iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** IX **Month of publication:** September 2017

DOI:

www.ijraset.com

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue IX, September 2017- Available at www.ijraset.com

Anti-Nutritional a factors in Three Varieties of Annona Species

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Abstract: ***Annona reticulata, Annona squamosa and Annona muricata, the three plants which come under family Annoneaceae, is reported to have anti nutritional properties such as oxalate, phytate, flavonoid, tannin, phenol, and alkaloid. This studyis doneto analyze the anti nutritional 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 reticulatais 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 reticulatais 0.032 mg/g and Annona squamosa is 1.646 mg/g.The total oxalate content in Annona muricata is 0.065 mg/g, Annona reticulatais 1.013 mg/g and Annona squamosa is 0.023mg/g.The total phytate content in Annona muricata is 0.027 mg/g, Annona reticulatais 0.012 mg/g and Annona squamosa is 0.013 mg/g.The total flavoniod content in Annona muricata is 0.093 mg/g, Annona reticulatais 0.053 mg/g and Annona squamosa is 0.030 mg/g. The total alkaloid content in Annona muricata is 1.001 mg/g, Annona reticulatais 0.047 mg/g andAnnona squamosa is 0.012mg/g.***

Keywords: ***Annona reticulata, Annona squamosa, Annona muricata, anti nutritional, oxalate, phytate, flavonoid, tannin, phenol, and alkaloid***

I. INTRODUCTION

Plants produce a series of anti-nutritional components as secondary metabolites. These compounds are useful to these plants in defense mechanisms although they diminish the nutritional qualities of plants. But the anti-nutritional factors are found to have effect on gastrointestinal tract and affect the microflora count of the intestine by promoting the growth of beneficial bacteria's. Anti-nutritional factors are chemical compounds synthesized within natural food or feedstuffs by the normal metabolism of species and by different mechanisms.Such chemical compounds, are frequently, but not exclusively associated with foods and feeding stuffs of plant origin. These anti-nutritional factors are also known as 'secondary metabolites' in plants. They are biologically active. These secondary metabolites are secondary compound produced as side products of processes leading to the synthesis of primary metabolites. One of the major factor limiting the wider food utilization of many tropical plants is, the ubiquitous occurrence in them of a diverse range of natural compounds capable of precipitating deleterious effects in man, and animals compound which act to reduce nutrient utilization and/or food intake are often referred to as anti-nutritional factors, (Shanthakumari *et al.*,2008).Anti-nutrients are chemicals evolved by plants for their own defense. Among other biological functions, it reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value. Some chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed at appropriate amounts, (Ugwu *et al.*,2006).To avoid predation, sedentary species (plants, fungi and bacteria) synthesize a range of low and high molecular weight compounds. These secondary metabolites play a role in defense against herbivorous, insects, pathogens or adverse growing conditions, (Harbourne 1983)

II. MATERIALS AND METHODS

A. Study Material

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

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue IX, September 2017- Available at www.ijraset.com



Plate 1: Annona reticulataPlate 2: Annona squamosal Plate 3: Annona muricata

B. Taxonomical information of selected plants

1) Annona muricata

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

2) Annona reticulata

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

3) Annona squamosa

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

III. COLLECTION AND PREPARATION OF SAMPLE

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

IV. ANALYSIS OF ANTINUTRITIONS

A. Determination of oxalate

One gram of sample was extracted twice with 0.25 N HCL in a water bath (60ºC) for one hour each. The centrifuge was collected in a conical flask (around 40 mL). Added 5mL tungsto-phosphoric acid and mixed well. It was kept overnight and centrifuged. The centrifuge was collected and neutralized with ammonia solution (1:1 diluted). Calcium oxalate was prepared by adding 5mL acetate buffer containing calcium chloride (pH 4.5). It was also kept overnight, centrifuged and washed twice with wash liquid(6mL each, pre cooled). The precipitate was then dissolved in 10 to 15 mL hot 2 N Sulphuric acids and transferred to a 100mL conical flask and titrated against 0.1N KMnO4 solution at 60ºC.

