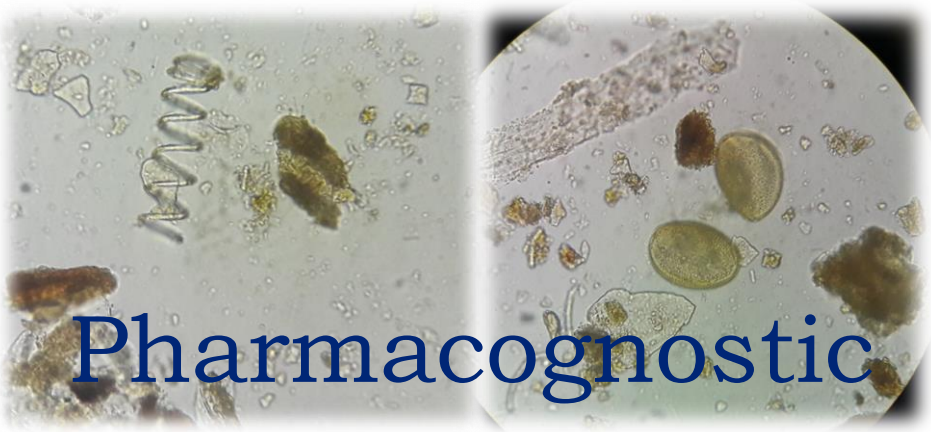
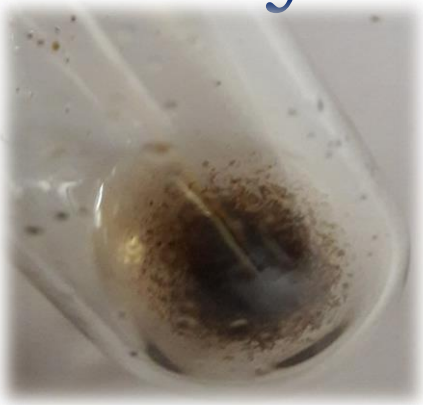


Pharmacognostic study



Introduction

Pharmacognosy is the study of crude drug closely related to botany of medicinal plants. Actually it is originated from the Greek word – "pharmakon" meaning a drug, and "gignosco" to acquire knowledge of. The terms 'pharmacognos' and 'pharmacodynamics' were first reported by Johann Adam Schmidt (1759- 1809) in his manuscript "Lehrbuch der Materia Medica", which was published in Vienna (Kar, 2003). In the beginning of the 20th century, the subject had developed mainly on the botanical side concerning the description and identification of drugs, both in the whole or in powder forms and also taking into accounts their history, economics, collection, preparation and storage. Such branches of pharmacognosy are still of fundamental importance, particularly for pharmacopoeial identification and quality control purposes, but rapid developments in other areas have immensely enriched the subject. The use of modern isolation techniques and pharmacological testing procedures usually help to find new plant drugs and making their way into medicine as purified material (Evan, 2002). The treasure of knowledge regarding the medicinal, narcotic and other properties of plants are still transmitted orally from generation to generation by tribal societies, particularly those of tropical Africa, North and South America and the Pacific countries. These are the areas containing the world's major repository of plant diversity. Similar records exist in books on medicinal plants and Ayurvedic, Unani and Siddha systems of medicines existing in India since long past. The quality of medicinally active chemicals from herbal sources is significantly influenced by some factors. The variation in the chemical profile of herbs is caused due to intrinsic and extrinsic factors like edapic factors, growth, harvesting, geographical source,

