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Physicochemical studies, phytochemical screening and microscopic evaluation of the dried powder of the stem bark of *Syzygium jambos* (L.) Alston.

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Abstract
Syzygium jambos (L.) Alston. (Myrtaceae) is a famous medicinal plant widely cultivated all over the world for its juicy fruits. The stem bark, leaves and seeds of *Syzygium jambos* (L.) Alston. are traditionally used for medicinal purpose. Establishing pharmacognostic profile of this plant will assist in correct identification of the plant drug and its standardization for maintaining quality and purity will be easier. The present work is therefore, an effort to evaluate the stem bark powder of *Syzygium jambos* (L.) Alston. By carrying out proximate analysis, phytochemical screening and studying microscopical powder characteristics.

Keywords: *Syzygium jambos* (L.) Alston, proximate analysis, phytochemical screening, microscopy

1. Introduction
Plants have been used in medicine since ancient times and have continued to play an important role in traditional as well as modern systems of medicine. Herbal medicinal products are the products derived from plant parts that elicit a pharmacologic effect. Herbal formulations involve use of fresh or dried plant parts. Correct identity of such crude drugs is an important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy, a term used since 19th century, is derived from two Greek words viz. Pharmacon (a drug) and Gignosco (to acquire the knowledge of). It is an objective study of crude drugs from natural sources, treated scientifically [1]. Pharmacognostical evaluation involves physicochemical or proximate analysis, phytochemical studies and microscopic study; these are together used in standardization of herbal products. Hence, in the present research work, pharamacognostical study has been carried out for the stem bark of *Syzygium jambos* (L.) Alston. which is used as a folklore medicine [2] These studies will help in identification and authentication of the plant material. In the literature, microscopy studies of the seed and leaves of *Syzygium jambos* (L.) Alston. have been reported [3, 4]. But no systematic report of any study of the microscopic parameters of stem bark of *Syzygium jambos* (L.) Alston. is available. Hence, an effort has been made to establish the pharmacognostic study of the stem bark of *Syzygium jambos* (L.) Alston.

2. Materials and methods
2.1 The plant material
The part of the plant material used in the present research work is stem bark of *Syzygium jambos* (L.) Alston. The stem bark was collected from a domestic garden in Alibaug, District Raigad, Maharashtra, India. Herbarium of *Syzygium jambos* (L.) Alston. was prepared and authenticated from Botanical Survey of India, Pune, India. (Certificate No. BSI/WC/Tech/2012/70) Duplicate herbarium was prepared and preserved in Ramnarain Ruia College. Dried plant material was then finely powdered using ice jacketed electric mixer grinder and then sieved through BSS mesh size 85 and stored in an airtight container at room temperature (28±2 °C).

2.2 Experimental reagents
Iodine, Phloroglucinol, Ruthenium Red and Sudan III stains were procured from Lobachemie, E. Merck (India) Ltd, Mumbai.
Glycerol (90% purified), Safranin stain, Glacial acetic acid (AR Grade), Ethanol (AR Grade), Potassium iodide (AR Grade), Lead acetate (AR Grade) were procured from E. Merck.
Hydrochloric acid, sulphuric acid, acetic anhydride, $FeCl_3$, Karl Fischer reagent was procured from Sigma Aldrich Chemie GmbH (Aldrich Division, Stein beim, Germany).

Distilled Water used, was purified with a Sartorious water purification unit. (Arium 61315, made in USA).

2.3 Instrumentation
Labomed 2000 microscope was used for the microscopical analysis of the stem bark under the magnification of 10X, 40X and 100X lenses of microscope. AV USB 2.0 Capture application software was used for image capturing.

2.4 Proximate analysis [5, 6]
The parameters such as foreign matter, total ash content, acid insoluble ash content, water soluble ash content, moisture content and loss on drying were evaluated for proximate analysis.

