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EXTRACTION AND CHARACTERIZATION OF STARCH FROM THE TUBERS OF ANTIGONON LEPTOPUS SPECIES

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ABSTRACT

Starch is a natural polymer and is a group of polysaccharides composed of glucopyranose units joined together by glycosidic linkages. Starch is an important polysaccharide extensively used in many industries like food, cosmetic, paper, textile, pharmaceutical etc. It is found to be present in appreciable quantity in stem, roots, leaves, seeds, fruits and tubers. Hence this study was conducted to isolate and characterize starch from tuber of Antigonon leptopus species. In this work starch from Antigonon leptopus tubure was extracted and % starch yield was calculated as well as evaluated for its macroscopic characters, Preliminary phytochemical screening of aqueous extract for the presence of carbohydrate, tannins , saponins and flavonoids. The result showed that there was a sufficient amount of starch yield. The compound microscope it showed the presence of round and oval shaped small and large starch particles, single as well as in groups. The diamond and prism shaped calcium oxalate crystals were also seen. Thus starch of tuber Antigonon leptopus species shows good properties and could serve as alternatives for the production of industrial products that may require starch.

KEYWORDS: Starch, Antigonon leptopus tuber, Microscopic characterization, phytochemical screening.

INTRODUCTION

Starch is a one of type of carbohydrate reserve in various plants part such as in leaves, flowers, fruits and different types of stems and roots. In a plants starch is used as a source of carbon and energy. Starch made up of glucose residues that are linked in two different forms such as glucose moiety link with two polymers Amylase (with α-1, 4 linkages) and

amylopectin (joined by α-1, 6 linkages). Starch mainly comprise of 70-80% of Amylopectin whereas amylose consists of 15-30% of starch.[1]

Starch acts as glucide reserve of plants found in maize, wheat and potato from which it is extracted, as well as in many other plants: rice, barley, vegetables, manioc, and sweet potato. Starch is synthesis in plants via photosynthesis process and this mechanism utilized by plants to produce and store the glucose (elementary sugar) which is necessary for their growth and reproduction.[2] The leaves of *Antigonon leptopus* was evaluated for its pharmacognostic evaluation and it showed the presence of both simple and compound form of starch grains.[3] The various parts of *Antigonon leptopus* (family Polygonaceae) like tubers and flowers of it consumed as food in several parts of the world. Tea preparation of aerial portion such as flowers used as a cold remedy.[4,5] The pharmacological action behind its use of functional food qualities and it was found that the methanol extract of the aerial parts of A. leptopus, inhibited lipid peroxidation (LPO) by 89% and cyclooxygenase enzymes, COX-1 and COX-2 by 50.4% and 72.5%, respectively, at 250 lg/ml. The extracted and purified methanolic extract of *Antigonon leptopus* yields n-hentriacontane, ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside, and kaempherol-3-glucoside along with b-sitosterol, b-sitosterol-glucoside and d-mannitol and hence shows as a antibacterial, anti-inlamatory, anti-oxidant activity, etc.[6] Also it has been used to treat diabetes, asthma, liver and spleen disorders, cough and throat constriction, flu-related pains, hypertension, antithrombin agent and used to reduced menstrual pains.[7-10]

Literature survey revealed that the extract of plant was evaluated for structural characterization, antioxidant and anticancer properties of gold nanoparticles of extract,[11] for new steroidal saponin,[12] as well as for extermination of fish bacterial pathogens.[13] Being a one of the tropical source of starch which has not been utilized for industrial application is *Antigonon leptopus* tuber. However, extraction of starch has not been reported previously in the literature, hence the present study is designed with the objective to study the extraction of starch from tubers *Antigonon leptopus* species and characterization of starch using microscopic and preliminary phytochemical screening for potential industrial applications.

MATERIALS AND METHODS

Sample collection and preparation: Fresh and healthy tuber was collected from near places in Kamptee, Durga Society-new Yerkheda and identified by Botany department of Nagpur. The fresh tubers were dried, pulverized and powdered for starch extraction.

Starch Extraction: 100mg of fresh tubers were collected, thoroughly washed with distilled water, cut into small pieces. Then these pieces were grinded in an electrical mixture to get a fine slurry .It was then filter through muslin cloth. It was allowed to settle overnight then the liquid decanted and settled residue was then washed with again the distilled water and allowed to settle for few hour after this the water was decanted and residue was allowed to dry. % yield was calculated and its morphological, microscopic characteristics were evaluated. Identification of chemical constituents was confirmed by means of chemical test.

