

# Pharmacognostical and Physicochemical Analysis of *Cleome viscosa* L. Seeds

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## ABSTRACT

**Aim:** The seeds of *Cleome viscosa* L. are used to treat tumor, emesis, diarrhea, epilepsy and hyperthermia. Seeds are used to heal wound also. In order to ensure the use of only genuine and uniform material in treatment of ailments by herbs and herbal formulations, pharmacognostical standardization and physicochemical parameters evaluation is needed. **Methods:** Morphological and powder microscopic characters of seed powder were studied. The physicochemical parameters studied were LOD, total ash, acid insoluble, water soluble, sulphated ash, extractives, volatile oil content and crude fibre content etc. Further, fluorescence analysis and element analysis of seed powder was performed by undergoing chemical reaction +UV method and EDAX method respectively. **Results:** Brown colored seeds with rough seed surface with seed size 0.1 cm were recorded. The pods were brown coloured when air dried. They were 4-5 cm long containing 8-12 small seeds. Powder microscopic study revealed the presence of fragment of epidermis of testa, groups of cells resembling like stone cells and irregularly shaped protein bodies were observed. Fluorescence Analysis revealed the presence of chromophores. EDAX analysis showed the presence of Mg, Al, Si, S, Cl, K, Ca and Cu etc in different amount. **Conclusion:** Pharmacognostical and physicochemical parameters were established which would facilitate quick identification and selection of the herbal drugs from various adulterants.

**Key words:** *Cleome viscosa* L., Macroscopic, microscopic, physicochemical, Crude fibre.

## INTRODUCTION

The study of plant drugs from the pharmacognostical stand point would include the study of the habitat, general characters of the plant from which the drug is derived, its place in the botanical system, the organ or the organs of the plant used, their gross, minute structures in the whole and in the powdered conditions and the chemistry of the constituents especially of those which may be used in therapeutics. The macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the purity of such materials.<sup>1</sup> This should be performed before any tests are undertaken. The importance of pharmacognosy has been widely felt in recent times. Unlike taxonomic identification, pharmacognostic study includes parameters which help in identifying adulteration from powdered drug also.<sup>2</sup>

Pharmacognostical and physicochemical studies are important because once the plant is dried and powdered, it loses its morphological identity and easily prone to adulteration. Pharmacognostic studies ensures plant identity, lays down standardization parameters which prevent the drug from adulterations. Such study helps in authentication of the plants and ensures reproducible quality of herbal products, which lead to safety and efficacy of natural products.<sup>3</sup>

On another hand, the physicochemical evaluation of the plant material is mandatory also in order to detect adulteration or improper handling of drugs. The parameters which are studied includes moisture content, loss on drying (LOD), total ash, acid-insoluble ash, water soluble ash value, alcohol and water-soluble extractive values, etc.<sup>4</sup>

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consists of mainly silica and indicates contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent.<sup>5</sup>

*Cleome* is well known for the therapeutic efficacy of its different species. One of the most important species of this genus is *Cleome viscosa* L. *Cleome viscosa* L. is called 'Hurhur' in India. Traditionally *Cleome viscosa* Linn. plant is an antimalarial drug and beneficial in blood diseases and uterine complaints also. The pungent seeds and seed pods are used as sub-

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stitute of mustard in curries. In traditional system of medicine, leaves, seeds and roots of the plant are widely used as an anthelmintic, anti-septic, cardiac stimulant, carminative, anticonvulsant, antidiarrhoeal, antipyretic and are also used to treat skin diseases.<sup>6,7</sup> In previous phytochemical studies, *Cleome viscosa* L. seeds have been reported to contain Oct-1-ene, Heptane-4-one, Heptane-2-one, Non-1-ene,  $\alpha$ -pinene, dehydrosabene, 6-Methylhept-5-ene-2-one, E-ocimene, myrcene, p-cymene, limonene, dehydrolinalool, undecan, limonene oxide,  $\alpha$ -tepeniol, benzoic acid, Deca-2,4-dien-1-ol, Decan-2-ol, Gerniol, Undec-10-e-1-ol.<sup>8</sup> The literature survey reveals that there is no systematic pharmacognostical and physicochemical study for *Cleome viscosa* L. seeds has been undertaken yet. In the present communication, the study was performed on the pharmacognostical and physicochemical parameters of the seeds of *Cleome viscosa* L.

## MATERIALS AND METHOD

All the chemicals and reagents used were of analytical grade purchased from CDH Laboratories Pvt. Ltd., New Delhi, India.

