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Evaluation of major phytochemical constituents of two edible fruit yielding species of Annonaceae: *Annona muricata* L. and *Annona reticulata* L.

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Abstract

The fruits of *Annona muricata* Linn. and *Annona reticulata* Linn. have been widely used as a potential source of edibles. In addition to that, they are also a valuable source of medicine to cure various diseases. The present study is mainly intended to evaluate some major phytochemical constituents which are present in the fruits of *Annona muricata* Linn. and *Annona reticulata* Linn. The results obtained during the study also highlights the value of these species in the field of pharmacology to develop new drugs.

Keywords: Phytochemical constituents, Edible fruits, *A. muricata* L., *A. reticulata* L.

Introduction

Traditional system of medicine consists of large number of plants with various medicinal and pharmacological applications and hence represents a priceless tank of new bioactive molecules. During past several years, there has been growing interest among the usage of various underutilized fruits as medicines in traditional system of medicine for the treatment of various ailments for human health (Singh *et al.*, 2014) [1]. In India drugs of herbal origin have been used in traditional system of medicines such as Ayurveda, Unani, Siddha, Yoga and Neuropathy. The drugs are derived from the whole plant or from different parts of plant and their products has been of importance in health care practices. Such active or natural compounds from fruits, vegetables and other plant materials for medicinal purposes is dependent on the ways they are extracted from natural products and the presence of antioxidants (Daniel, 2002) [2].

Plant based bioactive compounds are gaining attention due to their multi functional, therapeutic properties and for overall safety. The most convincing evidence for protective benefits is attributed to their antioxidant property or multiple activities against free radicals. Free radicals induce oxidative damage to lipids, proteins and nucleic acids which eventually causes atherosclerosis, ageing, diabetes, cancer, AIDS, and several degenerative diseases in humans are well documented (Halliwell, 1994; Maxwell, 1997) [3, 4]. Antioxidants from natural sources are preferred to use in food or medicine to replace synthetic ones, which are being restricted due to their carcinogenicity (Velioglu *et al.*, 1998) [5]. Antioxidants are rich in fruits, vegetables and other plant serve as nutraceuticals that alleviate the oxidative stress and there for prevent or reduce the risk of degenerative diseases (Kitts *et al.*, 2000) [6].

Nutraceutical property of fruits are attributed to the presence of phenolics, flavanoids and pigments (Kalyani and Kamaruz, 2013) [7]. Specifically, fruits are low in calories and fat with many anti-oxidants. They are the source of simple sugars, fiber, vitamins and plenty of soluble dietary fibers which are essential for optimizing our health (Forgacs *et al.*, 2010) [8]. The present study on characterisation of biochemical constituents of the fruits of *Annona muricata* Linn. and *Annona reticulata* Linn., which are the major two potential edible fruits yielding trees.

Materials and Methods

Sample collection

Fresh and healthy fruits of *Annona muricata* Linn. and *Annona reticulata* Linn. were procured from homestead gardens of Kuravilangad, Kottayam district.

Their correct identity were confirmed with the help of available Floras and Literature (Gamble, and Fischer, 1915-1936; Sasidharan, 2004)^[9, 10] (Fig.1).

Analytical approach

Preliminary phytochemical analysis of the methanolic and aqueous extract of two fruits were screened to detect the occurrence of different chemical groups such as alkaloids (Evans and Wagner, 2002)^[11] and phenols (Harborne and Alato, 1973)^[12]. Similarly the presence of protein, carbohydrate, saponin, flavanoid, terpinoid, tannin were also identified by using the same extract (Lowry, 1951; Pearson, 1976)^[13, 14]. The chemical tests were carried out for each group of chemicals by using standard reagents. Express the result in terms of catechol or any other phenol equivalent used as standard (Malick and Singh, 1982)^[15].

Analysis of Antibacterial activity

Microbial strains used for the study

The microbial strains which are used for the study are *Staphylococcus*, and *Bacillus*, (gram positive) along with *E. Coli*, *Proteus*, and *Klebsiella*. (gram-negative). Their cultural characteristics and morphological features were confirmed before experimentation. The test organisms were maintained in nutrient agar slants.

Sample preparation

Sample for antibacterial activity was prepared by adding 1g of the fruit powder of both *A. muricata* Linn. and *A. reticulata* Linn. was taken in 10 ml ethyl alcohol and placed in a boiling water bath for half an hour and then filtered using Whatman No.2 filter paper. This extract was subjected for antibacterial screening.

