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A comparative analysis of anti oxidant properties of three varieties of *Annona* sp

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Abstract
Annona reticulate, *Annona squamosa* and *Annona muricata*, the three plants which come under family Annoneaceae, is reported to have antioxidant properties such as tannin, phenol, alkaloid, etc. This study is done for comparing the total tannin and total phenol content in the fruits of these three varieties. Analyses were carried out in the laboratory of Department of Environmental Sciences of the institution and the following results were observed. The total phenol content in *Annona muricata* is 2.646 mg/g, *Annona reticulate* is 1.932 mg/g and *Annona squamosa* is 2.304 mg/g. The total tannin content in *Annona muricata* is 0.659 mg/g, *Annona reticulate* is 0.032 mg/g and *Annona squamosa* is 1.646 mg/g. *Annona muricata* had the maximum phenol content whereas the tannin content was of lower level. *Annona squamosa* had both phenol and tannin content at a higher level.

Keywords: *Annona reticulate, Annona squamosa, Annona muricata,* Antioxidant, Phenol, Tannin

1. Introduction

Annona fruits are one of the world's best tasting fruits, due to the sweet, creamy, flesh and fragrant flavor when fully ripe. The flesh has a pleasant blend of sweetness and mild acidity with a consistency of baked custard. Because of this characteristic, Annona fruits are called custard apple or sugar apple and frequently confused with each other (Lizana and Reginato 1990) [27]. Out of more than 100 species of Annona, only 5 species, namely the custard apple, cherimoya, soursop, bullock's heart and atemoya are of major commercial importance. Seventeen genera of these are distributed in tropical areas, and only four genera, Annona, Rollinia, Uvaria and Asimina produce edible fruits (Ochse *et al.,* 1974) [34].

Generally it is recognized that Annonas are climacteric fruits. The fruits and leaves contain numerous nutritional, anti-nutritional and antioxidants such as vitamin C, vitamin A, etc., oxalate, phytate etc., and tannin, phenol etc. respectively. *Annona muricata* fruit contain 85.5% pulp, 3.3% seeds, 8.9% skin and 2.9% of a fleshy receptacle, (Paull, 1982) [37]. The soursop fruit taste is the result of the combination of sugars and organic acids (0.65–0.85%), (Morton, 1987) [31]. The fruits of *Annona muricata* are extensively used to prepare syrups, candies, beverages, ice creams and shakes.

Annona reticulata flesh is creamy yellow, rich and sweet, with low acidity. However, Wu Leung and Flores, (1961) [49] indicated that the flavor of custard apple is not considered comparable to that of cherimoya or sweetsop, although the physiology seems to be very similar to that of the other Annona fruits. The custard apple edible pulp contained 18.7 g/100 g of carbohydrates and 45% of which 78.6 g/100 g is water, (Wu Leung and Flores, 1961) [49]. There is a thick, cream-white layer of custard-like flesh beneath the skin. Sugar apple was categorized as having very high antioxidant activity i.e >70mmol/100g edible part. Many studies including conducted in India, (Noichinda *et al.,* 2009; Kaur and Kapoor 2005) [33, 24] showed that extracts of *Annona squamosa, Annona cherimola* and *Annona muricata* have high anti-oxidant activity.

Annona squamosa pulp is slightly granular, creamy yellow or white, sweet with a good flavor and low acidity (Mowry *et al.,* 1941) [32]. It is considered the sweetest of the annona fruits (FAO, 1990) [15]. Unripe and dried Fruits are used as anti dysenteric. Despite the medicinal property of the fruits and reasonable taste, the species has not completely come into common use like many other fruits like jack, mangoes etc. Flowering and fruit setting of this plant occurs at hot dry climate.

Even though there are a lot of medicinal values for this fruit, the consumption and movement in the market is less.

Mattson and Cheng (2006) [30] states that antioxidants are compounds that protect cells from damaging effects of reactive oxygen species which results in oxidative stress leading to cellular damage. Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Uddin *et al.,* 2008; Jayasri *et al.,* 2009) [47, 22].

