

## A STUDY ON QUANTITATIVE ESTIMATION OF ANTI-NUTRITIONAL FACTORS OF *SYZYGIUM JAMBOS* FRUIT AND ITS SEED

### Pharmaceutical Science

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### ABSTRACT

*Syzygium jambos* (L.) Alston is an evergreen tree that belongs to the Myrtaceae family. It is commonly found in the South-East region of Asia. The fruit of this plant is known as "Rose apple", "Malabar plum" and "Golap-jam" in West Bengal (India). **Objective:** This fruit and its seed have been used in Ayurveda from ancient times. Although it is not very popular as fruit among people. This study was carried out to identify the anti-nutritional factors (oxalate, phytate, alkaloids, tannin and saponins) present in the fruit and its seed. **Results:** The total oxalate, phytate, alkaloid, tannin and saponin content of *Syzygium jambos* fruit was found to be 4.54 mg/100g, 5.68 mg/100g, 3.53 mg/100g, 32.43mg/100g, 386.49mg/100g respectively. The total oxalate, phytate, alkaloid, tannin and saponin content of *Syzygium jambos* seed was found to be 9.87 mg/100g, 7.34 mg/100g, 5.38 mg/100g, 168.29 mg/100g, 528.55 mg/100g respectively. **Conclusion:** A comparative study of anti-nutritional factors has been done among 3 fruits (Jackfruit, Mango, *Syzygium jambos*) and their respective seeds. The results show that *Syzygium jambos* (fruit & seed) has less quantity of antinutritional factors than commonly consumed fruits (Jackfruit & Mango) and their seeds. So, it may be concluded that *Syzygium jambos* fruit and its seed may be safe to include in the daily diet. These may not lead to any adverse health effects. Moreover, *S. jambos* fruit and its seed may be beneficial to meet daily dietary requirements for maintaining a good nutritional status for humans.

### KEYWORDS

*Syzygium jambos*, fruit, seed, Anti-Nutritional Factors (ANFs), health effects.

### 1. INTRODUCTION

All the parts of plants are important sources of macro and micronutrients. Plants can also synthesize some secondary metabolites to protect themselves from insects and pathogens. These are called Anti-nutritional Factors (ANFs). For an example - oxalate, phytate, tannin, saponin, alkaloid, trypsin inhibitors, cyanogenic glycoside, etc. These are mainly a compound or substance of natural or synthetic origin that binds with the nutrients and plays a key role in the reduction of bioavailability of the nutrients. These are commonly found in grains, beans, legumes and nuts with high concentrations. Leaves, roots, flowers and fruits have lesser quantities of these compounds<sup>[1]</sup>.



**Figure 1: *Syzygium jambos* fruit and its seed**

The genus *Syzygium* is very popular as a medicinal plant in Ayurveda, Unani and therapeutic medicine for a decade, however, *Syzygium jambos* (L.) Alston is comparatively less familiar. It is an evergreen tree that belongs to the Myrtaceae family. It is commonly found in South-East Asia like Indonesia, Malaysia and different parts of India. The fruit of this plant is known as 'Rose apple' and 'Malabar plum' in Karnataka, 'Pani Jamuk' in Assam and 'Golap-jam' in West Bengal, India. The fruit is yellow and nearly round or oval-shaped. It is sweet in flavor with crispy, crunchy in texture and has a delicate rose fragrance. In the center of the fruit, there is a light-brown seed with a rough seed coat. It is loosely bound with the inner wall of the fruit<sup>[2]</sup>.

In Indian folk medicine, *Syzygium jambos* fruit has been used as a tonic for the brain, liver and also used as a diuretic. The seeds are used to treat diarrhea, dysentery and catarrh<sup>[3]</sup>. It has been reported that *S. jambos* fruit has some volatile constituents like geraniol, citronellol, linalool, cis-rose oxide, nerol, etc<sup>[4]</sup> and the aroma of the fruit is due to the presence of hexanal, 3-penten-2-one, hexanol, (z)-3 hexen-1-ol, benzyl alcohol, 2-phenylethylalcohol and (E)- cinnamaldehyde<sup>[5]</sup>. In a recent study, it has been found that both *Syzygium jambos* fruit and its seed have significant quantity of micronutrients and trace elements - **Calcium** (fruit -12.71 mg/100g, seed - 23.72 mg/100g), **Sulphur** (fruit -10.31 mg/100g, seed -12.73 mg/100g) **Phosphorus** (fruit - 11.63 mg/100g, seed -14.62 mg/100g), **Manganese** (fruit -0.045 mg/100g, seed - 0.093 mg/100g) **Potassium** (fruit - 45.62 mg/100g,

seed - 329.32 mg/100g), **Iron** (fruit -0.73 mg/100g, seed -1.15 mg/100g), **Zinc** (fruit - 0.23 mg/100g, seed - 0.39 mg/100g) and **Copper** (fruit - 0.067 mg/100g, seed - 0.186 mg/100g)<sup>[6]</sup>.

