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Preliminary phytochemical, physicochemical and biochemical analysis in seeds of *Artocarpus heterophyllus* lam

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Abstract
Artocarpus heterophyllus is one of the most important and widespread trees in tropical region and its belongs to the family Moraceae. These plant seed have high medicinal values. Moracea is a large family comprising about 60 genera and nearly 1400 species, including important group such as Artocarpus, Morus, and Ficus. Phytochemicals are responsible for medicinal activities of plant. These are chemical that have protected human from various diseases. The physicochemical parameters such as ash value and determination of moisture loss was used to determine the quality and purity of a crude drug.

Keywords: substituted Li ferrite, magnetostatic and spin waves, microstrip array antenna, X-band frequency range

Introduction
Moraceae the mulberry family of the Rose order. *Artocarpus heterophyllus* is one of the most important and widespread trees in tropical region useful tree in significant genus *Artocarpus (*Raja Sekhar k.k. 2010) [14]. The tree is reported native to the rainforest of Malaysia, Western ghats of India and also found in Eastern &Southern Africa, Brazil, Florida, Australia. All parts of tree exude sticky, white milky latex when injured (Rahman AM 1999) [13]. The whole tree has valued place in research due to its medicinal and nutritive properties. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodiasic and also used brain tonic. The seeds are diuretic and conspitating. The wood is nervine, antidiabetic, and sedative useful in convulusions (Hemborn PP. 1996) [4].
Naturally occurring substances are of plants, animals and mineral origin. They are organic substances and could be obtained in both primary and secondary metabolic process. They also provide a source of medicine since the earliest time. The medicinal importance of plant is due to the presence of chemical constituents like alkaloids, glycosides, resins, volitale oils, gums and tannins (Kumar *et al.*, 2011) [87]. These compounds are synthesized by primary or rather secondary metabolism of living organisms.
These are many phytochemicals herbs and each works differently. These phytochemicals have various health benefits such as anti-oxidant, anti-inflammatory, anti-cancerous, anti-diabetic and anti-hypertensive effect (Savthramma *et al.*, 2011, Rupa Singh *et al., 2003*) [19, 17].
Biochemical analysis techniques refers to a set of methods says and procedures that enables scientists to analyze the substances found in living organisms and the chemical reactions underlying life processes. Nutrients are substances needed for growth, metabolism and other body functions. The main function of nutrients is to provide calories or energy.

Materials and Methods
Study area
In present study *Artocarpus heterophyllus* seeds are collected from Thalassery (Plate -1). It is a Thaluk and city in Kannur district in the state of Kerala in India. They contain 27^0C and 83% humidity so plant grows well.

Sample Collection
Fresh seeds of the selected plant materials were collected during April (Plate-2).They were cut in to small pieces, shade dried (Plate-3) and ground to fine powder and stored in air tight container for further analysis.

Physicochemical Analysis
The seed was evaluated for its physicochemical parameters like total ash and determination of moisture loss using standard procedures (Pulok Mukherjee, 2012; Anonymous, 2017, Kokate *et al.*, 1994) [11, 1].

Moisture Content
Fresh weight of the sample was determined and placed the sample in a hot air oven initially for one hour 100^0C and the dry weight of the sample become constant.

$$\text{Moisture content \%} = \frac{\text{Fresh weight-Dry weight x}}{\text{Fresh weight}} 100$$

Total ash
About 2g of powdered seed was accurately weighed and taken separately in silica crucible, which was previously ignited weighed. Powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight.

$$\text{Ash content\%} = \frac{\text{Fresh weight- Dry weight x}}{\text{Fresh weight}} 100$$

Preliminary phytochemical analysis
(Ramman, 2006, Karpagam *et al*., 2008; Kokate *et al*., 2001) [1].

Extraction
The powded seeds were collected and 15g of it were measured and introduced in to 100ml of ethanol, chloroform acetone extraction is carried out by shaker system for 48hrs. The nature and yield of the extract were noted. The extracts were stored in a refrigerator at 4^0c for further studied.
The extracts of the selected plant seed were tested for carbohydrates, proteins, aminoacids, steroids, glycosides, flavanoids, alkaloids, tannins, saponins, terpenoids and resins, This phytochemical screening of the extracts are carried out by standard methods. (Raaman, 2006; Karpagam *et al*., 2008: Kokate *et al*.; 2001) [15, 5, 7].