storage, drying conditions etc. (WHO, 2002). To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards this is the authentic identification of the plant and characterization of chemical contents, followed by a check in respect of standards for conformation (Agarwal, 2005). In 1996, WHO has set up the guideline for the pharmaceutical preparation of medicinal plant drugs. During last two decades, the pharmaceutical industry has made massive investments on pharmacological, clinical and chemical research all over the world in an effort to discover more potent plant drugs (Remya et al., 2012). For the purpose of standardization of herbs or herbal products pharmacognostic analysis make progress with the study of macromorphology, micromorphology, anatomy, physiochemical, phytochemicals, fluorescent behaviour of powder drug etc. Plant anatomy plays an important role in pharmacognostic study to discriminate desirable species from the fake ones. Anatomical investigation of medicinal plants for the authenticity and quality control of the drugs has come to much use (Banarjee et al. 2001, Gupta et al. 2001). According to World Health Organization, the macro and microscopic studies are required for the purpose of identification of any medicinal plant, prior to performing other tests with the plant products to control the purity of processed medicine (Anonymous, 1996). The gross morphological similarities confound the identification of this two species of *Crinum* L. However, details scrutiny on morphology revealed differences in two species. In consideration of the importance of pharmacognostic study different macro-morphological, anatomical, micro-morphological, phytochemical and physiochemical features of two species have been sorted out adulterants of them and will help to confirm their identity.

Materials and Methods

Collection of Plant Materials

The study has been dealt with two different species of *Crinum* L. Two different species of *Crinum* L. were collected from different parts of West Bengal and also from different states of India. Details of the studied species have been described in the previous chapter.

Morphological and Anatomical Study

Fresh plants parts of both the species were observed thoroughly under a dissecting microscope. The morphological characters of different parts of the plant were studied and verified from the book on Amaryllidaceae and taking help of the herbaria of Central National Herbarium, Howrah too. The voucher specimens were deposited at the herbarium of Department of Botany and Forestry, Vidyasagar University, Midnapore, West Bengal.

Fresh leaves and bulbs of both of the species were collected to make thin (20 μm) section and observed under the Leica DM 1000 microscope followed by Iyengar and Nayak 1988.

Microscopy of powder

Freshly collected bulbs from each species were dried on sunlight and homogenized with mortar and pestle to make fine powder. The powder smear was made on the slide and observed under a light microscope (Leica DM1000).

Physico-chemical Study

Organoleptic study of bulb powder, analysis of physico-chemical parameters, such as responses of the powder in presence of different chemicals under visible and fluorescence light (254 nm and 366 nm) with standard procedure (Laha, 1981; Anonymous, 1989; Evans, 2002).

Phyto-chemical Screening

Preliminary phytochemical screenings for the detection of various active chemical constituents were carried out by using standard procedures (Evans, 2002).

Results

The study showed valuable differences in morphology and anatomy of both species in their height, appearance of bulb, leaf length, leaf margin, perianth and androecium and in anatomy in regard of number of vascular bundle of leaf, layers of lower epidermis, number of xylem strand in roots, size of the cortex and stele etc. Leaves are lanceolate or oblanceolate, arranged in whorl from the bulb. In these species true stem is absent.

Transverse sections of leaves have shown similar characters, like closed collateral vascular bundles (Figure 3.7 & 3.8) and diacytic type of stomata surrounded with parenchymatous cells. Transverse sections of roots have shown little difference in number of xylem strand, diameter of cortex and stele of studied species (Table 3.8, 3.11 and Figure 3.9-3.26). Rhaphied bundles are profusely present in the cortex cells and in the pith parenchymatous cells of both species (Figure 4.1). Microscopic studies of bulb powdered have shown trachieds with spiral thickening and xylem fibers and spongy parenchymatous cells (Figure 4.1 & 4.2). *C. asiaticum* and *C. latifolium* have shown both side pinnate rhaphieds and both species contain a few stone cells (Figure 4.2).

Table 4.1: Organoleptic study of both species.

Parameters / Species	Colour	Odour	Taste	Texture
Crinum asiaticum L.	Yellowish	Old dry plant tissue	Tasteless	Smooth and cottony
Crinum latifolium L.	Brown	Old dry plant tissue	Tasteless	Smooth

Table 4.2: Presence of different chemicals in both the species.

Secondary metabolites	*Crinum asiaticum* L.	*Crinum latifolium* L.
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+
Steroids	-	-
Gums and mucilage	+	+
Fat and oils	-	-
Flavonoids	+	+
Tannins	-	-
Saponin	-	-
Protein and amino acids	+	+

Table 4.3: Fluorescence analysis of *Crinum asiaticum* L.