2.5 Foreign matter
The collected stem bark of *Syzygium jambos* (L.) Alston. was washed, drained and dried in shade. 250.0 g of this dried stem bark of *Syzygium jambos* (L.) Alston. were accurately weighed and spread on a white, clean muslin cloth. Foreign matter was sorted out by visual inspection using a magnifying lens (6X). The portions of the sorted foreign matter were weighed and the percent content of foreign matter of the sample was calculated. Percent foreign matter was calculated by the formula;

$$\% \text{ Foreign matter} = \frac{W2 * 100}{W1}$$

Where,
W1= Weight of plant sample in g.
W2= Weight of foreign matter in g.
The results obtained are given in Table 1.

2.6 Total ash
About 2.0 g of dried powder of stem bark of *Syzygium jambos* (L.) Alston. were accurately weighed and transferred to a Silica crucible and were ignited with a flame of Bunsen burner, for about 1 hour. The ignition was completed by keeping in a muffle furnace, at 550±20 °C, till a white carbon free ash was formed. The Silica crucible was then cooled in a desiccator and weighed. The percent total ash content was then calculated. Percent total ash content was calculated by the formula,

$$\% \text{ Total Ash} = \frac{\text{Weight of Total Ash } (W2 - W) * 100}{\text{Weight of dried plant powder } (W1 - W)}$$

Where,
W= Weight of empty crucible in g.
W1=Weight of crucible with dried plant material taken for test in g.
W2= Weight of crucible with total ash in g.
The results obtained are given in Table 1.

2.7 Acid insoluble ash
About 2.0 g of the dried powder of stem bark of *Syzygium jambos* (L.) Alston. were accurately weighed and transferred to a silica crucible and were ignited with a Bunsen burner, for about 1 hour. The silica crucible was then kept in a muffle furnace at 550±20 °C, till a white carbon free ash was formed. The ash obtained was taken in conical flask (capacity 50 mL) and 25 mL of dilute hydrochloric acid (2N HCl) was added to it. The conical flask was kept covered and heated on a water bath, for 10 minutes. It was allowed to cool and contents were filtered through Whatman filter paper no. 41 (E. Merck, Mumbai India).The residues were then washed with water, till washings were free from chloride (as tested with $AgNO_3$ solution). The filter paper along with the residue of stem bark powder was placed in a Silica crucible and ignited in a muffle furnace, at 550±20 °C, for 1 hour. The crucible was cooled and weighed to a constant weight. The percentage of acid insoluble ash was then calculated for the stem bark powder of *Syzygium jambos* (L.) Alston.
Percent acid insoluble ash content was calculated by using the formula,

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of acid insoluble ash } (W2 - W) * 100}{\text{Weight of dried plant material } (W1 - W)}$$

Where,
W= Weight of empty crucible in g.
W1=Weight of crucible with dried plant material taken for test in g.
W2= Weight of crucible with acid insoluble ash in g.
The results obtained are given in Table 1.

2.8 Water soluble ash
About 2.0 g of the dried powder of the stem bark of *Syzygium jambos* (L.) Alston. were accurately weighed and transferred to a Silica crucible and were ignited with a Bunsen burner, for about 1 hour. The crucible was then kept in a muffle furnace at 550±20 °C, till a white carbon free ash was obtained. After cooling, the ash was taken in a conical flask (capacity 50 mL) and 25 mL of distilled water was added to it. The conical flask was kept covered and heated on a water bath, for 10 min. It was allowed to cool and contents were filtered through Whatman filter paper no. 41 (E. Merck, Mumbai, India). The filter paper and the residue were placed in a Silica crucible and ignited in a muffle furnace, at 550±20 °C, for 1 hour. The crucible was cooled and weighed to a constant weight. The weight of the residue obtained was subtracted from the weight of total ash to get the value of weight of water soluble ash.
Percent water-soluble ash content was calculated using the formula;

$$\frac{\text{Weight of water soluble ash } (W2) * 100}{\text{Weight of dried plant powder } (W1 - W)}$$

Where,
W= Weight of empty crucible in g.
W1=Weight of crucible with dried plant material taken for test in g.
W2= Weight of water soluble ash in g.
The results obtained are given in Table 1.

