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DETECTION OF FLAVANOIDS, TANNINS, PHENOLS AND PHENOLIC FLAVANOIDS IN VARIOUS SOLVENT EXTRACTS OF *ANNONA MURICATA* USING LEAD ACETATE TEST

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ABSTRACT

This recent research results obtained from the qualitative screening using lead acetate test to detect phenolic flavonoids, tannins, phenols and flavonoids in various solvent extracts of *Annona muricata* rely on the visual color change reactions as a basic response to the presence of a specific phytochemical compound. *Annona muricata* belongs to Annonaceae which is now used as natural therapy for treating cancer. The different solvent extracts showed different phytochemicals by the analysis using lead acetate test. Flavanoids were detected in Methanol Fresh, Methanol Dry, Petroleum Ether Fresh and Hexane Fresh extracts, Tannins and Phenols were present in acetone fresh Aqueous dry and fresh extracts Phenolic flavonoids were present in Acetone Dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethylacetate Fresh, Ethylacetate Dry, Petroleum ether Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry, Hexane Dry and was absent in all other tested extracts.

KEYWORDS: Lead acetate test, *Annona muricata*, Phenolic Flavanoids, Tannins, Phenols, flavonoids.

1. INTRODUCTION

Plants are the most vibrant forms of life on earth from which humanity derives most of its food, shelter, oxygen and other essential goods and services. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world.[2] Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of wild plants. From ancient literature to modern scientific records of traditional medicinal Knowledge, it is evident that plants are the main source of medicine for human health care. The study on the medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as a source for the new drugs.[18] Use of plants and plant products as pharmaceuticals dates back to the beginning of human civilization

Plants have always been the principal form of medicine in India and presently they are becoming popular throughout the developed countries, as people strive to stay healthy in the face of chronic stress and pollution. The sacred Vedas dated back between 3500 B.C and 800 B.C gives many references of the utilization of the medicinal plants. "Virikshayurveda" one of the remotest works in the traditional herbal medicine was compiled before the beginning of Christian era. "Rig Veda" one of the oldest literatures mentions the use of Cinnamon (*Cinnamomum verum*), Ginger (*Zingiber officinale*), Sandalwood (*Santalum album*) which were used not only in the religious ceremonies but also in the medical preparations.[19] The relationship between food and medicine was quoted as "Let food be thy medicine and medicine be thy food".[6] Plants and plant-based medicaments are used as the basis of many of the modern pharmaceuticals we use today in order to treat our various ailments.[1]

Phytochemicals are biosynthesized by plants to protect themselves from insect attacks and plant diseases. They are secondary metabolites with no nutritional value and the natural combination of these secondary metabolites provides the general beneficial therapeutic effects of the plant. Phytochemical analysis with various reagents and chemicals are used in the identification of plant compounds with medicinal properties. The impact of different types of solvents have been studied and analyzed by scientists.[3,4]

Extraction is the first step to separate the phytochemicals from the desired plants. Solvent selection is crucial for solvent extraction [20] Solvents used for extraction of phytochemicals from medicinal plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will accurately dissolve the

solute. Multiple solvents can be used sequentially to limit the amount of analogous compounds.[3] Soxhlet Extraction is an automatic continuous extraction method with high extraction efficiency, less time and solvent consumption. The high temperature and long extraction time may some times lead to thermal degradation of sample.[20]

Inorder to extract different phenolic compounds from plants various solvents with different polarities must be used.[27] Methanol was found to be more effective to extract a large amount of Phenolic contents when compared to ethanol.[5,10,22] It has been reported that the bioactive compounds of Rhodiola rosea extracted in methanol showed a significant yield of phenolics.[17] It was reported that ethanolic extracts of Ivorian plants extracted higher concentrations/amount of phenolics compared to acetone, water and methanol.[10]

Based on the importance and its biomedical applications, a well known Indian medicinal plant *Annona muricata* was selected for the present study since it can treat a myriad of conditions including hypertension, diabetes and cancer. *Annona muricata* is a species of the Annonaceae family that has been used and widely studied in the last decades due to its therapeutic potential. More than 120 acetogenins have been isolated from extracts of different parts of *Annona muricate* which contribute mainly for its anticancer and antitumerogenic property.[12] Some of the key intermediates that are involved in the biosynthesis of these acetogenins has been isolated from this species recently.