Chemical for treatment	*Crinum asiaticum* L.		
	Visible Light	Visible Light	Visible Light
Dry powder	Gold (met)	Gold (met)	Gold (met)
water	Signal yellow	Signal yellow	Signal yellow
Absolute ethanol	Signal yellow	Signal yellow	Signal yellow
Methanol	Light brown	Light brown	Light brown
n-Butanol	Yellow	Yellow	Yellow
n-Hexane	Light brown	Light brown	Light brown
NaOH 1(N)	Pale gold	Pale gold	Pale gold
50% NaOH	Copper (met	Copper (met	Copper (met
KOH 1(N)	Yellow	Yellow	Yellow
Conc. HCl	Brimstone yellow	Brimstone yellow	Brimstone yellow
50% H2SO4	Signal yellow	Signal yellow	Signal yellow
50% HNO3	Cream	Light brown	Azure blue
Conc. HNO3	Cream	Signal yellow	Blue
Acetone	Light yellow	Light yellow	Light yellow
5% Iodine	Copper (met)	Copper (met)	Copper (met)
FeCl3	Nut brown	Nut brown	Nut brown

Table 4.4: Fluorescence analysis of *Crinum latifolium* L.

Chemical for treatment	*Crinum latifolium* L.		
	Visible Light	Short web length	Long web length
Dry powder	Yellow	Golden	Brown yellow
water	Signal yellow	Gold (met)	Traffic blue
Absolute ethanol	Signal yellow	Copper (met)	Dark grey met
Methanol	Light brown	Brown	Steel blue
n-Butanol	Yellow	Brown	Black
n-Hexane	Light brown	Brown	Steel blue
NaOH 1(N)	Pale gold	Peru	Deep blue
50% NaOH	Copper (met	Gold (met)	Cobalt blue
KOH 1(N)	Yellow	Signal yellow	Gentian
Conc. HCl	Brimstone yellow	Signal yellow	Turquoise blue
50% H2SO4	Signal yellow	Gold (met)	Azure blue
50% HNO3	Cream	Light brown	Azure blue
Conc. HNO3	Cream	Signal yellow	Blue
Acetone	Light yellow	Light brown	Cobalt blue
5% Iodine	Copper (met)	Brown	Deep sea blue
FeCl3	Nut brown	Brown	Black

Table 4.5: Microscopic studies of both species.

Elements	*C. asiaticum* L.	*C. latifolium* L.
Tracheid	Spiral	Spiral
Trachea	Reticulate and Scalariform	Reticulate and Scalariform
Xylem Fiber	Long and pitted	Long and pitted
Xylem parenchyma	Smooth	Smooth
Xylem strand	10 - 22	7 - 10
Raphide	Cortex	In cortex and pith
Stone cells	Present	
Schlerides	Absent	Rarely present in conjugative cells

