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PHYTOCHEMICAL EXTRACTION AND EVALUATION: CHEMICAL COMPOSITION TOTAL PHENOLIC CONTENT AND FLAVANOID CONTENT, ANTIOXIDANT ACTIVITY OF "*ANNONA MURICATA*" FRUIT PULP

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ABSTRACT

Annona muricata is one of the best plants, used as a traditional medicine due to its richness f phytomedicine metabolites identifying the appropriate solvent selection of extracting these bioactive components from Annona muricata. The percentage yield of extract was found to be 50% percentage. Phytochemical and Pharmacognstic investigation was carried out on the fruit pulp of Annona muricata. The Pharmacognostic analysis revealed total ash value of 11.70%w/w, Water soluble ash 43%w/w, & loss on drying 11.805%w/w. The Quantitative & Qualitative analysis is very essential for identifying compounds present in Proteins, Carbhydrates, Tannins, Flavonoids, Saponins, Terpenoids, & Alkaloids, while Steroids & Glycosides absent. The Antioxidant activity was evaluated by the method of DPPH Scavenging method using ascorbic acid as a Standard. The 20ug/ml concentration of *Annona muricata* extract shows 81.23% of DPPH Scavenging capacity using Ascorbic acid.

KEYWORDS: A. Muricata, DPPH, Ascorbic acid.

1. INTRODUCTION

1.1 HERBAL MEDICINE

Medicinal plants area rich source of bioactive molecules which are used approximately 80% of the world population for their basic health needs. Around 500 species are known

worldwide, 24 species of which are native to India!. In India, however, earliest references of use of plants as medicine appear in Rig-Veda, which is said to be written between 1600-3500 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians (an indigenous system of medicine) which are a basic foundation of ancient medical science in India.[2] The ethno botany provides a rich resource for natural drug research and development "Taditional" use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as "traditional herbal medicines".

Fig 1.1 a: Extracted dried pulp of Annona muricata.

Fig 1.1 b: Extracted pulp of Annona muricata.

Scientific Classification

- Kingdom: Plantae
- Clade: Tracheophytes
- Clade: Angiosperms

- Clade: Magnoliids
- Order: Magnoliales
- Family: Annonaceae
- Genus: Annona
- Species: A. muricata
- Binomial name :Annona muricataL.

Fig 1.1 c: FLOWER & FRUITS.

1.1.2. Advantage of Herbal Medicine

Herbal medicines have long history of use and better patient tolerance as well as public acceptance. Medicinal plants have a renewable source, which is our only hope for sustainable supplies of cheaper medicine for the worlds growing population.

1.2. Antioxidant

Antioxidants are compounds that inhibit oxidation / Oxidative Stress. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid(vitamin C)terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide: dismutase), produced internally, or the dietary antioxidants vitamin C, and vitamin E.

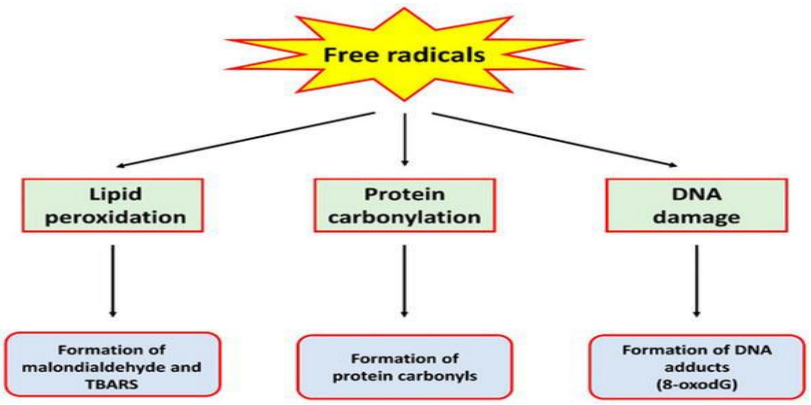


Fig 1.1 e: Effect Of Free Radicals.