The objective of this study was to find out the best solvents to extracts flavonoids, tannins, Phenols and phenolic flavonoids from *Annona muricata* a widely studied plant against cancer and was used traditionally as a food as well as medicine. An effort was made to discriminate between the fresh and dried plant extracts for extraction of bioactive compounds using various solvents.

2. MATERIALS AND METHODS

2.1 Selection of Plant for the study

Annona muricata L. belongs to the family Annonaceae was selected for this study based on ethno botanical information compiled through interviews from siddha and Ayurveda medical practitioners, traditional healers and old experienced people which form authentic and first-hand source of reference. The plant was identified by Dr. N. Vijayakumar Associate Professor, Department of Botany, S.T Hindu College Nagercoil. The Plant was collected from Siddha Vidya Abhyasalayam Arayoor during 2018 June - July month. Arayoor is a small Village in Neyyattinkara Taluk in Thiruvananthapuram District. It comes under Chenkal Panchayath. It belongs to South Kerala Division. Arayoor is located 5 km from Parassala, 6 km from Neyyattinkara and 30 km from Thiruvananthapuram. Arayoor lies between Global Positioning System co ordinates of 8.3615° N, 77.1299° E. Healthy and fresh leaves of *Annona muricata* was collected from locally grown plants.

2.2 Extraction

2.2.1 Fresh extract[15]

50 g of fresh plant material of plant was grounded with different solvents and soaked in 200 ml of different solvents for 3-7 days filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No. (41) to attain a clear filtrate. The filtrates were evaporated and dried at 40°C under reduced pressure using rotatory vacuum and stored in a small bottle in fridge at 5°C.

2.2.2 Dry Extract (Soxhlet extraction)[25]

The extraction procedure for the isolation of crude drug from plants has been practiced since long time. The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on type of substance that is being isolated. Normally the crude extract is taken from soxhlet apparatus with the help of non-polar to polar solvents.

2.2.3 Soxhlet apparatus

This apparatus mainly consists of three parts, round bottom flask in which the solvent is taken, main jar in which material from which the compounds to be extracted is kept loaded and condencer in which condensation of vapors of solvents takes place. 100 g of the powder of plant material from which the extract has to be taken is packed into soxhlet main jar. The solvent is poured into the round bottom flask and extract condensation under reduced pressure and controlled temperature of 60-80°C is set to boil through regulated heating mantle. The vapor of the solvent pass through drive tubes, enter the condenser through the main jar and get condensed where there is continuous flow of water in the condenser. The condensed solvent falls back on the packed material in the main jar before collecting in jar itself. The collection and extraction of material takes place simultaneously in the main jar as seen by the coloring of the solvent as compound of material get dissolved in the solvent. Thus, the crude extract of the plant material is obtained. For the isolation of all phytoconstituents from plant requires relatively large volume of organic solvents normally it took 7-8 hours for complete extraction. The solvent will be evaporated and finally it yields brown/green/waxy extract, this is stored in refrigerator for further usage.

2.3 Phytochemical Analysis

Phytochemicals, chemical compounds that occur naturally in plants (phyto means "plant" in Greek), are responsible for color and biological properties. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients.

The following test used for the analysis of phytochemicals was carried on different extracts of plant. The test was triplicated and confirmed.

2.3.1 Lead Acetate Test[4]

Extracts were treated with few drops of Lead Acetate solution. Formation of a yellow precipitate indicates the presence of flavonoids. A bulky white precipitate indicates presence of tannins and phenols. A brown precipitate indicates presence of phenolic flavonoids.

3. RESULT

Acetone Fresh, Acetone Dry, Aqueous Fresh, Aqueous Dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethylacetate Fresh, Ethylacetate Dry, Methanol Fresh, Methanol Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry, Petroleum Ether Fresh, Petroleum ether Dry, Hexane Fresh, Hexane Dry extracts of *Annona muricata* was screened by lead acetate test.