Preparation of inoculum

The suspension of all organisms were prepared by inoculating one colony of the strain in 30 mL of Muller Hinton broth in conical flask and incubated at 37 °C for 24 hours to activate the strain. Muller Hinton agar (Hi Media) was prepared for the study.

Screening of Antibacterial activity

Screening for antibacterial activity of dried fruit powder was carried out against various bacterial organisms using disc diffusion method. Muller Hinton agar medium prepared was poured into the sterile petriplates and allowed to solidify. The crude extracts of both *Annona muricata* Linn. and *Annona reticulata* Linn. were used to screen against different organisms. Plates were allowed to incubate at 37 °C for 24 hours. After 24 hours, the zone of inhibition was measured.



Fig 1: Images of Fruits and seeds of *Annona muricata* L. and *Annona reticulata* L.

Results and Discussion

Physical measurement

The weight of *Annona reticulata* Linn. was ranges between 200-250 g where, *Annona muricata* Linn. were 350-500 g. The size of both the fruits changes depend on its developmental stages. The size of *A. muricata* ranges between 7-12 cm and for *A. reticulata* were 4 - 8 cm. Compared to *A.reticulata* Linn. the weight and size of *A.muricata* Linn. were more.

Qualitative estimation

Preliminary qualitative investigation conducted with both aqueous and methanolic extracts of shade dried fruit powder of *Annona muricata* Linn. showed the presence of certain metabolites, like saponins, terpinoids, phenolics, tannins, carbohydrates, alkaloids, flavanoids and proteins. The methanolic extracts of *A.muricata* Linn. showed the presence of saponin, terpinoid, tannin, alkaloid, protein etc, where phenol and flavanoids were absent. While in case of *A. reticulata* Linn. test for terpinoid, phenol, tannin, carbohydrate, alkaloid, and protein gave the positive result. But saponin and flavanoids were absent (Table-1).

Quantitative estimation

The quantitative estimation was carried out by estimating the total quantity of, protein, phenol, sugars and reducing sugars, ascorbic acid, oxalate and alkaloids (mg/gm tissue). The detailed result of each has given in below (Table-2).

Total protein

The protein content was increasing as the fruit was developing from immature juvenile phase to mature fruit. In the eleventh week of maturity the protein content of *Annona muricata* Linn. was 0.28mg/gm tissue and for *Annona reticulata* Linn. was 0.21mg/gm tissue (Lowry, 1951) (Fig. 2).

Total phenol

Among the important plant chemicals; phenols are the most important antioxidant components which possess health promoting effects (Chaudhary and Mukhopadhyay, 2012)^[16].



The decline in phenols probably leads to a loss of astringency during ripening and it leads to a bland flavor of the slightly overripe fruit. Very over ripe fruit has an off-flavor due to low phenols (Paull, 1982) [17]. The estimated amount of phenol for *Annona reticulata* Linn. was 0.186 mg/gm tissue, while in case of *Annona muricata* Linn. was 0.176 mg / gm tissue (Fig. 3).

Total sugars and reducing sugars

The total sugars for both the fruits were found that 0.027mg/gm in *A. muricata* Linn. and 0.058mg/gm tissue in case of *A. reticulata* Linn. The total reducing sugar content in the fresh fruits of *Annona muricata* Linn. were 0.018 mg/gm tissue, where in *Annona reticulata* Linn. was 0.046mg/gm tissue. The major soluble sugars of the fruit pulp consist of fructose, glucose and sucrose. The increase in sucrose might be due to enzymatic break down of polysaccharides into sugars (Bolivar *et al.*, 2009) [18]. This increase in sucrose is very important as it improves the sweetness of the fruit (Othman *et al.*, 2014) [19]. (Fig. 4).

Total ascorbic acid

The analysis of all the fruit mesocarp showed considerable, but varying quantities of ascorbic acid. The calculated amount of ascorbic acid in *Annona muricata* Linn. was 22.5 mg/100mg tissue and for *Annona reticulata* Linn. was 15 mg/100mg tissue. The high values of ascorbic acid in soursoup also signify the occurrence of good antioxidant. The Recommended Daily Intake (RDI) of ascorbic acid is about 30 mg/day for adults and 17 mg/day for children. Therefore, these fruits could be considered as good sources of ascorbic acid for human dietary needs (Wall, 2006) [20] (Fig. 5).

