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Original Article

Physicochemical and Phytochemical Analysis of Different Plant Parts of *Annona muricata* L. (*Annonaceae*)

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ABSTRACT

Background: *Annona muricata* L. possesses multitudinous curative benefits and used traditionally to treat diverse ailments including cancer. The current study was undertaken to assess and investigate the physicochemical and phytochemical profile in different parts (rind, pulp, seed, leaf, bark and root) of *Annona muricata* L. collected from the State of Goa to determine requisite pharmacognostic standards for evaluating the plant material. **Methods:** Comparative assessment of physico-chemical parameters of plant parts viz. moisture loss, total ash, water soluble ash, acid insoluble ash and extractives were determined according to WHO recommended parameters for standardization. Phytochemical analysis to evaluate the effect of extractive solvents to determine difference of solvent polarity to phytochemical content, using aqueous, methanol, ethanol, ethyl acetate, chloroform, petroleum ether and hexane as solvents. **Results:** Physicochemical parameters revealed constants for identification and authentication of plant parts of *A. muricata*. Phytochemical analysis manifested the presence of salient classes of phytoconstituents. Among the solvents used for extraction, methanol showed the maximum yield of extract for all plant parts. Interestingly, major phytochemicals has polar properties largely extracted by methanol and part of them had semi-polar properties extracted by other polar and semi-polar solvents including alkaloids, flavonoid, saponin, phenol, tannin, proteins, amino acids, quinones and reducing sugars. Phytosterols, coumarins, fixed oils and fats were largely detected in non-polar solvents such as chloroform, petroleum ether and hexane. **Conclusion:** The findings of the present study form referential data for identification and standardization of the plant material for pharmaceutical applications.

Key words: Acetogenins, Curative, Pharmacognosy, Pharmacopoeia, Phytoconstituents.

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INTRODUCTION

Plant derived bio-active compounds and health care have evolved as inseparable domains of human activity to help sustain mankind, since the dawn of medicine.[1] According to WHO survey, 80% population living in developing countries relies mainly on traditional medicines for their primary health care needs.[2] Also, modern pharmacopoeia still comprises at least 25% drugs derived from plants and many others which are factitious analogues built on prototype compounds isolated from plants.[3] There has been an alarming upsurge in number of disease and disorders caused by synthetic drugs inducing a switch over to traditional herbal medicine. Studies of plants continue principally for uncovering novel secondary metabolites or phytochemicals derived from plants exhibiting protective functions for human consumers.[4]

Annona muricata L. (Soursop) belongs to the custard apple tree family Annonaceae which originated from tropical America is now widely cultivated in India. It possesses numerous traditional medicinal uses and has become popular nutritional medicinal supplement. Different parts of this plant possess multifarious medicinal properties. The fruit, seeds, bark, leaves and roots have been reported to treat coughs, intestinal parasites, liver ailments, inflammation, arthritis and diabetes, among many uses.[5-7] Furthermore, extensive phytochemical evaluations in Annonaceae family have resulted in identification of wide array of Annonaceous acetogenins which promising new antitumor agents are found unique in this plant family.[8] Until now, very few scientific investigations have been carried out on physico-chemical parameters of *A. muricata*. Hence, the present study framed to investigate the pharmacognostical and phytochemical potential of different plant parts of *A. muricata* L.

MATERIALS AND METHODS

Collection and authentication of plant materials

The plant materials were procured from KOCL Research Farm, Kirbhatt, Nuvem, South Goa district, Goa, during 2015-16. Study area lies at 15°18'11.15"N and 73°57'13.22"E. In addition, survey was carried out in the form of questionnaire and discussion with local people to understand the plant parts used, mode of consumption, shelf life and ethnic value of *A. muricata*. During this study different plant parts such as root, leaf, bark, pulp, rind and seeds were collected from the above location and used for the analysis. The collected plant samples were thoroughly washed and dried. Herbarium specimens were prepared and deposited in Goa University Herbarium located at the Department of Botany, Goa University, Goa, India. (Figure 1)

Loss on drying

2 g of precisely weighed dried plant parts of *A. muricata* was placed in a tared porcelain dish and dried at 100-105°C for 5 h and weighed. Drying and weighing are continued at an interval of one hour until two successive weighing is constant.[9]

Determination of total ash

2 g of coarsely powdered plant parts of *A. muricata* was taken in a tared silica crucible and incinerated at a temperature not more than 450°C until free from carbon. The ash obtained was cooled and weighed. The percentage of ash was calculated with reference to the air-dried sample.[9]

Acid-insoluble ash

The total ash obtained from 2 g powder of plant parts was boiled with 25 mL of dilute hydrochloric acid for 5 mins. Further, the insoluble matter obtained on an ashless filter paper was washed, ignited and weighed. The

percentage of acid insoluble ash was calculated with reference to the air-dried sample.[9]

Water soluble ash

The total ash obtained from 2 g of powder of plant parts was boiled with 25 mL of water for 5 minutes and the insoluble matter was collected on an ashless filter paper. It was washed, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried sample.[9]