Flavanoids were detected in Methanol Fresh, Methanol Dry, Petroleum Ether Fresh and Hexane Fresh extracts and was absent in Acetone Fresh, Acetone Dry, Aqueous Fresh, Aqueous Dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethylacetate Fresh, Ethylacetate Dry Extracts, Hexane Dry, Petroleum ether Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry.

Tannins and Phenols were present in acetone fresh Aqueous dry and fresh extracts and was absent in acetone dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethylacetate Fresh, Ethylacetate Dry, Methanol Fresh, Methanol Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry, Petroleum Ether Fresh, Petroleum ether Dry, Hexane Fresh, Hexane Dry extracts.

Phenolic flavonoids were present in Acetone Dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethylacetate Fresh, Ethylacetate Dry, Petroleum ether Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry, Hexane Dry and was absent in Methanol Fresh, Methanol Dry, Petroleum Ether Fresh, Hexane Fresh extracts, acetone fresh, Aqueous dry and fresh extracts.

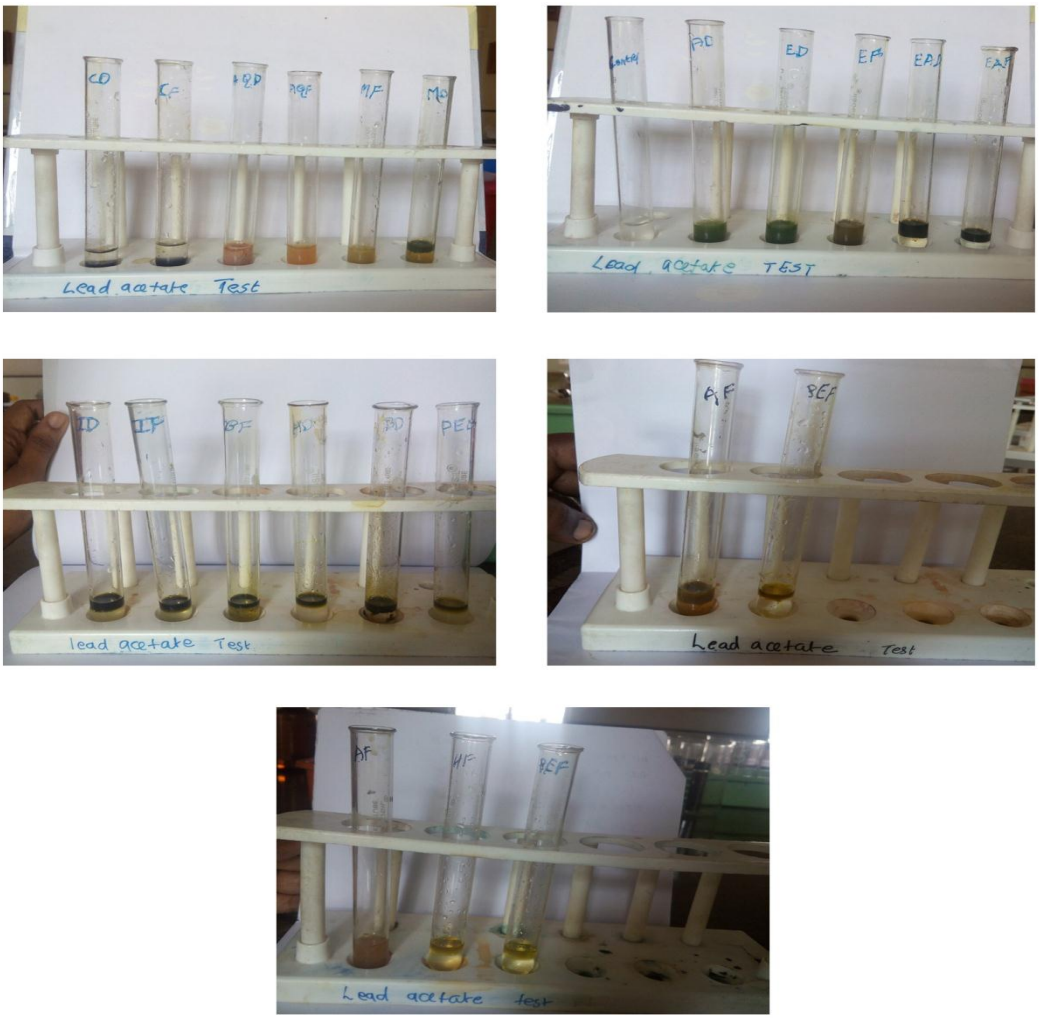

Fig. 1: Detection of Flavanoids, Tannins, Phenols And Phenolic Flavanoids In Various Solvent Extracts Of *Annona muricata* Using Lead Acetate Test.