B. 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.

C. 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.

D. Estimation of alkaloid

A total 0f 25mL of 20% acetic acid was added to 1g of samplestaken in a separate 100mL beaker and was covered to stand for 4 hours. The mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample concentrated ammonium hydroxide solution was added drop wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighted. The percentage of total alkaloid content was calculated.

E. Estimation of flavonoid

The total flavonoid content (mg/mL) was determined using $AlCl_3$ method. The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL of distilled water and 0.3 mL of 5% $NaNO_2$ was incubated for 5 minutes at 25°C. This was followed by the addition of 0.3 mL of 10% of $AlCl_3$ immediately. 2 mL of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

F. Estimation of Phytate

Two grams of sampleswere weighed into 250 mL conical flasks. 100 mL of 2% concentrated hydrochloric acid was used to soak the sample in the conical flask for 3 h and then the solution was filtered through double layered hardened filter papers. 50 mL of filtrate was placed in 250 mL beaker and 100 mL of distilled water was added to give proper acidity. 10 mL of 0.3% ammonium thiocyanate solution was added as indicator. Each solution was titrated with standard iron chloride solution, which contained 0.00195 g iron per mL. The end point colour was slightly brownish - yellow which persisted for 5 min. The percentage phytic acid was calculated.

V. RESULT AND DISCUSSION

A. Alkaloid

The Alkaloid content in *Annona muricata* is 1.001 mg/g, in *Annonareticulata* is 0.047 mg/g and *Annonasquamosa* is 0. 0.012 mg/g. *Annona muricata* have the highest alkaloid content (Figure 1).

According to Radji *et al.,* (2015) antimicrobial bioactivity of *Annona muricata* extracts is attributed to flavonoids, steroids and alkaloids present in the plant extracts). The mechanism of action is probably due to a synergism of these compounds. It has been reported that some alkaloids have the ability to bind with DNA of microorganisms and inhibit RNA synthesis (Roger *et al.,* 2015), and have shown antimicrobial activity by glycosidase inhibition (Mohanty *et al.,* 2008). The other compounds such as anthocyanin, alkaloids and tannins have been studied especially for their potential of anti-parasitic, anti-rheumatic, astringent and emetic effect and anti-hyperglycemic property. There are two previous reviews of the literature on alkaloids from the *Annona*ceae. The first was published by Le Boeuf *et al.*(1982) and the second by Saito (1990).

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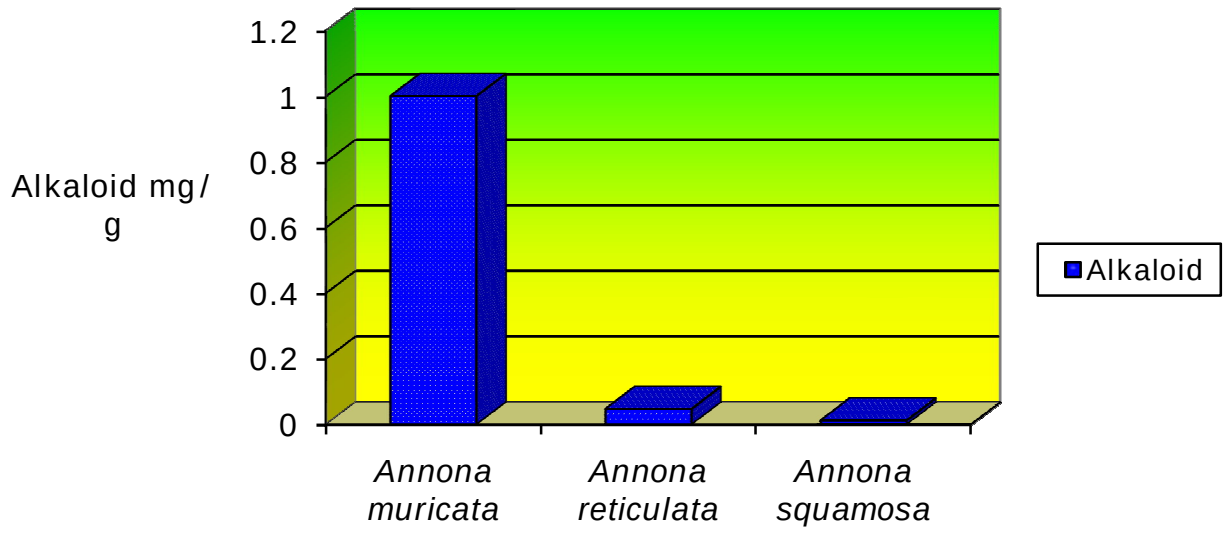