### Collection and Authentication

The mature seeds of *Cleome viscosa* L. were collected from nearby areas of Moradabad in month of September. Care was taken to select normal and even size seeds while collection. The collected seeds along with plant specimen were authenticated by renowned botanist Dr. G.P. Sinha (Scientist D), Botanical Survey of India (BSI), Allahabad, India and a voucher specimen (TR No. GG 950204) was deposited in office of Botanical Survey of India, Allahabad, India.

### Macroscopic Studies

Macroscopical evaluation refers to evaluation of drugs by color, odour, taste, shape and special features like touch and texture. It is a technique of qualitative evaluation based on the study of morphological profiles of the seeds. The dried seeds were taken for macroscopical evaluation.<sup>9-11</sup>

In order to evaluate the macroscopical features of seeds, number of seeds in the pods, size and color of pods of the *Cleome viscosa* L. species were observed (Figure 1). Findings of macroscopic characters are presented in Table 1.

### Microscopic Studies

In powder microscopic study, powder of dried seeds was taken. *Cleome viscosa* L. seeds were powdered using mixer grinder. The coarse powdered material thus obtained, sieved with the help of 40 mm sieve for

powder microscopy. For microscopical characters of purified powder samples, slides were prepared by using water, chloral hydrate as a clearing agent and glycerin as mounts. All powder samples were stained with various reagents as per the textual reference.<sup>12</sup>

### Physicochemical Parameters

Physicochemical parameters were studied for the seeds of *C. viscosa*. The procedures recommended in WHO guidelines were followed to evaluate the physicochemical constants.<sup>13</sup> Findings are presented in Table 1.

### Fluorescence Analysis

0.5 g of seed powder were taken into clean test tubes. In each tube, 5 ml of different solvents were added and the tubes were shaken. The samples in test tubes were allowed to stand for about 20-25 m. The solutions obtained were observed under the UV visible light for their characteristic colour reaction and were compared with a standard colour chart. The observed colors were recorded. Same procedure was repeated for different extracts obtained from seeds of *Cleome viscosa*. The results are presented in Table 2.

### Elemental Analysis

For seeds of *Cleome viscosa* L. spectra of the various micro mineral elements and macro mineral elements viz. carbon (C), oxygen (O), magnesium (Mg), aluminium (Al), silicon (Si), sulphur (S), chloride (Cl), potassium (K), calcium (Ca) and copper (Cu) were generated by energy dispersive X-ray spectroscopy (EDAX) technique. The results are presented in Table 3.

## RESULT AND DISCUSSION

### Macroscopic Studies

*Cleome viscosa* L. seeds were brown coloured. They were odourless, tasteless, looking like snail shell. Their surface was rough. Size was 0.1 cm. The pods were brown colored when air dried. They were 4-5 cm long and contained 8-12 small seeds. These macroscopic characters of *Cleome viscosa* L. seeds are presented in Figure 1.

### Microscopic Studies

For microscopy, seed powder was taken on glass slide and observed under light microscope. Number of fragments of epidermis testa consisting of thin-walled, polygonal cells and groups of cells, resembling like stone cells of reddish-brown color were observed. Large number of oval, rounded or irregularly shaped protein bodies were also observed. Starch

**Table 1: Physicochemical parameters of the seeds of *Cleome viscosa* L.**

S. No	Physicochemical Parameters	Percentage (Mean $\pm$ SEM)
1.	Loss on Drying (LOD)	4.85 $\pm$ 0.321%
2.	Total ash value	5.2 $\pm$ 0.385%
3.	Acid insoluble ash value	0.51 $\pm$ 0.0211%
4.	Water soluble ash value	1.78 $\pm$ 0.0213%
5.	Sulphated Ash	4.98 $\pm$ 0.366%
6.	Alcohol soluble extractive value	38.8 $\pm$ 0.852%
7.	Water soluble extractive value	15.8 $\pm$ 0.514%
8.	Ethyl acetate soluble extractive value	10.50 $\pm$ 0.986%
9.	Petroleum ether soluble extractive value	18.67 $\pm$ 0.881%
10.	Volatile oil content	0.88 $\pm$ 0.0034%
11.	Crude fibre content	28.9 $\pm$ 0.889%



**Figure 1: *Cleome viscosa* L. seeds showing its macroscopic characters.**

grains and crystals of calcium oxalate were found to be absent. Figure 2(A-C) (10X45X)

### Physicochemical Studies

The results of physicochemical characters such as LOD, total ash, acid insoluble ash, water soluble ash, sulphated ash, percentage of extractive values in various solvents such as alcohol, water, ethyl acetate, petroleum ether, volatile oil content and crude fiber content of the seeds powder of *Cleome viscosa* L. are presented in Table 1.