**Therefore, the aims and objectives of this study are –**

- To estimate the quantity of anti-nutritional factors present in *Syzygium jambos* fruit and its seed that have not been documented yet.
- To make a comparative study between anti-nutritional factors of *Syzygium jambos* (fruit, seed), Jackfruit (fruit, seed) and Mango (fruit, seed).
- To promote the goodness and health benefits of this underutilized fruit and its seed among people.

### 2. MATERIALS AND METHODS

#### 2.1 Sample Collection and Identification

Fruits of *Syzygium jambos* were collected from the market of Baruipur, near Kolkata (West Bengal, India). The sample was identified by the Botanical Survey of India, Shibpur, Howrah. The specimen No. is UC/SD-01, dated on 30.12.2019

#### 2.2 Preparation of Sample Extract

At first, fruits and seeds were separated. The outer layer of the seeds was removed and cleaned thoroughly. Then, a paste of both the samples was made for freeze-drying by using (Laboratory Freeze Dryer, Model- DPRG-01). The methanolic extract of the fruit and its seed was prepared by adding 1gm of each freeze-dried sample powder with 80% methanol. The infusions were stirred on the magnetic stirrer at room temperature for 5 hrs. Then centrifuged at 6000 rpm at -4°C for 10 mins by using a cold centrifuge (Eppendorf, centrifuge 5430R). Finally, the mixtures were filtered through Whatman No.1 filter paper. Then the filtrates were stored at -4°C for further studies<sup>[7]</sup>.

#### 2.3 Chemicals Used:

All the reagents and chemicals used in the experiments were of good analytical grade.

#### 2.4 Quantitative Analysis of Anti-Nutritional Factors:

##### 1. Determination of total oxalate content by titrimetric method<sup>[8]</sup>:

Oxalate content was determined by the method of Day and Underwood (1986). 1 g of sample (fruit and seed) was weighed in the electric weighing balance and transferred to 30 ml of 0.5 (N) H<sub>2</sub>SO<sub>4</sub> and was boiled in a water bath for 15 minutes. Then the extract was filtered with Whatman No. 1 filter paper. An equal volume of deionized water was added. Then 10ml of the filtered extract was taken and 40 ml 0.5 N H<sub>2</sub>SO<sub>4</sub> was added. The final 50 ml of the mixture was heated to 60° C and was titrated against 0.05 (N) KMnO<sub>4</sub>. The endpoint was determined by the permanent appearance of light pink colour. The amount was calculated by the following equation:

1ml of 0.05 (N)  $\text{KmnO}_4 = 2.2 \text{ mg oxalate}$

## II. Determination of total phytate content by titrimetric method<sup>[8]</sup>:

The phytate of each of the samples was determined by Lucas and Markaka (1975). 2 g of each sample was taken into a 250 ml conical flask. 100 ml of 2% conc. HCl was used to soak the samples in the conical flask for 3 hrs and then filtered through a double layer filter paper. 50 ml of each of the sample filtrate was placed in a 250 ml beaker and 107 ml of distilled water was added to improve proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added to each sample solution as an indicator and titrated with standard iron chloride solution which contained 0.00195 g iron/ml. The endpoint was determined by brownish-yellow colouration that persisted for 5 mins.

The amount of phytate was calculated with the equation below:

$$\% \text{ Phytic acid} = y \times 1.19 \times 100$$

Where, y = titre value  $\times 0.00195\text{g}$

## III. Determination of total alkaloids content by titrimetric method<sup>[9]</sup>:

From the prepared sample, 10 ml was taken into a 100 ml separating funnel. Then, 10 ml of 0.1 (N) HCl was added and shaken thoroughly for 2-3 mins. The lower layer contains alkaloids neutralized with 0.1 (N) HCl and the upper layer contains n-butanol. 10 ml HCl portion was collected in a beaker and 2-3 drops methyl-red was added to it, which turns the solution into a slightly reddish colour. The contents of the beaker were titrated against 0.1 (N) NaOH, till the colour changed from red to pale yellow. The neutralization point was determined.