Figure 1: Alkaloid

B. Flavonoid

The Flavonoid content in *Annona muricata* is 0.093mg/g, in *Annonareticulata* is 0.053 mg/g and *Annonasquamosa* is 0.030 mg/g (Figure 2). *Annona muricata* have the highest flavonoid content. It has also been reported that flavonoids act by inhibiting both cytoplasmic membrane function and DNA synthesis, such as quercetin that binds to GyrB subunit of *E. coli* DNA gyrase and inhibits the enzyme ATPase activity, (Radji *et al.*, 2015). The presence of flavonoids in the soursop pulp and drink is desirable. Flavonoids are large group of compounds widely distributed in plant foods. They have antioxidant properties to protect the body against cardiovascular diseases and some form of cancer.

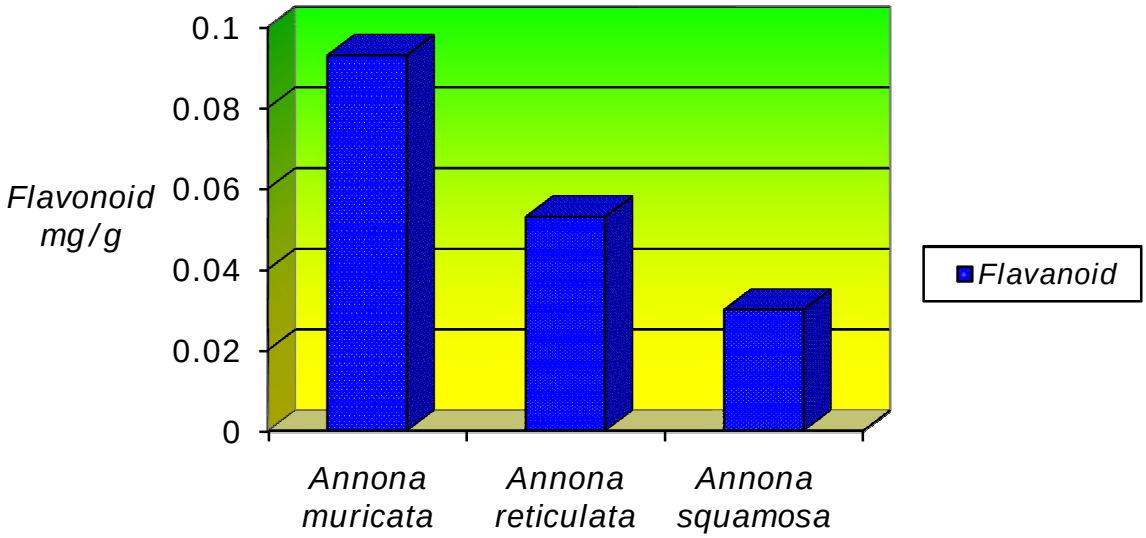

Figure 2: Flavonid

C. Phenol

Among these bioactive compounds, Fernandez et al., (1996), Shoko et al., (1999) and Baydar et al., (2004) confirmed that phenolics were the most important activecompounds against bacteria.

The total phenol content was determined according to the method of Singleton et al., (1999). The results of determination of phenol are given in the figure 3. The total phenol content in 1gm of ground sample of Annonamuricata is 2.646 mg/g, Annona reticulata is 1.932 mg/g and Annona squamosa is 2.304 mg/g. It is found that Annona muricata have high amount of phenol content compared to Annona reticulata and Annonasquamosa, and Annona reticulata have less amount of phenol content compared to Annona muricata and Annonasquamosa. As per Adefegha and Oboh, (2012) 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). Numerous studies have shown that majority of the antioxidant activity of plants food is from phenolic compounds. Lima de Olivera etal., (1994) reported that this physiopathy in soursop had occurred by the oxidation of phenolic compounds because of polyphenoloxidase (PPO) activity. In Annona squamosaseveral 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). In Annona reticulata 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). Barreca et al., (2011) 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.