Starch Yield: Starch yield was measured in percentage by comparing the weight of obtained starch (dry basis) with the weight of dry matter sample (*Antigonon leptopus* tuber). The Starch Yield (SY) was determined by the equation:

$$SY\ (\%) = \frac{W1}{W2} \times 100$$

Where W1 is weight of dried starch and W2 is the weight of original sample (*Antigonon leptopus* tuber).

Starch Granules Microscopic Evaluation (Morphology):- Smear of isolated starch powder as well as smear staind with dilute Iodine solution was prepared on a glass slide and observed under compound microscope.

Preliminary phytochemical screening[14]: Tubers of *Antigonon leptopus* were dried, pulverized to coarse powder and aqueous extract was prepared by maceration. It was filtered and subjected to Preliminary phytochemical screening.

Tests for carbohydrates

1. **Molish's test:** To 3 ml of test solution, two drops of alcoholic solution of α-Naphthol were added. The mixture was shaken well and few drops of concentrated sulphuric acid were added slowly along the sides of test tube. A violet ring at the junction of two lights indicates the presence of carbohydrates.
2. **Benedict' s test:** To 3 ml of test solution, 3 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

3. **Fehling's test :** 1ml of Fehling's A solution and 1ml of Fehling's B solution were mixed, boiled for 1 minute, equal amount of test solution was added to it. Reaction mixture was

heated in boiling water bath for 5-10 minutes. First yellow, then red precipitate of cuprous oxide indicates the presence of sugar.

4. **Iodine test:** 3 ml of test solution was mixed with few drops of dilute Iodine solution Formation of deep blue colour indicates the presence of starch. It disappears on boiling and reappears on cooling.

Tests for proteins

1. **Millon's test:** To 3 ml of test solution, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins.

2. **Biuret test:** To 3ml test solution, 2ml Biuret reagent was added, violet colour indicates presence of proteins.

3. **Test with trichloroacetic acid:** To 3ml of test solution, few drops of trichloroacetic acid were added; appearance of precipitate indicates the presence of proteins.

4. **Xanthoproetic test:** To 3ml of test solution, 1ml of concentrated nitric acid was added and boiled, yellow precipitate is formed. After cooling it, 40% sodium hydroxide solution added, orange colour indicates presence of proteins.

5. **Lead acetate test:** To 3ml the test solution, few drops of lead acetate solution were added. Appearance of yellow color precipitate indicates the presence of proteins.

Tests for amino acids

1. **Ninhydrin test:** To 3ml of test solution, 2 ml of ninhydrin solution was added. Appearance of purple colour indicates the presence of amino acids.

2. **Millon's test:** To 3ml test solution, about 2ml of Millions reagent was added, a white precipitate indicates presence of amino acids.
3. **Tests for fixed oils and fats:** Spot test:- A small quantity of powder was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Tests for alkaloids

1. **Mayer's test:** To 3ml of test solution, few drops of Mayer's reagent were added. Appearance of white creamy precipitate indicates the presence of alkaloids.

2. **Wagner's test:** To 3ml of test solution, few drops of Wagner's reagent were added. A reddish- Brown precipitate indicates the presence of alkaloids.

3. **Dragendorff's test:** To 3ml test solution, few drops of Dragendorff's reagent were added. Formation of reddish brown precipitate indicates the presence of alkaloids.

4. **Hager's test:** To 3ml test solution, few drops of Hager's reagent were added. Formation of yellow orange precipitate indicates the presence of alkaloids.

Tests for glycosides

- **Anthraquinone glycosides**

1. **Borntrager's test:** To 3ml of test solution, dilute hydrochloric acid was added, it was then boiled and filtered. Then equal volume of chloroform was added and shaken, chloroform layer was separated and equal volume of dilute ammonia solution was added to it. Appearance of pink or red colour indicates presence of anthraquinone glycosides.

2. **Modified Borntrager's test:** 5 ml of test solution, 5ml of 5% aqueous ferric chloride solution and 5 ml of dil. HCL were heated for 5 minutes in boiling water bath, cooled, and shaken with equal volume of chloroform. The chloroform layer was separated and 10% ammonia solution was added to it. Appearance of pink or red color indicates presence of C type of Anthraquinone glycosides.