1. Test for Carbohydrates
To 2ml of test solution adds two drops of the Molish reagent (a solution of α napthol in 95% ethanol). The solution is then poured slowly into a test tube containing 2ml of conc. Sulphuric acid so that two layers form. The formation of a purple product at the interface of the two layers indicates the presence of carbohydrates.

2. Test for Proteins
It is used to determine the presence of peptide bonds in protein. To 3 ml of test sample add 3%NaOH and few drops of 1 % $CuSO_4$. The solution turns from blue to violet (purple) or to pink. That indicates the presence of protein.

3. Test for starch
Mix 3ml test solution and few drops of dilute iodine solution. Blue colour appears. It disappears on boiling and reappears on cooling.

4. Test for Amino Acids
To 5ml of test sample solution add a few drops of 40 % NaOH and 10% lead acetate boiled the solution formation of black precipitates show the presence of amino acid.

5. Test for Steroids
To 2ml of extract add 2ml chloroform and 2 ml conc. Sulphuric acid. Shake well, chloroform 1 layer appear red and acid layer show greenish yellow florescence which indicate the presence of steroids.

6. Test for Glycosides
To the solution of extract add glacial acetic acid, few drops 5% ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and bluish green colour in upper which indicates presence of glycosides.

7. Test for Flavonoids
To 2 ml of extract add few drops of 1% Ammonia solution. A yellow colouration was observed for the presence of flavaonoids.

8. Test for Alkaloids
To 0.5g of each extracts adds 5ml of 1% aqueous hydrochloric acid and kept in water bath: 1ml of the filtrate is to be treated with Mayer's reagent (Potassium Mercuric Iodide).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

9. Test for Tannins
To 0.5ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green balck for catecholic tannins.

10. Test for Sponins
To 1ml extract solution, 1ml of water and shake it. Persistent foam indicates presence of saponins.

11. Test for Terpenoids
2ml extract was mixed with 2ml chloroform in a test tube. To this 3ml Conc. Sulphuric acid was carefully added along the wall of the tube to form a layer. An interface with a reddish brown colouration confirmed the presence of terpenoids.

12. Test for Gums
To 1 ml of extract add 3ml of Dil. HCL; Feling's solution is added drope by drope till red colouration visualizes the presence of gums.

Biochemical analysis
The biochemical analysis is performed on *Artocarpus heterophyllus Lam*. The powder of selected plant seed where tested for estimation of carbohydrate (Anthrone method), protein (Lowry's method) by Sadasivam and Manikam (2008) [18] and starch.

Estimation of Carbohydrate by Anthorne Method
100mg of dried powdered seed was hydrolysed in a boiling water bath for 30 minutes with 80%ethanol in water and centrifuge 8000rpmfor 15 minutes. And preserved 4ml of supernatant. From it 1ml of the supernatant dried and dissolved in 50 ml distilled H_2O. Anthrone reagent is prepared by mixing 300gm anthrone with 150ml icecold H_2SO4.0.2ml of sample made up to 1ml with distilled H_2O add 4ml of Anthrone reagent and rapidly cooled in ice bath. OD values which was read at 630nm using Bovine Serum Albumin (BSA).

Protein estimation by Lowry's method 1951
1gm of the sample weighed and grained well with a pestle and motor in 1ml of the buffer. Add 5% of TCA and kept in cooled for 1hour. Centrifuge at 3500rpm for 20 minutes. Dissolved precipitated proteinin 0.1N NaOH (Reagent A). 0.5% $Cuso_4$ in 1% potassium sodium tartarate (Reagent B). 50ml of reagent A and B was mixed prior to use and reagent C was obtained. Which was immediately added in to the test tube was mixed well and allowed to stand for 10minutes. 0.5ml of reagent D (Folin Ciocalteau reagent) was added, mixed well and incubated at room temperature in the dark for 30 minutes to develop blue colour. OD values read at 660 nm using glucose as standard and calculate the amount of protein (Lowry *et al*. 1951)

Estimation of starch by Hedge and Hotreiter 1962

The total soluble carbohydrates from the selected sample were extracting and estimated by the Anthrone reagent method (Hedge and Hotreiter, 1962). Using glucose as standard at 620nm in a spectrophotometer the OD values were expressed as mg/100gm on dry weight basis.

Result

The present investigation was carried to find out the phytochemical and biochemical constituents present in *Artocarpus heterophyllus*. Bioactive substances from this plant can there for employed in the formation of drugs for the treatment of various diseases.