Total oxalate

Oxalates are strong chelators that react with minerals such as calcium, magnesium, zinc, copper, iron etc. to form complexes that cannot be absorbed by the intestine (Akanke *et al.*, 2010) [21]. Studies showed that lethal dose of oxalate is between 200 and 300 mg/gm tissue. Here, the observed amount of oxalate in *Annona muricata* Linn were 1.5 mg/ 100 gm tissue. and for *Annona reticulata* Linn. were 0.9 mg/100gm tissue (Fig. 6).

Total alkaloid

Alkaloids provide many pharmaceutical activities; they act as drug precursor and antihypertensive, antibiotic among many other therapeutic uses (Cordell, 1983) [21]. The amount of alkaloid estimated for *Annona muricata* Linn. is 0.403 mg/100 gm tissue and for *Annona reticulata* Linn. is 0.07 mg/100 gm tissue (Fig. 7).

Antibacterial activity

Annona muricata Linn. and *Annona reticulata* Linn. tested against five different bacterial strains showed different prototype of inhibition zone. The result of antibacterial screening by agar disc diffusion method designates that elevated zone of inhibition was shown by the ethanolic fruit extract of *Annona reticulata* Linn. for *E. Coli*, 1.8 cm and lowest for *Proteus* 0.8 cm. The *Annona muricata* extract highest zone of inhibition for *Klebsiella* 1.6 cm and lowest for the *Proteus* 0.9 cm. The present observation concluded that, both *Annona muricata* Linn. and *Annona reticulata* Linn. were shows significant activity against both gram positive and gram negative bacterial strains (Table-3; Fig. 8&9).

Table 1: Qualitative analysis of methanolic extract of *A. muricata* L. and *A. reticulata* L.

Sl No.	Metabolites	Test Name	<i>A. muricata</i>	<i>A. reticulata</i>
1	Saponin	Foam test	+	-
2	Terpinoid	Salkowski test	+	+
3	Phenol	Ferric chloride test	-	+
4	Tannin	Gelatin test	+	+
5	Carbohydrate	Benedicts reagent test	-	+
		Fehling's test	+	+
6	Alkaloid	Mayer's test	+	+
7	Flavanoid	Alkaline reagent test	-	-
		Lead acetate test	-	-
8	Protein	Xanthoproteic test	+	+

Table 2: Quantitative analysis of biochemical constituents of *A. muricata* L. and *A. reticulata* L.

Sl no.	Name of the biochemical compound	Amount of compounds (mg/gm tissue)	
		<i>Annona reticulata</i> Linn.	<i>Annona muricata</i> Linn.
1	Ascorbic acid	15 mg/100gm tissue	22.5 mg/100 gm tissue
2	Reducing sugar	0.46	0.18
3	Phenol	0.326	0.176
4	Protein	0.21	0.28
5	Oxalic acid	0.9 mg/100 gm tissue	1.5 mg/100 gm tissue.
6	Alkaloid	0.40 mg/100 gm tissue	0.07 mg/100gm tissue
7	Total carbohydrate	0.58	0.27

Table 3: Antibacterial activity of fruit extract of *Annona reticulata* L. and *Annona muricata* L. against different microbial strains

Sl No.	Test organism	Zone of inhibition (cm)	
		<i>Annona reticulata</i> L.	<i>Annona muricata</i> L.
1	<i>Bacillus</i>	1.2	1.5
2	<i>Proteus</i>	-	-
3	<i>Klebsiella</i>	1.2	1.6
4	<i>Staphylococcus</i>	-	-
5	<i>E. coli</i>	1.8	1.4

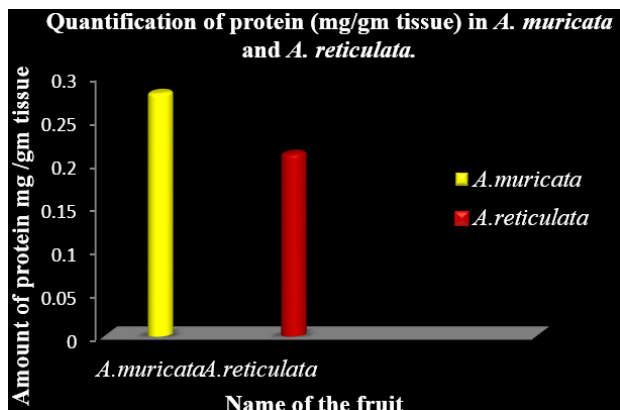


Fig. 2: Quantification of amount of protein (mg/gm tissue) present in *Annona muricata* Linn. and *Annona reticulata* Linn.