- **Cardiac glycosides**

1. **Keller-Killiani test (for deoxy-sugars):** To 2 ml of test solution, few drops of glacial acetic acid and 1 drop of 5% ferric chloride were added and the contents were then transferred to a small test tube, 0.5ml of concentrated sulphuric acid was added carefully by the side of the test tube. A reddish brown colour appears at the junction of two liquids layer and upper layer becames bluish green it indicates presence of deoxy-sugar.
2. **Raymond's test:** The test solution was treated with hot methanolic alkali; violet colour indicates presence of cardiac glycoside.

3. **Legal's test:** The test solution was treated with 1ml of pyridine and 1ml of sodium nitroprusside solution, Pink to blood red colour indicates presence of cardiac glycoside.

4. **Baljet's test:** The 2ml of test solution was treated with sodium picrate; formation of orange colour indicates presence of cardiac glycoside.

- **Coumarin glycosides**

1. Drugs containing coumarins possess Aromatic odour. A small quantity of test solution was placed in a test tube and was covered with filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes, the paper was removed and exposed to ultraviolet (UV) light, presence of green fluorescence indicates the presence of Coumarin glycosides.

2. Alcoholic test solution when made alkaline shows blue or green fluorescence.

- **Saponin glycosides**

1. **Froth formation test:** 3ml of test solution was shaken well with water in a test tube; formation of stable froth (foam) indicates the presence of saponin glycoside.

2. **Haemolysis test:** To 3ml test solution, 1 drop of blood was added and allowed to stand for 15 minutes, settling down of RBCs indicates the presence of saponin.

Tests for phenols and tannins

1. **Ferric chloride test:** 3 ml test solution was treated with 5 % ferric chloride solution, appearance of blue colour indicates presence of hydrolysable tannins and appearance of green colour indicates the presence of condensed tannins

2. **Phenazone test:** To 5ml of test solution, 0.5gm to sodium acid phosphate was added, it was warmed and filtered. To the filtrate, 2% Phenazone solution was added, bulky precipitate indicates presence of tannin.

3. **Lead acetate test:** To 3 ml of aqueous test solution, few drops of lead acetate solution was added, formation of precipitate indicates the presence of tannins.

4. **Potassium dichromate test:** To the 3 ml of test solution, few drops of potassium dichromate solution was added, appearance of red precipitate indicates the presence of tannin.

Tests for steroids

1. **Liebermann-Burchard test:** To 3ml of test solution, few drops of acetic anhydride were added, boiled and cooled. Then concentrated sulphuric acid was added from the side of the test tube, brown ring was formed at the junction two layers and upper layer turns

green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

2. **Salkowski test:** To 3ml of test solution, chloroform was added and concentrated sulphuric acid was added from the side of test tube, the chloroform layer shows red to blue colour and acid layer shows greenish yellow florescence.

Tests for flavonoids

1. **Shinoda test:** To 3ml of test solution, 5ml of 95% ethanol, few drops of conc. hydrochloric acid and 0.5 g magnesium turnings were added, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes which indicate presence of flavonoids.

2. **Alkaline reagent test:** To the test solution, few drops of sodium hydroxide solution was added, there is formation of intense yellow colour which becomes colourless on addition of few drops of dilute acid indicates presence of flavonoids.

3. **Lead acetate Test:** To 5ml of test solution, few drops of lead acetate solution were added. Yellow precipitate indicates presence of flavonoids.

Test for terpenoids: 1 ml of test solution was treated with 1% CuSO4 solution; formation of green colour indicates the presence of Diterpene.

Test for gum and mucilage: Few ml of test solution was dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicates the presence of Gums and Mucilage.

RESULT AND DISCUSSION

Macroscopic characterization

The fresh tubers of A. leptopus were evaluated for its macroscopic as well as microscopic characters.

Table no. 1: Macroscopic characteristics of antigonon leptopus tubers.

Sr. no	Property	Observation
1	Colour	Whitish pink
2	Texture	Slippery
3	Odour	Slight
4	Taste	Bitter and areca nut like

As literature survey revealed that the tuberous plants contains a starch in a sufficient amount and *Antigonon leptopus* is one of the tuberous plant hence its tuber is rich in starch. The starch was extracted from *Antigonon leptopus* tuber then it was evaluated for its macroscopic evaluation and % starch yield was calculated.