Determination of alcohol-soluble extractive

4 g of accurately weighed coarsely powdered plant parts were taken and macerated with 100 mL of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to stand for 18 h. After 24 h, 25 mL of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.[9]

Determination of water-soluble extractive

4 g of accurately weighed coarsely powdered plant parts were taken and macerated with 100 mL of water for 24 h. The contents were frequently shaken during the first 6 h and allowed to stand for 18 h. After 24 h, 25 mL of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.[9]

Determination of Ether soluble extractive

4 g of accurately weighed coarsely powdered plant parts were taken and macerated with 100 mL of ether for 24 h. The contents were frequently shaken during the first 6 h and allowed to stand for 18 h. After 24 h, 25 mL of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.[9]

Preparation of extract and Phytochemical Screening

The crude extracts of plant parts of *A. muricata* viz. rind, pulp, seed, leaf, bark and root were prepared in different solvents by boiling 50 g of air-dried powdered material in 250 mL of hexane, petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water (aqueous) respectively with occasional stirring between 50-55°C for 4 hrs to avoid denaturation of the active ingredients. The hot extracts were then left to stand for 48 hours and filtered through muslin cloth on a plug of glass wool in a glass column.[10] The extracts were then subjected to phytochemical screening for the detection of class of phytoconstituents like alkaloids, carbohydrates, saponins, proteins and amino acids, phytosterols, coumarins, quinones, fixed oils and fats, phenolic and flavonoids, gums and mucilage using standard procedures.[11]

Statistical analysis

The experiments were carried out in triplicate and the results are reported as mean ± standard error of mean (SEM).

RESULTS AND DISCUSSION

Physicochemical parameters

The analysis of physicochemical parameters of different parts of *A. muricata* is represented in Table 1. Physical constant evaluation of the drugs is a vital parameter in determining the purity and quality of drugs which aids in formulation of pharmacopoeial standards.

The moisture loss in various plant parts studied was considerably high as represented in Table 1, ranging from 11-15%. Pulp showed the highest moisture content followed by rind, seed, leaf, root and bark in decreasing order which partially corroborates the findings of Agu and Okolie, 2017.[12] Lower moisture content is preferable for better stability against decomposition and should be minimal to prevent deterioration by chemical change or microbial growth.[13] Among the various physicochemical characteristics evaluated in the present study, ash value is one of the common attributes used to determine the identity and purity of the plant material, especially in powder form for use in future study or application.

The ash value was determined by three different forms viz., total ash, water-soluble ash and acid insoluble ash. The total ash in all parts studied was ranged from 8.27-13.34%, while water-soluble ash and acid insoluble ash were found between 7.37-9.37% and 1.53-3.17% respectively. High total ash content was noted in root and least in pulp and similar trend was observed in water soluble and acid insoluble ash contents which is attributed to greater fluid content of the pulp and the propinquity of the root to the soil as source of mineral elements. Further, the capillary action against gravity by conducting vessels in plants could also be a paramount contributory consequence to transportation of minerals from root, via stems and branches and finally to storage sites such as leaves and fruits for metabolic utilization.[12] Among the plant parts studied, the ash content values found in decreasing order as follows: Root >Bark >Leaf > Seed > Rind > Pulp as represented in Figure 2.

The ash values indicate the presence of varied impurities like silicate, oxalate, phosphate and carbonate which may be derived from plant (natural or physiological ash) and extraneous matter, especially sand and soil adhering to surface of plants (non-physiological ash).[14] The water-soluble ash gives an extent of the amount of inorganic compounds in plant parts and indicates that almost half of the total ash is soluble in water. The acid insoluble ash measures the amount of silica present and indicates contamination with earthy particles. Less amount of these three variables indicate that inorganic matter and silica were less in *A. muricata* plant parts.

Soluble extractive value

Extractable matter determination of *A. muricata* plant parts was carried out to determine the drug active constituents. Substances are generally extracted with water, methanol, petroleum ether and other solvents to determine extractive matters. The extractive value of different plant parts of *A. muricata* is provided in Table 2. The extractive yield of plant parts was found to be comparatively high in methanol, followed by water and minimum yield was in petroleum ether. In all studied parts, soluble extractive values found in the rank of petroleum ether < aqueous < methanol as represented in Figure 3. The extractive values are useful to evaluate the chemical compounds present in the crude drug and also helpful in estimation of specific constituents soluble in a particular solvent.[15] The variation in yield of extractable matter in various solvent is attributed to polarity of different compounds present in plant parts and indicative of the fact that the genesis of bioactive principle of medicinal plants may be influenced by intrinsic and extrinsic factors.[16] High alcohol soluble and water-soluble extractive values unveil the presence of polar substance like phenols, tannins and glycosides, as reported in the literature concerning secondary metabolites.[17,18] Due to the higher yield of extract, use of hydro-alcohol alternatively of water for the preparation of herbal formulations can be considered and it may lead to judicious use of raw materials.[19]

