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Extraction of Phytochemicals of *Artocarpus altilis* (Parkinson) Fosberg (seedless) fruit pulp using Non-polar and Polar Solvents

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Abstract

Aim: Selection of a suitable solvent is important and utilized in the extraction of desirable chemical components in medicinal plants.

Study Design: Chemical analysis of various extracts of *Artocarpus altilis* fruit pulp using standard analytical procedures.

Methodology: Fruit pulp of *Artocarpus altilis* (Parkinson) Fosberg or breadfruit (seedless) were extracted with six solvents categorized into polar (Acetone, Methanol and Aqueous) and non-polar (Ethyl acetate, Hexane and Chloroform) types using cold maceration method, the qualitative and quantitative phytochemical assay was done on the respective extracts using the standard methods.

Results: Phytochemical screening of the non-polar solvent extract revealed the presence of Steroid, Terpenoids, Flavonoids, carbohydrates, oil and fat for all the solvents, except petroleum ether crude extract. The quantitative analysis has revealed that ethanolic extract of *A.altilis* fruit pulp is very rich in phenols (11.16 ±0.13); alkaloids (9.42±0.21); flavonoids (2.50 ±0.09) and Steroid (1.21±0.05) which is in the order of phenols >alkaloids> flavonoids >Steroid which gives a very strong reason to select this plant for future evaluation of cytotoxic intern anticancer and other pharmacological properties.

Conclusion: The phytochemical constituents detected in varying quantities depend on the polarity of the substances, *Artocarpus altilis* could be exploited and extracted very well using a polar solvent like ethanol, acetone and aqueous. Further investigations regarding more biological activities of the ethanolic extract need to be conducted.

Keywords: *Artocarpus altilis* (Parkinson) Fosberg, polar, non polar, phytoconstituents

1. Introduction

Phytonutrients also known as Phytochemicals are naturally occurring substances in plant [1]. Any plant where one or more of its part contains substances that can be utilized for medicinal purposes or serves as starting materials for the creation of functional medication is called medicinal plant[2]. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential and fixed)[3]. Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat

various health ailments, hungry and chronic diseases as well [4].

Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world [5-9]. For Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Extraction is a vital activity in the process of phytochemical isolation for the detection of pharmacologically active components in plant materials [10, 11]. The selection of an appropriate solvent system for extraction is vital for medicinal product standardization as it is used in the isolation of the required constituents while excluding the unwanted matrix.

Artocarpus altilis (Parkinson) Fosberg, commonly known as breadfruit (seedless), is a tree from the Moraceae family which consists of approximately 60 species native to the Indian subcontinent, Southeast Asia, and Australia and primarily used as a food source. Breadfruit tree is an evergreen multipurpose and traditional agroforestry species. Its starchy fruits are a staple food in the Pacific Islands. The name breadfruit is due to the flavour of the fruit after being cooked which reminds of freshly cooked bread [12]. Breadfruit has many ethno medicinal uses [13].All parts are used medicinally in the Pacific and Caribbean, especially the latex, leaf tips, and inner bark. The latex is massaged into the skin to treat broken bones and sprains and is bandaged on the spine to relieve sciatica. It is commonly used to treat skin ailments and fungus diseases such as “thrush,” which is also treated with crushed leaves. Diluted latex is taken internally to treat diarrhoea, stomach-aches, and dysentery. The sap from the crushed stems of leaves is used to treat ear infections or sore eyes. The root is astringent and used as a purgative; when macerated it is used as a poultice for skin ailments. The bark is also used to treat headaches in several islands. In the West Indies the yellowing leaf is brewed into tea and taken to reduce high blood pressure and relieve asthma. The tea is also thought to control diabetes. A syncarpus yellow green coloured soft, sweet, creamy flesh fruit is a store house of minerals, vitamins, antioxidants and other nutrients, besides that, it is also a valuable source for medicinally important compounds. Despite its nutritional attributes and usage in the traditional medicinal system, limited studies have been conducted to demonstrate the various biological effects of *A. altilis* and these studies do not indicate or demonstrate the mode of action [14].