Table no. 2: Macroscopic characteristics of Isolated starch of antigonon leptopus tubers.

Sr. no	Property	Observation
1	Colour	White
2	Consistency	Solid
3	Odour	Odourless
4	Taste	Tasteless
5	% Yield	4.73 % w/w

Microscopic characteristics of Isolated starch of A. leptopus tubers

Smear of isolated starch powder was prepared on a glass slide and observed under compound microscope showed the presence of round shaped small and large starch grains, single as well as in groups. The diamond and prism shaped calcium oxalate crystals were also seen.

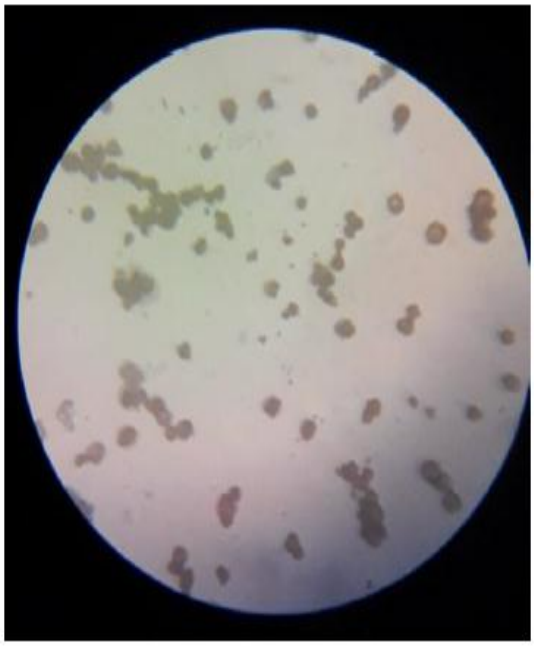


Fig. no.1: Starch grains with Iodine stain

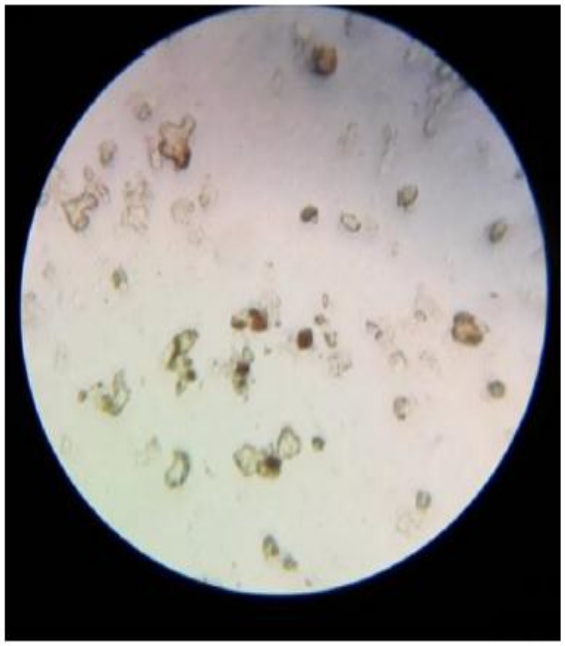


Fig. no.2: Starch grains without Iodine stain

Smear of isolated starch powder was prepared on a glass slide was, stained with dilute Iodine solution and observed under compound microscope, showed the presence of dark bluish black colour, round shaped small and large starch grains, single as well as in groups. The diamond and prism shaped calcium oxalate crystals were also seen. (Fig no. 3-6)