In another term antioxidant is "any substance that, when present at low concentrations compared with of that an oxidizable substrate, significantly delays or inhibits oxidation of that substrate.

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body.

Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietlary sources like fruits, vegetables and tea, etc. Regular consumption of anti-oxidative vegetables and fruits has been recognized as reducing the risk of chronic diseases. It is a well-known fact that citrus fruits (oranges, lemons, etc.) contain a high amount of natural antioxidants, such as vitamin C.

Blueberries, strawberries, grapes, plums, prunes, red beans, spinach, kale, broccoli flowers, alfalfa sprouts, and more have been proven to contain a high amount of antioxidants and have been incorporated into many dietary menus.

Recent studies also suggested that fruit-like jackfruit, araticu-domato, pindo palm, and mandacaru-de-tresquinas are good sources of vitamins C and A and phenolic compounds.

Dietary antioxidants: The dictary antioxidants such as ascorbates, tocopherols and Icarotenoids are well known and there is a surplus of publications related to their role in health.

Vitamin C, vitamin E, and beta carotene, Beta carotene and other [carotenoids and oxycarotenoids, c.g. lycopene and lutcinare among the most widely studied dietary antioxidants.

In extracellular fluids vitamin C is considered the most important water- soluble antioxidant. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated.

Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E.
Flavanoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity synthetic antioxidant.

Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation .These antioxidants fall into two major categories depending on their mode of action Primary antioxidants and Secondary antioxidants.

1.2.1 Antioxidant mechanism of action

An antioxidant is a substance that at low concentration delays or prevents oxidantion of a substance. antioxidant compounds act through compounds act through several chemical mechanisms: Hydrogen atom transfer(HAT), single electron transfer (SET)and the ability to chelate transition metals.

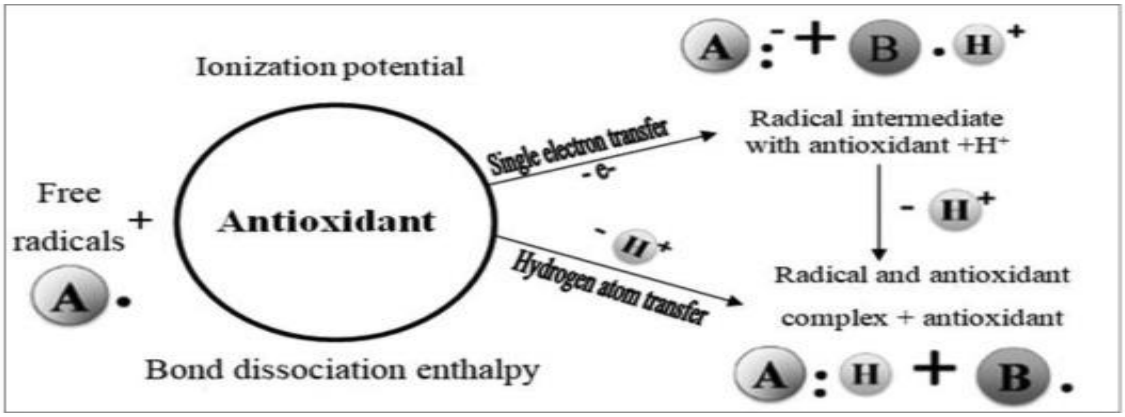


Fig 1.2.1 a: 1Antioxidant mechanism of action.

Annona Muricata

Annona muricata F. the extract origin is unknown; it is native to the tropical regions of the Americans and the Caribbean and is widely propagated. It is in the same genus, Annona, as cherimoya and is in the Annonaceace family.