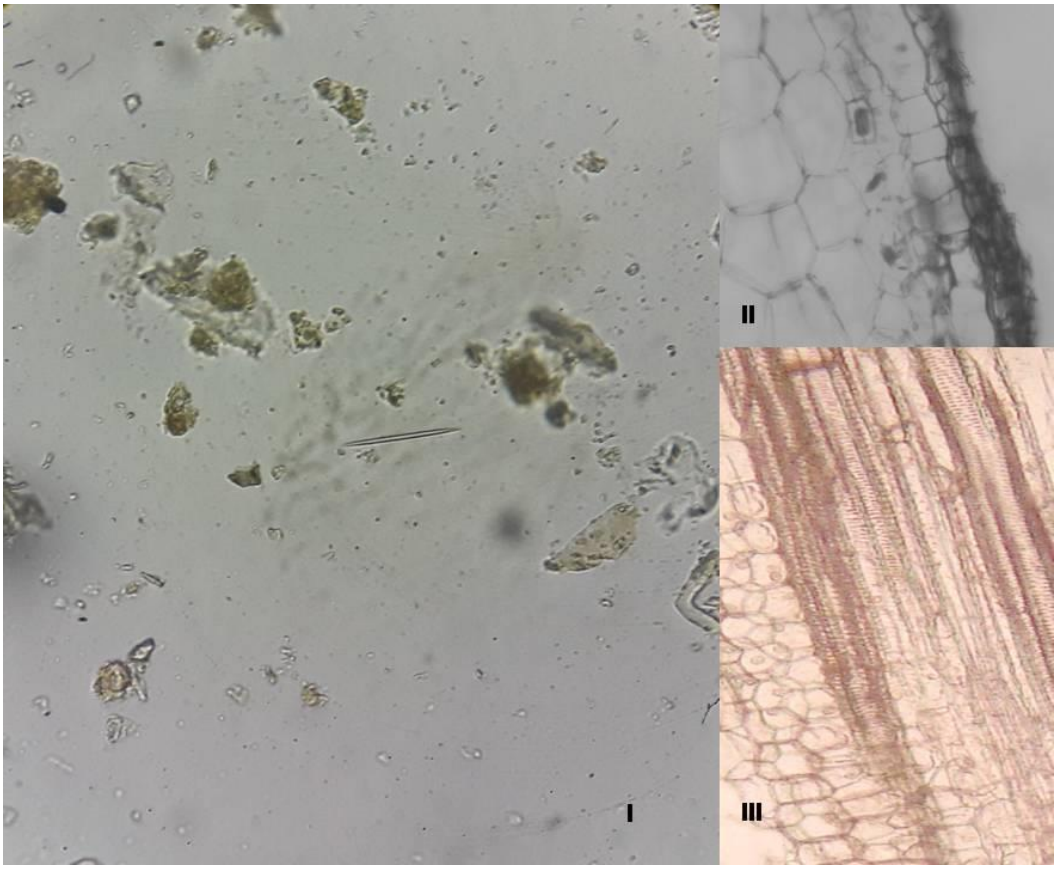


Figure 4.1: Powder microscopic study of *C. asiaticum*. (I – Acicular rhaphide , II – Rhaphides in cortex of root, III – Spiral tracheids).