Table 1 shows that result of phytochemical analysis of *Artocarpus heterophyllus* of seed. The result provides potential use of these plants in making new drugs. Phytochemical analysis of *Artocarpus heterophyllus* ethanolic extract showed positive result for carbohydrate, protein, saponins, terpenoids, glycosides, tanin. While in methanolic extract shows carbohydrate, proteins, flavonoids, alkaloids, sapnonis, Terpenoids, and glycosides, tanin. And in acetone extract shows carbohydrate, proteins, steroids, glycosides, alkaloids, saponins, terpenoids, tanin

The moisture content of dry seed *Artocarpus heterophyllus* seed is 10% and total ash content is 15%. (Table 2)

Table 3 shows that result of biochemical analysis showed that total carbohydrate content in the seed of *Artocarpus heterophyllus* Contain 9.68mg carbohydrate and in protein was 24mg and starch is 21.6mg.

Table 1: Phytochemical analysis of *Artocarpus heterophyllus*

SI. No	Phytochemical constituent	Ethanol extract	Methanol extract	Acetone extract
1.	Carbohydrate	+	+	+
2.	Protein	+	+	+
3.	Starch	+	+	+
4.	Amino acid	-	-	-
5.	Steroid	-	-	+
6.	Glycoside	+	+	+
7.	Flavonoid	-	+	-
8.	Alkaloid	-	+	+
9.	Tannin	+	+	+
10.	Saponin	+	+	+
11.	Terpenoid	+	+	+
12	Gum	-	-	-

+= Present; - = Absent

Table 2: Physicochemical analysis of dry seed in *Artocarpus heterophyllus*

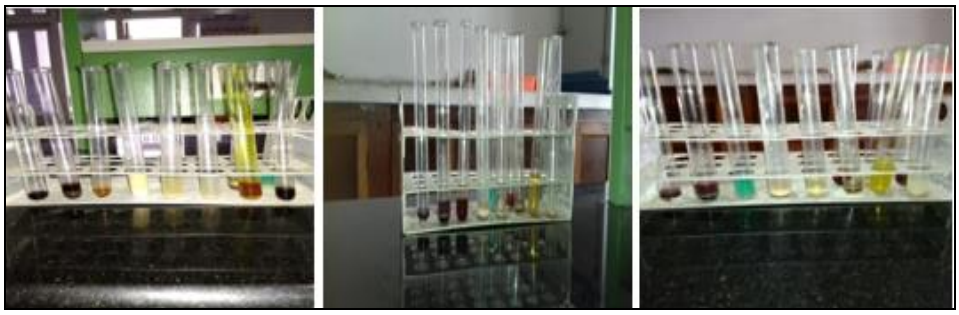
SI. No	Parameter analysed	*Artocarpus heterophyllus %*
1.	Moisture content	10%
2.	Total ash	15%

Table 3: Carbohydrate, proteins and starch content in the seeds of *Artocarpus heterophyllus*

SI. No	Biochemical constituent	*Artocarpus heterophyllus*, Lam. *mg/g*
1.	Carbohydrate	58.75mg
2.	Protein	24mg
3.	Starch	52.87mg

Fig 1: Habit and fresh seed of *Artocarpus hertophyllus*

Fig 2: Dried sample and seed extract



(a) Ethanolic extract (b) Methanolic extract (c) Acetone extract

Fig 3: Phytochemical screening of *Artocarpus heterophyllus*

Discussion

The medicinal plants are used for healing and curing of human disease because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants leaves, vegetables, and roots. Phytochemicals are primary and secondary compounds chlorophyll, proteins, and common sugars are including primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds The present investigation was carried to find out phytochemical and biochemical constituent present in *Artocarpus heterophyllus*. The ethonolic extract of *Artocarpus heterophyllus* seed contain saponins, tannins, terpenoids, and flavanoids, tanin. Similar result was reported by Delphin *et al.,* (2014) [3]. Alkaloids are the class of naturally occurring organic nitrogen containing bases. Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants and animals. They often have pharmacological effects and used as medications (Stray *et al.*, 1998).

The methanolic extract of *Artocarpus heterophyllus* seed contain carbohydrate, protein, saponins, glycosides, flavanoids, terpenoids, tanin similar result were reported by Vaishnavi Bhat & Vijaya Poojitha (2017) [8]. Flavanoids most important secondary metabolites and bioactive compounds in plants (Kim, 2013) [6].

The acetone extract of *Artocarpus heterophyllus* seed contain carbohydrate, protein, steroids, saponins, tanin whereas these secondary metabolites were absent in Sreeletha *et al.,* (2017) Glycosides are naturally cardio-active drugs used in the treatment of congestive heart failure and cardiac disease. The presence of glycosides indicates that may be potent in curing cardiac insufficiency, cough and circulatory problems (Sule 2010) [24].

