Comparative pharmacognostical analysis through quantitative micrometry and analytical study on *Mridu* and *Tikshna Apamarga Kshara*

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Abstract

Introduction: *Kshara* is derived from the word "*Ksharana"* that means as something that mobilizes and removes the deformed flesh, skin, tissue, etc., due to its corrosive nature (*Ksharanata*). *Pratisarniya Kshara* has been further classified into three types on the basis of its potency – *Mridu* (mild), *Madhyama* (moderate) and *Tikshna* (strong). This study aims at comparison between (*Mridu* and *Tikshna*) *Apamarga Kshara* on the basis of pharmacognostical and pharmaceutical evaluation. **Materials and Methods:** *Apamarga Panchanga* (whole plant of *Achyranthes aspera* Linn.) was collected, and authentication was done by the expert. *Mridu Apamarga Kshara* (MAK) and *Tikshna Apamarga Kshara* (TAK) were prepared as proposed by Sushruta Samhita. Pharmacognostical and pharmaceutical analyses were carried out according to standard protocol. **Observation and Results:** Both the *Kshara* showed their own peculiar crystal system and analytical findings showed higher pH value (10.65) and calcium content (6.1%) in TAK as compared to MAK. **Discussion:** Quantitative micrometric microscopy showed more amount of crystals in TAK (13/mm^2) than MAK (6/mm^2), which may be due to *Kapardika* and *Chitrakamoola* (roots of *Plumbago zeylenica* Linn.). pH of MAK and TAK was 10.2 and 10.65, respectively. This result showed that TAK is more alkaline, which may be also due to *Kapardika* and *Chitrakamoola*. **Conclusion:** Sodium and potassium ion concentration was higher in MAK (Na^+ = 26%, K^+ = 45%) as compared to TAK (Na^+ = 12.6%, K^+ = 32.5%). Calcium ion estimation was lower (2.31%) in MAK and higher (6.1%) in TAK. These findings can be further used for the standardization purpose of *Tikshna Kshara* which may enrich the Ayurvedic Pharmacopoeia of India.

Keywords: *Achyranthes aspera* Linn*, Apamarga Kshara*, *Mridu Kshara*, quantimetric, *Tikshna Kshara*

Introduction

"*Kshara"* has scope in *Shalya Tantra* (surgical and para-surgical) to its *Chedana* (incising), *Lekhana* (scraping) and *Ropana* (healing) qualities.[1] Sushruta has dedicated a whole chapter to *Kshara* and narrated *Kshara* as an *Anushastra* (secondary instrument), *Upayantra* (secondary appliance), and one of the *Upakrama* of *Vrana* (interventions for wound care).[2-4] Dalhana; the renowned commentator of Sushruta Samhita defined "*Ksharana*" as something that mobilizes and removes the deformed flesh, skin, tissue etc. It removes the debris from their location due to its corrosive nature (*Ksharanata*). *Kshara* possesses dominance of *Katu Rasa* (pungent taste) and *Lavana* (salt) as *Anurasa* (secondary taste).[5] On the basis of application, Sushruta has mentioned two types of *Kshara,* i.e., *Pratisarniya* (external application) and *Paniya* (internal application).[6] *Pratisarniya Kshara* has been further classified into three types on the basis of its potency – *Mridu* (mild), *Madhyama* (moderate) and *Tikshna* (strong).[7] *Kshara* are penetrating in nature, hot in potency, antihelminthic, vitiate *Pitta* and *Rakta* and help in the digestion of substances help in the breakdown of hard masses and perforating the tissues. Being pungent in smell and salty in taste, it has ill effect on heart, semen, *Oja*, hair and eyes.[8]

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Sushruta has mentioned three types of *Pratisaraniya Kshara* as per potency, but indications of these three different types are not mentioned. However, *Tikshna Kshara* is used for treating piles, fistula-in-ano, rectal prolapse, pilonidal sinus, infected wound, and some types of skin disease, whereas *Mridu Kshara* is used in the preparation of *Ksharasutra*, for local application in hypergranulated or fresh wounds and for internal use in diseases such as renal calculi, asthma and diseases of alimentary canal.[9-11] *Mridu* and *Tikshna Kshara* are traditionally used in different conditions, and their effect on body tissue varies as observed clinically. This study aims to compare between the types of *Apamarga Kshara* (*Mridu* and *Tikshna*) on the basis of their pharmacognostical and pharmaceutical evaluation.