Some medicinal plants including *Artocarpus altilis* exploited for medicinal purposes have to undergo phytochemical screening and bioassay as steps towards drug developments [11]. There are few studies concerning the phytochemical profile of *Artocarpus altilis*. In light of this, the objective of this study is to comparatively assess the

extraction efficiency of six solvents type in terms of the qualitative and quantitative phytochemical assay with a view to provide information on the best solvent type for the extraction of phytocompounds from the fruit pulp of *Artocarpus altilis*.

2. Materials and Method

2.1Chemicals:

All chemicals and reagents were analytical or HPLC grade.

2.2 Collection of Plant material (Identification, Authentication and Plant sample Preparation)

The fruits of *Artocarpus altilis* (Parkinson) Fosberg, (Breadfruit) of the family *Moraceae* were collect from Gobichettipalayam, Erode, Tamilnadu, India. The plant was authenticated by Botanical Survey of India, Coimbatore; letter No BSI/SRC/5/23/2017/Tech/1610.

Mature breadfruits were inspected for blemishes and cleaned with potable water to clear away latex and dirt from the skin. After washing, breadfruits were air dried to remove excess water. For whole breadfruit flour (WBF), fruits were peeled and chopped into small (<4mm diameter) rough chunks and then dried in room temperature for 7 days in sealed tray; product was then packed into polyethylene bags. Dried fruits were then milled into flour and passed through an 80-mesh sieve. Breadfruit flour samples were stored in double layered airlock plastic bags.

2.3 Method of Extraction: Cold Maceration

The powdered Fruit samples (20 g) is subjected to maceration extraction with the incubation period of 3-7 days with various solvents viz petroleum ether (PE), Chloroform, Ethyl acetate, Acetone, Ethanol and Distilled water. The extracts were evaporated at 40º C under reduced pressure to dryness in a rotary evaporator, stored in air-tight container at 4ºC until use. Yield of extracts are given in Table 1.Extraction yield of all extracts were calculated using the following equation below:

Total extract yield, Y (%) = $\frac{\text{Total mass of extract}}{\text{Total mass of sample}} \times 100$ [15]

Table 1: Yield of extracts

Solvent	Weight of powdered plant material	Volume of solvent	Weight of extract(g)	% of Yield extraction
Petroleum ether	20 g	200 ml	1.34	6.7
Ethyl acetate	20 g	200 ml	1.66	8.3
Chloroform	20 g	200 ml	1.12	5.6
Acetone	20 g	200 ml	2.46	12.3
Ethanol	20 g	200 ml	4.84	24.2
Aqueous	20 g	200 ml	3.72	18.6

2.4. Phytochemical Assay

Preliminary phytochemical analysis was carried out for various solvent extracts of *Artocarpus altilis* fruit pulp as per standard methods described by Brain and Turner 1975 and Evans 1996 in Table 2[16, 17].

2.4 (a) Quantitative Phytochemical Analysis

1. Estimation of Alkaloids:

Alkaloids were determined using Harborne method. Five grams of the sample was weighed into a 250 ml beaker, 200 ml of 10% acetic acid

in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [18]. All the experiments were run in triplicate.

2. Estimation of flavonoids:

Flavanoids were determined using Swain and Hill's method.The plant extracts (50mg each) were dissolved separately in 50ml of methanol. These solutions were serially diluted with methanol to obtain lower dilutions. Phloroglucinol (50mg) was dissolved in 50ml of distilled water and it was serially diluted with water to obtain lower dilutions.0.2ml of the extract was taken in a test tube and the final volume was made up to 2ml with distilled water and to this 4ml of vanillin reagent was added rapidly. Exactly after 15min, the absorbance was recorded at 500nm against blank. Using different concentrations of Phloroglucinol, the unknown was read from a standard curve prepared [19]. All the experiments were run in triplicate.

