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Isolation of starch from Ginger rhizome (*Zingiber officinale)*

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Abstract
Ginger contains a many constituent like starch, fat, Gingerol, volatile oil. And it is used traditionally for dizziness, arthritis, menstrual pain. While isolation of starch from ginger rhizome will be used for tablet and other purposes and simultaneously for pharmacological effect.

Keywords*:* *Zingiber officinale*, Ginger, starch.

1. Introduction
Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the usage of the agents in the traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.
India has several traditional medical systems, such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. The material medica of these systems contains a rich heritage of indigenous to herbal practices that have help to sustain the health of most rural people of India. The ancient texts like the Rig Veda (4500-1600BC) and the Atharva Veda mention the use of several plants as medicine. The books on ayurvedic medicine such as Charaka Samhita and Sushruta Samhita refer to the use of more than 700 herbs.
According to the World health organization (WHO, 1977) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purpose or which, are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to a thorough investigation. The term "Herbal drug" determines part/parts of plant (leaves, seeds, roots, rhizomes) used for preparing medicine. Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain Khandelwal DKR [12].

2. Aim and Objective:
Isolation of starch from ginger rhizome (*Zingiber officinale).*
Ginger having a large number of reviews about the chemical constituents are extracted. The starch mainly used as an Indicator, diluents in tablet and other purposes. Hence, choosing the isolation of starch from ginger rhizome. Therefore, in the present work following aspect of Ginger was planned for isolation and confirmation test. The main object behind to isolate starch from the ginger rhizome. Ginger is the rhizome of the plant *Zingiber officinale*, Starch is one of the most abundant organic chemicals on earth and it is synthesized in the amyloplasts of seeds, grain, roots and tubers of many plants where it serves as the chemical storage form of energy from the sun. It is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by all green plants as an energy store. It is the most common carbohydrate in the human diet. Starch generally contains 20 to 25% amylase and 75 to 80% amylopectin by weight. Glycogen, the glucose store of animals, is a more branched version of amylopectin. Starch is an important ingredient in various food

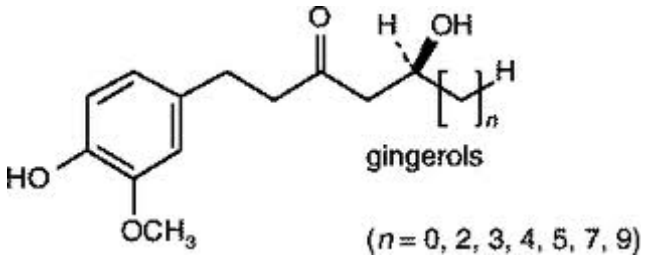
systems as thickening, gelling and binding agents. It imparts texture to a great diversity of foodstuffs such as soups, potages, sauces processed foods etc. Starch is one of the most widely used biomaterials in the food, textile, cosmetics, and plastic, adhesives, paper and pharmaceutical industries. The diverse industrial usage of the material is premised on its availability, low cost, high caloric value, inherent excellent physicochemical properties and the ease of its modification to other derivatives. The versatility of starch in industrial applications is clearly defined by its physicochemical properties; therefore a thorough evaluation of the necessary parameter is important in elucidating its industrial use. The morphology and physicochemical characteristics of starch are typical of its biological origin; hence starch from each plant source will vary somewhat in appearance, composition and properties.

1) Selection, Collection and authentication of medicinal plant
2) Microscopical study of ginger rhizome.
3) Preparation of extract of ginger rhizome.
4) Chromatographic and spectrophotometric characterization of extract.

Regional names
Marathi: Adrak
Hindi: Aal

Macroscopical characters: Kokate CK [4]
Colour: Externally, it is buff coloured.
Odour: Agreegable and aromatic.
Taste: Agreegable and pungent.
Size: Rhizomes of ginger generally are about 5 to 15 x 1.5 to 6.5 cm.
Shape: The rhizomes are laterally compressed, bearing short flat ovate and olique branches on the upper side, with bud at the apex.
Fracture: Short and fibrous.