Qualitative phytochemical analysis

The qualitative chemical tests give a wide-ranging suggestion regarding the nature of phytochemical constituents present in the crude drug. The phytochemical screening of rind, pulp, seed, leaf, bark, root extracts in different solvents viz., aqueous, methanol, ethanol, ethyl acetate, chloro-

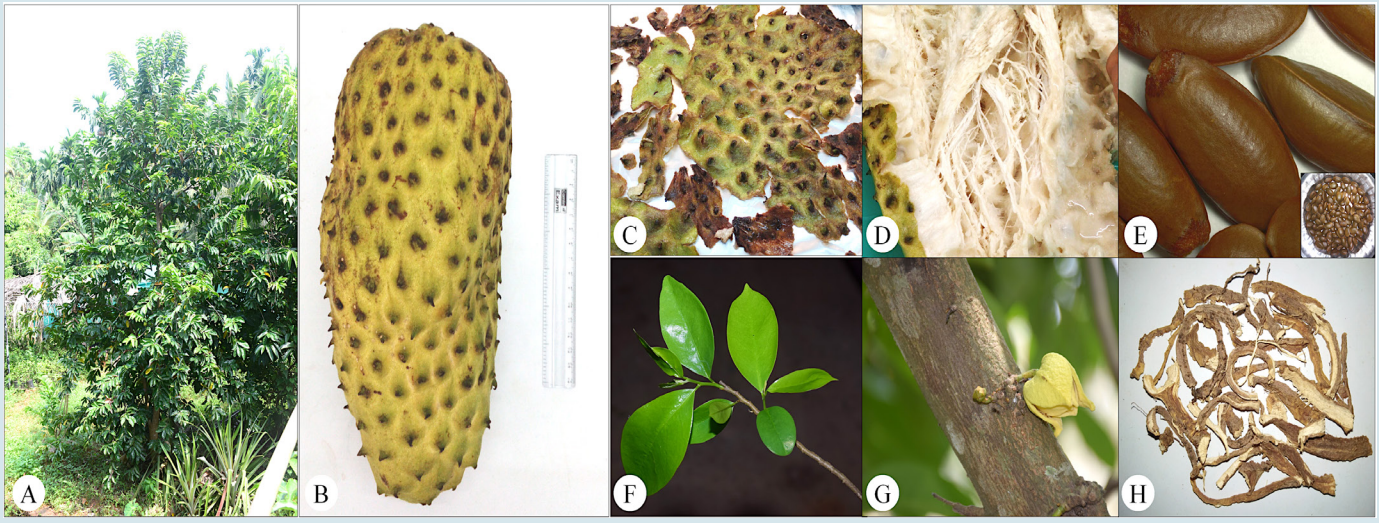

Figure 1: *Annona muricata* (A) Habit; (B) Fruit; (C) Rind; (D) Pulp; (E) Seed; (F) Leaves; (G) Bark with flower; (H) Root.

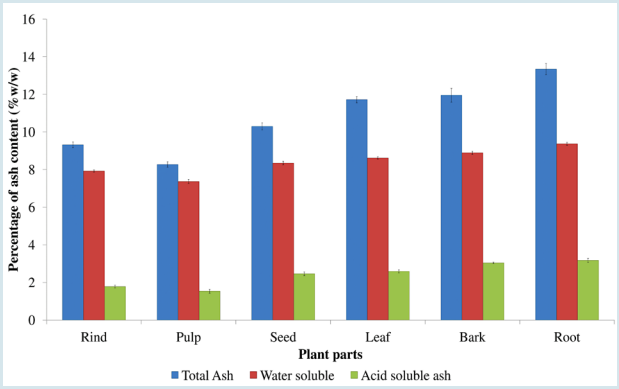

Figure 2: Comparative ash content averages obtained from plant parts of *A. muricata.*

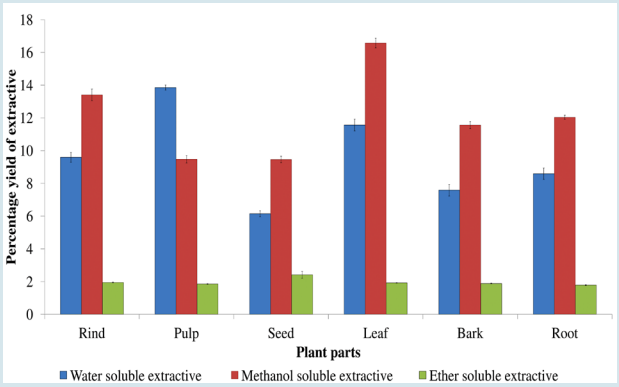

Figure 3: Comparative percentage yield of extractive of *A. muricata* plant parts.

Table 1: Quantitative physicochemical parameters of *A. muricata* plant parts.