Fig 1.2.1 a: SEEDS

Fig 1.2.1 b: FRUITS

1.3. SELECTION Of PLANTS: Botanical Description

Annona muricata is a slender, evergreen tree, 5-10 m in height and 15 cm in diameter; trunk straight; bark smooth, dull grey or grey-brown, rough and fissured with age; inner bark pinkish and tasteless; branches at first ascending with the crown forming an inverted cone, later spreading; crown at maturity spherical due to lack of apical dominance; twigs brown or grey, bearing minute raised dots(lenticels);root system extensive and superficial, spreading beyond the diameter of the crown although shallow rooted; juvenile plants have a taproot that is eventually lost.

Leaves

Leaves alternate, 7.6-15.2 cm long, 2.5-7.6 cm wide, leathery, obovate to elliptic, glossy on top, glabrous on underside, simple; stipules absent; blade oblanceolate, green on top, paler and dull on under side with fine lateral nerves; a strong, pungent odour; petioles short, 3-10 mm long.

Flowers

Flower terminal or lateral, large; stalks stout, green, 1.3-1.9 cm long; 3 sepals, minute, inconspicuous, broad, green,3 mm long, triangular; petals yellowish-green, 6 in 2 whorls of 3, outer petals larger, ovate-acute, valvate, cordate with pointed apex (heart shaped), 4-5x3-4 cm, 3mm thick and fleshy, fiting together at edges in bud and rough on the outside; 3 inner petals, narrow, smaller, nearly 3.8 cm long, thinner, rounded, concave with fingernail-shaped base and overlapping edges; stamens numerous, shield shaped, united below; anthers parallel and opening longitudinally; carpels numerous, overtopping the stamens, each with 1 ovule; pistils white, narrow, 5 mm long, with sticky stigmas.

Fruit

Fruit 14-40x10-18 cm, weighing up to 7 kg, ovoid, heart shaped, an oblong syncarp composed of numerous united pistils, pistils end in a fleshy spine or short base of spine 1.5 mm or more in length, which grows from the style; often asymmetric due to incomplete fertiliration of the ovules; epidermis often shining, dark green, with short, fleshy spines covering each carpel; pulp white, fibrous and juicy; seeds shiny, dark brown or black, oblong, up to 2 cm long, 0.7 cm wide. The genus name 'Annona' is from the Latin word anon', meaning 'yearly produce', referring to the fruit production habits of the various species in this genus.

AIM AND OBJECTIVES

Phytochemical Extraction And Evaluation Of: Chemical Composition, Total Phenolic Content And Flavanoid Content Of *Annona muricata* Fruit Pulp.

OBJECTIVES

- ✓ To procurement and Authentification of plant material
- ✓ Drying and size reduction of plant material, Extraction of fruit pulp of *Annona muricata* by using the sovlent.

Ethanol.

- ✓ To perfrom preliminary phytochemical screening.
- ✓ To perform pharmacognostical Investigation, Ash value, Water soluble ash value, Loss on drying.
- ✓ ·To evaluate antioxidant activity
- ✓ ·To determine Total phenolic content
- ✓ ·To determine Total flavanoid content

2. EXPERIMENTAL PROCEDURE

2.1. Extraction & Phytochemistry

2.1.1. MATERIALS AND METHODS: Plant Materials: Fruit of *Annona muricata* Family of Annonaceae were procured from the local areas of Mahabubnagar. Freshly collect fruit pulp were dried at 50c in hot air oven and then subjected for remove of moisture content. The reduction of fruit pulp and then for extraction by soxhlet method by using ethanol as a solvent extracted drug was stored in a packed polythene bag.

Plant Materials of Annona muricata

Identification and Authentification of Plant Material

Annona muricata is an evergreen, terrestial, erect tree reaching 5-8m in height and features an open, roundish canopy withlarge, glossy, dark green leaves. The edible fruits of the tree are large heart-shaped and green in color, and the diameter varies between 15 and 20cm.

Chemicals and Reagents

- ✓ Ethanol
- ✓ Mayer's reagent
- ✓ Ferric chloride
- ✓ Fehling's solution A and Fehling's solution B

- ✓ Benedict's reagent
- ✓ Concentrated HNO_3
- ✓ Lead acetate
- ✓ Glacial acetic acid

Phytochemical Screening of Extracts

The phytochemical analysis of extracts was caricd out by the method mentioned by Khandelwal.

