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ISOLATION AND CHARACTERIZATION OF STARCH FROM MANGROVES *Aegiceras corniculatum* (L.) Blanco and *Cynometra iripa* Kostel.

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ABSTRACT: The present investigation deals with the isolation and characterization of starch from mangroves *Aegiceras corniculatum* and *Cynometra iripa*. *Aegiceras* is one of the important mangrove genera, which is an indicator of fresh water influence. *Cynometra iripa* is critically endangered species from west coast of Maharashtra. The propagules of *A. corniculatum* are starchy, therefore the starch from these propagules was isolated and properties of starch were studied. From the fruits of *Cynometra iripa* starch was isolated. Isolated starch was employed to techniques such as scanning electron microscopy (SEM), gelatinization and X-ray diffraction. Starch granules are variable in size and shape. Starch granules of *A. corniculatum* are round, oval and lenticular in shape with an average size 5µm, while in *C. iripa* starch granules are round and oval in shape with an average size 7 µm. The gelatinization temperature range varies from 60^0C to 90^0C; while in *C. iripa* range varies from 50^0C to 100^0C. The range of temperature in both the mangroves is comparatively higher than other starch sources.

Key words: Starch, mangroves, gelatinization, SEM, XRD

INTRODUCTION

Mangroves are tropical and subtropical in occurrence. Mangroves are salt tolerant plants & they form very special type of vegetation protecting our sea coast. They play an extremely important role in maintain the quality & productivity of coastal water. Mangroves have many economic & ecological uses. *Aegiceras corniculatum* (L.)Blanco is a mangrove, which occurs in the middle & distal zone in the mangrove communities. It is an indicator of fresh water influence. *Cynometra iripa* Kostel is one of the critically endangered species from west coast of Maharashtra. Only taxonomic account of this species is available (Percival & Womersley, 1975; Chapman, 1976; Tomlinson, 1986). Bhosale *et.al,* (2002) have reported both the species in Sindhudurg & Ratnagiri districts of Maharashtra. Germination & other related aspects are carried out by Gokhale M. V. (2004). Starch has key role in the process of seed germination & seedling physiology. In the seedling of Aegiceras corniculatum 32% starch has been reported (Joshi *et. al*., 1972). Starch is a bimolecule & its structure, granule size, crystallinity as well as other properties like gelatinization are determined by the genetical make-up of species. Starch represents first basic form of reserve carbohydrates in general; possibly take part in osmo-regulatory processes in mangrove to overcome the potentially harmful effects of salts in the substratum (Bhosale & Mulik, 1992). Starch is one of the most important plant products to man. It is an essential component of the daily calorific intake and is important in non-food uses such as in adhesives, papers, textiles etc. Starch is a versatile, cheap, & has many uses such as thickener, water binder, emulsion stabilizer and gelling agent.

Microscopically, starch grains are fine crystals or lumps, the precise form of these grains varies within the plant kingdom. Chemical formula of starch $(C_6H_{10}O_5)n$ is a large polysaccharide of glucose monosaccharide units joined together by glycosidic bonds. It is used by plants to store excess glucose. Depending upon the plants, starch generally contains 20-25% amylose and 75-80% amylopectin. Starch grains are generally made up of successive layers of amylase & amylopectin (Banks & Muir, 1980). Banks & Muir (1980) found that the size & morphology of starch grains vary between species & can be useful in species identification. In the present investigation an attempt has been made to isolate & characterization of starch from *Aegiceras corniculatum* (L.) Blanco *& Cynometra iripa* Kostel.

MATERIAL AND METHODS

Material:

Propagules of *Aegiceras corniculatum*(L.)Blanco and fruits of *Cynometra iripa* Kostel were collected from Sindhudurg district along West coast of Maharashtra. Mature propagules and fruits of same size were selected for analytical work.

Methods:

1. Isolation of starch:

Propagules of *Aegiceras corniculatum* and fruits of *Cynometra iripa* were cut into pieces and crushed to obtain the extract in distilled water. The starch in the extract was allowed to settle down and the supernatant was discarded. The starch powder was washed repeatedly with 80% ethanol. Finally, the powder was air-dried and stored at 4°C. The basic method of isolation of starch is given by Badenhuizen (1964). This method is used for present investigation of starch and under laboratory condition isolation protocol was standardized.