Excessive amount of water present in plant drugs is the cause for bacterial and fungal growth as well as responsible for the hydrolysis of constituents. The pharmacopoeial monographs limit the water content, especially in drugs that have the facility to absorb it, or in which the excessive amounts of water cause deterioration. In the present study, loss on drying was found to be  $4.85 \pm 0.321\%$ .

Ash determination includes determination of total ash, sulfated ash (residue on ignition) and acid insoluble ash. The total ash involves the determination of physiological and the non-physiological ash. After drug calcinations, the amount of non volatilized residue was found to be  $5.2 \pm 0.385\%$ . After calcination with concentrated sulfuric acid, non volatilized residue represents sulphated ash. The metals present in the drug gets converted to sulfates, as these are more stable to heat and allow more accurate results than those obtained by simple calcination. The sulfated ash value was found to be  $4.98 \pm 0.366\%$ . The acid insoluble ash is the residue, obtained after boiling total ash with dilute hydrochloric acid, filtrating to remove the soluble and igniting the remaining insoluble matter. Thus by this procedure, level of silica is determined, specially sand and siliceous earth content present in the drug. Acid insoluble ash value was found to be  $0.51 \pm 0.0211\%$ . Any significant deviation in the % of ash value reported in present research work clearly shows adulteration of the drug. Extractable matter determination was carried out to determine the drug active constituents. Substances are generally extracted with water, methanol, petroleum ether and other solvents to determine extractive matters. *Cleome viscosa* L. seed's extractive value was found to be  $38.8 \pm 0.852\%$  in alcohol,  $15.8 \pm 0.514\%$  in water,  $10.50 \pm 0.986\%$  in ethyl acetate and  $18.67 \pm 0.881\%$  in petroleum ether. The maximum value was found to be with alcohol.

In many plants, the volatile constituents i.e. essential oils are present and characterized the mixture of terpenes, sesquiterpenes and their oxygenated derivatives, aromatic compounds, which are oily and volatilize at room temperature. Pharmacologically active substances may also pres-

ent in essential oils. The volatile content was found to be  $0.88 \pm 0.0034\%$  in *Cleome viscosa* L. seeds.

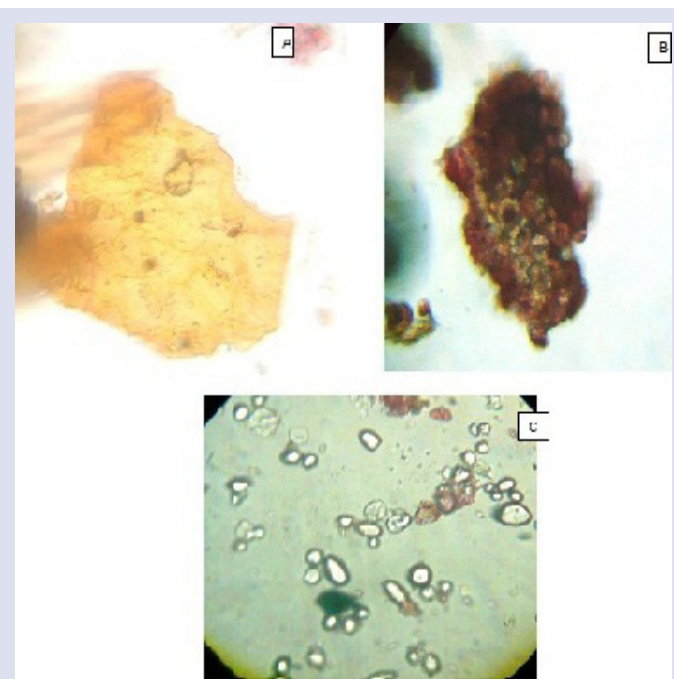
### Fluorescence Analysis

Fluorescence analysis of powdered seeds and different extracts of the seeds of *Cleome viscosa* L. with different reagents were carried out to observe the color reactions (Table.2) and to record presence of chromophore.

### Elemental Analysis

Elemental analysis of *Cleome viscosa* L. seeds was done to find out weight % of elements present in seeds (Table.3, Figure 3).

The mineral constituent present in *Cleome viscosa* L. seeds indicates its ethnopharmacological and nutritional importance. High peaks of carbon, oxygen, showed that these elements are present in very large



**Figure 2:** A) Fragments of epidermis of testa, B) Groups of cells resembling like stone cells, C) Irregularly shaped protein bodies.