2.9 Moisture content
The moisture content in plant material can be determined by using the Karl Fischer titrimetric method. The moisture in the reaction vessel has to be neutralized before any analysis. The dry reaction bottle was filled with approximately 30 mL of methanol. The autotitrator was filled with pyridine-free Karl Fischer reagent. Instrument equipped with magnetic stirrer was switched on. Karl Fischer reagent was added from the burette. The end point is reached once methanol in reaction vessel is moisture free and then addition of Karl Fischer reagent is stopped. The instrument display gives us the

amount of Karl Fischer reagent, in mL, which gets consumed to neutralize the traces of moisture in the vessel.

2.10 Calibration

The dry reaction bottle was filled with methanol. The autotitrator was filled with pyridine free Karl Fischer reagent. The instrument equipped with magnetic stirrer was switched on. About 0.1 gm Disodium Tartarate (water content 15.66%) was added to the reaction vessel containing moisture free methanol and Karl Fischer reagent was added to the reaction vessel, till the end point was reached. The titre factor was then calculated using the following formula.

$$\text{Titre Factor (Water Equivalent Factor)} = \frac{\text{mg of Disodium Tartarate added}}{\text{Karl Fischer reagent consumption in mL}}$$

About 100 mg of accurately weighed dried powder of stem bark of *Syzygium jambos* (L.) Alston. were transferred to the reaction vessel. Titration with Karl Fischer reagent was carried out as described above. Display reading for Karl Fischer reagent in mL was recorded.

Percent moisture was calculated by using the following formula;

$$\% \text{ Moisture} = \frac{\text{Amount of Karl Fischer reagent consumed in mL} \times \text{Titre Factor} \times 100}{\text{Weight of sample in g} \times 1000}$$

The results obtained are given in Table 1.

2.11 Loss on drying

About 3.0 g of dried powder of stem bark of *Syzygium jambos* (L.) Alston. were accurately weighed, in a dry wide mouthed flat weighing bottle. The bottle was then placed in an air oven, maintained at 100±2 °C, for 2 hours. The bottle was then removed, covered and placed in a desiccator. The bottle was weighed after cooling to room temperature and was reheated until two consecutive weights did not differ by more than 5 mg. The percent loss on drying was then calculated.

Percent loss on drying was calculated using formula,

$$\% \text{ Loss on drying} = \frac{\text{Loss of weight (W1} - \text{W2)} * 100}{\text{Weight of dried plant powder (W1)}}$$

Where,

W1=Initial weight of dried plant material before heating in oven in g.

W2= Weight of dried plant material after heating in oven in g.

W1- W2= Loss of weight in g.

The results obtained are given in Table 1.

2.12 Preliminary phytochemical analysis

Following tests were carried out for preliminary phytochemical analysis of dried powder of stem bark of *Syzygium jambos* (L.) Alston [7, 8]

2.13 Tannins

About 0.2 g of the dried powder of stem bark of *Syzygium jambos* (L.) Alston. was weighed; 10 mL of distilled water was added to it. The solution was filtered through Whatmann filter paper no. 41. The aqueous filtrate was collected. 2 mL of alcoholic $FeCl_3$ was added to 2 mL of the above aqueous filtrate. Formation of blue precipitate indicates the presence of tannins.

2.14 Alkaloids

About 0.2 g of the dried powder of stem bark of *Syzygium jambos* (L.) Alston. was weighed; 10 mL methanol was added to it. The solution was filtered through Whatmann filter paper no. 41. The methanolic filtrate was collected. 1mL of 1% HCl and 6 drops of Dragendroff's reagent were added to the above methanolic filtrate. Formation of orange precipitate indicates presence of alkaloids.