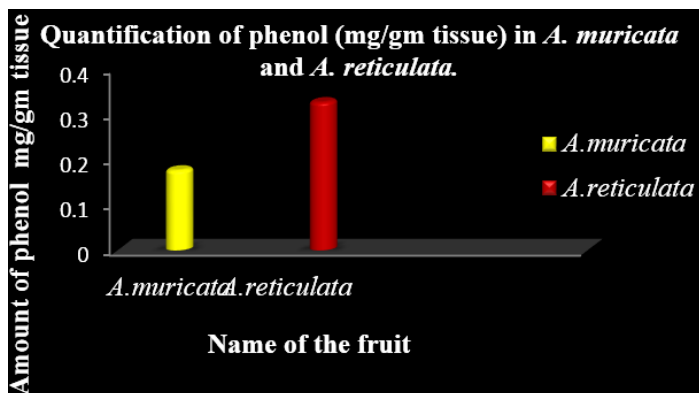


Fig 3: Quantification of amount of phenol (mg/gm tissue) present in *Annona muricata* Linn. and *Annona reticulata* Linn.

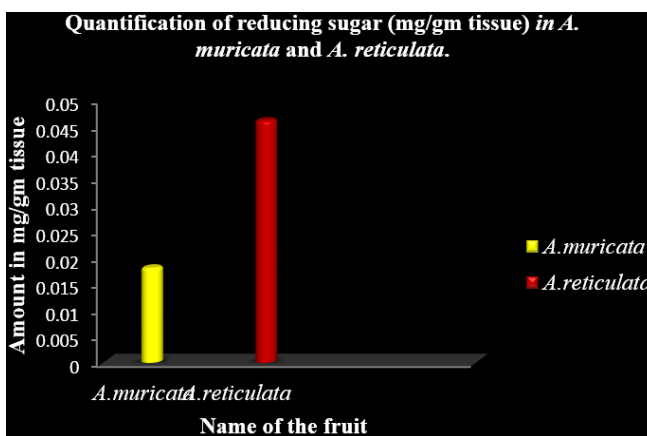


Fig 4: Quantification of amount of reducing sugar (mg/gm tissue) present in *Annona muricata* Linn. and *Annona reticulata* Linn.

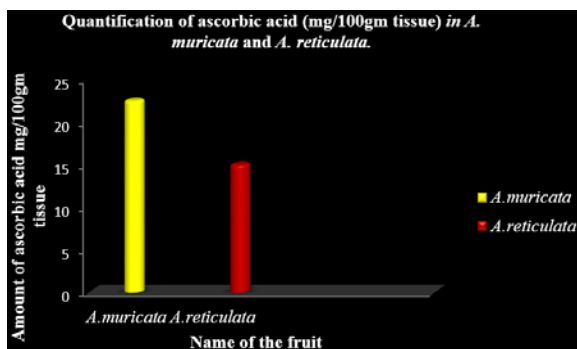


Fig. 5: Quantification of amount of ascorbic acid (mg/ 100 gm tissue) present in *Annona muricata* Linn. and *Annona reticulata* Linn.

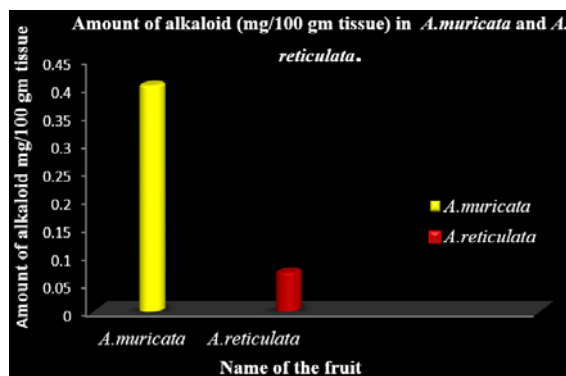


Fig 7: Quantification of amount of alkaloid (mg/100gm tissue) present in *Annona muricata* Linn. and *Annona reticulata* Linn.