**Table 2: Findings of Florescence Analysis**

S. No.	Reagents	Day light	Short UV (254nm)	Long UV (365nm)
1	Distilled water	Brown	Brown	Dark brown
2	Powder + 1 N HCl	Pale Yellow	Pale Green	Pale Green
3	Powder + 1 N NaOH	Red	Brownish Green	Brownish Green
4	Powder + 50% HCl	Brownish Green	Fluorescent Green	Fluorescent Green
5	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Dark brown	Brown	Brown
6	Powder + Methanol + 50% HNO <sub>3</sub>	Greenish	Greenish	Dark brown
7	Powder + Methanol + 1 N NaOH	Brown	Greenish	Greenish
8	Aqueous extract	Light Brown	Brown	Brown
9	Methanolic extract	Greenish brown	Greenish fluorescence	Greenish fluorescence
10	Pet. Ether extract	Brown	Greenish brown	Greenish brown
11	Ethyl acetate extract	Brown	Dark brown	Dark brown

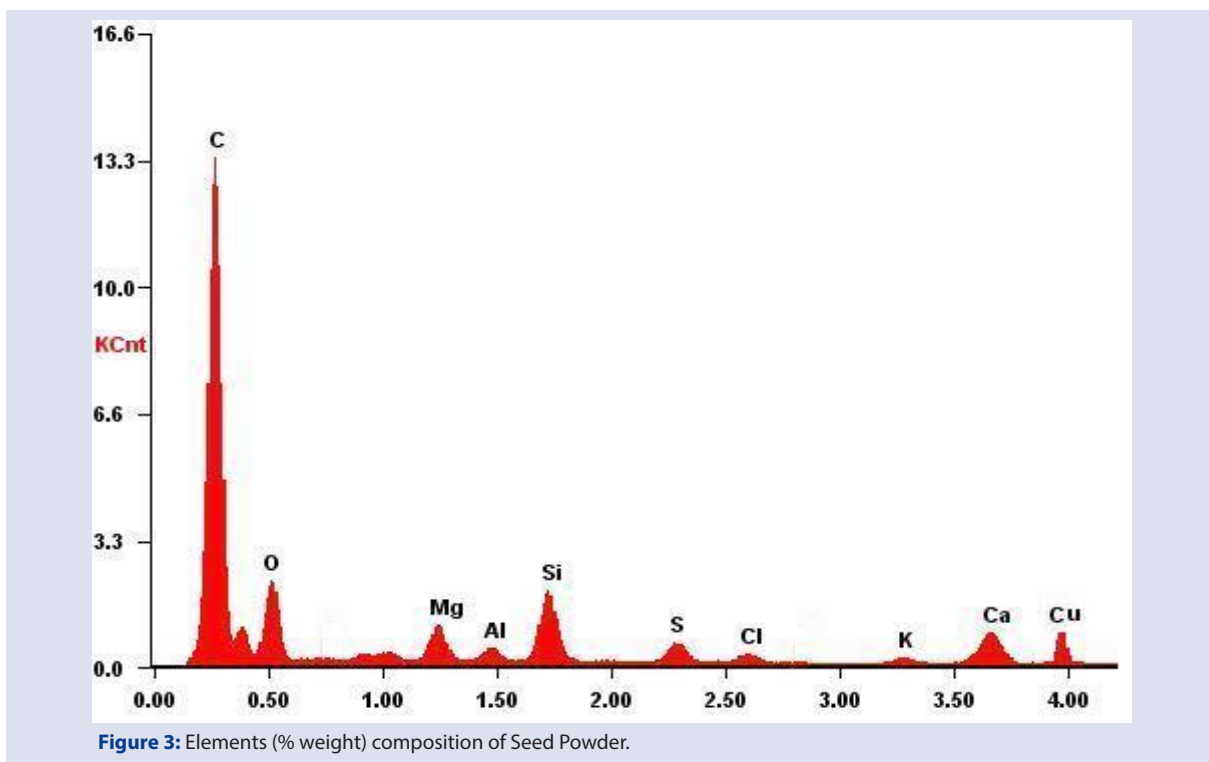


Figure 3: Elements (% weight) composition of Seed Powder.

Table 3: Elements Analysis Result

S. No.	Element	Weight %
1	C	66.80
2	O	12.40
3	Mg	03.20
4	Al	01.90
5	Si	07.60
6	S	01.20
7	Cl	00.70
8	K	00.90
9	Ca	03.20
10	Cu	02.10

amount. For instance, magnesium, calcium, copper and silica were in limited amounts. Aluminium and sulphur were found in less amounts (Figure 3). All of these mineral elements have different metabolic roles in human body.