Test Details

The following procedures were adopted to test for prescnce of various chemical constituents in the isocratic extracts of leaves.

Test solutions

Igm of powder extract was dissolved in 100ml of ethanol.

Test for glycosides

Keller-Killiani test: To 2ml test solution add few drops of glacial acetic acid, feric chloride solution and concentrated H_2 SO_4 through the sides of test tube which shows the separation between two layers, lower layer shows reddish brown and upper acetic acid layer tums bluish.

Legal's test

To the test residue add 1ml pyridine and 2-3 drops of 0.5%aqueous sodium nitroprusside solution. The solution is made alkaline. Pink color indicates presence of the cardiac glycosides.

Tests for alkaloids

Mayer's test

Test solution with Mayer's reagent (potassium mercuric iodide)gives cream colored precipitate.

Dragendroff's test

The acidic solution with Dragendroffs reagent (potassium bismuth iodide).shows orange brown precipitate.

Tests for flavanoids

Ferric-chloride test

Test soution with few drops of ferric chloride solution shows blackish red color.

Shinoda test

Test solution with few fragments of magnesium ribbon and concentrated HNO_3 , shows pink to magenta red colour.

Lead acetate solution test

Test solution with few drops of lead acetate(10%)solution gives yellow precipitate.

Test for steroids

Salkowaski test

When a few drops of concentrated sulphuric acid is added to the mixture of chloroform and test solution, shaken and allowed to stand, lower layer turns red indicating the presence of sterols and formation of yellow color in the lower layer indicates the presence of triterpenoids.

Libermann's test

To a few mg of the residue in a test tube, add few ml acctic anhydride and heat gently. Cool the test tube. Add few drops of concentrated sulphuric acid form the side of the test tube. A blue color indicates presence of sterols.

Test for terpenoids

Licbermann-Buchardt test

The test solution is treated with few drops of acetic anhydride and mixed. When concentrated sulphuric acid is added from the sides of the test tubes, it shows a brown ring at the junction of the two layers and upper layer turns green.

Test for carbohydrates

Fehling's test

Mix 1ml Fchling's solution A and Fchling's solution B boil for Imin; add equal volume of test solution, heat in boiling water bath or 10 minutes. First yellow, then brick red precipitate is observed.

Benedict's test

Test solution treated with Benedicts reagent and heating on a boiling water bath solution appears green, yellow red depending on amount of reducing sugar present in test solution.

Barfoed's test

Test solution treated with Barfoed's reagent, on boiling on a water bath shoes brick red precipitate at the bottom of the test tube.

Test for proteins

Biuret test

Test solution treated with 4%NaOH and dilute $CuSO_4$ (1%)solution gives violet or pink color.

Millon's test

Mix 3ml of test solution with 5ml Millon's reagent. White precipitate is obtained which turns brick red iato or red colored solution on warming. If a carbon free ash cannot be obtained in this way exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, until the ash is white or nearly so, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°C. The percentage of ash was calculated with reference to the air-dried drug.

Ash(%)=Loss in Weight /W×100

Where W=weight of Leaves and fruits powder in gm

Determination of loss on drying

Loss on drying is the loss in weight in %, w/w. resulting from water and volatile matter of any kind that can be driven off under specified conditions.

Procedure: Accurately weighed 10g of drug taken in a tarred evaporating dish and initial weight of sample with evaporating dish was taken. The sample was heated at 105C in an oven and weigh. This procedure was repeated until constant weight was obtained. The moisture content of the sample was calculated with reference to air dried drug.

Loss on drying (%)=Loss in weight /W×100

Where W=weight of leaves powder in grams.

Water soluble ash value

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed C. The °with hot water and ignited for 15 minutes at a temperature not exceeding 450 weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water soluble ash calculated with reference to the air-dried drug.