Materials and Methods

Preparation of *Mridu Apamarga Kshara* and *Tikshna Apamarga Kshara*

Apamarga Panchanga (whole plant of *Achyranthes aspera* Linn.) and *Chitrakamoola* (root of *Plumbago zeylenica* Linn.) were procured from the pharmacy of Gujarat Ayurved University. Authentication of both the samples was done on the basis of macro- and micro-morphological characteristics at Pharmacognosy laboratory, IPGT and RA, Jamnagar, Gujarat [Figure 1]. The dried *Apamarga Panchanga* was burnt along with crude lime stone, and the ash was collected. The ash was weighed and transferred to a clean vessel [Figure 2] and dissolved in water in 1:6 proportion; the mixture was mixed well and then allowed to settle down for 24 h. After 24 h, the solution was filtered 21 times, and a soapy, viscous, red-tinted liquid (*Ksharajala*) was obtained. This liquid was subjected to mild continuous heating with intermittent stirring [Figure 2].[12] For the preparation of MAK, *Ksharajala* was further boiled till water evaporated completely and a white powder (MAK) was obtained [Figure 2]. [13]

For the preparation of TAK, some quantity of *Ksharajala* was taken and red-hot *Kapardika* (1/10 part of ash) was quenched in it. This solution was filtered and mixed with rest of the *Ksharajala,* and then paste of root of *Chitraka* (1/100 part of ash) was added and heated with intermittent stirring, from which a reddish semi-solid substance (TAK) was obtained [Figure 2].[14]

Organoleptic evaluation

Organoleptic characteristics such as color, odor, taste and touch were noted down by sensory observations for both the *Kshara* (MAK and TAK).

Pharmacognostical evaluation

Pharmacognostical study was carried out in two phases. First, the microscopy of raw drugs and then the final products, i.e., MAK and TAK was done for standardization purpose at the Pharmacognosy laboratory, IPGT and RA, Jamnagar, as per the standard procedure. The drug was placed on a slide and distilled water was added to observe the specific characteristics of drugs. Microphotographs were taken with the help of Carl Zeiss trinocular microscope attached to the camera.[15,16]

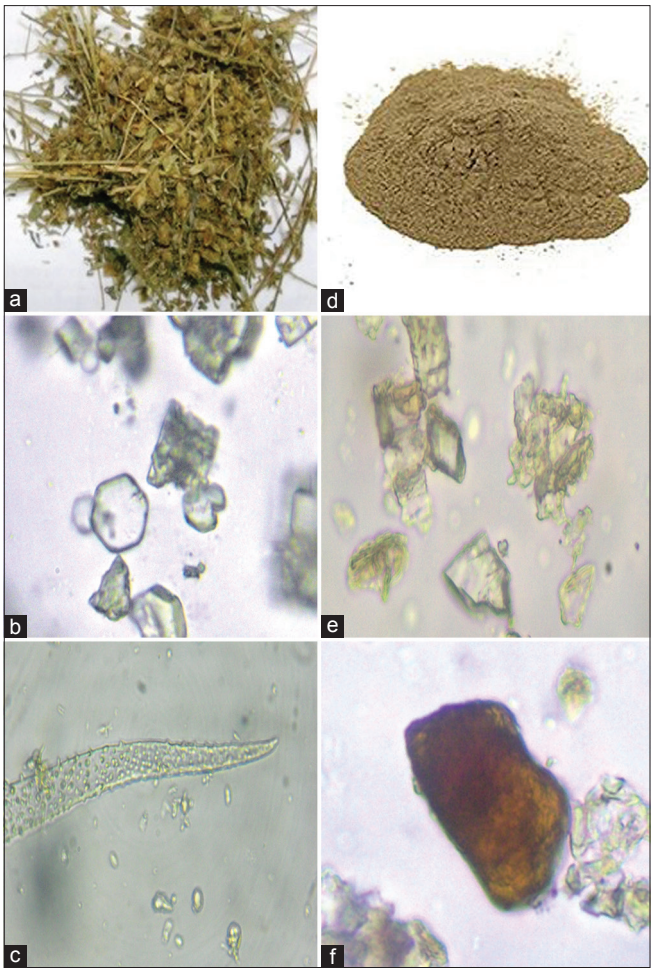

Figure 1: Raw drug authentication (a) Whole plant of *Apamarga* (b) Crystal of *Apamarga* (c) Warty trichomes of *Apamarga* (d) *Chitrakamoola* powder (e). Prismatic crystal of *Chitrakamoola* (f) Tannin contain of *Chitrakamoola*