AF – Acetone Fresh Extract, AD – Acetone Dry Extract, AQF – Aqueous Fresh Extract, AQD – Aqueous Dry Extract, BF – Butanol Fresh Extract, BD – Butanol Dry Extract; CF – Chloroform Fresh Extract, CD – Chloroform Dry Extract; EF – Ethanol Fresh Extract, ED – Ethanol Dry Extract, EAD – Ethyl acetate dry Extract, EAF - Ethyl acetate fresh Extract, HF – Hexane Fresh Extract, HD – Hexane Dry Extract, ID – Iso amyl alcohol dry extract, IF - Iso amyl alcohol fresh extract; MD – Methanol dry extract, MF – Methanol Fresh Extract, PEF – Petroleum Ether fresh extract, PED - Petroleum Ether dry extract

Table 1: Detection of Flavanoids In Various Solvent Extracts of *Annona muricata* Using Lead Acetate Test.

Sl.No	Solvent extracts	Presence of Flavanoids
1	Acetone Fresh	Absent
2	Acetone Dry	Absent
3	Aqueous Fresh	Absent
4	Aqueous Dry	Absent
5	Butanol Fresh	Absent
6.	Butanol Dry	Absent
7.	Chloroform Fresh	Absent
8.	Chloroform Dry	Absent
9	Ethanol Fresh	Absent
10	Ethanol Dry	Absent
11	Ethylacetate Fresh	Absent
12	Ethylacetate Dry	Absent
13	Methanol Fresh	Present
14	Methanol Dry	Present
15	Iso amyl Alcohol Fresh	Absent
16	Isoamyl Alcohol Dry	Absent
17	Petroleum Ether Fresh	Present
18	Petroleum ether Dry	Absent
19	Hexane Fresh	Present
20	Hexane Dry	Absent

Table 2: Detection of Tannins and Phenols In Various Solvent Extracts of *Annona muricata* Using Lead Acetate Test.

Sl.No	Solvent extracts	Presence of Phenols and Tannins
1	Acetone Fresh	Present
2	Acetone Dry	Absent
3	Aqueous Fresh	Present
4	Aqueous Dry	Present
5	Butanol Fresh	Absent
6.	Butanol Dry	Absent
7.	Chloroform Fresh	Absent
8.	Chloroform Dry	Absent
9	Ethanol Fresh	Absent
10	Ethanol Dry	Absent
11	Ethylacetate Fresh	Absent
12	Ethylacetate Dry	Absent
13	Methanol Fresh	Absent
14	Methanol Dry	Absent
15	Iso amyl Alcohol Fresh	Absent
16	Isoamyl Alcohol Dry	Absent
17	Petroleum Ether Fresh	Absent
18	Petroleum ether Dry	Absent
19	Hexane Fresh	Absent
20	Hexane Dry	Absent

Table 3: Detection of Phenolic flavonoids In Various Solvent Extracts of *Annona muricata* Using Lead Acetate Test.

Sl.No	Solvent extracts	Presence of Phenolic Flavanoids
1	Acetone Fresh	Absent
2	Acetone Dry	Present
3	Aqueous Fresh	Absent
4	Aqueous Dry	Absent
5	Butanol Fresh	Present
6.	Butanol Dry	Present
7.	Chloroform Fresh	Present
8.	Chloroform Dry	Present
9	Ethanol Fresh	Present
10	Ethanol Dry	Present
11	Ethylacetate Fresh	Present
12	Ethylacetate Dry	Present
13	Methanol Fresh	Absent
14	Methanol Dry	Absent
15	Iso amyl Alcohol Fresh	Present
16	Isoamyl Alcohol Dry	Present
17	Petroleum Ether Fresh	Absent
18	Petroleum ether Dry	Present
19	Hexane Fresh	Absent
20	Hexane Dry	Present

4. DISCUSSION

Phytochemical Sccreening of *Annona muricata* using Lead acetate test revealed some differences in extraction of phytochemicals according to solvents used.