Plant Part	Physical State	Loss on drying (%)	Colour of ash	% of Ash Content			pH of ash
				Total Ash	Water Soluble Ash	Acid Insoluble Ash	
Rind	Coarse powder	12.525±0.14	White	9.322±0.15	7.923±0.06	1.782±0.07	9.67±0.22
Pulp	Coarse powder	14.655±0.31	Light pink	8.273±0.14	7.372±0.11	1.535±0.11	8.95±0.19
Seed	Coarse powder	12.1±0.04	Light brown	10.296±0.18	8.348±0.1	2.458±0.1	10.64±0.29
Leaf	Fine powder	11.875±0.2	White	11.721±0.17	8.622±0.06	2.585±0.08	10.94±0.09
Bark	Fine powder	10.92±0.35	Greyish	11.953±0.36	8.893±0.08	3.04±0.04	10.95±0.25
Root	Fine powder	11.558±0.32	Greyish	13.341±0.3	9.372±0.08	3.175±0.11	11.01±0.15

form, petroleum ether and hexane of *A. muricata* were carried out for their presence of different classes of bio-active components.

The efficiency of different solvents for the extraction of phytochemicals from various plant parts (rind, pulp, seed, leaf, bark and root) was compared. The results obtained by qualitative phytochemical screening for primary and secondary metabolites of plant parts in different solvents have been summarized in Table 3-8.

Rind: Phytochemical analysis of rind in various solvent extracts revealed the presence of alkaloids, carbohydrates, saponins, proteins, amino acids, phytosterols, coumarins, quinines, fats, oils, phenols, flavonoids, whereas gums and mucilage were completely absent. Rind extracts of water and methanol showed rich in alkaloids and carbohydrates, whereas in ethanol and ethyl acetate small quantities were present. The methanol extract exhibited strongly for proteins and amino acids compared to other extracts. Phytosterols were strongly detected in chloroform, petroleum ether and hexane extracts and found feebly in methanol and aqueous extracts, while coumarins were present high amount in chloroform

Table 2: Percentage yield of extractive obtained from plant parts of *A. muricata* using different solvents.

Plant Part	Water soluble extractive	Methanol soluble extractive	Ether soluble extractive
Rind	9.597±0.31	13.41±0.36	1.947±0.03
Pulp	13.853±0.15	9.477±0.22	1.853±0.02
Seed	6.147±0.18	9.463±0.2	2.413±0.21
Leaf	11.567±0.36	16.573±0.29	1.923±0.02
Bark	7.587±0.36	11.563±0.21	1.883±0.03
Root	8.593±0.35	12.037±0.13	1.786±0.03

extract and moderately found in other solvent extracts barring aqueous. Spot test for fixed oils and fats are weakly present in all extracts except aqueous. Phenolic and flavonoids were strongly intensified in methanol extract compared to other extracts and completely absent in hexane extract. Alkaloids, carbohydrates and saponin test of petroleum ether and hexane extract were negative.

Pulp: Phytochemical analysis of various solvent extracts for pulp revealed the presence of alkaloids, carbohydrates, proteins, amino acids, phytosterols, coumarins, fats, oils, phenols and flavonoids, whereas saponins, gums and mucilage were completely absent. Carbohydrates were strongly detected in aqueous and methanol compared to other solvent extracts. Phytosterols, coumarins, fats and oils were less in solvent extracts and completely absent in aqueous extract. Similarly, proteins and amino acids were found very feebly in all solvent extracts. Phenolic and flavonoids showed maximum solubility in methanol compared to other extracts and showed negative tests in aqueous, petroleum ether and hexane solvent extracts.

Seed: Different solvent extracts of seed indicated the presence of alkaloids, carbohydrates, saponins, proteins, amino acids, phytosterols, coumarins, quinines, fats and oils, phenolic and flavonoids, whereas gums and mucilage were absolutely absent. High concentrations of alkaloids, carbohydrates, proteins, amino acids, phenolic and flavonoids were present in methanol and aqueous extracts, while petroleum ether and hexane solvent extracts were found to be negative. Presence of phytosteroids and coumarins were strongly positive in methanol, chloroform, petroleum ether and hexane extracts whereas aqueous extract showed negative tests. Quinones were detected in methanol and ethanol extracts. All solvent extracts showed the presence of saponins, fats and oils.

Leaf: Phytochemical screening of all solvent extracts of leaf revealed the presence of alkaloids, carbohydrates, saponins, proteins, amino acids, phytosterols, coumarins, quinines, fats and oils, phenolic and flavonoids, whereas gums and mucilage were completely absent. Alkaloids, carbohydrates, phenolic and flavonoids were predominantly found in methanol and aqueous extracts compared to other extracts, while hexane and petroleum ether showed a complete absence of carbohydrates, phenolic and flavonoids. Analysis of proteins and amino acids in methanol extract were strongly positive, whereas weakly detected in other extracts. Saponins were screened positive in methanol and aqueous extract. Phytosteroids and coumarins were strongly detected in chloroform and petroleum ether, feebly present in other extracts and showed complete absence in aqueous extract. Whereas quinones were weakly positive in methanol, ethanol and ethyl acetate extract. Except for aqueous extract, all extracts gave positive spot test for the presence of fixed oils and fats.