Pharmaceutical analysis

Physicochemical parameters

Preliminary physicochemical parameters such as loss on drying (LOD) at 110°C, ash value, acid-insoluble ash and pH value were analyzed as per the Ayurvedic Pharmacopoeia of India (API).[17]

Estimation of sodium and potassium ions

Estimation of sodium and potassium ions was done by flame photometer at the Analytical Laboratory, IPGT and RA, Jamnagar.[18]

Estimation of calcium and iron

Estimation of calcium and iron was carried out by standard protocol at the Analytical Laboratory of IPGT and RA, Jamnagar.[19]

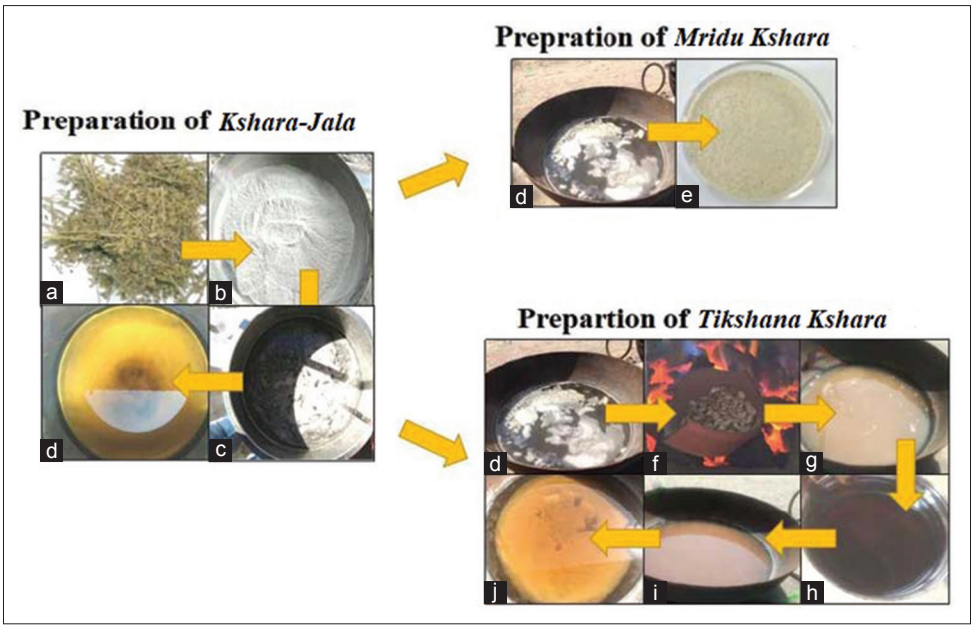

Figure 2: Preparation of *Mridu* and *Tikshna Apamarga Kshara* (a) *Apamaraga Panchanga* (whole plant of *Achyranthes aspera* Linn.) (b) Ash of *Apamarga Panchanga* (c) Solution of ash in six times of water (d) *Kshara-Jala* (e) *Mridu Apamarga Kshara* (f) Red-hot *Kapardika* ($CaCO_3$) (g) Quenching of red-hot *Kapardika* in *Kshara-Jala* (h) *Chitrakamoola* (root of *Plumbago zeylenica*) paste (i) Mixing of *Chitrakamoola* paste with *Kshara-Jala* (with *Kapardika*) (j) *Tikshna Apamarga Kshara*

Observations and Results

Organoleptic characteristics

Comparison of organoleptic characteristics of prepared MAK and TAK such as color, odor, taste, touch and sound was done and the values were noted down and the results are depicted in Table 1.

Pharmacognostical observation

Raw powder of *Apamarga Panchanga* and *Chitrakamoola* was taken for microscopy and microphotographs were taken. *Apamarga Panchanga* comprised of stellate, warty and simple trichomes, cluster, prismatic and rod-shaped crystals of calcium oxalate. *Chitrakamoola* powder comprised of simple fibers, tannin contents and rosette crystals [Figure 1].