The total amount of alkaloids was calculated by considering the following equivalent:

$$1\text{ml } 0.1 \text{ (N) HCl} = 0.0162 \text{ g alkaloid}$$

## IV. Determination of total tannin content by spectrophotometric method<sup>[10]</sup>:

Tannin content of the samples was measured by Folin-Denis method. Accurately Weighed 1 g of the powdered sample was transferred to a 250ml conical flask and 75ml of water was added. The flask was gently heated and boiled for 30 min, centrifuged at 2,000rpm for 20 min and the supernatant was collected in 100ml volumetric flask and the volume made up. Then, 1ml of the sample extract was transferred to a 100ml volumetric flask containing 75ml water. 5ml of Folin-Denis reagent, 10ml of sodium carbonate solution were mixed and diluted to 100ml with water. Absorbance was measured at 650 nm after 30 min. The tannin content was expressed in terms of mg of tannic acid equivalents (TE)/ g of dried sample from the standard curve.

$$\% \text{ Tannin (mg/100g)} = \frac{A \times C \times Df}{A_s \times W \times 100}$$

Where; An = absorbance of test sample, As = absorbance of standard tannic acid, C = concentration of standard tannic acid (mg/ml), Df = dilution factor, W = weight of the test sample (mg)

## V. Determination of total saponins content by spectrophotometric method<sup>[11]</sup>:

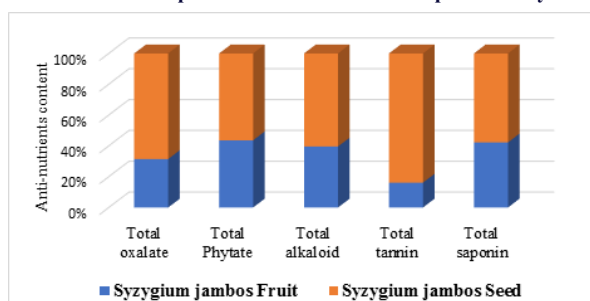
Total saponin content was determined by using anisaldehyde reagent. For the estimation of total saponins, 500  $\mu\text{l}$  of the extracted sample and 500  $\mu\text{l}$  of 0.5% anisaldehyde reagent were mixed and kept aside for 10 min. Later, 2 ml of 50% sulphuric acid was added and tubes were shaken properly. Then tubes were kept in a water bath at the temperature of 60°C for 10 mins. After that, the tubes were cooled in an ice-cooled water bath for 3-4 mins. Then the absorbance was measured at 544 nm. The saponin content was calculated from the standard curve and expressed as mg of diosgenin equivalents (DE) / g dried sample.

## 3. RESULTS AND DISCUSSION

**Table 1: Anti-Nutritional Factors present in Syzygium jambos fruit and its seed**

Anti-Nutritional Factors	<i>Syzygium jambos</i> Fruit (mg/100g)	<i>Syzygium jambos</i> Seed (mg/100g)
Total Oxalate	4.54 $\pm$ 0.81	9.87 $\pm$ 0.65
Total Phytate	5.68 $\pm$ 0.68	7.34 $\pm$ 0.71
Total Alkaloid	3.53 $\pm$ 0.73	5.38 $\pm$ 1.22
Total Tannin	32.43 $\pm$ 1.36	168.29 $\pm$ 0.79
Total Saponin	386.49 $\pm$ 0.83	528.55 $\pm$ 1.19

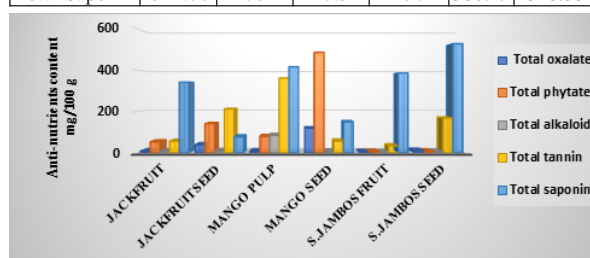
Note: Values are expressed as Mean  $\pm$  SEM of triplicate analysis



**Figure 2: Graphical Representation of Anti-Nutritional Factors of Syzygium jambos Fruit and Its Seed**

**Table 2: A Comparative study among the anti-nutritional factors of Jackfruit (fruit & seed), Mango (fruit & seed) and Syzygium jambos (fruit & seed)**