3. Estimation of Phenol

The total phenolic content was estimated by Folin Ciocalteu method as described by Singleton et al., (1965) with slight modifications. The extract (1 mg/mL) was mixed with 5 mL of distilled water, 1 mL of sodium carbonate (20%) and 1 mL of Folin Ciocalteu reagent. The mixture was allowed to stand in a water bath for 30 min at 40°C. The content of total phenolic compounds was expressed as mg of gallic acid equivalents per g dry matter (mg GAE. g-1DM). The absorbance was measured at 765 nm using a UV-Vis spectrophotometer T60 U. [20]. All the experiments were run in triplicate.

4. Estimation of Steroids

Flavanoids were determined using Evans method. 1ml of ethanolic extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 20 ^{o}C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank [21]. All the experiments were run in triplicate.

Table 2: Preliminary phytochemical tests for plant extracts

Phytoconstituents	Test	Observation
Alkaloids		
Mayer's test	2ml extract + few drops of Mayer's reagent	Cream color
Wagner's test	2ml extract + few drops of Wagner's reagent	Reddish brown solution/ precipitate
Flavonoids		
Lead acetate test	1ml extract + 1ml Pb $(OAc)_4$ (10%)	Yellow coloration
H_2SO_4 test	1ml extract + 1ml H_2SO_4 test	Reddish brown / Orange color precipitate
Steroids		
Liebermann-Burchard test	2ml extract + 2ml $(CH_3CO)_2O$ + 2ml H_2SO_4 (conc.)	violet to blue or green
Terpenoids	2ml extract + 2ml $CHCl_3$+ 2-3 drops conc.	Reddish brown coloration of the
Salkowski Test	H_2SO_4	inner face
Anthraquinones	3ml extract + 3ml Benzene + 5ml NH_3 (10%)	Pink, Violet or Red coloration in
Borntrager's Test		ammonical layer
Tannins	2ml extract + 2ml H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate
Braymer's Test		
Carbohydrates	2ml extract + 10ml H_2O + 2 drops Ethanolic α-	Reddish violet ring at the jun ction
Molisch's Test	naphthol (20%) +2ml H_2SO_4 (conc.)	
Saponins (Foam Test)	1ml extract + 5ml H_2O + heat	Froth appears
Phenol		
Ferric chloride test	1ml extract +few drops of 3% ferric chloride	Deep blue to Black color formation
Oil and fats	Small extract in between two filter paper	Oil stain produced

2.5 Statistical Analysis

Quantitative data were expressed as Mean ±SD of triplicate measurement; analysis of variance (ANOVA) was used to test significant difference between the mean of phytochemicals from each extract,

while specific differences were identified using Duncan Multiple Range Test, where $p<0.05$ was considered significant. IBM SPSS version 2.0 was used for the statistical analysis.

3. Result and Discussion

Extraction step is the initial step prior to analysing phytochemical component of the herbs. Cold maceration is still a better option because some of herbal components are heat sensitive. Cold maceration methods of extraction are performed. Hence, effects of solvents and extraction methods are studied in terms of extraction yield as shown in table 1.The results indicated a wide range of extraction yield for different solvents (5.6% - 24.2%). Among all of the solvents selected, all herbs showed a tendency to dis-solve in ethanol. In cold maceration, *Artocarpus altilis* fruit pulp extract yields highest percentage of phenolic content followed by flavonoids, alkaloid, steroid, carbohydrate and terpenoids [22].