Bark: Preliminary phytochemical evaluation of different solvent extracts of bark revealed the presence of all phytoconstituents undertaken in this study except gums and mucilage. Alkaloids, proteins, amino acids, phenolic and flavonoids were more strongly detected in methanol, followed by aqueous extracts and weakly detected in ethanol, ethyl acetate and chloroform extracts. Carbohydrates were weakly detected in all extracts except chloroform, petroleum ether and hexane extracts. Phytosteroids and coumarins showed more colour reaction in hexane, petroleum ether and chloroform extracts while completely absent in aqueous extract. Saponins and quinones were found poorly in methanol and ethanol extract and absent in other extracts. Spot test for fixed oils and fats gave a weakly

Table 3: Phytochemical screening of powdered rind extract of *Annona muricata* L.

PHYTO CHEMICAL TESTS (Rind)	Aq	M	E	Ea	C	Pe	H
1. ALKALOIDS							
a. Mayer's reagent	+	+	+	-	-	-	-
b. Wagner's reagent	++	+	+	-	-	-	-
c. Hager's reagent	+	+	-	-	-	-	-
d. Dragendorff's reagent	++	+	+	+	+	-	-
2. CARBOHYDRATES							
a. Molisch's test	++	++	+	+	-	-	-
b. Fehling's test	++	++	+	+	-	-	-
c. Barfoed's test	++	++	-	-	-	-	-
d. Benedict's test	++	++	+	+	-	-	-
e. Borntrager's test	++	++	-	-	-	-	-
f. Legal's test	++	++	+	+	-	-	-
3. SAPONINS							
Foam test	+	+	-	-	-	-	-
4. PROTEINS And AMINO ACIDS							
a. Millon's reagent	+	+	+	-	-	-	-
b. Biuret reagent	+	++	+	-	-	-	-
c. Ninhydrin reagent	+	++	+	+	+	+	+
5. PHYTOSTEROIDS							
Liebermann- Burchard's test	-	+	+	+	++	++	++
6. COUMARINS	-	+	+	+	++	+	+
7. QUINONES	-	+	+	-	-	-	-
8. FIXED OILS AND FATS							
a. Spot test	-	+	+	+	+	+	+
b. Saponification test	-	-	-	-	-	-	-
9. PHENOLIC AND FLAVONOIDS							
a. Ferric chloride	+	++	+	+	-	-	-
b. Gelatin test	+	++	+	+	+	+	-
c. Lead acetate	+	++	+	-	-	-	-
d. Alkaline reagent	+	+	+	+	-	-	-
e. Shinado's test (Mg and HCl reduction)	+	++	+	+	-	-	-
10. GUMS and MUCILAGE							
Absolute 95% test	-	-	-	-	-	-	-

Where (++) is highly present; (+) is sparingly present; (-) is completely absent "M"-Methanol; "E"- Ethanol; "Ea"- Ethyl acetate; "C"- Chloroform; "Pe"- Petroleum ether; "Aq"- Aqueous

Table 4: Phytochemical screening of powdered pulp extract of *Annona muricata* L.

PHYTO CHEMICAL TESTS (Pulp)	Aq	M	E	Ea	C	Pe	H
1. ALKALOIDS							
a. Mayer's reagent	+	+	-	-	-	-	-
b. Wagner's reagent	+	+	-	-	-	-	-
c. Hager's reagent	+	+	-	-	-	-	-
d. Dragendorff's reagent	+	+	-	-	-	-	-
2. CARBOHYDRATES							
a. Molisch's test	++	++	++	+	+	-	-
b. Fehling's test	++	++	+	+	+	+	-
c. Barfoed's test	++	++	+	+	-	-	-
d. Benedict's test	++	++	+	+	+	+	+
e. Borntrager's test	++	++	+	+	-	-	-
f. Legal's test	++	++	+	+	+	-	-
3. SAPONINS							
Foam test	-	-	-	-	-	-	-
4. PROTEINS and AMINO ACIDS							
a. Millon's reagent	+	+	+	-	-	-	-
b. Biuret reagent	+	+	+	-	-	-	-
c. Ninhydrin reagent	+	+	+	+	+	+	+
5. PHYTOSTEROIDS							
Liebermann- Burchard's test	-	+	+	+	+	+	+
6. COUMARINS	-	+	+	+	+	+	+
7. QUINONES	-	+	+	-	-	-	-
8. FIXED OILS and FATS							
a. Spot test	-	+	+	+	+	+	+
b. Saponification test	-	-	-	-	-	-	-
9. PHENOLIC and FLAVONOIDS							
a. Ferric chloride	+	++	+	+	-	-	-
b. Gelatin test	+	++	+	+	+	-	-
c. Lead acetate	+	++	+	+	-	-	-
d. Alkaline reagent	+	+	+	+	-	-	-
e. Shinado's test (Mg and HCl reduction)	+	+	+	+	-	-	-
10. GUMS and MUCILAGE							
Absolute 95% test	-	-	-	-	-	-	-

Where (++) is highly present; (+) is sparingly present; (-) is completely absent "M"-Methanol; "E"- Ethanol; "Ea"- Ethyl acetate; "C"- Chloroform; "Pe"- Petroleum ether; "Aq"- Aqueous

Table 5: Phytochemical screening of powdered seed extract of *Annona muricata* L.