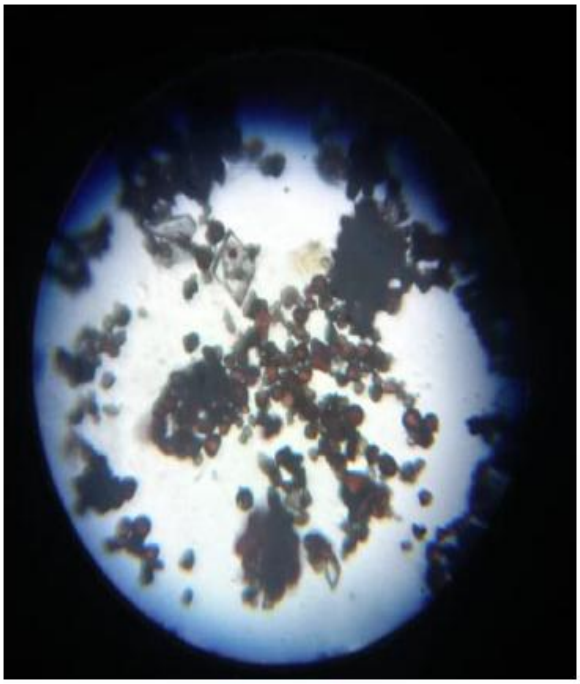


Fig. no. 3.

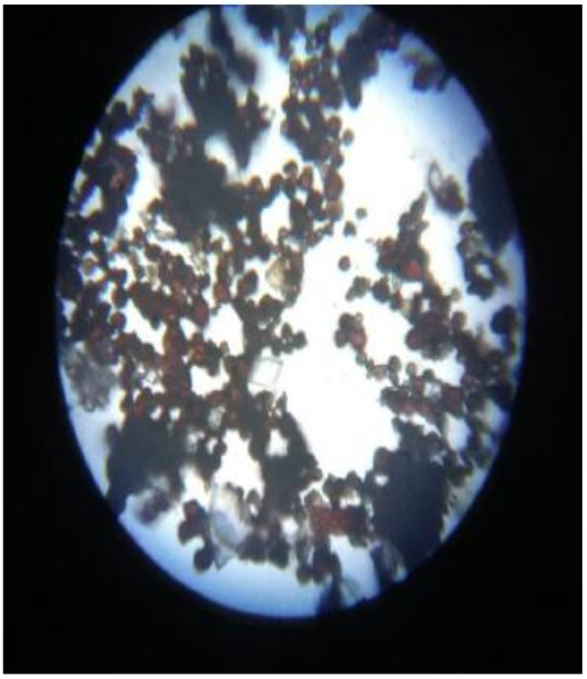
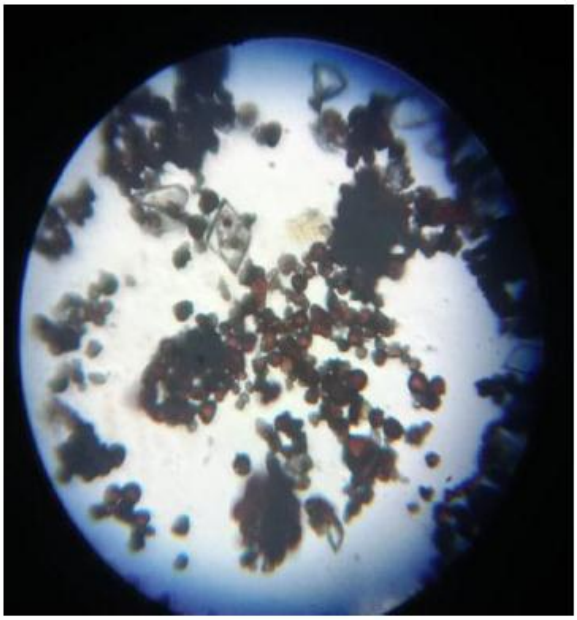


Fig. no. 4.



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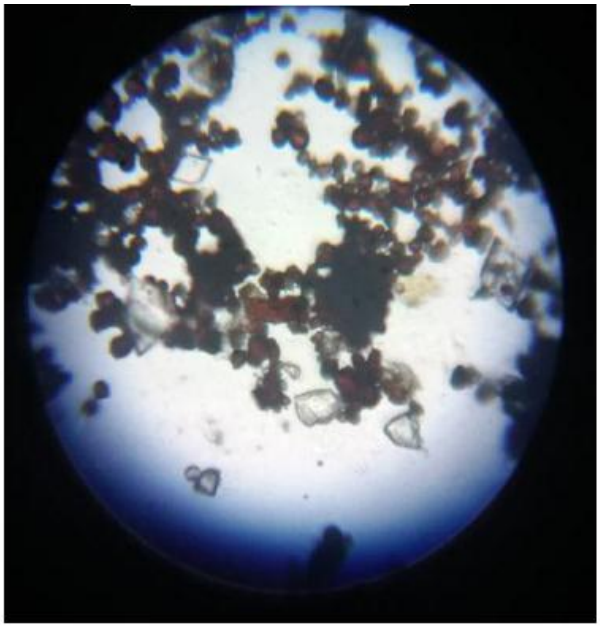

Fig. no. 6.