Chemical constituents:
Ginger consist of volatile oil (1-4%), Starch (40-60%), fat (5%), inorganic material (6%), residual matter (10%) and resinous matter (5-8%). Ginger oil is constituted of monoterpene hydrocarbons, sequiterpene hydrocarbons, oxygenated mono and sequiterpenes, and phenyl propanoids Kokate CK *et al.* [4, 13]



Structure 1: Gingerols

3. Material and Method
Ginger has taken for dissertation work on the basis of literature survey, leaf of rhizome of the plant had been selected. In the present study mature plant materials were collected from local area of Nashik district (Maharashtra) in January 2014 and authenticated by Mr. A. Benniamin, Deputy Director Botanical Survey of India, Koregaon road Pune, by comparing morphological features of reference (Voucher specimen number-PBT 01). After the authentification plants were ginger are cutted pieces used in subjected for further study Kolawole SA *et al.* [10].

Pharmacognostic study
The part of a plant selected in this study is confounding as a rhizome. Therefore, microscopic study was carried out on fresh sample to ascertain its correct nature.

Chemical and equipments
The staining reagent such as phloroglucinol, conc. hydrochloric acid and iodine solution were used. Digital images captured using a Binocular microscope fitted with 4 mega pixels camera imaging accessory.

Transverse section of rhizome
The ginger were thoroughly washed with water to remove dust. Free hand sections were prepared from fresh ginger and finally stained with various staining reagents as per standard procedures.

Procedure
The section were taken by placing the rhizome cut along with very thin section. Transfer the section into watch glass containing water and section were stained with phloroglucinol, conc. hydrochloric acid and iodine solution. And observe under Binocular microscope.

Extraction process
The fresh roots of ginger of about 250 gm were peeled and washed and the sample were chopped into small pieces and soaked in sodium metabisulphite solution (700 ml 1% w/v) at room temperature (25 ^{0}C). Thereafter, the pieces of root were removed and wet milled into slurry using a greater. The paste was dispersed in a large volume of 1% sodium metabisulphite and filtered through muslin cloth. The suspension was centrifuged at 3500 rpm for 10 min to facilitate the removal of dirty, the supernatant was carefully decanted and the mucilage scraped off. The process was repeated for four times with the mucilage on the starch scraped continuously until a pure starch was obtained. The resulting starch was further dried at 60 ^{0}C in hot air oven, pulverized, weighed and stored in a sample bottle for analysis Kolawole SA *et al.* [10]

Chromatographic study of ginger extracted starch [11]
Steps involved in TLC of starch
Preparation of TLC plate
Prepared the slurry of adsorbent media (silica gel-G) in distilled water and poured the slurry on the TLC glass slide plates to obtain thin layer.

Activation of TLC plate
Heating in oven for 30 min. at 105 ^{0}C activated plate.

Mobile phase
Water, butanol, acetic acid (5:4:1).

Sample application
Dipping the capillary touched into the solution to be examined and applied the sample by capillary touched to the thin layer plate at point about 2 cm from the bottom. Air dried the spot.

Chamber saturation
The glass chamber for TLC should be saturated with the mobile phase. Mobile phase was poured into the chamber and capped with a lid. Allowed saturating for 30 min.

Chromatogram development
After the saturation of chamber and spotting of sample on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not to be above spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10-15 cm on the silica plate.

Visualization
Plates were removed and examined visually by spraying the ninhydrin solution on plate.

$$R_f = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

Spectral characterization of isolated compound
A Spectrascan U.V. 2600 and quartz cuvettes are used. The concentrations of the starch slurry between 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm were made in different boiling tubes and heated in a water bath for 30 minutes. The transmittance was determined at 580 nm using a UV spectrophotometer.

Determination of Swelling Power
The starch sample (0.1 g) was weighed into a test tube and 10 ml of distilled water was added. The mixture was heated in a water bath at a temperature of 50 ^{0}C for 30 min with continuous shaking. The test tube was centrifuged at 1500 rpm for 20 min in order to facilitate the removal of the supernatant which was carefully decanted and weight of the starch paste taken. The swelling power was calculated as follows:

$$\text{Swelling power} = \frac{\text{Weight of the starch paste}}{\text{Weight of dry starch sample}}$$

This was carried out over a temperature range of 50 ^{0}C-100 ^{0} C. [10]