Antioxidants exert their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a fairly 'stable radical'. The free radicals are metastable chemical species, which tend to trap electrons from the molecules in the immediate surroundings. These radicals if not scavenged effectively in time, they may damage crucial bio molecules like lipids, proteins including those present in all membranes, mitochondria and, the DNA resulting in abnormalities leading to disease conditions (Uddin *et al.,* 2008) [47]. Thus, free radicals are involved in a number of diseases including: tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases, AIDS and even early senescence (Chen *et al.,* 2006; Uddin *et al.,* 2008) [14, 47]. Plants contain extensive diversity of free radicals including phenols, flavonoids, vitamins; terpenoids are rich in antioxidant activity (Madsen & Bertelsen, *et al.,* 1995; Cai and Sun 2003) [28, 12]. Phytochemicals provide protection to the plants agaist several diseases as they have antioxidant properties, (Hertog and Feskens 1993; Anderson and Teuber *et al.,* 2001) [20, 6].

Phenolics, phenols or polyphenolics are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. They are very important to plants and have multiple functions. The significant role of plant are defence against pathogens, herbivore and predators, and thus are applied in the control of human pathogenic infections (Puupponen *et al.,* 2008) [38]. Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents.

Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e. to convert things into leather. They are acidic in reaction and the acidic reaction is attributed to the presence of phenolics or carboxylic group. They form complexes with proteins, carbohydrates, gelatin and alkaloids. Tannins are divided into hydrolysable tannins and condensed tannins. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Tannin rich medicinal plants are used as healing agents in a number of diseases.

This piece of work looks for the content of phenol and tannins as antioxidants in three Annona speices.

2. Materials and methods

2.1 Study Material

Three species of fruits of the family Annoneacea were selected for the study: *Annona reticulate*, *Annona squamosa* and *Annona muricata.*

Plate 1: *Annona reticulate*

Plate 2: *Annona squamosal*

Plate 3: *Annona muricata*

Taxonomical information of selected plants

Annona muricata

Kingdom : Plantae
Order : Magnoliales
Family : Annonaceae
Genus : Annona
Species : Muricata
Vernacular names:
English: Sour soup, prickly custard apple

Annona reticulate

Kingdom : plantae
Order : Magnoliales
Family : Annonaceae
Genus : Annona
Species : reticulate
Vernacular names:
English : Custard apple, wild sweet sop

Annona squamosa

Kingdom : Plantae
Order : Magnoliales
Family : Annonaceae
Genus : Annona
Species : Squamosa
Vernacular names
English: Custard apple, sugar- apple, sweetsop

2.2 Collection and preparation of sample

The fresh samples of three varieties of Annona fruits were collected from local market of Attingal, Trivandrum district (Kerala state). Well matured slightly yellow to green coloured fruits were selected which were free from blemishes and mechanical injuries. Fruits were washed under running tap water, hand-peeled, cored, and deseeded and the pulp was macerated. One gram of the fruit pulp was used for analyses.

3. Analysis of antioxidants

3.1 Determination of Tannin

One gram of the sample was extracted using 50 % methanol. It was mixed occasionally by swirling. After 20-28 hours, the mixture was centrifuged and the supernatant was collected. One ml of the supernatant was pipette out. Vanillin hydrochloride reagent was quickly added to the reaction mixture. Absorbance was read in a spectrophotometer (UV-1800 Shimadzu) at 500 nm after 20 minutes. A blank was prepared with vanilllin hydrochloride reagent alone. Result was calculated by preparing a standard graph with 20-100µg catechin using the diluted stock solution.