Fresh samples of MAK and TAK were taken for detailed quantitative micrometric study. Both the samples showed different types and number of crystals. The results of micrometric evaluations of MAK and TAK are depicted in Table 2 and Figures 3, 4.

Quantitative micrometric analysis

The quantitative analysis of TAK and MAK showed the distribution and amount of crystals under the microscope, which were found to be 13/mm^2 and 6/mm^2, respectively.

Pharmaceutical analysis

Physicochemical parameters and ion estimation

Physicochemical parameter analysis, as well as ion estimation of both the samples, were carried out and the results are depicted in Table 3.

Table 1: Organoleptic characteristics of *Mridu* and *Tikshna Apamarga Kshara*

Characters	MAK	TAK
Nature/state	Dry	Semi-solid
Color	Dull white	Reddish brown
Odor	Slightly irritant	Slightly offensive
Taste	Salt	Concentrated salt
Touch	Coarse	Coarse
Sound	Crispy	No audible sound

MAK: *Mridu Apamarga Kshara*, TAK: *Tikshna Apamarga Kshara*

Discussion

Pharmacognostical study for the authentication, i.e., microscopic study, showed some distinctive characteristics of the drug such as *Apamarga Panchanga* comprised of stellate, warty and simple trichomes, cluster, prismatic and rod-shaped crystals of calcium oxalate and *Chitrakamoola* powder comprised of simple fibers, tannin contents and rosette crystals.

Apamarga Kshara Jala was prepared as per classical text and further used for preparing MAK and TAK. The finished product was white powder for MAK and reddish, semi-solid for TAK. The pharmacognostical study reveals that TAK showed certain peculiar characteristics of ingredients, i.e., fibers and vessels of *Chitrakamoola* and many crystals of various shapes and sizes as compared to MAK. Quantitative microscopy showed more crystals in TAK (13/mm^2) than MAK (6/mm^2), which may be because of further processing of *Kshara Jala* with *Kapardika* and *Chitrakamoola*.

Table 2: Micrometric measurement of *Mridu* and *Tikshna Apamarga Kshara*

Shape of crystals	MAK (μm)	TAK (μm)
Crystal mass	5.5×4.3	Not found
Irregular-shaped crystals	0.7×0.6	Not found
Prismatic crystals	0.5×0.4	Not found
Square-shaped crystals	1.1×0.6	0.1×0.1
Rectangular crystals	1.6×0.4	Not found
Hexagonal-shaped crystals with blunt angle	Not found	0.4×0.5
Hexagonal-shaped crystals with sharp angle	Not found	1.5×1.3
Hexagonal crystals 2D	Not found	1×0.9
Hexagonal crystals 3D	Not found	5.5×3.9
Rosette crystals	Not found	1.6×1.7
Blunt, elongated crystalline material	Not found	7×2
Round-shaped pitted crystals	Not found	0.9
Dumbbell-shaped crystals	Not found	1.2×1.3
Dumbbell-shaped and acicular crystals	Not found	1.8
Prismatic 2D crystals	Not found	0.6×0.5
Group of crystals	Not found	+

MAK: *Mridu Apamarga Kshara*, TAK: *Tikshna Apamarga Kshara*, 2D: Two dimensional, 3D: Three dimensional

Table 3: Physicochemical analysis of *Mridu* and *Tikshna Apamarga Kshara*

Test	MAK	TAK
Loss on drying (%)	97.49	61.27
Acid-insoluble ash (%)	0.1	0.1
pH value	10.2	10.65
Ion estimation (%)	Na^+ ion=12.6 K^+ ion=45 Ca^+ ion=2.31 Fe^+ ion=Not detected	Na^+ ion=12.6 K^+ ion=32.5 Ca^+ ion=6.1 Fe^+ ion=Not detected