PHYTO CHEMICAL TESTS (Seed)	Aq	M	E	Ea	C	Pe	H
1. ALKALOIDS							
a. Mayer's reagent	++	+	+	-	-	-	-
b. Wagner's reagent	++	+	+	-	-	-	-
c. Hager's reagent	++	+	-	-	-	-	-
d. Dragendorff's reagent	++	++	+	+	+	-	-
2. CARBOHYDRATES							
a. Molisch's test	++	++	+	+	+	-	-
b. Fehling's test	+	+	+	+	-	-	-
c. Barfoed's test	+	+	-	-	-	-	-
d. Benedict's test	++	+	+	+	+	-	-
e. Borntrager's test	+	+	-	-	-	-	-
f. Legal's test	+	+	+	+	-	-	-
3. SAPONINS							
Foam test	+	+	+	+	+	+	+
4.PROTEINS and AMINO ACIDS							
a. Millon's reagent	+	+	+	-	-	-	-
b. Biuret reagent	+	++	+	-	-	-	-
c. Ninhydrin reagent	++	++	+	+	+	-	-
5. PHYTOSTEROIDS							
Liebermann- Burchard's test	-	++	+	+	++	++	++
6. COUMARINS	-	++	+	+	++	++	++
7. QUINONES	-	+	+	-	-	-	-
8. FIXED OILS and FATS							
a. Spot test	+	+	+	+	+	+	+
b. Saponification test	-	-	-	-	-	-	-
9. PHENOLIC and FLAVONOIDS							
a. Ferric chloride	++	++	+	+	-	-	-
b. Gelatin test	+	+	+	+	+	-	-
c. Lead acetate	+	+	+	+	-	-	-
d. Alkaline reagent	+	+	+	+	-	-	-
e. Shinado's test (Mg and HCl reduction)	+	+	+	+	-	-	-
10. GUMS and MUCILAGE							
Absolute 95% test	-	-	-	-	-	-	-

Where (++) is highly present; (+) is sparingly present; (-) is completely absent "M"-Methanol; "E"- Ethanol; "Ea"- Ethyl acetate; "C"- Chloroform; "Pe"- Petroleum ether; "Aq"- Aqueous

positive test for all extracts except aqueous extract.

Root: Phytochemical screening of root extracts showed the presence of diverse phytochemical constituents in different solvents. Test performed for alkaloids were strongly positive for methanol extract followed by aqueous and weakly positive in other extracts studied. Carbohydrates were strongly positive in methanol and aqueous extract and completely absent in ethyl acetate, chloroform, petroleum ether and hexane extracts. Phytosteroids, coumarins, fixed oils and fats were weakly detected in all solvent extracts except aqueous. Test for proteins and amino acids were found to be positive in methanol, aqueous and ethanol extracts while quinones were feebly detected in methanol and ethanol extracts. All solvent extracts of root revealed low amounts of phenolic and flavonoids

Table 6: Phytochemical screening of powdered leaf extract of *Annona muricata* L.

PHYTO CHEMICAL TESTS (Leaf)	Aq	M	E	Ea	C	Pe	H
1. ALKALOIDS							
a. Mayer's reagent	++	++	+	-	-	-	-
b. Wagner's reagent	++	+	+	+	+	+	-
c. Hager's reagent	+	+	-	-	-	-	-
d. Dragendorff's reagent	++	++	+	+	+	+	-
2. CARBOHYDRATES							
a. Molisch's test	++	++	+	+	+	-	-
b. Fehling's test	++	++	+	+	+	-	-
c. Barfoed's test	++	++	+	+	-	-	-
d. Benedict's test	++	++	+	+	+	-	-
e. Borntrager's test	++	++	-	-	-	-	-
f. Legal's test	++	++	+	+	-	-	-
3. SAPONINS							
Foam test	+	+	-	-	-	-	-
4. PROTEINS and AMINO ACIDS							
a. Millon's reagent	+	+	+	-	-	-	-
b. Biuret reagent	+	++	+	+	-	-	-
c. Ninhydrin reagent	+	++	+	+	+	+	+
5. PHYTOSTEROIDS							
Liebermann- Burchard's test	-	+	+	+	++	+	+
6. COUMARINS	-	+	+	+	++	++	+
7. QUINONES	-	+	+	+	-	-	-
8. FIXED OILS and FATS							
a. Spot test	-	+	+	+	+	+	+
b. Saponification test	-	-	-	-	-	-	-
9. PHENOLIC and FLAVONOIDS							
a. Ferric chloride	+	++	+	+	-	-	-
b. Gelatin test	+	+	+	+	+	-	-
c. Lead acetate	+	++	+	-	-	-	-
d. Alkaline reagent	+	+	+	+	-	-	-
e. Shinado's test (Mg and HCl reduction)	+	++	+	+	-	-	-
10. GUMS and MUCILAGE							
Absolute 95% test	-	-	-	-	-	-	-

Where (++) is highly present; (+) is sparingly present; (-) is completely absent
"M"-Methanol; "E"- Ethanol; "Ea"- Ethyl acetate; "C"- Chloroform; "Pe"- Petroleum ether; "Aq"- Aqueous

Table 7: Phytochemical screening of powdered bark extract of *Annona muricata* L.