2.15 Saponins

To 0.5 mL of above methanolic filtrate, 5 mL of distilled water was added. Formation of persistent frothing on shaking indicates the presence of saponins.

2.16 Terpenoids

To 2.0 mL of methanolic filtrate, 2 mL acetic anhydride was added followed by slow addition of 1 mL of conc. H_2SO_4. Formation of blue ring indicates presence of terpenoids.

2.17 Cardiac glycosides

To 2.0 mL of methanolic filtrate, 1 mL glacial acetic acid was added followed by addition of 1 drop of alcoholic $FeCl_3$ and 1 mL of conc. H_2SO_4. Formation of green ring indicates presence of cardiac glycosides.

2.18 Steroids

About 0.2 g of the dried powder of stem bark of *Syzygium jambos* (L.) Alston. was weighed; 10 mL of chloroform was added to it. The solution was filtered through Whatmann filter paper no. 41. The filtrate was collected. In 2 mL of filtrate, 2 mL of acetic anhydride and 1 mL of conc. H_2SO_4 was added. Formation of blue ring indicates presence of steroids.

2.19 Flavonoids

About 0.2 g of the dried powder of stem bark of *Syzygium jambos* (L.) Alston. was weighed; 10 mL of ethanol was added to it. The solution was filtered through Whatmann filter paper no. 41. The filtrate was collected. In 2 mL of filtrate, 1 mL conc. HCl and a small piece of magnesium ribbon was added. Formation of tomato red color indicates presence of flavonoids.

The results of preliminary phytochemical analysis are discussed below.

3. Microscopical evaluation of the stem bark of *Syzygium jambos* (L.) Alston.

3.1 Staining and mounting of plant powder [8]

The desired stain was taken on a clean watch glass and was diluted with water if needed. A clean glass slide was taken and a pinch of powdered plant material was placed on the slide. Few drops of stain were added to the powdered plant material with the help of dropper. The powdered plant material was allowed to stain properly by mixing the powder in the stain solution with the help of a needle. The specimen i.e. powdered plant material on the slide was then slowly covered with cover slip with the help of forceps, to avoid any air bubbles entrapped between the cover slip and slide. The excess of liquid outside the cover slip was wiped with blotting paper. The specimen was then placed and observed under the magnifying lenses of 10X, 40X and 100X magnification of the microscope. The images of characters found under microscope are discussed below.

4. Results and Discussion

Table 1: Results for proximate analysis of the dried powder of stem bark of *Syzygium jambos* (L.) Alston.

Parameters	Stem bark of *Syzygium jambos* (L.) Alston. Mean ±S.D. (n=3)
% Foreign matter	0.337±0.0197
% Total ash	7.670±0.170
% Acid insoluble ash	0.665±0.0273
% Water soluble ash	1.448±0.0492
% Moisture Content	10.489±0.188
% Loss on drying	7.280±0.155

4.1 Preliminary phyto chemical analysis

In the dried powder of stem bark of *Syzygium jambos* (L.) Alston, tannins, alkaloids, saponins, terpenoids and flavonoids were found to be present whereas, cardiac glycosides and steroids were found to be absent.

4.2 Microscopic Study

In the dried powder of stem bark of *Syzygium jambos* (L.) Alston. Various characters were observed. Slightly elongated, compact epidermal cells with polygonal shape (Figure 1) and cork cells having thick wall, flat polygonal shape were observed. (Figure 2). Vessels with spiral thickenings (Figure 3) and Tracheids (Figure 4) were distinct. Simple starch grains (Figure 5) and fibres with lignified, tapering ends were observed. (Figure 6). Multicellular trichomes were also seen. (Figure 7).

