consistency as shown in fig.8

Fig 8: *Karanjadi lepa*

Analytical Study

Analytical study was done to evaluate the basic parameters for *Karanjadi lepa* as there is no pharmacopeial standards. The formulation was tested for organoleptic parameters, Physicochemical analysis and microbial specification tests. Analysis of samples were conducted as per API standards**.**

3. Results

The results obtained from the analysis of raw drugs were found within normal limits mentioned in API.

PHYSICOCHEMICAL PARAMETERS OF RAW DRUGS:

Karanja beeja, Kushta moola and *Chakramarda beeja* were analysed for physicochemical parameters and the result obtained were within the normal limit as per the standards of API and are mentioned in table III

Table III: physicochemical parameters of raw drugs:

Sr. no	Parameter	***Karanja beeja***	***Chakramarda beeja***	***Kushta moola***
		Result	**Result**	**Result**
1	Colour	Reddish leathery	Greenish brown to brownish black	Greyish to dull brown
2	Taste	Bitter	Bitter	Slightly bitter
3	Odour	Characteristic	Odourless	Characteristically aromatic
4	Foreign matter	NA	Nil	NA
5	Ash value	2.664%	1.941%	2.488%
6	Acid insoluble Ash	0.098%	0.149%	0.746%
7	Water soluble extractive	19.404%	17.939%	34.952%
8	Alcohol soluble extractive	27.606%	9.688%	18.827%

Karanja beeja, Kushta moola and *Chakramarda beeja* were analysed for Preliminary Phytochemicals and the results are tabulated in table IV

Table IV: Preliminary Phytochemical Screening

	Karanja beeja	***Chakramarda beeja***	***Kushta moola***

TESTS	Water soluble extract	Alcohol soluble extract	Water soluble extract	Alcohol soluble extract	Water soluble extract	Alcohol soluble extract
Reducing sugar	+	+	+	+	+	+
Monosaccharides	-	-	+	+	+	+
Proteins	-	-	-	-	-	-
Steroids	-	-	-	+	-	-
Flavonoids	-	+	+	+	+	+
Cardiac glycosides	+	+	-	+	-	-
Anthraquinone glycosides	-	-	+	+	-	-
Saponin glycosides	-	-	+	-	-	-

The physicochemical parameters of *gomutra* like colour, taste, pH etc and Inorganic element Analysis were analysed. As there is no standard parameters available in API the following were the standard results obtained which are tabulated in Table V and VI

Table V: Physicochemical parameters of *Gomutra*

TESTS	RESULT
Form	Liquid
Colour	Yellow
Taste	Characteristic
Odour	Characteristic strong
Specific Gravity	1.019
pH	8.30
Total Solids	4.821%

Table VI: The Inorganic element Analysis of *gomutra*

Tests	Results
Calcium	+
Magnesium	+
Sodium	+
Potassium	-
Iron	+
Sulphate	+
Phosphate	+
Chloride	-
Carbonate	-
Nitrates	-

The intermediate product (*Karanjadi choorna*) was analysed for physicochemical parameters. The API standards are not available for this and the obtained results are mentioned in table VII

Table VII: Physicochemical parameters of *Karanjadi choorna*:

Sr. no	Parameter	Result
1	Form	*Churna*
2	Colour	Greenish
3	Taste	Bitter
4	Odour	Aromatic
5	Loss on drying	7.043%
6	Ash value	5.062%
7	Acid insoluble ash	1.269%
8	Water soluble extractive	24.491%
9	Alcohol soluble extractive	22.404%
10	pH	4.97

The finished product *(Karanjadi lepa)* was analysed for physicochemical parameters. The API standards are not available for this and the obtained results are mentioned in table VIII

Table VIII: Physicochemical parameters of finished product:

Parameter	Result
Form	*Lepa*
Colour	Dark brownish
Odour	Strong/ Characteristic
Moisture Content	70.533%
Spreadability	50mm
pH	6.06
Homogeneity	Passes

Karanjadi choorna and *Karanjadi lepa* were analysed for Preliminary Phytochemical Screening.
The API standards are not available for this and the obtained results are mentioned in table IX
Microbial limit test and test for specified Micro-Organisms/Microbial contamination test of *Karanjadi Choorna* are mentioned in table X and XI

Table IX: Preliminary Phytochemical Screening of *Karanjadi choorna* and *Karanjadi lepa*

	Karanjadi choorna		*Karanjadi lepa*	
TESTS	**WATER SOUBLE EXTRACT**	**ALCOHOL SOUBLE EXTRACT**	**WATER SOUBLE EXTRACT**	**ALCOHOL SOUBLE EXTRACT**

Test for Carbohydrates	+	+	+	+
Test for Reducing sugar	+	+	+	+
Test for Monosaccharides	+	+	+	+
Test for Pentose sugar	-	-	-	-
Test for Non reducing sugar	-	-	-	-
Test for hexose sugar	-	-	-	-
Test for proteins	-	-	-	-
Test for Amino acids	-	-	+	+
Test for steroids	-	-	-	+
Test for flavonoids	+	+	+	+
Test for alkaloids	-	-	-	-
Test for tannins	+	+	-	-
Cardiac glycosides	-	+	-	-
Anthraquinone glycosides	-	-	-	-
Saponin glycosides	+	-	-	-

Table X: Microbial limit test for *Karanjadi choorna*:

Parameter	**Results**
Total Bacterial Count	No growth
Total Fungal Count	No growth

Table XI: Test for specified Micro-Organisms/Microbial contamination test of *Karanjadi Choorna*:

Organism	Result
E coli	Absent
Staphylococcus aureus	Absent
Pseudomonas aeruginosa	Absent
S abony	Absent

4.Discussion:

Pharmaceutical part:

While preparing *churnas* it is observed that 71.33 % loss of drug of *karanja beeja*, 66.66%loss of *chakramarda beeja* and 63.33% loss of *kushta mula*. This may be because of presence of more oil content in *karanja beeja* and *chakramarda beeja*.