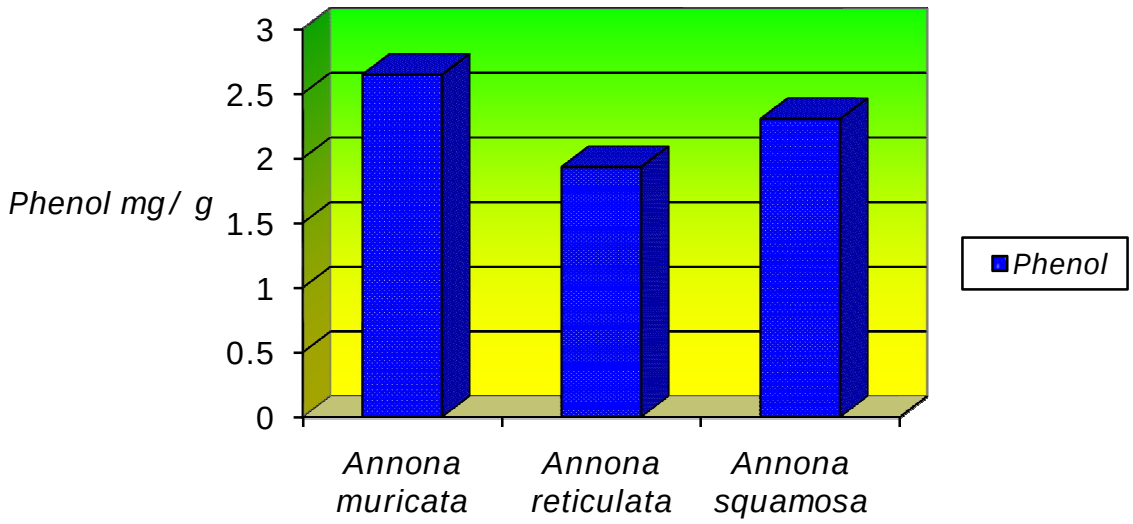

Figure 3: Phenol

D. Phytate

The Phytate content in *Annonamuricata* is 0.027 mg/g, in *Annonareticulata* is 0.012 mg/g and *Annonasquamosa* is 0.013 mg/g (figure 4). Anti-nutritional factor such as oxalate and phytate are also present in *Annona muricata*, (Dahouenon-Ahoussi *et al.*, 2012). Phytate diet of 1 to 6% over a long period decreases the bioavailability of mineral elements in monogastric animals. Phytic acid can bind to mineral elements such as calcium, zinc, manganese, iron and magnesium to form complexes that are indigestible, thereby decreasing the bioavailability of the element for absorption, (Erdman, 1979). Phytic acid also has a negative effect on amino acid digestibility, (Merken and Beecher 1998). The phytate, is capable of chelating divalent cationic minerals, like Ca^{2+}, Fe^{2+}, Mg^{2+}, and Zn^{2+} thereby inducing dietary deficiency. Several workers have attributed the incidence of several mineral deficiency symptoms

in animals to the occurrence of phytate in seeds, (Balogun and Fetuga, 1988). Despite some of the drawback, high phytate content in the body had been known to enhance the activity of natural killer cells and inhibit tumor growth.