Anti-Nutritional Factors	<i>Artocarpus heterophyllus</i> [13, 21, 25, 28] (mg/100g)		<i>Mangifera indica</i> [13, 14, 30, 31, 32] (mg/100g)		<i>Syzygium jambos</i> (Sample) (mg/100g)	
	Fruit	Seed	Fruit	Seed	Fruit	Seed
Total Oxalate	8.45	38.51	8.09	119	4.54	9.87
Total Phytate	52.94	141	79.46	487.30	5.68	7.34
Total Alkaloid	7.88	8.85	85	6.30	3.53	5.38
Total Tannin	56.17	212	362	56.5	32.43	168.29
Total Saponin	342.76	78	417.32	150	386.49	528.55



**Figure 3: Graphical Representation of a Comparison among Anti-Nutritional Factors of 3 Different Fruits and their Respective Seeds**

Table 1 shows the quantity of anti-nutritional factors present in Syzygium jambos fruit and its seed. A comparison among the anti-nutritional factors of 3 different fruits (Jackfruit, Mango, syzygium jambos) and their respective seeds are presented in Table 2. Graphical representations of both the tables are given in Figure 2 and 3. Anti-nutritional factors can play dual role by giving adverse health effects when consumed in excess and it also has a wide range of beneficial biological effects when used wisely. According to Table 1, it has been found that the *S. jambos* fruit and its seed have oxalate, phytate, alkaloids, tannins, and saponin in various quantities. Total saponin content was highest in both fruit and seed i.e., 384.49mg/100g and 528.55mg/100g respectively. Total alkaloid content was found to be the lowest in both fruit (3.53mg/100g) and seed (5.38 mg/ 100g). Oxalate, phytate and tannin content were found to be in the lower range.

### Oxalate:

Oxalates are mainly soluble / insoluble forms of salt or esters of oxalic acid. An excess amount of oxalate is commonly found in leafy vegetables, pulses, grains and nuts<sup>[12]</sup>. In this study, the total oxalate content of *Syzygium jambos* fruit and its seed was found to be 4.54mg/100g and 9.87mg/100g respectively. The oxalate content of other fruits and seeds which people normally consume such as jackfruit, mango, etc. are higher than Syzygium jambos fruit and its seed<sup>[13,14]</sup>. Daily intake of any food that has >20 mg oxalate in every 100 gm should be taken in moderate amount<sup>[15]</sup>. Calcium absorption will be hampered if the ratio of oxalate to calcium is >9:4. Muscle weakness, paralysis, gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals and hypocalcemia are the reasons of oxalate overdose<sup>[16]</sup>. The oxalate content in Syzygium jambos is within the permissible limit. Hence it may not create any adverse effect on people.

**Phytate:** Phytate or phytic acid is the principal storage form of phosphorus in plant tissues. It is present in considerable quantities within legumes and oilseeds - soybean, cottonseed, and rapeseed. Dietary phytate has many health benefits by lowering the blood glucose level in diabetic patients. It regulates insulin secretion and prevents renal stone development. Other than that, it may also reduce blood clots, cholesterol, triglycerides, and thus it can lower the chances of coronary heart diseases<sup>[17]</sup>. Consumption of an excessive amount of phytate may cause poor bone growth, short stature, rickets, narrow jaws, tooth decay in infants, and reduces mineral (iron, zinc) absorption in adults. Recommended daily allowances of phytate is 25 mg/100 g or less<sup>[18]</sup>. In this study, it has been found that the phytate content of *Syzygium jambos* fruit and seed are 5.64 mg/100 g and 7.34 mg/100 g respectively (**Figure 2**). These values are comparatively less in quantity than Jackfruit & seed, mango pulp & seed.

**Alkaloid:** Alkaloids are one of the most important classes of secondary metabolites that are synthesized by plants and found as salts of plant acids such as oxalic, malic, tartaric, or citric acid<sup>[19]</sup>. The richest sources of alkaloids are cocoa, coffee, tea, black pepper, honey and grains. It has been reported that the alkaloid (caffeine) content of an average cup of black tea is 47 mg whereas, green tea contains 20-45 mg and white tea 6-60 mg per cup<sup>[20]</sup>. The present showed that the total alkaloid content of *Syzygium jambos* fruit and its seed was found to be 3.53 mg/100gm and 5.38 mg/100gm respectively (**Figure 2**). These are less in quantity while compared to (Jackfruit - 7.88 mg/100g, its seed - 8.85 mg/100g) and (Mango pulp -85 mg/100g, its seed 6.30mg/100g)<sup>[21,14]</sup>. Plant alkaloids and their synthetic derivatives are also used as medicinal agents from ancient times which possess important biological properties like antioxidant, analgesic and antiseptic. But an overdose of certain alkaloids has shown reverse effects such as disturbance in the nervous system, asphyxia, paralysis<sup>[22]</sup>. The studied sample has a low amount of alkaloid which is very much beneficial for human health.