MAK: *Mridu Apamarga Kshara*, TAK: *Tikshna Apamarga Kshara*

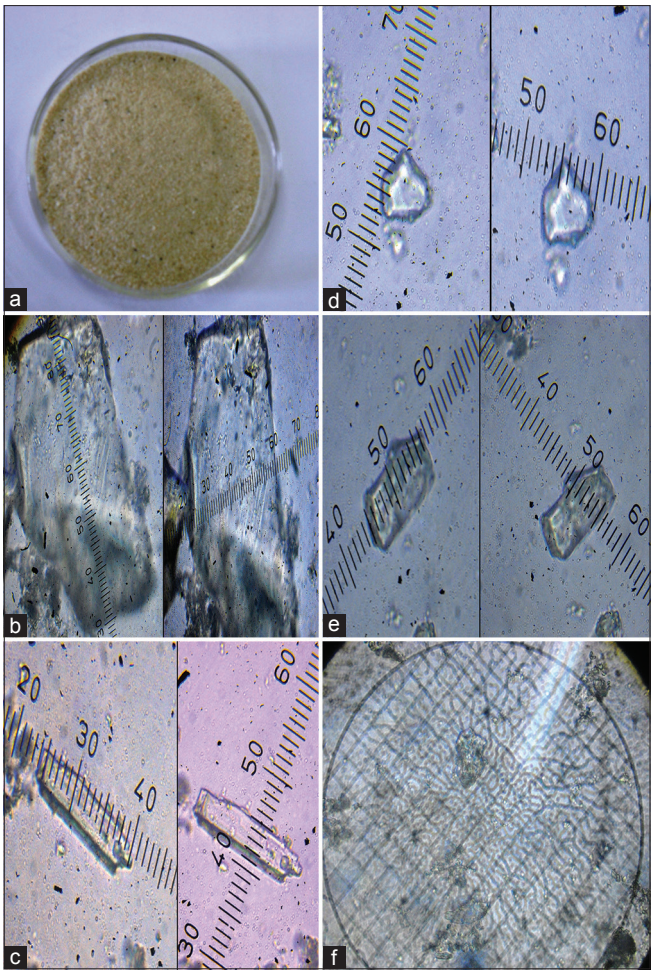

Figure 3: Micrometry of *Mridu Apamarga Kshara* (a) *Mridu Apamarga Kshara* (b) Crystal mass (c) Rectangular-shaped crystals (d) Irregular-shaped crystals (e) Square-shaped crystals (f) Quantitative analysis

The pharmaceutical analysis showed lesser value of LOD in TAK as compared to MAK. As TAK is semisolid in consistency, it has more water content as compared to MAK. pH of 5% solution of both the samples at room temperature was 10.2 and 10.65 for MAK and TAK, respectively. This result showed that TAK is more alkaline, which may be because of its processing with *Kapardika* and *Chitrakamoola*. Sodium and potassium ion concentration was higher in MAK (Na^+ = 26%, K^+ = 45%) as compared to TAK (Na^+ =12.6%, K^+ =32.5%). Calcium ion estimation was lower (2.31%) in MAK and higher (6.1%) in TAK, which might be due to the addition of *Kapardika* (cowry, $CaCO_3$) in TAK.

In this study, an attempt has been made to differentiate MAK and TAK on the basis of quantitative micrometry and analytical parameters such as LOD value, pH value, and Na+, K+, and Ca^{2+} concentration. Sushruta has described three different *Kshara* on the basis of the potency which may be because of their different actions on the body tissue, which can be justified by more alkalinity of TAK over MAK. Thus, TAK can be used at places where more debridement is expected such as in hemorrhoids, fistula-in-ano, and unhealthy wounds, whereas MAK can be used at places where less debridement is present as for internal administration in calculi, bronchial asthma, gastrointestinal tract disorders, etc. TAK can be used in wound care as it has more calcium ion concentration, as some research studies report that calcium ion promotes wound healing in an early stage.[20]

Conclusion

In this study, first attempt has been made to generate the analytical profile and to differentiate two types of *Apamarga Kshara* (*Mridu* and *Tikshna*) on the basis of pharmacognostical and pharmaceutical evaluation. The findings can be further used for the standardization purpose of *Tikshna Kshara* which may enrich the API. The limitation of this study is that the *Kshara* of *Apamarga Panchanga* may vary from place to place.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