The acetone of *Artocarpus heterophyllus* seed contain protein, flavanoids, saponins, terpenoids where these secondary metabolites absent in Prasad *et al.,* (2014) [10] Terpenoids are large and diverse class of naturally occurring organic chemicals. It is very important in attracting useful mites and consumes the herbivores insects. It is found to be useful in the prevention and therapy of several diseases, including cancer, and also have anti-microbial, antifungal, anti-parasitic, anti-viral, anti-allergenic, anti-plasmodic, anti-hyperglycemic, anti-inflamatory and immunomodulatory properties (Rabi *et al.*, 2009) [12].

Tanins have stringent properties widely used as an application to sprains, superficial wounds. They are responsible for anti-dysenteric, anti-diarrheal, anti-helmintics, anti-microbial and antivirals, anti-oxidants and chelate dietary iron (Prakash *et al*., 2013) [7]. Steroids have reported antibacterial properties, analgesic properties, and act on nervous activities (Sayyah *et al*., 2004) [20].

Saponins a diverse range of properties which include property of precipitating and coagulating red blood cells (Sodipo *et al*., 2000) [22], anti-microbial, insecticidal, activites (Attele *et al*., 1999) [2]. Saponins are also used in anticancer and anti-inflamatory activities (Rathore *et al*., 2012) [16].

Physicochemical analysis of *Artocarpus heterophyllus* showed the moisture content 10%.It was higher than the same plant species seed (22.4%) reported by (Nilanjana Nandlal *et al.*, 2012) [9] *Artocarpus heterophyllus* contain total ash content it was lower than the same plant seed (15%) of ash content.

The biochemical analysis showed that total carbohydrate content in the seed of *Artocarpus heterophyllus*, Lam. contain 58.75mg carbohydrate and in protein was 24mg and starch is 52.87mg. (Table 3)

Carbohydrate perform numerous roles in living organisms found in a wide spread organic substances in nature and are essential constituents of all living things. They are said to be chemically simpler nucleotides or aminoacid because they contain just three elements namely carbon, hydrogen and oxygen).

Starch is the most abundant constituent for plants, it is stored mainly in fruit, rhizome and seed. Starch is extensively used in food, textile and paper industries, other applications are reported in fields such as pharmacy, hygiene products, environmental management, agricultural, biomedial engineering and biofuel production depending up on properties (Valencia *et al.,* 2012) [26].

Proteins are large complex molecules that play many roles in the body, proteins are made up of hundreads or thousands of smaller units called aminoacids. Protein, another class of food often times referred to as the nitrogen containing natural product has been proved to be essential for te survival of human being and anmals (Voet *et al.,* 2008) [29].

Summary

Artocarpus heterophyllus belongs to the family Moraceae. These plant seed have high medicinal values. *Artocarpus heterophyllus* in the present study reveals the presence of various potential phytochemical constituents.

Phytochemical analysis of *Artocarpus heterophyllus* ethanolic

extract shows the presence of carbohydrate, protein, saponins, terpenoids, and glycosides, Tanin. In the case of methanolic extract shows the carbohydrate, protein, flavanoids, alkaloids, saponins, terpenoids, and glycosides, tanin. The acetone extract shows the carbohydrate, protein, steroids, glycosides, alkaloids, saponins, terpenoids. Tanin The moisture content in dry seed of *Artocarpus heterophyllus* is 10% and total ash content is 15%.The physicochemical parameters such as ash value and determination of moisture loss was used to determine the quality and purity of a crude drug. Biochemical analysis of *Artocarpus heterophyllus* revealed 58.75mg of carbohydrate, 24 mg of protein and 52.87 mg of starch.

Jack fruit rich source of vitamin, minerals, phytonutrients, carbohydrate, fiber, fat and protein. Jack fruit lowers risk of heart disease, keeps thyroid healthy, regulation of blood sugar levels, improve eyesight, digestion, maintain blood pressure, and promote hair growth. Jack fruit is a sweet, delicious it contain many nutrients, and consume for the best taste nutrition and many health benefits. Seeds contain a good amount of protein this can be added to different dishes.

Therefore the phytochemical investigation of *Artocarpus heterophyllus* in the present study reveals the presence of various phytochemical constituents which may be useful for pharmaceutical industries and are potential use of these plants in making new drugs.

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