3.2 Determination of Phenol

One gram of the sample was reflexed it in 80% methanol for 20 minutes. It was ground thoroughly and filtrate was collected. Then the filtrate was subjected to centrifugation at 1000 rpm for 10 minutes, and the supernatant was collected. Then it was makeup to a known volume by using methanol. After that an aliquot of 0.1 ml is taken. Again it was makeup to 3 ml using methanol. Then 0.5 ml of Folin reagent is added. At last 2 ml 20% of Na_2CO_3 is added and keep in a boiling water bath for 5 minutes. Finally a white precipitate was formed. The mixture was centrifuged at 5000 rpm for 5 minutes. Absorbance was read in a spectrophotometer (UV-1800 Shimadzu) at 650 nm after 20 minutes. A blank was prepared with Folin reagent alone.

3.3 Phenol Content

The total phenol content was determined according to the method of Singleton et al., (1999) [43]. The results of determination of phenol are given in the table (Table 1). The total phenol content in 1gm of ground sample of *Annona muricata* is 2.646 ± 0.002 mg/g, *Annona reticulate* is 1.932 ± 0.002 mg/g and *Annona squamosa* is 2.304 ± 0.001 mg/g. It is found that *Annona muricata* have high amount of phenol content compared to *Annona reticulate* and *Annona squamosa*, and *Annona reticulate* have less amount of phenol content compared to *Annona muricata* and *Annona squamosa*.

As per Adefegha and Oboh, (2012) [2] in *Annona muricata*, polyphenolic compounds have shown antioxidant properties by reduction of Fe^{3+} to Fe^{2+}, chelation of Fe, and mopping of radicals. Recently, polyphenolic compounds have become subjects of interest because of their beneficial effects on human health (Ademiluyi *et al*., 2015) [3]. Numerous studies have shown that majority of the antioxidant activity of plants food is from phenolic compounds. Lima de Olivera *et al.,* (1994) [26] reported that this physiopathy in soursop had occurred by the oxidation of phenolic compounds because of polyphenoloxidase (PPO) activity. In *Annona squamosa* several studies reported the relationships between phenolic content and antioxidant activity; some authors found a high correlation between the phenolic content and the antioxidant activity (Kuskoski *et al*., 2005; Mahattanatawee *et al*., 2006; Reddy *et al*., 2010; Silva *et al*., 2007; Thaipong *et al.,* 2006) [25, 29, 39, 45, 46]. In *Annona reticulate* there are many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain types of cancer in which polyphenol is linked to the antioxidant properties (Barros *et al*., 2007; Jagadish *et al*., 2009) [8, 21]. Barreca *et al.,* (2011) [7] reported polyphenol content in Annona reticulata, which did not coincide with the data of total polyphenolic content found in our experiments for control fruits, because the former authors quantified EP (Extractable Polyphenols) only.

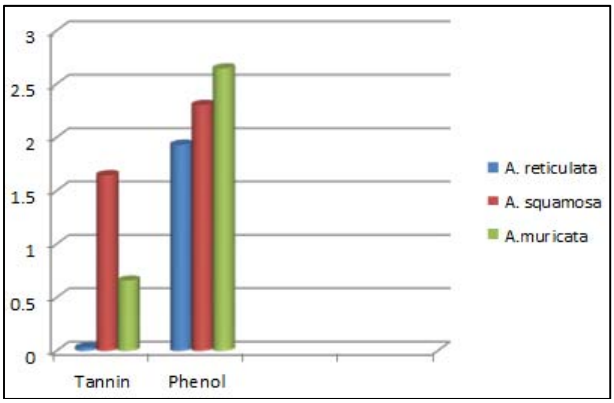
3.4 Tannin Content

The total tannin content in 1gm of ground sample of *Annona muricata* is 0.659 ± 0.001 mg/g, *Annona reticulate* is 0.032 ± 0.002 mg/g and *Annona squamosa* is 1.646 ± 0.002 mg/g (Table 1), Tannin content was greatest in *Annona squamosa* compared to *Annona reticulate* and *Annona muricata*, and *Annona reticulate* have less amount of phenol content compared to *Annona muricata* and *Annona squamosa.*