2.2. Antioxidant activity

2.2.1. MATERIALS AND METHODS

Chemicals: Analytical laboratory grade chemicals, solvents were used for the studies, which were procured.

- DPPH
- Methanol
- Ascordic acid
- Standard Drugs
- Ascorbic acid

- **Apparatus**

Weighing balance, stand flask, beaker etc.

Instruments

- Analytical UV-Visible spectrophotometer
- Soxhlet apparatus
- Heating mantle

22.2. Antioxidant activity of A. muricata extracts

DPPH scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)scavenging activity of soursop extracts was determined. Firstly, the methanol (2mL) and methanolic solution of 1 mM DPPH radicals (0.25 mL) were mixed. Then, extracts (0.1mL) in different concentrations (0.4-2.0 mg/assay) were added. After the reaction in dark (20min), the absorbance was measured at 517 nm. The EC50 value (the half-maximal effective concentration) was determined on the basis of the plot of absorbance vs. extract concentrations.

FORMULA: E%=Abs control-Abs sample/A Control*100

23 Total phenolics content (TPC)[18]

Procedure: 1ml of plant extract or standard of different concentration solution was taken in a test tube 5ml of folin-ciocaltetus(diluted 10 times with water)reagent solution was added into the test tube,5ml of sodium corbonate 7.5% solution was added into the test tube. The test tube was incubated for 20 minutes at 25C to complete the reaction. Then the absorbance of the test solution was measured at 760nm using a spectrophotometry against blank Atypical blank solution contained all reagents except plant extract or standard solution.

Formula: A=C*V/M

24 Total flavonoids content (TFC)'[9]

Procedure: 1.0 ml of plant extract or standard of different concentration solution was taken in a test tube·3ml of methanol was added into the test tube 200ul of 10% aluminium chloride solution was added into test tube, 5.6ml of distilled water was added into the test tube. The test tube was then incubated at room tenmperature for 30 minutes to complete the reaction. Then the absorbance of the solution was measured at 420nm using a spectrophotometer against blank A typical blank solution contained all reagents except plant extract or standard solution.

Formula: C=X*V/NS

3. RESULTS AND DISCUSSION

3.1 Extraction

The percentage yield of extract was found to be 50%. Yield of Ethanolic extract of A. muricata determine yield was more when compared to Methanolic & other organic solvents extraction.

Table 3.1: Percentage Yield of Extract.

S.NO	Extract	Appearance	Consistency	Yield(%)
1	Soxhlet extract (fruit pulp: Ethanol 1:10)	Light green	Water	50%

S.NO	Standardiztion parameter	Values obtained
1	Total ash value	11.70%
2	Water soluble ash	3.43%
3	Loss on drying	11.8%

3.2. Phytochemical Screening

The Phytochemical screening revealed that the presence of alkaloids, saponins, flavanoids, Tannins, proteins, carbohydrates and terpenoids, while glycosides and steroids were absent.

Pharmacognostical Investigation: The powdered fruit of *Annona muricata* was investigated for the physical constants which revealed that total ash 11.70%w/w, Water soluble ash 3.43%w/w, loss on drying 11.80%w/w.

Table 3.2: Phytochemical Screening & Pharmacognostical Investigation.

Sl.No	Phytochemicals	Results
1	Alkaloids	+
2	Saponins	+
3	Flavanoids	+
4	Glycosides	+
5	Tannins	+
6	Proteins	+
7	Carbohydrates	+
8	Terpenoids	+
9	Steroids	-

3.3: ANTIOXIDANT ACTIVITY

The antioxidant activity revealed that the present of concentration of 0.4to 2.0 mg/ml and the absorbance and E% are given in table.