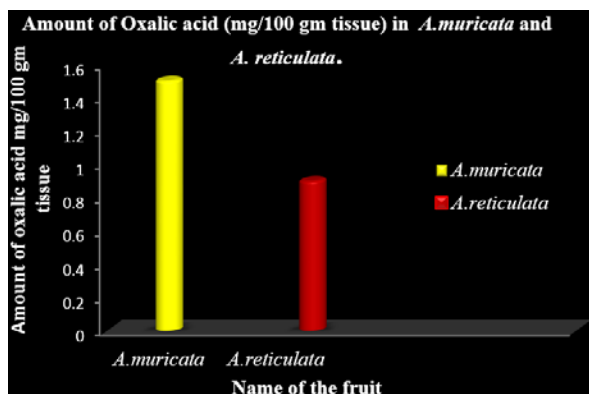


Fig. 6: Quantification of amount of Oxalic acid (mg/100gm tissue) present in *Annona muricata* Linn. and *Annona reticulata* Linn.

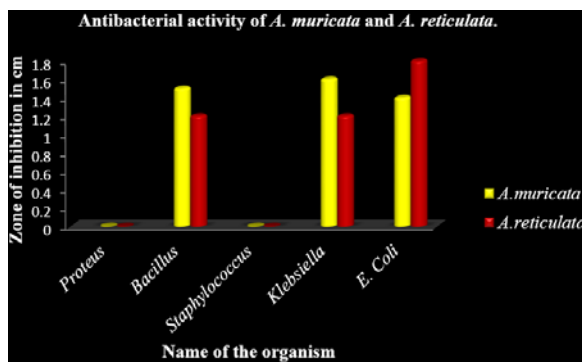


Fig 8: Antibacterial activity of *Annona muricata* Linn. and *Annona reticulata* Linn.

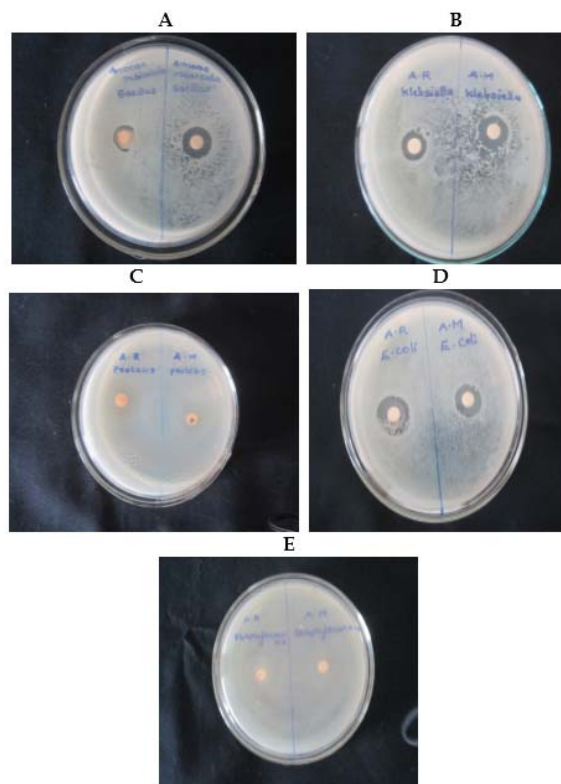


Fig 9: Antibacterial activity of *Annona muricata* L. and *Annona reticulata* L.

- A- Ethanol extract of fruit of *A. muricata* and *A. reticulata* against *Bacillus*.
- B- Ethanol extract of fruit of *A. muricata* and *A. reticulata* against *Klebsiella*.
- C- Ethanol extract of fruit of *A. muricata* and *A. reticulata* against *Proteus*.
- D- Ethanol extract of fruit of *A. muricata* and *A. reticulata* against *E. Coli*.
- E- Ethanol extract of fruit of *A. muricata* and *A. reticulata* against *Staphylococcus*.

Conclusion

Medicinal plants were the potent source of human health due to their active phytochemical compounds that is responsible for various pharmacological activities. The phytochemical analysis of fruits both *Annona muricata* Linn. and *Annona reticulata* Linn. reveals that, the potential bio active compounds which are present in these species indicates their value in the field of pharmacology. In addition to that, these fruits are also prospective edibles with high nutrient values. The results obtained from the present study may useful to standardize some herbal formulations from the fruits. It also gives some clues for the development of potential drugs to cure some diseases.

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