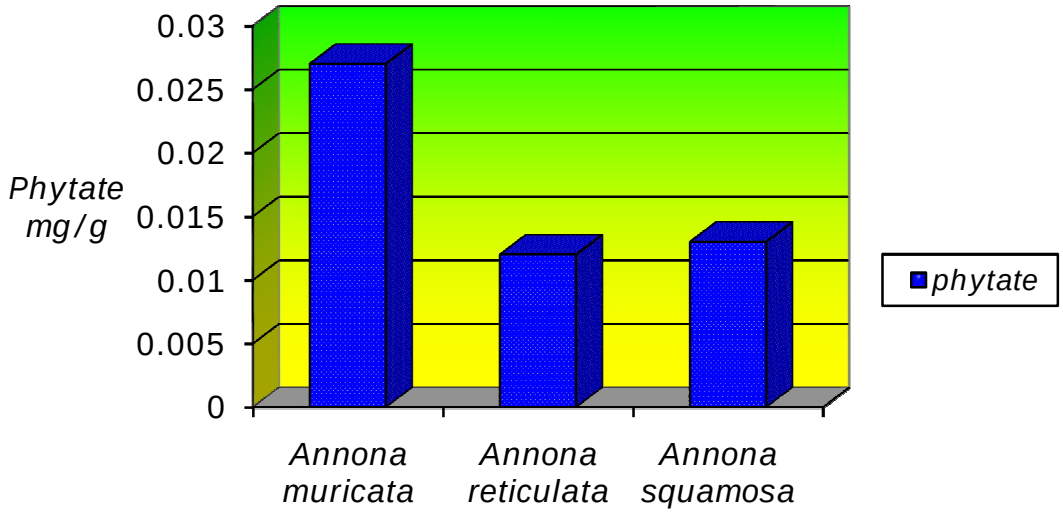

Figure 4 : Phytate

E. Total Oxalate

The Oxalate content in Annona muricata is 0.065 mg/g, in Annona reticulata is 1.013 mg/g and Annona squamosa is 0.023 mg/g (figure 5). Annona reticulata have the highest oxalate content. According to Oke (1969), oxalate have the ability to bind calcium present in food, thereby rendering calcium occupied for normal physiological and biochemical role such as the maintenance of strong bone, teeth, cofactor in enzymatic reaction, nerve impulse transmission and as clotting factor in the blood. The calcium oxalate, which is insoluble, may also precipitate around soft tissues such as kidney, causing kidney stones. Anti-nutritional factor such as oxalate and phytate are also present in the Annona muricata (Dahouenon-Ahoussi et al., 2012).

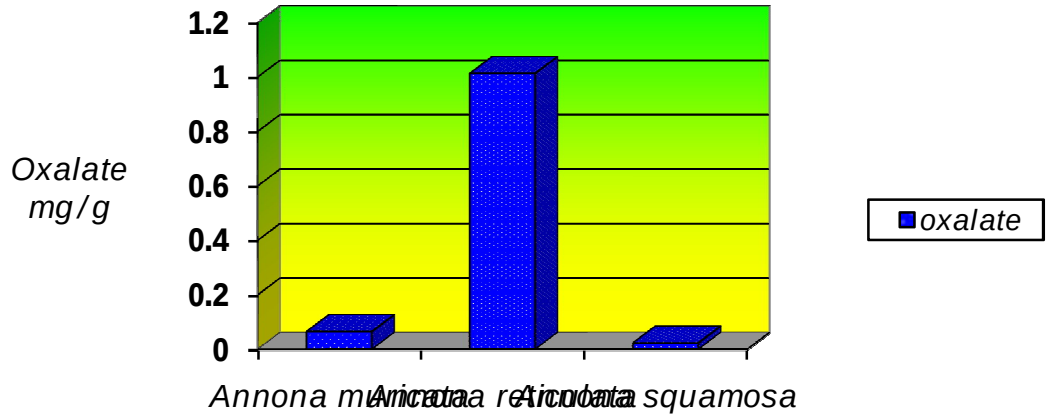

Figure 5: Oxalate

F. Tannin

The total tannin content in 1gm of ground sample of *Annona muricata* is 0.659 mg/g, *Annona reticulata* is 0.032 mg/g and *Annona squamosa* is 1.646 mg/g (figure 6). Tannin content was greatest in *Annona squamosa* compared to *Annona reticulata* and *Annona muricata*. In *Annona squamosa*, the antibacterial activity of the plantextracts might be attributed to the presence bioactive

plantcompounds such as tannins, phenolic compounds,polyphenols and flavonoids (Ouattara *et al*., 2011).Adetuyi *et al*., (2010) reported that *Annona muricata* contain antioxidants such aspolyphenols, tannins, and ascorbic acid.