Determination of Solubility Power
Solubility index was determined over a temperature range of 50 ^{0}C-100 ^{0}C as follows:
Starch sample (0.5 g) was added to 10ml distilled water in a test tube. This was subjected to heating in a water bath with a starting temperature of 50 ^{o}C for 30 min. Thereafter, it was centrifuged at 1500 rpm for 30 min. 5 ml of the supernatant was decanted and dried to constant weight. The solubility was expressed as the percentage (%) by weight of dissolved starch from heated solution [10].

$$\% \text{ Solubility} = \frac{\text{Weight of the starch paste}}{\text{Weight of sample on dry basis}} \times 100$$

Gelatinization Temperature
The starch sample (1 g) was put in a 20 ml beaker and 10 ml of distilled water was added. The dispersion was heated on a hot plate. The gelatinization temperature was then read with a thermometer suspended in starch slurry [10].

Preliminary phytochemical screening of for extract of rhizome of ginger [13]
Preliminary phytochemical test of ginger rhizome extract was perfomed for phytochemical analysis of alkaloids, glycosides, steroids, tannins, phenolic compounds, proteins, amino acid, saponins, flavonoids, terpenoids.

Characterization of plant for various chemical constituents by chemical methods
Test for carbohydrate
Molishs test
2 ml of extract solution was treated with a few drop of molishs reagent in a test tube and 2 ml of conc.H_2S0_4 was added carefully along the side of tubes the formation of reddish violet ring at the junction of two layers indicate the presence of carbohydrate.

Test for reducing sugar
Benedicts test
To 2 ml of benedicts reagent, 1 ml of extract was added, warmed and allowed to stand. formation of a red precipitate indicates the presence of sugar.

Felhing's test
5 ml of extract solution was mixed with 5 ml fehling solution (equal mixture of felhing solution A and B) and boil. Development of brick red precipated indicates the presence of reducing sugar.

Test for monosaccharides
Barfoed's test
Nicks equal volume of barfoeds reagent test solution. Heat for 1-2 min. In boiling water bath and cool. Red precipitate indicates the presence the monosaccaharides.

Test for pentose sugar
Mix equal volume of test solution and HCl heat. Add a crystal of phloroglucinol. Read color appears.

Test for hexose sugar
Selwinoff's test
Heat 3 ml selwinoff's reagent and 1 ml test solution in bearing water bath for 1-2 min. Red color is formed.

Test for non-reducing sugars
Benedicts test
To 2 ml of benedicts reagent, 1 ml of extract was added, warmed and allowed to stand. Formation of red precipitate indicates the presence of sugar.

Test for non-reducing sugars
Iodine test
Mix 3 ml test solution and few drop of dilute iodine solution. Blue color appears, it disappears on boiling and reappears on cooling.

Tannic acid test for starch
With 20% tannic acid, test solution give ppt.

4. Result
Pharmacognostic studies
In the pharmacognostic study of ginger rhizome the morphology, microscopical, physical parameters were studied.

Morphology

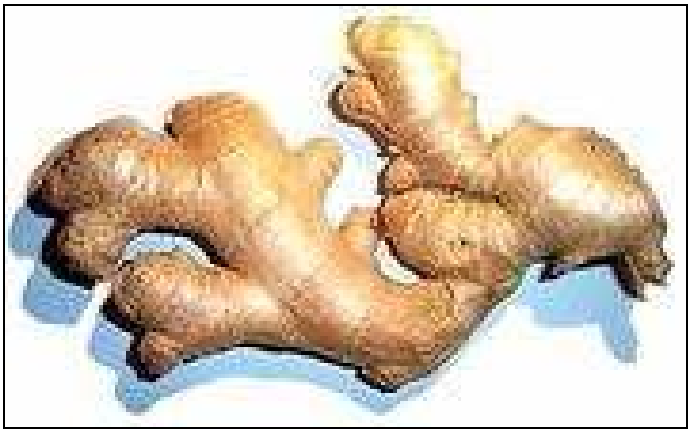


Fig 1: Ginger rhizome

Table 1:

Morphological parameters	Observation
Condition	Fresh
Size	Rhizome of ginger are about 5 to 15 x 1.5to 6.5
colour	Externally it is buff coloured and internally yellowish coloured
odour	characteristics
taste	characteristics

