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**Proximate analysis on biochemical study of
Phyllanthus acidus, *Phyllanthus emblica* and *Citrus limon***

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Abstract

The present study was under taken to investigate the biochemical characterization of *Phyllanthus acidus*, *Phyllanthus emblica* and *Citrus limon*. The results suggest that among the three varieties the level of storage polysaccharides (Total Carbohydrate & Starch) and total protein seems to be higher in *Phyllanthus emblica* than *phyllanthus acidus* and *citrus limon*. The amount of cholesterol in *Phyllanthus acidus* showed better level than the rest of the two varieties. When compared to *Phyllanthus acidus* and *Phyllanthus emblica*, the ascorbic acid content was found to be higher in *Citrus limon*. *Phyllanthus emblica* contain high amount of calcium than the other two varieties. The amount of phosphorous and iron in *Citrus limon* posses better level than *phyllanthus acidus* and *emblica*. Thereby to conclude the fruits selected *Phyllanthus acidus*, *Phyllanthus emblica* and *Citrus limon* varieties showed very impressive biochemical components which is of therapeutic and economic importance.

Key-Words: *Phyllanthus acidus*, *Phyllanthus emblica*, *Citrus limon*, Iron.**Introduction**

Medicinal plants are the most important source of life saving drugs for the majority of the World's population.⁹ Plants have been an important source of medicine for thousands of years.^{8, 13, 14} *Phyllanthus* is the largest genus in the family *Phyllanthaceae*. *Phyllanthus acidus* is an annual erect little branched herb, 10-50 cm high.⁷ The fruits are fleshy, acid and astringent.¹ *Aonla* (*Phyllanthus emblica*) is a medium – sized deciduous tree belonging to the family *Euphorbiaceae*. It is commonly known as Indian gooseberry and grows in the plains and sub-mountain tracts all over the Indian subcontinent from 200 to 1300m .altitude.¹² The genus *citrus*, which includes few of the most important fruits Worldwide, belongs to the family *Rutaceae*, which comprises 140 genera and 1300 species throughout the world.³ The lemon is a small evergreen tree (*Citrus limon*) originally native to Asia and is also the name of the tree's oval yellow fruit. The fruit is used for culinary and non-culinary purposes throughout the world.

The study was undertaken to assess the biochemical constituents (carbohydrates, starch, protein, iron, phosphorous, calcium, cholesterol, ascorbic acid) of *Phyllanthus acidus*, *Phyllanthus emblica* and *citrus limon*.

Material and Methods

The fruits selected were *Phyllanthus acidus*, *Phyllanthus emblica* and *Citrus limon*. These fruits were collected from Kanjamalai, Salem district, Tamilnadu, India.

**Preparation of fruit extract for biochemical analysis
Quantitative Estimation**

Different methods of extraction have been used for the quantification using standard protocols.

Extraction of fruit sample**Extraction in hydrochloric acid for the estimation of total carbohydrate**

Weighed 2g of the sample into a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3 hours. Add 15 ml of 2.5 N Hydrochloric acid and cool to room temperature. Neutralize it with solid sodiumcarbonate until the effervescence ceases, make up the volume to 100 ml and centrifuge and save the supernatant.

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Extraction in 80% ethanol for the estimation of starch

Homogenized 1.5g of the sample in hot 80% ethanol to remove sugars. Centrifuge and retain the residue. Wash the residue repeatedly with hot 80% ethanol till the washings do not give colour with anthrone reagent. Dry the residue well over a water bath. The residue is used for further estimation.

Extraction in phosphate buffer for the estimation of protein

Extraction is usually carried out with buffers used for the enzyme assay. Weighed 2g of the sample and grind well with a pestle and mortar in 10 ml of the phosphate buffer. Centrifuge the homogenate at 1000 rpm for 20 minutes and use the supernatant.

Extraction in oxalic acid for the estimation of ascorbic acid

Grind 0.5 to 5g of sample material either mechanically or using a pestle and mortar in 25 to 50 ml of 4% oxalic acid solution. Centrifuge or filter and collect the liquid. Transfer an aliquot (10 ml) to a conical flask and add bromine water drop wise with constant mixing. The enolic hydrogen atoms in ascorbic acid are removed by bromine. When the extract turns orange yellow due to excess bromine, expel it by blowing in air. Make up to a known volume (25 or 50 ml) with 4% oxalic acid solution.