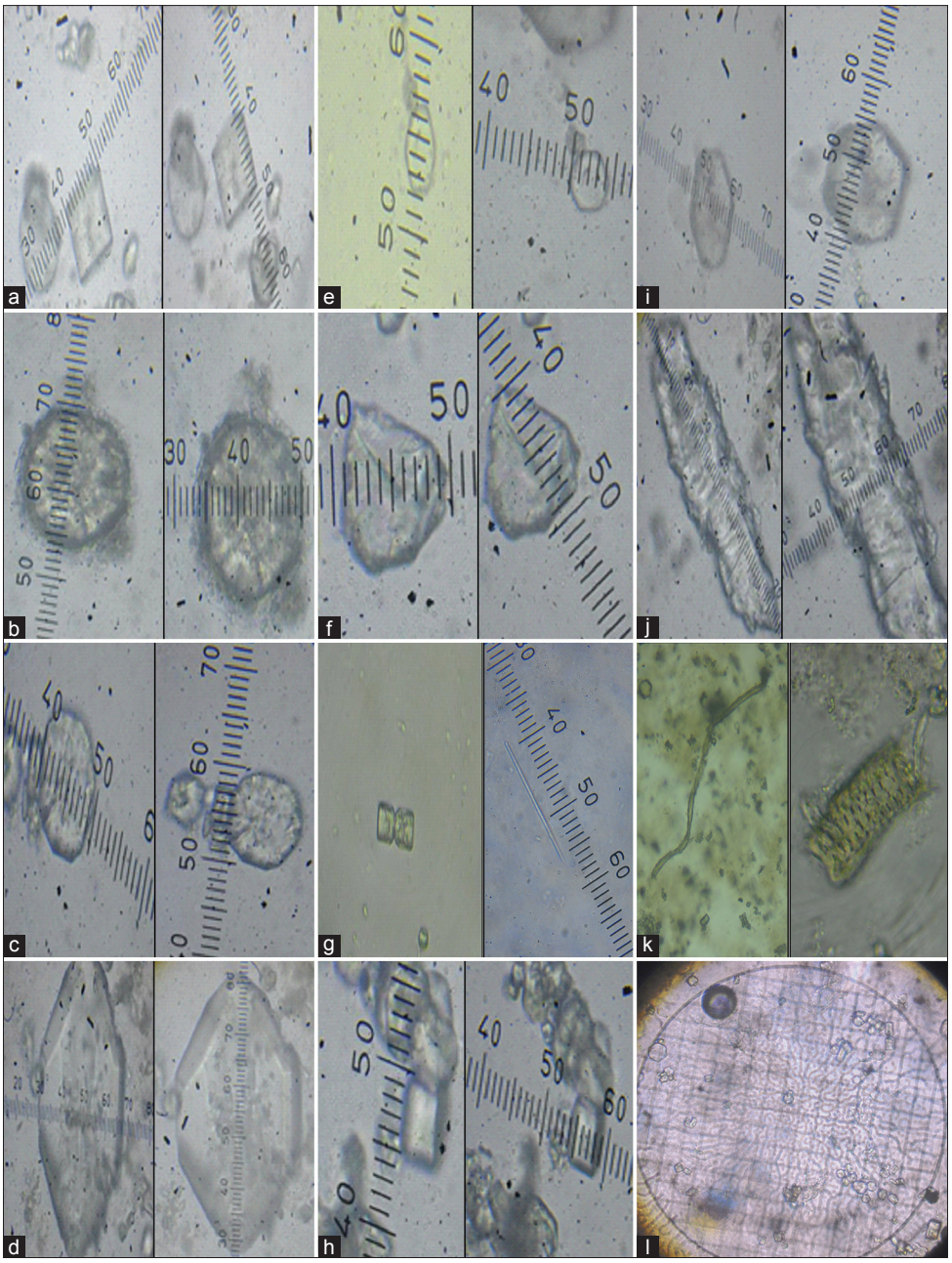

Figure 4: Micrometry of *Tikshna Apamarga Kshara* (a) Square Crystal (b) Rosette Crystal (c) Round-shaped pitted crystal (d). Hexagonal crystal three dimensions (e) Hexagonal with blunt angle (f) Hexagonal crystal two dimensions (g) Dumbbell-shaped and acicular crystal (h) Prismatic crystal (i) Hexagonal with sharp angle (j) Blunted elongated crystalline material (k) Fiber and vessel of *Chitrakamoola* (l) Quantitative analysis

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