2. Gelatinization:

Starch from different sources differs in their range of gelatinization temperature. Gelatinization of starch was carried out by following the method given by Daniel (1911). Granule morphology was studied microscopically. Micrometry technique was employed for starch granule measurement.

3. X-ray diffraction (XRD):

The crystalline structure of starch granule is highly conserved in plants at molecular level (Jenkins et.al, 1993) as well as microscopic level.

The starch isolated from both the species was used for X-ray diffraction studies. The experiment was carried out on Philips/Panalytical X-ray diffractometer-3710. The continuous scan of starch with angle 2Θ (10-50) was done on diffractometer.

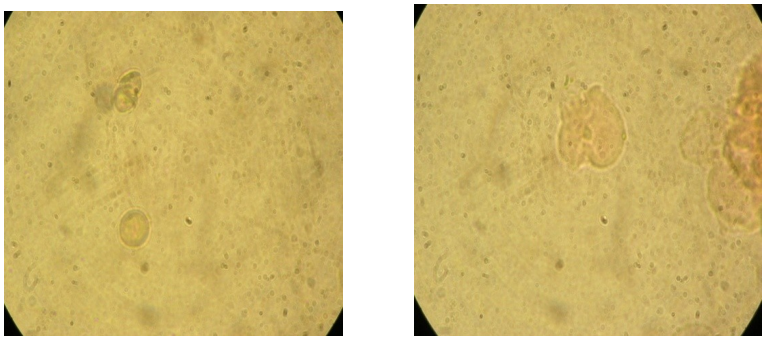
4. Scanning Electron Microscopy (SEM):

Electron microscopy was carried out with scanning electron microscope JEOL-JSM-360. A thin layer of starch granules was prepared on a glass slides and were scanned to obtain the granule size and shape variations.

RESULTS AND DISCUSSION:

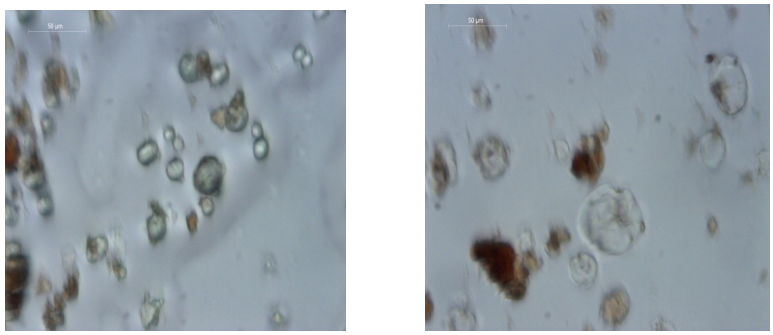
The studies revealed that, the isolated starch of *Aegiceras corniculatum* and *Cynometra iripa* is amorphous in nature.

Isolated starch further used for gelatinization. Gelatinization temperature ranges from 60°C to 90°C in *A. corniculatum* (Plate-1a) and 50°c to100°C in *C. iripa* (Plate-1b). The range of temperature is comparatively higher than other sources. So, the starch is more stable to high temperature. As soon as the gelatinization starts the starch granules loses its birefringence followed by increase in viscosity and translucency (Daniel, 1991). Gelatinized starch used as a thickener, as an adhesive and for sizing of textile and paper (French, D. 1957). The temperature range over which gelatinization is observed depends on property during determined (Ziegler *et.al,* 1993). Since, each starch is having a definite temperature that is used in the identification of starch from different sources (Daniel *et.al,* 1991) as well as definite temperature is distinctive for different types of starch (Whistler and Corbett, 1957). Based on photo-plate 1A&B, a graphical analysis is represented. Present results suggests that, there is no effect of temperature between 40°C-50°C on the size of starch granules in *A. corniculatum* and also in *C. iripa*. However, there is a swelling from 60°C in *A. corniculatum* and from 50°C in *C. iripa* onwards and attains maximum swelling at 90°C and 100°C respectively. There was bursting effect on granule. Thus, the present investigation suggests the stability of starch granules of *A. corniculatum* and *C. iripa* at higher temperatures (Figure-1, A & B).