Table 3: Results of phytochemical screening of non-polar and polar solvent crude extract of *A.altilis*

Phytochemicals	**Non-Polar solvents**			**Polar solvents**		
	Petroleum ether	chloroform	Ethyl acetate	Acetone	Ethanol	Aqueous
Alkaloids Mayer's test Wagner's test	-	-	-	+	+	+
Flavonoids Lead acetate test H_2SO_4 test	+	+	+	+	+	-
Steroids Liebermann-Burchard test	-	+	+	-	+	-
Terpenoids Salkowski Test	+	+	+	+	+	-
Anthraquinones Borntrager's Test	-	-	-	-	-	-
Tannins Braymer's Test	-	-	-	-	-	-
Carbohydrates Molisch's Test	+	+	+	+	+	+
Saponins (Foam Test)	-	-	-	-	-	+
Phenol Ferric chloride test	-	-	-	+	+	+
Oil and fats	+	+	+	-	-	-

(+) = Presence, (-) = Absence

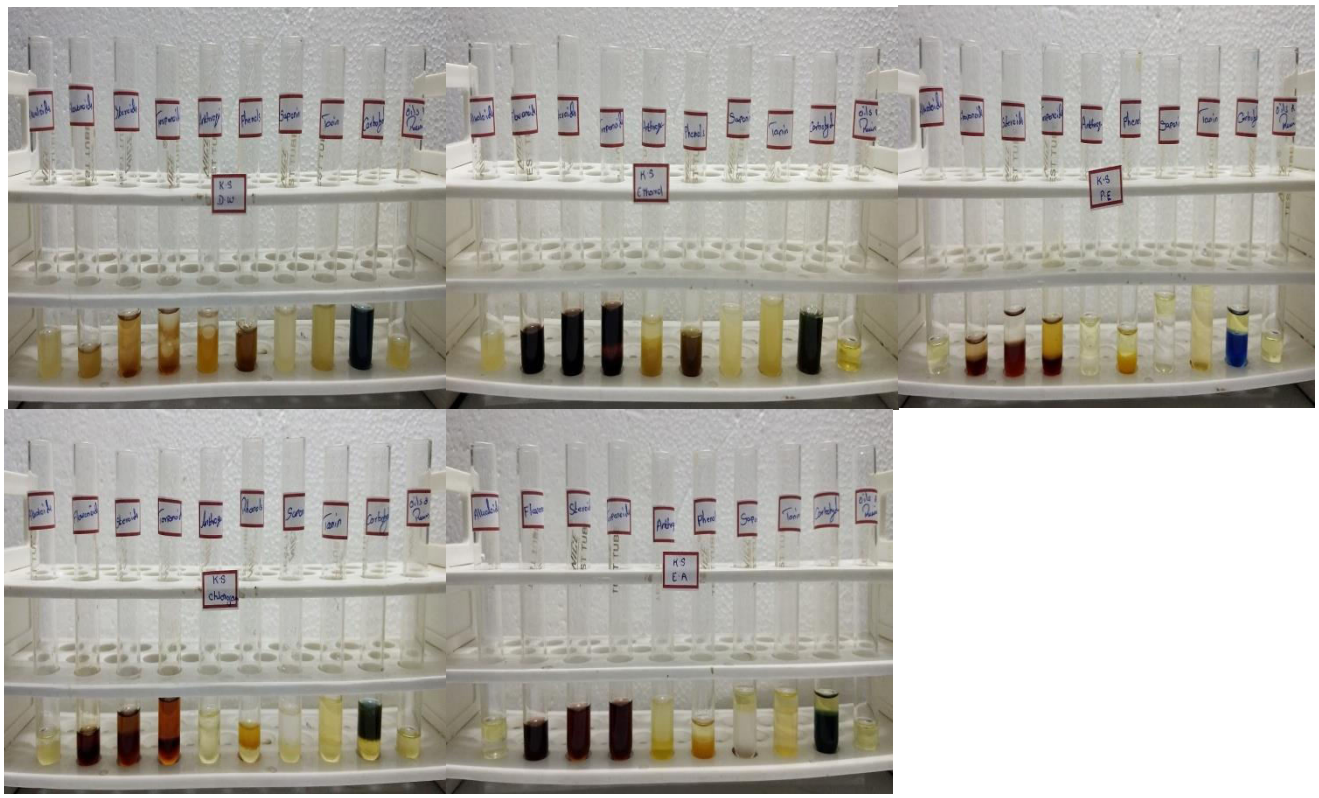

Figure: 1 phytochemical screening of non-polar and polar solvent crude extract of *A.altilis*