Adetuyi *et al*., (2010) found that the decrease of tannins during ripeningof fruit was caused by PPO (polyphenoloxidase), whichturned tannins into simple phenols. The phytochemicalstudy showed presence of terpenes and steroids in petroleumether extract, alkaloids and flavonoids in ethyl acetateextract while tannins, flavonoids and glycosides wereobserved in methanol extract (Bhalke and Chavan 2011).According Sousa *et al*., (2007) in *Annona reticulata* thephenolic compounds are distributed in the followingcategories: Simple phenolics, phenolic acids (benzoic andcinnamic acid derivatives), coumarins, flavonoids,hydrolysable and condensed tannins, stilbenes, lignans, andlignins. Phytochemical analysis of the plant revealed thepresence of tannins, steroids and cardiac glycosides whichare the major phytochemical compounds, (Gajalakshmi *et al*., 2012)

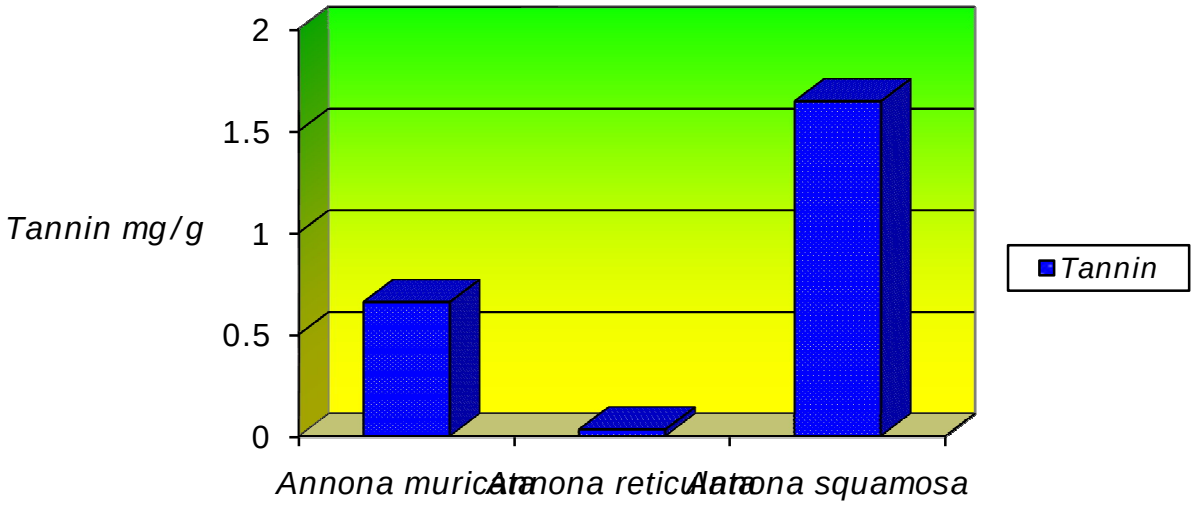

Figure6: Tannin

Sl no.	Anti nutritional	Annona muricata	Annona reticulata	Annona squamosa
1	Phenol	2.646	1.932	2.304
2	Phytate	0.027	0.012	0.013
3	Oxalate	0.065	1.013	0.023
4	Alkaloid	1.001	0.047	0.012
5	Tannin	0.659	0.032	1.646
6	Flavonoid	0.093	0.053	0.030

VI. CONCLUSION

The Mother Nature has provided us with an enormous count of flora and fauna. Annona reticulata is the best example of it. Thisstudy shows thatAnnona reticulata, is an important medicinal plant with diverse pharmacological spectrum. Some chemical constituent are isolated from the Annona reticulata showed anti-cancer, properties for bladder cancer and various cancer cell lines also. It's found to be a chemo preventive agent in cancer therapy. Further assessment is needed to be carried out on Annona reticulata in order to explore concealed areas and their practical clinical application, which can be used for the welfare of the mankind**.** Annona muricata is a coveted tropical tree, and a wealth of phytochemical investigations has been conducted for this fruit plant. In addition to being an important source for the food industry and an indigenous medicinal plant, Annona muricata is proven to possess a wide spectrum of biological activities. Among all former studies on Annona, the most promising activities are found to be its anticancer, ant parasitic and insecticidal activity. Because the majority of the previous studies were focused on the biological activities of the plant extract, further investigations on the biochemical and physiological functions of active compounds and the detailed mechanisms underlying these activities are completely pivotal for the development of pharmaceutical and agricultural products. Custard apple or the sugar apple is the fruit of Annona squamosa, which is one of the most widely grown species of Annona. The fruit pulp has shown frequent medicinal properties which include antioxidant, anti-diabetic, anti-infective and anti

dyslipidemic properties. Still the pulp of the fruit is not very easy for intake. There are a variety of recipes to overcome the hitch and increase intake.