For example, calcium is an important element which is known for reducing the risk of large variety of non-infectious diseases like maintenance of bones, teeth and muscles.<sup>14,15</sup> Acid–base balance and blood pressure is regulated with an important mineral which is potassium.<sup>16</sup> Magnesium is an important “helper molecule” of many enzymes mainly involved in energy metabolism, production of protein and for maintaining electrical potential of nerve tissue and cell membranes.<sup>17</sup> There is an important role of sulphur in synthesis of protein, regeneration of cells and cleansing of blood.<sup>18</sup> The presence of these important elements in *Cleome viscosa* Linn. seeds accounts for the therapeutic use as antipyretic, antimicrobial, analgesic, antiemetic, anticonvulsant and psychopharmacological, anthelmintic, carminative, anticonvulsant, wound healing.

## DISCUSSION

The evaluation of pharmacognostical parameters was performed on the basis of detailed botanical evaluation of the seeds which included morphology and microscopy as well as WHO recommended physicochemical parameters.<sup>19</sup> The findings of the pharmacognostical evaluation may be useful to identity of the seeds of *C. viscosa* (Linn.) which may produce a solid basis for the authenticity of the plant and also to distinguish the seeds of *C. viscosa* seeds other similar species.<sup>20,21</sup> The macroscopic characters were examined to identify the seed of the *C. viscosa*. The powder characters of a drug are mainly used in the identification of the drug in the powder form. The seeds powder was dark brown in colour with no specific odour and taste. The microscopical features of the powder which were observed, which showed numerous epidermis of testa, groups of cells resembling like stone cells and irregularly shaped protein bodies (Figure 3.3-3.5).

The physicochemical parameters play a vital role in evaluating the purity and quality of the drug.<sup>22</sup> Ash values are useful in evaluating the quality and purity of crude drugs, especially in powder form.<sup>23</sup> The ash values are important since ash may be derived from the plant itself (physiological or natural ash) as well as from the extraneous matter, especially sand and soil adhering to the surface of the drug (non physiological ash).<sup>24</sup> The determination of physiological and non physiological ash is termed as total ash.<sup>25</sup> The total ash may vary within wide limits for specimen of genuine drug due to variable natural or physiological ash,<sup>26</sup> in such cases the ash obtained is treated with acid in which most of the natural ash is soluble leaving the silica as acid-insoluble ash which represents most of the ash from the contaminating soil.<sup>27</sup> The ash values of the powdered seeds suggests about a high percentage of sulphated ash. Any significant variation in the percentage of ash as reported in the present work may show the sign of adulteration of the drug. In present study, all the above parameters were studied.<sup>28</sup>

Extractive values indicates about the chemical constituents present in the drug as well as useful in the determination of exhausted or adulterated drugs.<sup>29,30</sup> The results indicates that the powdered seeds have high alco-

hol soluble extractive value. The loss on drying reveals the percentage of moisture present in the drug, since moisture facilitates the enzyme hydrolysis or growth of microbes which leads to deterioration. Fluorescence analysis of powdered seeds was studied in both UV and day light. The powder showed green fluorescence with methanol in UV light at 254 nm, which indicates the sign of chormophore in the drug. The elemental analysis revealed the presence of some important elements.

## CONCLUSION

The detailed pharmacognostical studies on the seeds of *C. viscosa* may substantiate as an essential data for the identification of raw material and also used to differentiate the plant from its allied species and adulterants.

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## CONFLICT OF INTEREST

None

## ABBREVIATIONS USED

EDAX: Energy Dispersive X-ray Spectroscopy; UV: Ultra Violet, LOD: Loss on Drying.

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## SUMMARY

- In order to ensure the use of only genuine and uniform material in treatment of ailments by herbs and herbal formulations, pharmacognostical standardization and physicochemical parameters evaluation is required.
- Morphological and powder microscopic characters of seed powder were studied. The physicochemical parameters studied were LOD, total ash, acid insoluble, water soluble, sulphated ash, extractives, volatile oil content and crude fibre content etc.
- Further, fluorescence analysis and element analysis of seed powder was performed by undergoing chemical reaction +UV method and EDAX method respectively.
- Brown colored seeds with rough seed surface with seed size 0.1 cm were recorded. The brown coloured pods were 4-5 cm long containing 8-12 small seeds. Powder microscopic study revealed the presence of fragment of epidermis of testa, groups of cells resembling like stone cells and irregularly shaped protein bodies were observed.
- EDAX analysis showed the presence of Mg, Al, Si, S, Cl, K, Ca and Cu etc in different amount.

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