The data shown in Table 3 and Figure 1 gives results of the qualitative phytochemical screening of the crude extract of *A.altilis* fruit pulp using non-polar (Ethyl Acetate, Chloroform and Hexane) and polar (Methanol, Acetone and Aqueous) solvents based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes.Extracting adequate quantities of chemical compounds rely majorly on the type of solvent used during the extraction process; during this process, the solvents percolate into the matrix of the plant material where phytochemicals that are of the same polarity with the solvent are dissolved. The phytochemical screening shows the presence and absence of some phytochemicals determined.

Steroid, Terpenoids, Flavonoids, carbohydrates, oil and fat are present in all the non-polar solvents, except petroleum ether crude extract. However, phytochemicals such as phenol, saponins, tannin, alkaloid and anthraquinones were absent in the screening test of the non-polar extracts. Thus, petroleum ether, chloroform and ethyl acetate extracts of *A.altilis* fruit pulp were good sources of Steroid, Terpenoids, Flavonoids, carbohydrates, oil and resins. This may suggest that these solvents are

selective in the isolation of bioactive compounds due to their non-polar nature.

In the polar solvents, the presence of alkaloid, phenol and carbohydrates were evaluated in all except for tannins, anthraquinones, oil and fat. Steroid and saponins were absent in acetone extract. Flavanoids, steroid and terpenoids were present in the Ethanol extract. Flavanoids, steroid and terpenoids was absent where as saponins were present in the aqueous extract was observed in the phytochemical screening.

A.altilis fruit pulp extract was a fair source of saponins and alkaloids, whereas flavonoids, β-carotene and α-tocopherol were below detectable limits. The *Artocarpus* genus is known to produce a large number of secondary metabolites, and is specifically rich in phenylpropanoids such as flavonoids and flavones [23]. *Artocarpus altilis* (breadfruit) is no exception with over 130 compounds identified in various organs of the tree, more than 70 of which are derived from the phenylpropanoids pathway. Many of the isolated compounds have been found to exhibit biological activity including inhibition of platelet aggregation, anti-bacterial, anti-fungal, inhibition of leukaemia cells and as an anti-tumor agent. These data support the claim that the breadfruit tree may be an effective medicine with the potential to treat an assortment of medical conditions [24, 25].

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Alkaloids were observed to be present in the crude extract of all the polar solvent; it has been reported for its analgesic, antispasmodic and bactericidal, and antimalarial activities. Flavonoids were detected in all the non-polar solvents, which is following the same observation in the result obtained by Khanam et al. on the stem and root of E. longifolia using ethyl acetate, chloroform and methanol as solvent. Flavonoids belong to the group of polyphenolic compounds and are characteristically recognized for health promoting activities such as anti-inflammatory, anti-cancer, anti-allergic, antioxidant, and antimicrobial properties. They are commonly found in many plants; a positive correlation between ingestion of plants rich in flavonoids and reduced risk of cardiovascular diseases and cancer has been reported. Terpenoids have also been shown to possess antimicrobial activities [26].

The term phenolic compounds constitute widely distributed and one of the main groups of secondary metabolites. They consist of a wide range of plant substances which are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups. They are responsible for the major organoleptic characteristics of plant-derived foods and beverages, particularly colour and taste properties and they also contribute to the nutritional qualities of fruits and vegetables [27]. Phenolic compounds also have a wide range of pharmaceutical activities such as anti-

inflammatory, analgesic, antitumour, anti-HIV, antiseptic (Thymol), vasodilatory,immunostimulant and antiulcerogenic. Thus they may be used with therapeutic purposes [28].Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells [29] and steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune response [30].

3.1 QUANTITATIVE ANALYSIS

Depending on the above qualitative results the quantitative assay is carried out for Alkaloids, Flavanoids, Phenols, and steroids.