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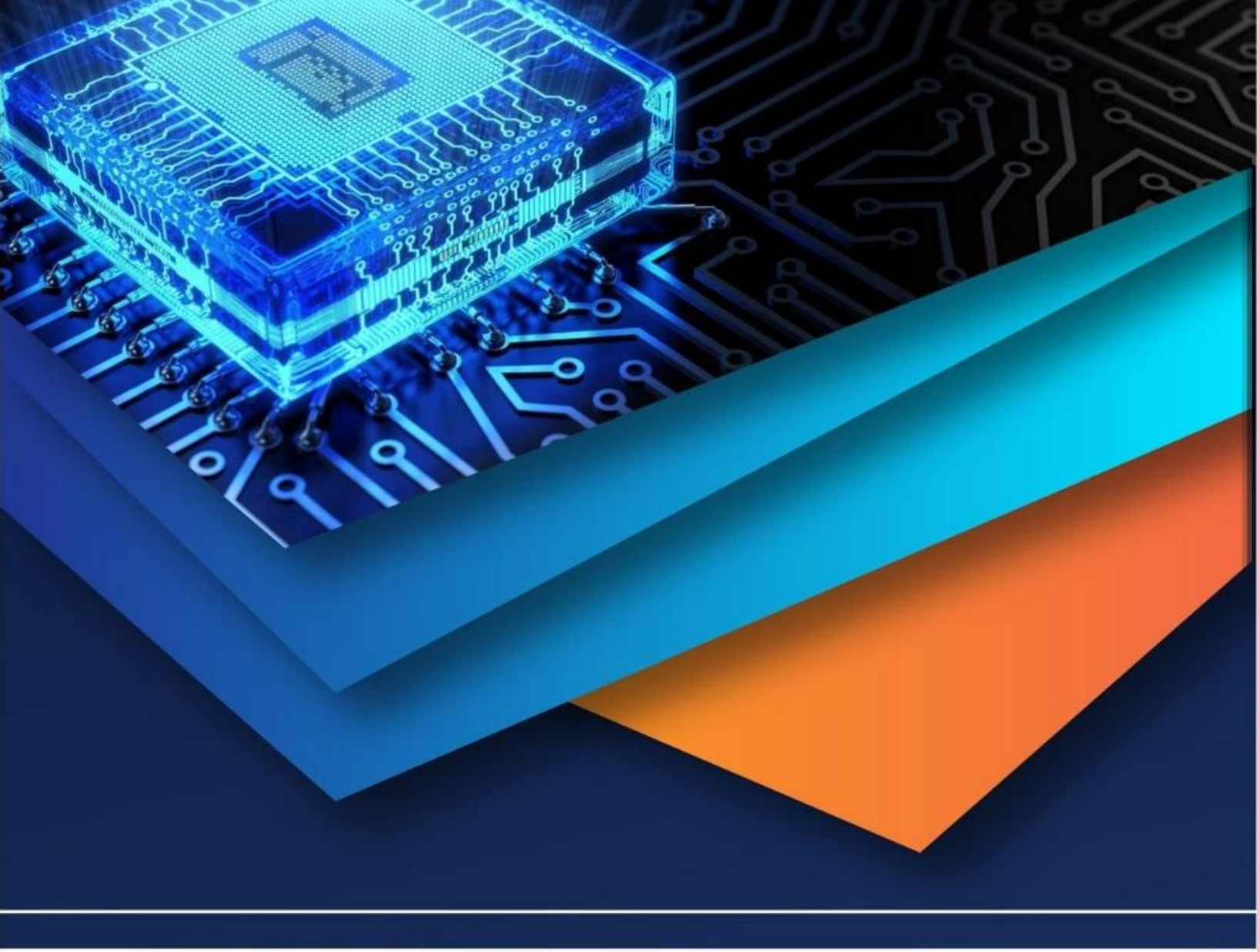

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