60° C 90° C

Plate 1(a): Gelatinization of Aegiceras corniculatum (L.) Blanco



50^0 C 100^0 C

Plate 1(b): Gelatinization of *Cynometra iripa* Kostel.

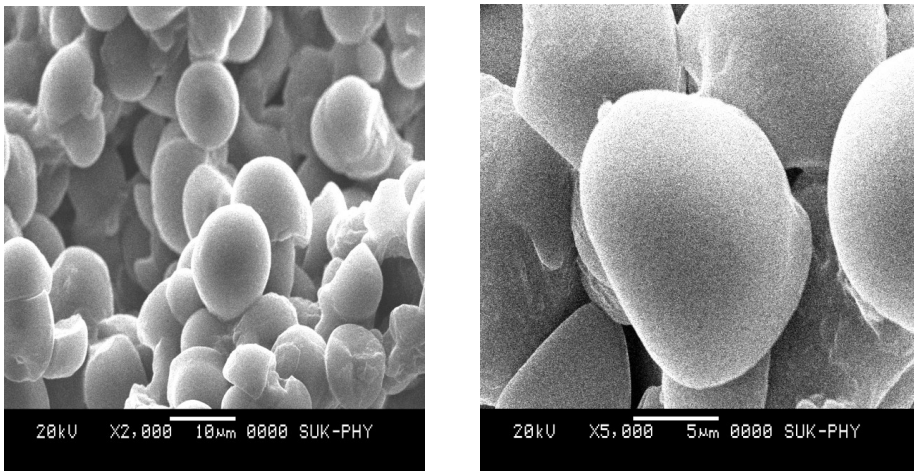


Plate 2(a): Scanning Electron Microscopy (SEM) of *Aegiceras corniculatum* (L.) Blanco starch granules

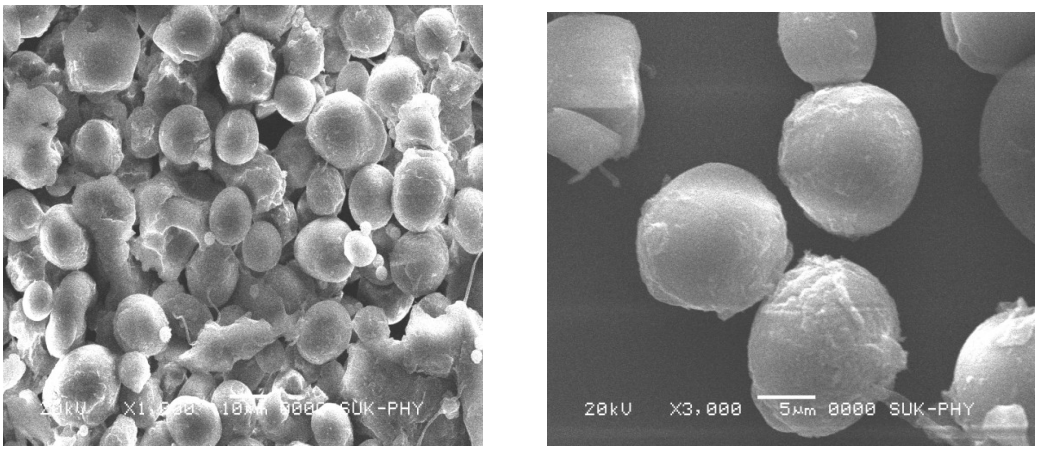


Plate 2(b): Scanning Electron Microscopy (SEM) of *Cynometra iripa* Kostel. starch granules

The starch isolated from *A. corniculatum* and *C. iripa* used for X-ray diffraction. Both shows total 4 peaks (Figure-2, I & II). X-ray diffraction is a very important method for the determination of structure in molecule (Jayraman & Jayraman, 1974). The particular advantage of diffraction analysis is that, it discloses the presence of substance, as the substance exists in that sample (Cullity, 1978). X-ray diffraction technique has found wide application in many diverse area's as a tool to determine the local atomic environment in many classes of materials for structure defined analysis by standard technique like diffraction (Gauns, 1996). The technique is used primarily to examine the crystalline structure of materials and to determine bond length and angles (Pomeranz and Meloan, 1971).