Starch grains and calcium oxalate crystals stained with Iodine solution shown in fig 3-6.

Confirmation of presence of starch: The presence of starch is confirmed by performing iodine test on a glass slide. The observation is as follows:-

Table no. 3: Chemical test of starch with iodine solution.

Test	Observation	Inference
Iodine + starch grains in a powder	Blue colour observed	Starch is present.

Preliminary phytochemical screening

The aqueous extract was prepared by maceration. It was filtered and subjected to Preliminary phytochemical screening.

Table no. 4: Preliminary phytochemical screening.

Phyto-Constituents	Name of tests	Result
Alkaloids	i) Mayers reagent test ii) Wagners reagent test iii)Dragendorff's Test iv) Hagers test	-Ve -Ve -Ve -V
Glycosides	i) Brontrager test ii)Killer killani test iii) Legal test iv)Baljet test	-Ve -Ve -Ve
Tannins and phenolic compounds	i)Lead acetate test ii) Ferric chloride test iii) Gelatin test	+Ve +Ve +Ve
Flavonoids	i)Shinoda test ii)Lead acetate test iii)Alkaline reagent test	+Ve +Ve +Ve
Saponins	i)Froth formation test	+Ve
Carbohydrates	i)Molisch reagent test ii)Benedict test iii)Fehling reagent test	+Ve +Ve +Ve

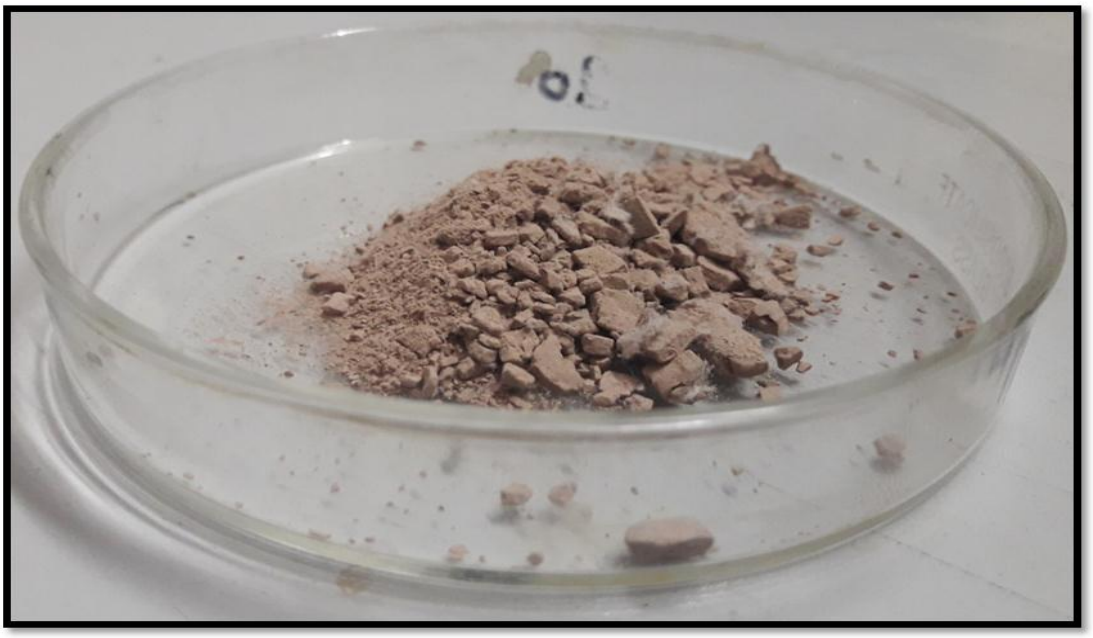

Fig. 7: Antigonon leptopus starch.

CONCLUSION

From the present studies it may be concluded that the quantity of starch isolated is very less as compared to the quantity isolated from other sources of starch. However, the tubers showed the presence of carbohydrates, tannins, saponins and flavonoids so these compounds can be isolated and evaluated for their therapeutic potential and can be formulated into suitable dosage form.

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