Table: 4 Quantitative analysis of phytochemical constituents (%w/w)

S.No	Phytoconstituents	Ethanolic extract
1	Phenol	11.16 ±0.13
2	Flavonoids	2.50 ±0.09
3	Steroids	1.21±0.05
4	Alkaloids	9.42±0.21

Note: Values of means of three independent determinations±SD

Several studies have reported variations in the phytochemical composition as well as the biological activities of extracts prepared using different extraction solvents [31-34], therefore, selection of appropriate solvent for extraction of phytochemicals from the plant is very essential based on some characteristics such as chemical properties of the analytes, matrix analyte interaction, sample matrix properties, efficiency and desired properties [35,36].

Artocarpus altilis fruit pulp ethanolic extract was found to possess phenols (11.16 ±0.13); alkaloids (9.42±0.21); flavonoids (2.50 ±0.09) and Steroid (1.21±0.05) is represented in Table.4 which is in the order of phenols >alkaloids> flavonoids >Steroid. The maceration with ethanol showed the highest TPC, TFC and TA in the extract.

Phenolic compounds are ubiquitous secondary metabolites in plants. They are known to have antioxidant activity and it is likely that the activity of these extracts is due to this compound [37, 38]. These results indicated the influence of the extraction solvent on the total content of phenolic compounds were high. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [39].Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherol etc.

Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colors of plants. In

addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities. Nowadays, flavonoids have attracted attention due to the discovery of their pharmacological activities[40].Limasset et al. (1993) reported that antioxidant properties, reactive oxygen species scavenging and cell function modulation of flavonoids could account for the large part of their pharmacological activity [41]. Polar extracts (ethanol, butanol and water extracts) showed more flavonoids than apolar extracts.

Consequently, *A.altilis* fruit pulp by its richness in different secondary metabolites may have several medical importances such as anti-tumor especially the ethanol extract due to the presence of flavonoids and antioxidant due to its richness in phenolic compounds.

The alkaloids begin to reduce and their concentrations weaken as the fruits mature and ripen. Environmental influences such as soil type, growing season, geographic location, and mineral status are known to impact intensities of plant secondary metabolites[42].Alkaloids have many medicinal properties such as cytotoxicity [43], analgesic [44], antispasmodic and antibacterial [45]. Alkaloids formed as metabolic byproducts and be responsible for the antibacterial activity [46].

Steroids have the fundamental structure of four carbon rings called the steroid nucleus [47]. The addition of different chemical groups at different positions on backbone leads to the formation of many different types of steroidal compounds including sex hormones progesterone and testosterone, the anti-inflammatory steroids like corticosteroids, cardiac steroids digoxin and digitoxin, animal steroid like cholesterol, steroidal glycosides[48,49]. Plant steroids synthesized by cyclisation of 2, 3-epoxysqualene into cycloartenol are further metabolized owing to the enzymatic conversion to produce biologically active steroid. Plant steroids possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiotonic activity [50].

4. Conclusion

The present study showed that ethanolic extract of *A.altilis* fruit pulp is rich in basic nutrients. Qualitative phytochemical screening showed that it is abundant in phytochemicals such as alkaloids, carbohydrates, saponins, flavonoids, phenols, and terpenoids. The quantitative analysis has revealed that ethanolic extract of *A.altilis* fruit pulp is very rich in phenols (11.16 ±0.13); alkaloids (9.42±0.21); flavonoids (2.50 ±0.09) and Steroid (1.21±0.05) which is in the order of phenols >alkaloids> flavonoids >Steroid which gives a very strong reason to select this plant for future evaluation of

cytotoxic intern anticancer and other pharmacological properties. Based on the results of investigations, *A.altilis* fruit pulp is a potent source of novel bioactive compounds. From the findings of the study, it may be concluded that the extracts contain diversity of phytochemicals in appreciable amount that can expertly keep the body against oxidative stress triggered by free radicals and therefore be used as a source of potent natural products. Further investigations regarding more biological activities of the ethanolic extract need to be conducted.

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