PHYTO CHEMICAL TESTS (Bark)	Aq	M	E	Ea	C	Pe	H
1. ALKALOIDS							
a. Mayer's reagent	++	++	+	-	-	-	-
b. Wagner's reagent	++	+	+	+	+	-	-
c. Hager's reagent	+	+	-	-	-	-	-
d. Dragendorff's reagent	++	+	+	+	+	-	-
2. CARBOHYDRATES							
a. Molisch's test	+	+	+	-	-	-	-
b. Fehling's test	+	+	+	+	-	-	-
c. Barfoed's test	+	+	-	-	-	-	-
d. Benedict's test	+	+	+	+	-	-	-
e. Borntrager's test	+	+	-	-	-	-	-
f. Legal's test	+	+	+	+	-	-	-
3. SAPONINS							
Foam test	+	+	-	-	-	-	-
4. PROTEINS and AMINO ACIDS							
a. Millon's reagent	+	+	+	-	-	-	-
b. Biuret reagent	+	++	+	-	-	-	-
c. Ninhydrin reagent	++	++	++	+	+	-	-
5. PHYTOSTEROIDS							
Liebermann- Burchard's test	-	+	+	+	++	++	++
6. COUMARINS	-	+	+	+	++	++	+
7. QUINONES	-	+	+	-	-	-	-
8. FIXED OILS and FATS							
a. Spot test	-	+	+	+	+	+	+
b. Saponification test	-	-	-	-	-	-	-
9. PHENOLIC and FLAVONOIDS							
a. Ferric chloride	++	++	+	+	-	-	-
b. Gelatin test	+	+	+	+	+	-	-
c. Lead acetate	+	++	+	+	-	-	-
d. Alkaline reagent	+	+	+	+	-	-	-
e. Shinado's test (Mg and HCl reduction)	+	+	+	+	-	-	-
10. GUMS and MUCILAGE							
Absolute 95% test	-	-	-	-	-	-	-

Where (++) is highly present; (+) is sparingly present; (-) is completely absent
"M"-Methanol; "E"- Ethanol; "Ea"- Ethyl acetate; "C"- Chloroform; "Pe"- Petroleum ether; "Aq"- Aqueous

while strongly detected in methanol extract and completely absent in hexane extract. Test performed for the presence of gums and mucilage were screened negative in all solvent extracts.

Phytochemical screening assay is a quick and inexpensive procedure in detecting bio-active components. Bioactive compounds present in plant parts are composed of multi-constituent mixtures, while their separation and identification still create complications. Virtually, the majority of them have to be purified by combination of various purification and chromatographic techniques to isolate the bio-active compounds.[20,21]

Ethno medicinally the fruit of *A. muricata* have been used abundantly in the treatment of various diseases especially cancer without knowing any pharmacognostical validation. The present work was undertaken to

Table 8: Phytochemical screening of powdered root extract of *Annona muricata* L.

PHYTO CHEMICAL TESTS (Root)	Aq	M	E	Ea	C	Pe	H
1. ALKALOIDS							
a. Mayer's reagent	+	++	+	-	-	-	-
b. Wagner's reagent	+	++	+	+	+	+	-
c. Hager's reagent	+	+	+	-	-	-	-
d. Dragendorff's reagent	++	++	+	+	+	+	+
2. CARBOHYDRATES							
a. Molisch's test	++	++	+	-	-	-	-
b. Fehling's test	++	++	+	-	-	-	-
c. Barfoed's test	++	++	+	-	-	-	-
d. Benedict's test	++	++	+	-	-	-	-
e. Borntrager's test	++	++	+	-	-	-	-
f. Legal's test	++	+	+	-	-	-	-
3. SAPONINS							
Foam test	+	+	-	-	-	-	-
4. PROTEINS and AMINO ACIDS							
a. Millon's reagent	+	+	-	-	-	-	-
b. Biuret reagent	+	+	-	-	-	-	-
c. Ninhydrin reagent	+	++	+	-	-	-	-
5. PHYTOSTEROIDS							
Liebermann- Burchard's test	-	+	+	+	+	+	+
6. COUMARINS	-	+	+	+	+	+	+
7. QUINONES	-	+	+	-	-	-	-
8. FIXED OILS and FATS							
a. Spot test	-	+	+	+	+	+	+
b. Saponification test	-	-	-	-	-	-	-
9. PHENOLIC and FLAVONOIDS							
a. Ferric chloride	+	++	+	+	-	-	-
b. Gelatin test	+	+	+	+	+	+	-
c. Lead acetate	+	++	+	+	-	-	-
d. Alkaline reagent	+	+	+	-	-	-	-
e. Shinado's test (Mg and HCl reduction)	+	+	+	+	-	-	-
10. GUMS and MUCILAGE							
Absolute 95% test	-	-	-	-	-	-	-