In *Annona squamosa*, the antibacterial activity of the plant extracts might be attributed to the presence bioactive plant compounds such as tannins, phenolic compounds, polyphenols and flavonoids (Ouattara *et al*., 2011) [36]. Among these bioactive compounds, Fernandez *et al.* (1996) [16], Shoko *et al.,* (1999) [42] and Baydar *et al.,* (2004) [9] confirmed that phenolics were the most important active compounds against bacteria. Adetuyi *et al.,* (2010) [4] reported that *Annona muricata* contain antioxidants such as polyphenols, tannins, and ascorbic acid. Adetuyi *et al.,* (2010) [4] found that the decrease of tannins during ripening of fruit was caused by PPO (polyphenoloxidase), which turned tannins into simple phenols. The phytochemical study showed presence of terpenes and steroids in petroleum ether extract, alkaloids and flavonoids in ethyl acetate extract while tannins, flavonoids and glycosides were observed in methanol extract (Bhalke and Chavan 2011) [10]. According Sousa *et al.,* (2007) [45] in *Annona reticulata* the phenolic compounds are distributed in the following categories: Simple phenolics, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, hydrolysable and condensed tannins, stilbenes, lignans, and lignins. Phytochemical analysis of the plant revealed the presence of tannins, steroids and cardiac glycosides which are the major phytochemical compounds, (Gajalakshmi *et al.,* 2012) [17].

Table 1: Phenol and Tannin content of the three species of Annona

Sl No.	Antioxidants	*Annona reticulata*	*Annona squamosa*	*Annona muricata*
1	phenol	1.932 ± 0.002	2.304 ± 0.001	2.646 ± 0.002
2	Tannin	0.032 ± 0.002	1.646 ± 0.002	0.659 ± 0.001



4. Conclusion

Three different species of Annonacea family have taken for the study for finding out the antioxidant properties of each fruit. According to Jiménez *et al.,* (2014) [23] *Annona muricata* fruit have shown the presence of various phytoconstituents and compounds, including phenolics. Tannin content was determined using the method of Harborne. According to Onimawo, (2002) [35] tannins have been studied especially for their potential of anti-parasitic, anti-rheumatic, astringent and emetic effect and anti-hyperglycemic property. According to Shivprasad *et al.,* (2012) [41] tannins are anti-nutrients factor that inhibit the activities of digestive enzymes and the implication of which is some dietary diseases. The pericarp of the fruit has phenol content (Saravanan and Parimelazhagan 2014, Ademiluyi *et al.,* 2015) [40, 3] In *Annona muricata* fruit Polyphenolic compounds have shown antioxidant properties (Adefegha and Oboh, 2012) [2].

It is worth noting that the higher phenolic contents in the pericarp compared to the pulp and seed of the *Annona muricata* fruit could be due to the fact that the pericarp is more exposed to the environmental stress factors such as ultraviolet ray from the sunlight (Soumaya *et al.,* 2013) [44]. Stress factors provoke intense synthesis of phenolic compounds in the plant in order to prevent oxidative damage where the stress factors could present to the plant cellular structures (Chanwitheesuk *et al.,* 2005) [13] unlike the pulp and seed that are protected by the edible portion of the *Annona muricata* and therefore have less exposure to such stress factors. However, the values obtained for the extracts are lower than what was reported in some edible plant obtained in Iran and India (Aberoumand *et al.,* 2010) [1] but higher than phenolics content in some selected tropical fruits from Malaysia (Alothman *et al.,* 2009). In the Taiwanese study (Chen *et al.,* 2006) [14], the antioxidant activity in mature *Annona squamosa* fruits of 36 species and varieties produced in Taiwan was analyzed by the ferric reducing antioxidant power (FRAP) assay. They found that, sugar apple was having very high antioxidant activity i.e > 70mmol/100g edible part. Many studies including conducted in India (Noichinda *et al.,* 2009; Kaur and Kapoor 2005) [33, 24] showed that extracts of *Annona squamosa, Annona cherimola* and *Annona muricata* have high anti-oxidant activity.

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