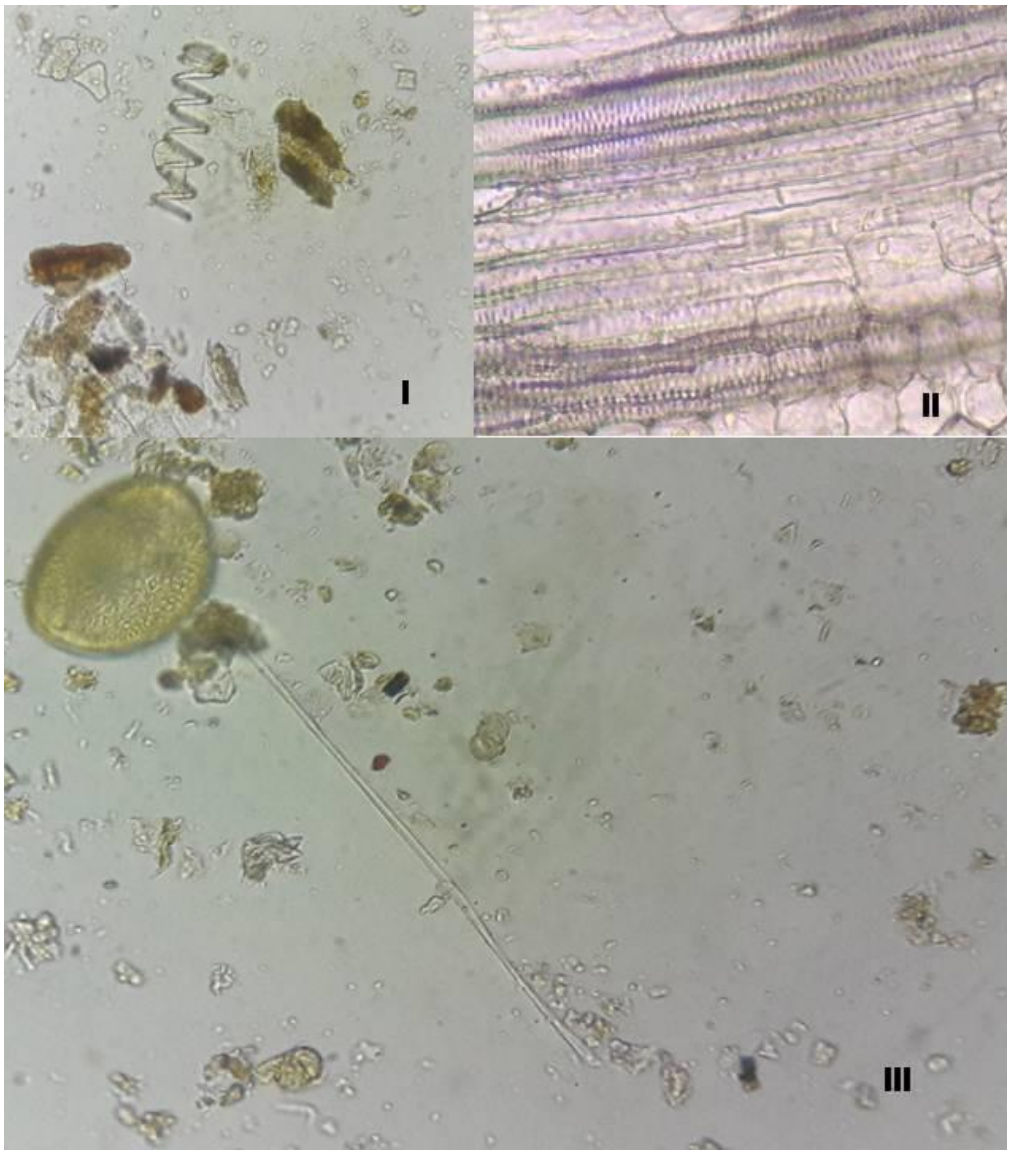


Figure 4.2: Powder microscopic study of *C. latifolium*. (I – Spiral tracheides, II – Spiral tracheids and III – Phloem fiber, stone cell).

Discussion

Flowers and bulb morphology of the species has been noted to be the key part for their identification (Figure 3.2 and 3.3), without this discrimination of these species become difficult. Leaves and bulbs have shown some differences in gross appearance as well as in other details. Leaf margin of *C. asiaticum* is wavy, while *C. latifolium* is plain and the bulb of *C. asiaticum* is longer than the other (Table 3.6 and Figure 3.2).

Tuber anatomy has shown some distinctive features on the basis of number of xylem strands, diameter of cortex, stele (Table 3.4 and Figure 3.6). Present study, *C. asiaticum* has shown highest number of xylem strand (12 - 13). Bundles of raphides have been recorded to be occasionally present in the parenchyma cells of pith and cortex cells only (Figure 4.2). Tuber powders of both species have also shown difference in colour, odour, taste and texture (Table 4.1). Microscopic studies have revealed the trachea of *C. asiaticum* and *C. latifolium* to be reticulate and scalariform, wich findings comply with the earlier records (Dolai and Nandi 2020). Both species has similar type of Lycorine present.

Treating the powders with different chemicals in presence of visible and ultraviolet light 254 nm and 366 nm has been noted to develop a variety of colours, also helpful in discriminating the species (Table 4.3 and 4.4). The two species have contained same secondary metabolites. They show the only different character in the study of physico chemical nature.

Conclusion

The detail Pharmacognostic study both *Crinum asiaticum* L. and *Crinum latifolium* L. have shown some similarity and differentiation in respect to morphology and anatomical study also. Detail Pharmacognostic study can help to identify the right species. The micro morphological study of the leaf and bulb also support the diverse nature. Micro morphological, physicochemical character and organoleptic study can help to identify the adulterant of *Crinum asiaticum* L. *Crinum latifolium* L.