Flavonoids were present in the aqueous and methanolic extracts of *Annona muricata* leaf extract.[23,26] In the present work flavonoids were present in Methanol Fresh, Methanol Dry, Petroleum Ether Fresh and Hexane Fresh extracts and was absent in Acetone Fresh, Acetone Dry, Aqueous Fresh, Aqueous Dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethylacetate Fresh, Ethylacetate Dry Extracts, Hexane Dry, Petroleum ether Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry, of the phytochemicals, flavonoids and their derivatives which are formed from the process of glycosylation, hydroxylation, methylation and alkylation have been widely studied, mainly due to their broad spectrum of pharmacological activities such as anti- inflammatory, anti- pyretic, anti-diabetic, anti-allergic and anti-hypertension in vitro and in vivo.[13,14]

Phenolic compounds consist of a wide range of plant substances which are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups. Phenolic compounds are responsible for the major organoleptic characteristics of plant-derived foods and beverages, particularly colour and taste properties and they also contribute to the nutritional qualities of fruits and vegetables. Plant Phenolic compounds are a chemically heterogenous group: Some are soluble only in organic solvents; some are water-soluble carboxylic acids and glycosides. Another group of phenolics are insoluble polymers.[8,22,24] Tannins have been reported to possess astringent properties, hasten the healing of wound and inflamed mucous membranes.[11,16] In the present study Tannins and Phenols were present in acetone fresh Aqueous dry and fresh extracts and was absent in acetone dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethyl acetate Fresh, Ethyl acetate Dry, Methanol Fresh, Methanol Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry, Petroleum Ether Fresh, Petroleum ether Dry, Hexane Fresh, Hexane Dry extracts. Tannins were present in aqueous and methanolic extracts of *Annona muricata.*[7 9 23] Presence of phenols in methanolic extracts of leaf and fruit were also reported.[26]

No studies on the detection of phenolic flavonoids of *Annona muricata* has been reported yet. Phenolic flavonoids are present in small traces in gooseberry aqueous extract.[4] In the present investigation Phenolic flavonoids were present in Acetone Dry, Butanol Fresh, Butanol Dry, Chloroform Fresh, Chloroform Dry, Ethanol Fresh, Ethanol Dry, Ethyl acetate Fresh, Ethyl acetate Dry, Petroleum ether Dry, Iso amyl Alcohol Fresh, Isoamyl Alcohol Dry, Hexane Dry and was absent in Methanol Fresh, Methanol Dry, Petroleum Ether Fresh, Hexane Fresh extracts, acetone fresh, Aqueous dry and fresh extracts..

Generally, researchers prefer dried extracts for phytochemical studies but sometimes few constituents are higher in fresh plant material, which degrades after drying. In the present study there was also distinct differences between fresh and dry extracts. Phenolic flavonoids, tannins and phenols were present in acetone

dry extracts but absent in acetone fresh extracts. Phenolic flavonoids were present in Petroleum ether dry and hexane dry extracts but absent in their fresh extracts. Flavonoids were present in petroleum ether fresh and hexane fresh extracts but absent in its dry extracts.

Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers and hence this plant is a good anticancer agent. These phytochemical compounds are the key candidates in the medicinal value of the plant. This data can also help us to choose the appropriate solvents to extract bioactive compounds in greater quantities for medical and therapeutic purposes.

5. CONCLUSION

The research postulates that the extraction of different phenolic compounds and flavonoid need different solvents with different polarities. The wise utilization of *Annona muricata* will be an advantage to mankind. Detailed study should be carried out on the isolation of the bioactive compounds present in *Annona muricata* and their mechanism of action. It can be useful for treating many life threatening diseases.

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