Extraction in triple acid for the estimation of minerals (calcium, phosphorus, iron)

A quantity of 3g of sample was taken in conical flask and digested with a mixture of 10 ml concentrated nitric acid, sulphuric acid and perchloric acid in 9:2:1 ratio. Initial digestion was done in a cold state and then digested over a hot plate until an ashy white digest was obtained. The triple acid extract was made up to 100 ml after filtration with distilled water.

Parameters to be analysed for the quantitative estimation

- Estimation of Total carbohydrate by Anthrone method-Hedge & Hofreiter 1962
- Estimation of Starch by Anthrone method-Hedge & Hofreiter 1962
- Estimation of Protein by Lowry's method-Lowry's et al., 1951
- Estimation of Cholesterol by Zak's method-Zak 1957
- Estimation of Ascorbic acid by Harris method-Harris & Ray 1935
- Estimation of Calcium by Clark & Collip method-Clark & Collip 1925
- Estimation of Phosphorus by Fiske Subbarow method-Fiske, Subbarow 1925

- Estimation of Iron by Wong's method-Wong 1928

Results and Conclusion

The results of the biochemical parameters suggested that the total carbohydrate and starch content seems to be significantly high in *Phyllanthus emblica* when compared to *Phyllanthus acidus* and *Citrus limon*. The fruit *Phyllanthus emblica* possesses very good store of protein which validates its nutritive and biochemical potential indulged in it, when compared with other two varieties. *Phyllanthus acidus* contains enhanced level of cholesterol than *Phyllanthus emblica* and *Citrus limon*. *Citrus limon* contains very impressive level of ascorbic acid than the other two *Phyllanthus* species. The *Phyllanthus* and *Citrus* varieties can be recommended as source of natural anti oxidant to fight against vulnerable diseases. The amount of calcium is high in *Phyllanthus emblica* than *Citrus limon* and *Phyllanthus acidus*. *Citrus limon* showed very good amount of phosphorous and iron than the *Phyllanthus acidus* and *emblica*. The level of calcium is high in *Phyllanthus emblica*, phosphorous and iron is high in *Citrus limon* variety which signifies its nutritive potential of this fruit.

Using fruits as an inspiration for new drugs provides infusion of novel compounds or substances for healing diseases. Evaluating the fruits from the traditional system of medicine provides us with the clues how these fruits can be used as nutritive source. The present study comprehensively and conclusively validates the *Phyllanthus* and *Citrus* varieties found to contain commendable store of biochemical constituents.

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Table 1: Levels of biochemical parameters in *Phyllanthus acidus*, *Phyllanthus emblica* & *Citrus limon*

Species	Carbohydrate (mg/g)	Starch (mg/g)	Protein (mg/g)	Cholesterol (mg/g)	Ascorbic acid (mg/g)	Calcium (mg/g)	Phosphorus (mg/g)	Iron (mg/g)
<i>Phyllanthus acidus</i>	20.75 ± 0.012	0.498 ± 0.006	4.013 ± 0.012	0.208 ± 0.0002	2.505 ± 0.0041	3.807 ± 0.0016	0.504 ± 0.0033	0.635 ± 0.0041
<i>Phyllanthus emblica</i>	23.25 ± 0.016	0.916 ± 0.0001	9.005 ± 0.004	0.063 ± 0.0001	3.501 ± 0.0018	4.804 ± 0.0029	0.716 ± 0.0001	0.606 ± 0.0001
<i>Citrus limon</i>	16.75 ± 0.017	0.251 ± 0.0012	2.004 ± 0.002	0.146 ± 0.0002	5.002 ± 0.0022	4.303 ± 0.0027	1.103 ± 0.0022	0.653 ± 0.002