After the preparation of *churnas*, the yields of each *churna* varied greatly. *Karanja beeja* has lot of oil content so it got sticked and clumped during grinding which made it difficult to grind finely. And while sieving also it got adhered to the mesh because of which the final yield obtained is comparatively less than the other two drugs. Moderate loss of 66.66% was seen in *Chakramarda beeja* which might be because of moderate oil content in it. On the other hand, *Kustha moola* is dry and more brittle which increased the grindability and yield. Thus, we can say that fat content in the drugs interfere with the grinding and has its significant impact on the total yield. This implies that materials with lower levels of moisture and oil are better suited for making fine powder using conventional methods.

Comparison of phytochemicals between raw drugs and the intermediate product

When comparing *Karanjadi Choorna* to its raw ingredients, *Karanja beeja, Kushta moola*, and *Chakramarda beeja*, the initial phytochemical screening shows that certain chemicals are retained and changed during preparation. Flavonoids, reducing sugars, and monosaccharides were consistently found in all raw drugs and remained significant in *Karanjadi Choorna*, indicating the durability and compatibility during processing. Steroid and anthraquinone glycosides, though present in the raw drugs, were not found in the *choorna*. This may be because of degradation, or loss during the manufacturing of *churna*.

Comparison of phytochemicals between the intermediate and final product

There were significant variations in the presence and transformation of constituents during the formulation process between *Karanjadi Choorna* and *Karanjadi Lepa*, according to the phytochemical screening. Carbohydrates, reducing sugars, monosaccharides and flavonoids were present in *churna* and *lepa* indicating their stability even after the *choorna* getting transformed into *lepa*.

Amino acids, steroids were found in the *lepa* whereas they were not present in *churna.* This may suggests that, during the preparation of *lepa, gomutra* was added and this may facilitated the release or transformation of precursors into amino acids and steroids.

Even though the *churna* was having tannins, sasponin glycosides, cardiac glycosides, *lepa* did not got these and this could be the result of degradation or change during the preparation of *lepa*. There was a change in phytoconstituents after the preparation of *lepa* procedure. So in each stage of formulation, phytochemical analysis varies which highlights the pharmacological potential of each product of the formulation, such as tannins and glycosides, reduce during processing, others, such as amino acids and steroids, develop.

Skin is the largest organ of human body that protects entire organism against external threats like mechanical injuries, radiation, allergens, chemicals and infections. The breakage of this barrier by chronic diseases extremely impacts every metabolic occurrence and plays a big role in the further degenerative conditions of the affected people. Moreover, in recent studies, it has been shown that environmental factors play an important role in the rise of autoimmune diseases including skin manifestations[3]. Flavonoid-rich plants have a host of beneficial actions that not only can prevent disease from developing, but also treat the condition very effectively. The main mechanism affecting the preventive use of flavonoids in skin diseases development is the antioxidant and photoprotective activities[4].

Numerous flavonoids have been isolated and studied in relation to their antifungal properties; they may be effective, economical, and promising agents for the prevention of fungal infections[5]. The structural characteristics of a number of flavonoids may be crucial for providing antibacterial activities[6]. Flavonoids are known to have antioxidant properties and to be highly efficient at scavenging free radicals. Flavonoids prevent membrane lipid oxidative reactions from releasing arachidonic acid. 5-lipoxygenase and cyclooxygenase, which are crucial for converting arachidonic acid into proinflammatory leukotrienes and prostaglandins, are rendered inactive by flavonoids with chelation capabilities. Their activity on the skin and their soothing qualities are correlated with their antioxidant qualities[7].

Flavonoids are having strong anti-inflammatory, and anti-allergic properties, making them helpful in preventing allergic reactions, inflammation, and skin irritation[8].Amino acids have great potential in the treatment of inflammatory diseases[9]. Because of their inherent moisturizing qualities, amino acids are a common component in skin care and anti-aging products[10]. Steroid moieties found in cow urine are responsible for their analgesic effects[11].*Gomutra's* primary function is to increase penetration. Chemicals and solvents such as urea, N,Ndiethyl-M toluamide, and others are penetration enhancers that directly affect the permeability of the skin. Additionally, it's important to note that *Gomutra* has over 30% urea. *Gomutra* can therefore be proposed as an Ayurvedic penetration booster[12]. It was observed that certain phytochemicals have changed after adding *gomutra* which indicates its additional effects. Evidence based pharmacotherapeutic many studies on cow urine validated its various therapeutic uses in numerous diseases. The therapeutic effects includes antioxidant, anti-diabetic, immunomodulating, anti-cancerous, anti-microbial, anthelmintic, analgesic, wound healing, bio enhancing, etc[13].

5. Conclusion:

Thus in this study, the raw drugs, intermediate and final products were analysed for physicochemical and phytochemical screening. The intermediate product *(Karanjadi churna)* was analysed for physicochemical and preliminary phytochemical screening, microbial limit test, microbial contamination test for specific organisms. Fresh Gomutra was analysed for physicochemical and inorganic elements before adding it into *lepa*. Final product *(Karanjadi lepa)* was analysed for physicochemical and preliminary phytochemical screening.The standard parameters are not available for *gomutra*, intermediate and final product. So this study would help to evaluate the parameters. Along with this it was observed that there is difference in the phytochemicals between intermediate and the final product which has its effects of therapeutics. And finally, the positive phytochemical findings of *lepa*, because of their actions proved to be useful in skin diseases. So *Karanjadi lepa*, one of the important *yoga* and this study would be helpful for the further study like dosage modification.

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