Microscopy

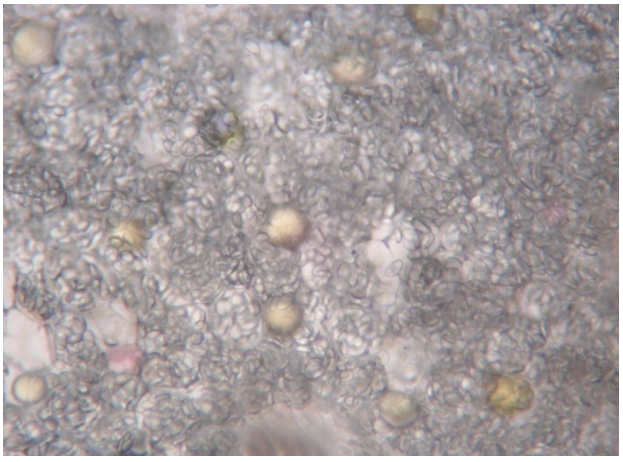


Fig 2: Ginger rhizome T.S. with oleoresin.

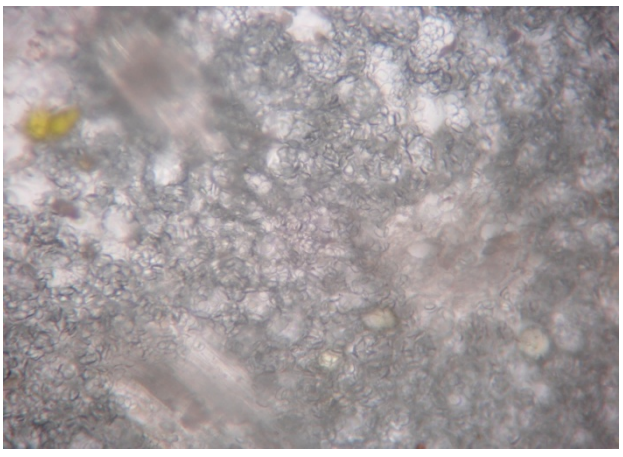


Fig 3: T.S .of ginger rhizome with parenchyma.

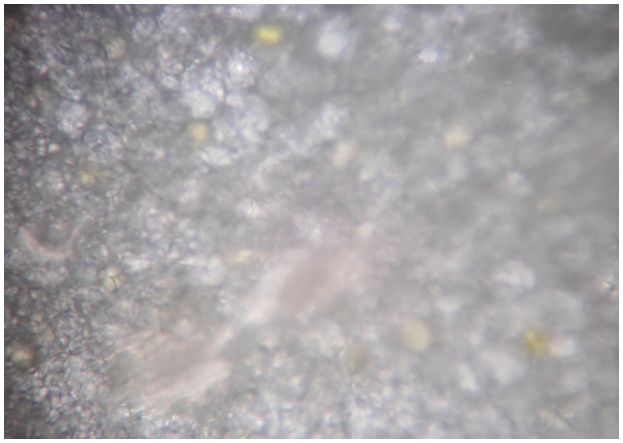


Fig 4: T.S. of ginger rhizome with fibres.

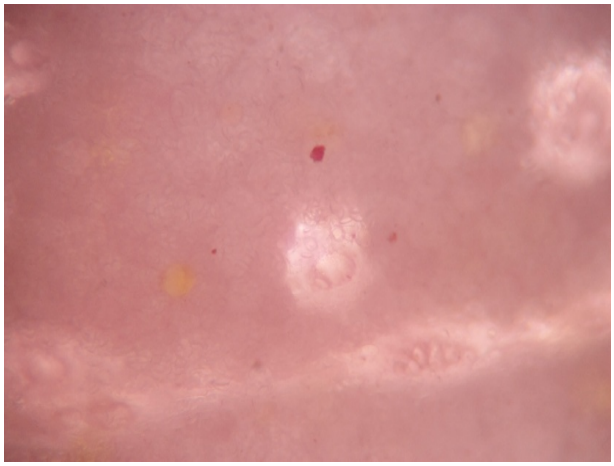


Fig 5: T.S. of ginger rhizome stained by phloroglucinol, conc. Hydrochloric acid.