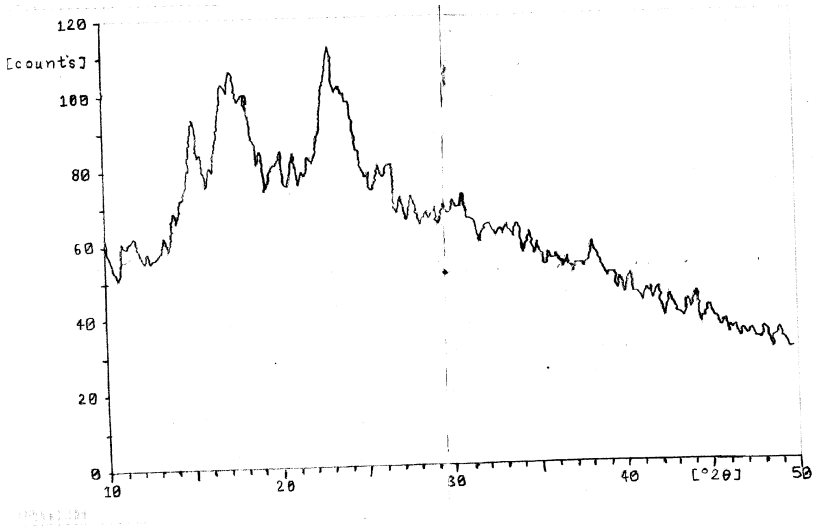


Figure 2(I): X-Ray Diffraction (XRD) of *Aegiceras corniculatum* (L.) Blanco

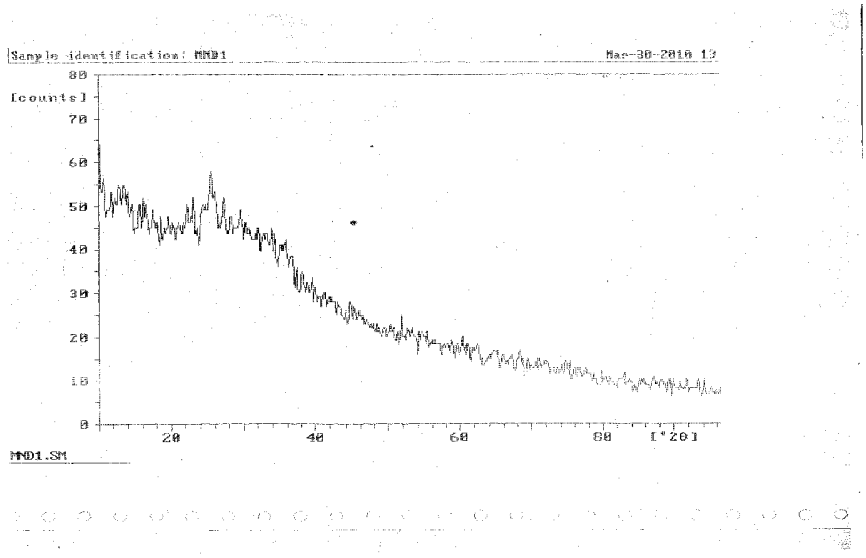


Figure 2(II): X-Ray Diffraction (XRD) of starch of *Cynometra iripa* Kostel.

The isolated starch powder is utilized for scanning electron microscopy; starch granules observed are variable in size and shape. In *A. corniculatum*, most of the starch granules are lenticular in shape, some are round or oval. The length of the granule ranges between 12-15µm and 8-10µm in breadth [Plate-2(a)]. In *C. iripa*, most of the granules are oval in shape. The length of the granule ranges between 10-15µm and 10-12µm in breadth [Plate-2(b)].

CONCLUSION:

A protocol for isolation of starch has been developed. The further confirmation is through X-ray diffraction technique. Shape of starch granules are variable viz; oval, round or lenticular. X-ray diffraction studies and scanning electron microscopy confirms the purity of starch. Therefore, simple method of isolation is standardized.

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