4.3 Microscopic characteristics of dried stem bark powder of *Syzygium jambos* (L.) Alston.

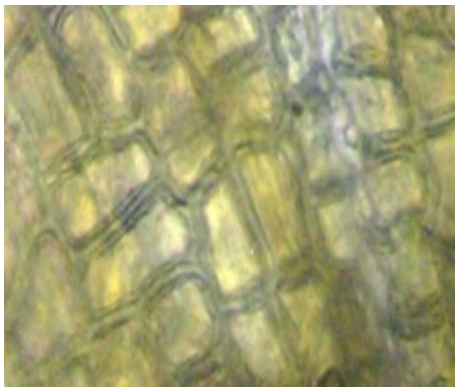

Fig 1: Epidermal cells

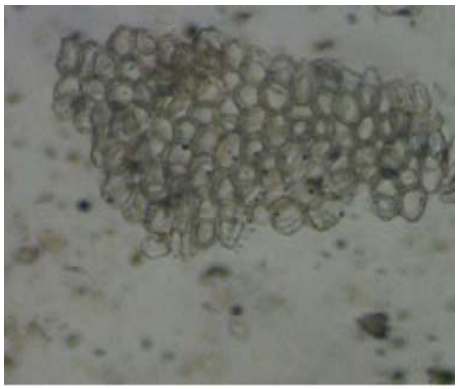

Fig 2: Cork cells

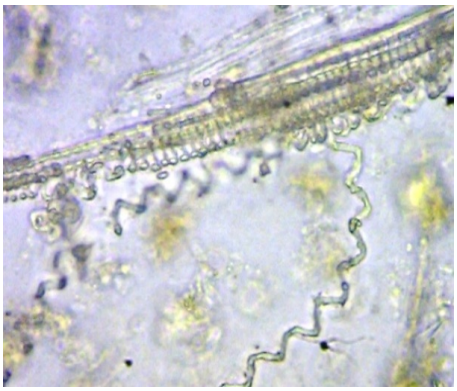

Fig 3: Vessels with spiral thickening

Fig 4: Tracheid

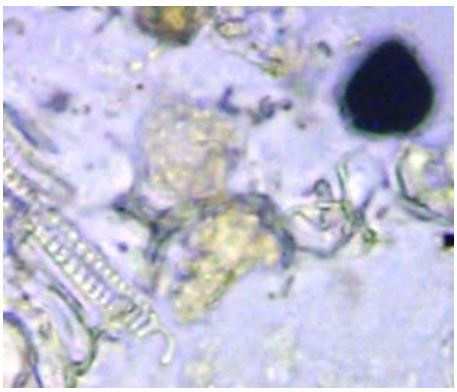

Fig 5: Starch grain

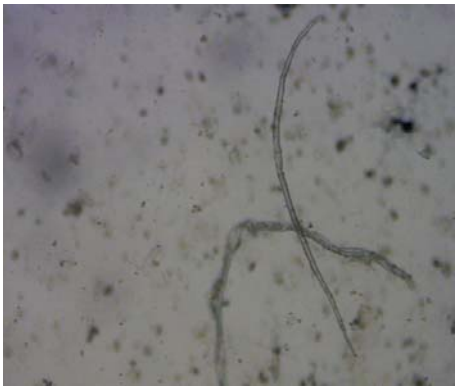

Fig 6: Fibers

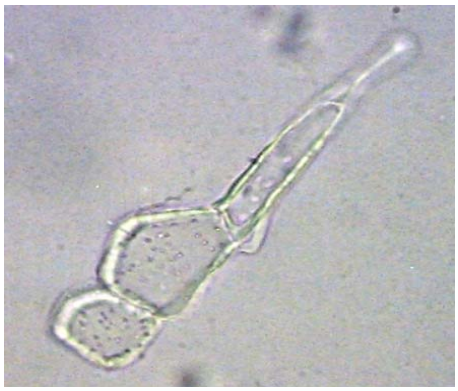

Fig 7: trichome

5. Conclusion

The methods carried out in the present research work namely, proximate analysis, phytochemical studies and microscopic powder analysis can be used for distinguishing the stem bark powder of *Syzygium jambos* (L.) Alston.

This work would assist in the identification of the crude drug in future.

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