Values are mean ± S.D, Statistical significance: p<0.01, p<0.005

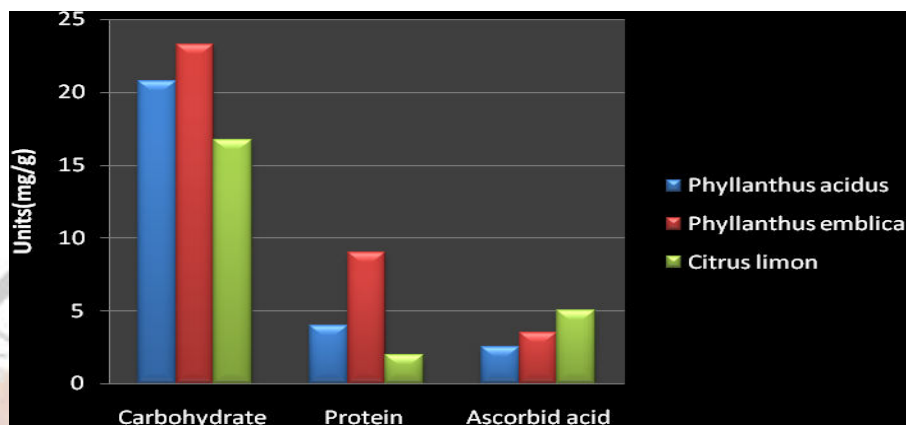


Fig.1: Levels of carbohydrate, protein and starch in *Phyllanthus acidus*, *Phyllanthus emblica* & *Citrus limon*

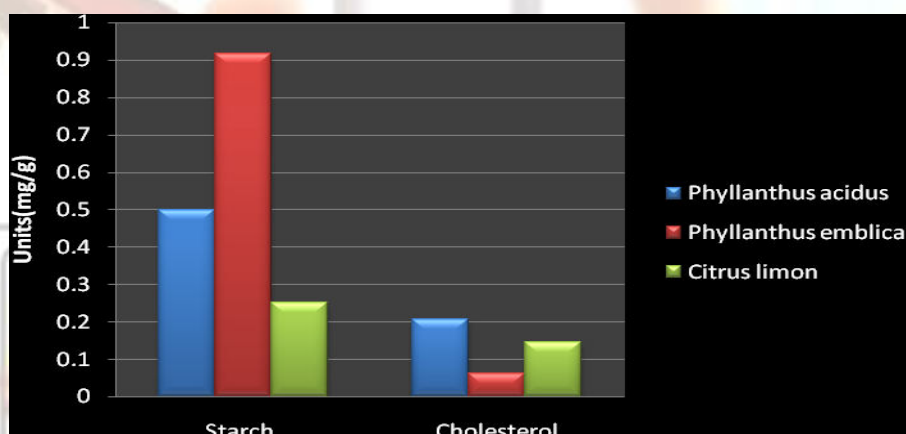


Fig.2: Levels of starch and cholesterol in *Phyllanthus acidus*, *Phyllanthus emblica* & *Citrus limon*

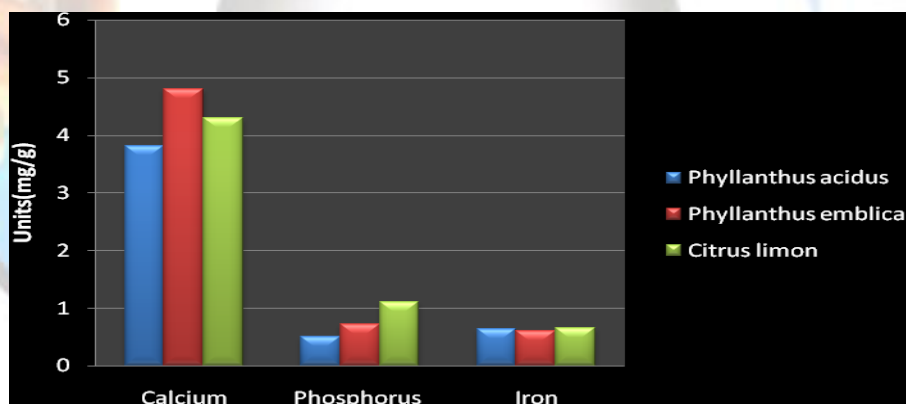


Fig.3: Levels of calcium, phosphorous and iron in *Phyllanthus acidus*, *Phyllanthus emblica* & *Citrus limon*