Where (++) is highly present; (+) is sparingly present; (-) is completely absent "M"-Methanol; "E"- Ethanol; "Ea"- Ethyl acetate; "C"- Chloroform; "Pe"- Petroleum ether; "Aq"- Aqueous

attain the standards which could be useful for establishing authenticity. Findings on comparative analysis of phytochemical profile of the different parts of *A. muricata* are very limited. Thus this study forms the base and provides an additional standardization data for pharmaceutical value. The various extracts of *A. muricata* plant parts have revealed the presence of diverse phytoconstituents whereas gums and mucilage were found to be absent in all solvent extracts as reported in the previous study.[22,23] In the present study, petroleum ether and hexane extracts for all plant parts showed presence of phytosterols, coumarins, while most of the phytoconstituents were absent in chloroform, petroleum ether and hexane extracts. The methanol extract of *A. muricata* plant parts found to have more phytoconstituents compared to other extracts as reported by previous researchers.[24,25] Also, in all plant parts aqueous extract showed the presence of carbohydrates in high concentration.[26] Among the extracts tested, saponification test for fats and oils and test for gums and mucilage exhibited negative results. Interestingly, all the plant parts exhibited similar phytochemicals in all the solvents examined and the trend of presence/absence of phytoconstituents was almost the same with slight variations.[12] From the analysis, it was observed that methanol was the best solvent to extract most phytochemicals followed by ethanol, water, ethyl acetate, chloroform, petroleum ether and hexane.[18] These secondary plant metabolites possess various pharmacological effects and might be responsible for the actions exerted by the plant.[27]

CONCLUSION

Due to the lack of comparative account of physicochemical and phytochemical data in different plant parts of *A. muricata*, the present study was carried out in view of laying down standards to establish the authenticity of the medicinally useful plant parts. Results of the current study are important in setting up diagnostic indices for identification and preparation of monograph according to pharmacopoeia. Generally, the presence of secondary metabolites confirms that extracts of plant parts of *A. muricata* contain polar-phytoconstituents largely extracted by methanol and semi-polar or non-polar properties extracted by other solvents except aqueous extract which could be of extensive use in the medical field both traditionally and pharmaceutically. The use of *A. muricata* in traditional medicine is validated by the presence of these phytochemicals of known health benefits and thus the interest in further studies on this species and related species as a potential source of useful therapeutics. Further studies are going on in order to isolate, identify, characterize and elucidate the structure of bioactive molecules along with their pharmacological potential.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization.

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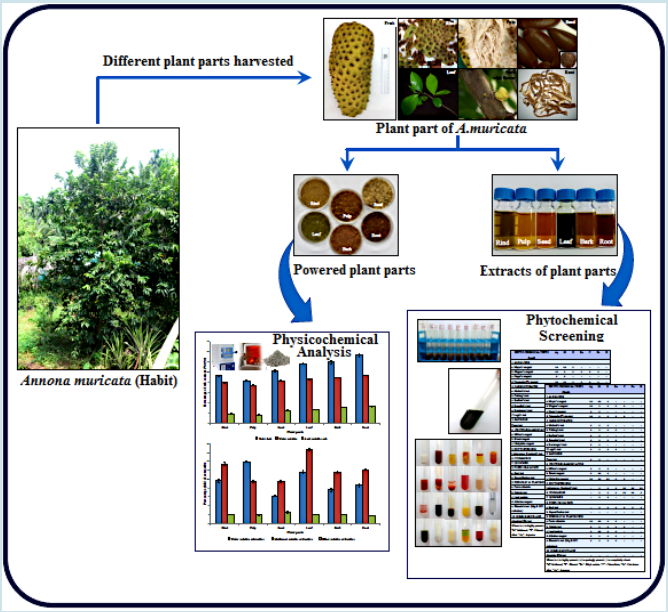
PICTORIAL ABSTRACT



SUMMARY

- Comparative physicochemical analysis of different plant parts of *Annona muricata* viz. rind, pulp, seed, leaf, bark and root were carried out.
- Difference of solvent polarity to solvent extraction using aqueous, methanol, ethanol, ethyl acetate, chloroform, petroleum ether and hexane were carried out to determine the best solvent for phytoconstituent extraction of plant parts.
- Physicochemical evaluation revealed highest ash content in roots. The ash content values were found in decreasing order as follows: Root >Bark >Leaf > Seed > Rind > Pulp.
- Soluble extractive values were found in the rank of petroleum ether < aqueous < methanol.
- Physicochemical analysis revealed constants for identification and standardization of Soursop plant.
- Phytochemical analysis of plant parts manifested presence of salient classes of phytoconstituents largely extracted by methanol as solvent.
- Further exploration of plant parts may reveal novel compounds of specialized kind with vast medicinal properties.

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