Obtain extracted solution [ml]	Concentrations mg/ml	Absrbance[A]	E%
Control	0	0.301	
1	0.4	0.014	5.86%
2	0.8	0.011	10.7%
3	1.2	0.009	17.26%
4	1.6	0.006	35.52%
5	2.0	0.004	65.82%

3.3 a: Calibration curve of *Annona muricata* for Antioxidant activity

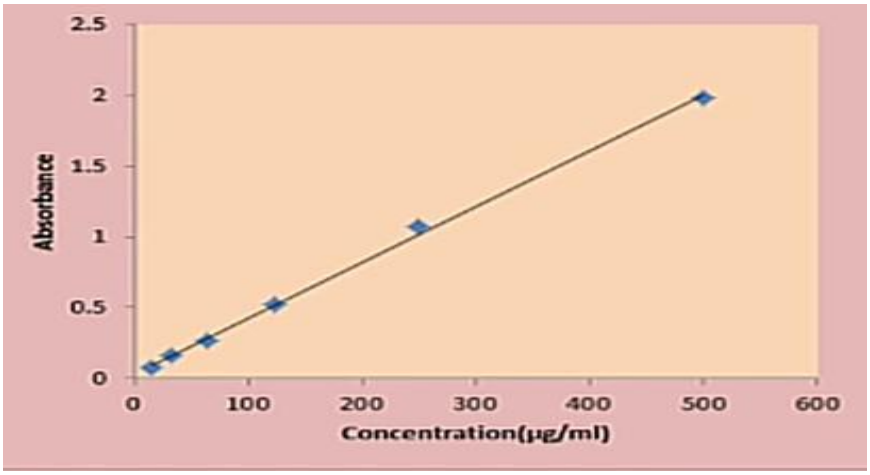
TOTAL PHENOLIC CONTENT

Total phenolic content: the total phenolic content revealed that the concentration (0.001), 0.281 absorbance and 82.67%.

Table 3.3 a: Total Phenlic content.

sample extract of A.muricata	Absorbance	TPC
0.001	0.281	82.67
0.001	0.277	81.33
0.001	0.28	82.33

3.3b: Calibration curve of *Annona muricata* for total phenolic content



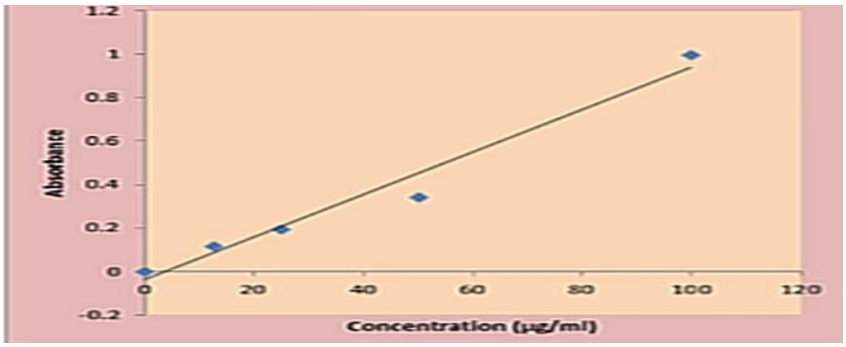
TOTAL FLAVANOID CONTENT

The flavanoid content revealed that the concentration (0.001), 0.672absorbance and 73.5%.

Table 3.3 a: Total Flavnoidcontent.

sample extract of A.muricata	Absorbance	TFC
0.001	0.669	73.2
0.001	0.672	73.5
0.001	0.67	73.3

3.3.c: Calibration curve of *Annona muricata* for total flavonoids content



CONCLUSION

- The genus *A.muricata* is widely used for various medicinal purposes such as Antioxidant, Anti bacterial, Cytotoxic, Anti-inflammatory etc.
- Bio active compounds extracted from fruit of *A.muricata* by using the solvent ethanol.
- The scope of the present work is also included.
- The phytochemical screening and pharmacognostical investigation of *A.muricata* extraction are presented and the relavant data are tabulated.
- Antioxidant activity is evaluated by DPPH scavenging assay.
- Ethanolic extract of *A.muricata* is found to be 50% yield.
- Phytochemical investigation relived that the presence of phenolic compound and flavanoid compounds.

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