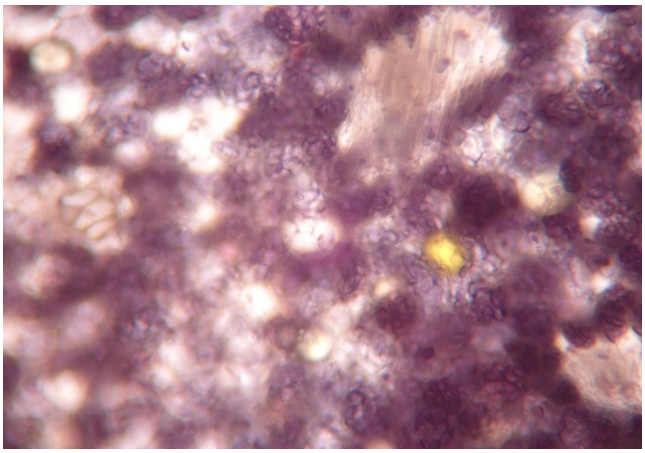


Fig 6: T.S. of ginger rhizome stained by dil. Iodine solution.

Microscopic characteristers of ginger rhizome

Observation table for microscopic character of ginger rhizome T.S.:

Table 2:

Reagent	Observation	Characteristics
Phloroglucinol + conc. hydrochloric acid	Pink	vascular bundle, sclerenchymatous fibres.
dil. Iodine solution	Blue	starch

Thin layer chromatography of starch:

Stationary phase: Silica gel G (activated)

Mobile phase: Water, butanol, acetic acid (5:4:1).

Spraying reagent: Ninhydrin reagent.

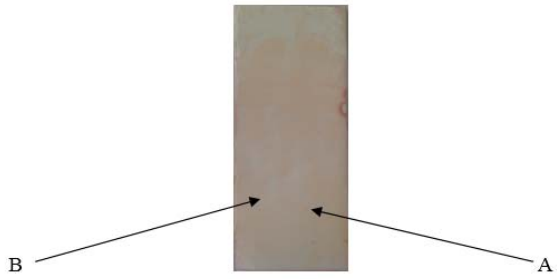


Fig 7: TLC of starch

A=Standard Starch
B=Extracted starch

Result
The TLC of B R_f Value =0.46
The TLC of B R_f Value =0.48

U.V. Spectroscopy:
The spectrophotometry of starch:
1) The concentration at 30 ppm:

Table 3

Wavelength	Absorbance
580	0.082

1) Spectrum of 30 ppm

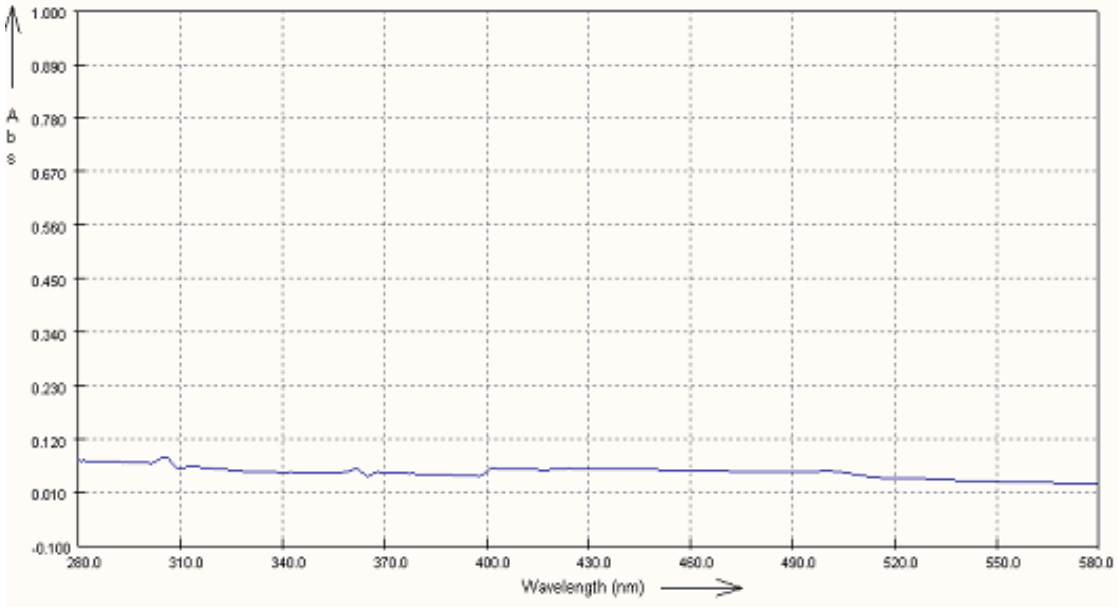


Fig 8: spectrum of 30 ppm solution

U.V. observation of isolated starch

Table 4

Sr.no	Concentration in ppm	Absorbance at 580 nm
1	10ppm	0.012
2	20 ppm	0.018
3	30 ppm	0.029
4	40 ppm	0.038
5	50 ppm	0.047

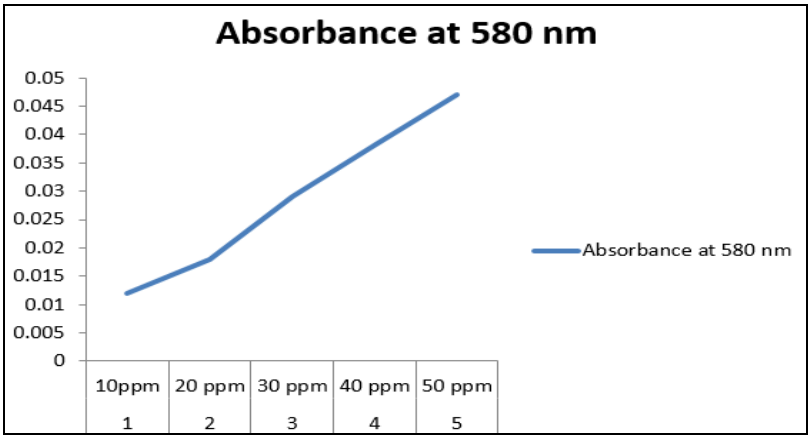


Fig 9: Graphical representation of starch concentrations

Swelling power

$$\text{Swelling power} = \frac{\text{Weight of starch power}}{\text{Weight of dry starch sample}}$$

$$= \frac{0.15}{0.1} = 1.5$$

Solubility power

$$\% \text{ Solubility} = \frac{\text{Weight of the starch paste}}{\text{Weight of sample on dry basis}} \times 100$$

$$= \frac{0.70}{0.5} \times 100 = 35\%$$

Gelatinization power

The Gelatinization power of starch was found to be $=82^0$C

Preliminary phytochemical screening of extracts of rhizome of ginger

Table 5

Sr.no.	Chemical test	observation
1)	Test for carbohydrate: a)Molishs test: b)Felhing test: c)Benedicts test:	 + - -
2)	Test for monosccharides; a)Barfoed's test:	 -
3)	Test for pentose sugar:	-
4)	Test for hexose sugar: a)selwinoff's test:	 +
5)	Test for non-reducing sugar; a)Benidicts test:	 -
6)	Test for non-reducing polysaccharides : a)Iodine test: b)Tannic acid test for starch	 + +

+: test shows present of test.
-: test shows no observation.

5. Result

From the above preliminary phytochemical screening for extract of starch from ginger were carried out extract content carbohydrates.

6. Conclusion

In the present study of ginger rhizome is investigated for pharmacognostic, phytochemical screening. In pharmacognostic study the plant material was authenticated by A. Benniamin Deputy Director botanical survey of India Koregaon road, Pune. By comparing morphological features and as confirm that plant is ginger and family Zingiberaceae in pharmacognostic study of ginger morphology, microscopy was studied. In microscopy of rhizome consist of rhizome presence of parenchyma. In micropscopical powder characteristic of plant shows presence of starch grain and fibres were observed under binocular microscope when stain with diluted iodine, phloroglucinol and conc. Hcl(1:1). The extracted starch for TLC mobile phase was used as Water, butanol, acetic acid (5:4:1). The solubility power, swelling power, gelatinization temperature and paste clarity were studied. The phytochemical test shows the presence of carbohydrate that means starch.

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