**Tannin:** Tannin is an astringent, bitter taste-like plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids<sup>[23]</sup>. Some good and most common dietary sources of tannins are tea, coffee, wine and chocolate. Tannin has a great role in boosting the mood, alertness as well as the performance of an individual. In India, the daily intake of tannin below the range of 1500-2500 mg is safe for consumption and do not cause any side effect<sup>[24]</sup>. In this study, the total tannin content of *Syzygium jambos* fruit was found to be 32.43 mg/100 g and its seed 168.29 mg/100 g (**Figure 2**), which is comparatively less in amount than (Jackfruit - 56.17mg/100g and its seed - 212 mg/100g) and (Mango pulp-362mg/100g and its seed-56.5 mg/100g)<sup>[25]</sup>. An excessive amount of tannin present in food products inhibits the activities of trypsin, chymotrypsin, amylase, and lipase. It may also decrease the protein quality of foods and interfere with dietary iron absorption. Other toxic effects of tannins are – low intake of food, inhibition of digestive enzymes, increases excretion of endogenous protein and digestive tract malfunctions<sup>[26]</sup>. Both *Syzygium jambos* fruit and seed may be consumed due to their goodness.

**Saponin:** Saponins are non-volatile, secondary metabolites and surface-active compounds that are extensively dispersed in nature. The richest sources of it are - legumes, onion, garlic, tea, oats, yam and spinach. The range of total saponin found in various legumes is about 1000-7000 mg/100g<sup>[13]</sup>. Several studies have revealed that saponin has many different pharmacological and biological effects such as cholesterol-lowering properties, acts as an anti-cancer agent. It can also stimulate the immune system<sup>[27]</sup>. The total saponin content of *Syzygium jambos* fruit and its seed was 386.49 mg/100g and 528.55 mg/100g respectively (**Figure 2**). These are comparatively higher than the fruit and seed of Jackfruit and mango<sup>[28,31,32]</sup>. Saponin has many beneficial effects on health but it has also been documented that a high saponin diet can reduce nutrient bioavailability and prevent enzyme activity. It inhibits various digestive enzymes such as chymotrypsin and trypsin. A large quantity of saponin intake may cause growth impairment. It can also decrease the intake of food and causes throat irritation<sup>[29]</sup>.

An excess amount of anti-nutritional factors can be removed by different traditional and technological methods from food such as soaking, milling, roasting, pressure cooking, boiling, germination and fermentation. Pressure cooking is one of the best methods to remove anti-nutrients from the food. It has been found that blanching, cooking

and soaking can significantly reduce oxalate, phytate and saponin content from the foodstuff. Germination and fermentation also help to increase mineral content and its bioavailability in food<sup>[33]</sup>.

#### 4. CONCLUSION

*Syzygium jambos* is not very popular among people in West Bengal (India). It has been found that *S. jambos* fruit and its seed have a considerable amount of micronutrients and trace elements. Apart from its Ayurvedic benefits and composition of nutrients, there is no such information about the anti-nutritional properties of this underutilized fruit and its seed. The results obtained from the quantitative analysis show that *Syzygium jambos* fruit and the seed have less quantity of anti-nutritional factors compared to the other common fruits and their respective seeds. The oxalate, phytate, alkaloid, tannin and saponin content of *Syzygium jambos* and its seed are found within the permissible limit. Therefore, it can be said that the anti-nutritional factors present in *Syzygium jambos* fruit and seed may not cause any health problems to consumers. Moreover, this fruit and its seed may help to meet daily dietary requirements for maintaining a good nutritional status for humans and may keep diseases at bay. Further study is needed to know about its medicinal properties and formulation of new food products from this fruit and its seed.

#### 5. ACKNOWLEDGEMENT:

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#### 6. CONFLICT OF INTEREST:

There is no conflict of interest regarding the research article.

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