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UTILISATION OF DYE ISOLATED FROM *Artocarpus heterophyllus* TREE BARK FOR BOTANICAL MICROTECHNIQUE

Fathima sumaya[1], Dr. Rohini[2].A, Dr. Moossa P.P[3,]A.Lakshman[4]V.Priyanka[5]

1. II M.Sc Biochemistry,Rathnavel Subramaniam College of Arts and Science, Coimbatore,Tamil Nadu-641402,fathimasumaya148@gmail.com,ph: 7561800221

2. Assistant Professor,Department of Biochemistry, Rathnavel Subramaniam College of Arts and Science,Coimbatore, Tamil Nadu-641402

3. Professor, Department of Crop Production, Regional Agricultural Research Station, Pattambi,Kerala,

4, II M.Sc Biochemistry,Rathnavel Subramaniam College of Arts and Science, Coimbatore,Tamil Nadu-641402

5. III B.Sc Biochemistry,Rathnavel Subramaniam College of Arts and Science, Coimbatore,Tamil Nadu-641402

Corresponding Author: Dr. Rohini[2,] rohini@rvsgroup.com,9894559991

Abstract

One of the main methods for plant anatomy study is the analysis of thin, transparent, and stained tissue sections. Synthetic dyes traditionally used in anatomical studies might be expensive and produced by specific companies. In contrast, the use of alternative dyes can both represent an inexpensive substitute as well as an environmentally friendly option for conducting plant anatomy studies. In this study, powder of *Artocarpus heterophyllus* tree bark were used, Transversal sections of plants obtained using the freehand cutting technique were stained using ethanol solution and this powder mixed with 85% ethanol solution showed higher efficiency in tissue contrast, allowing greater solubility of dye powder and better solution interaction with the plant tissues. Quantitative phytochemical analysis of this species exhibited the presence of glycoside, phytosterol, phenol, flavonoid. The results pertaining to GC-MS analysis lead to the identification of a number of compounds from gas chromatography fractions of the ethanolic extract of *Artocarpus heterophyllus* .The results revealed that the presence of different phytoconstituents .This findings suggest that the developed method can be useful in mixed practical classes of plant anatomy, chemistry, and/or biochemistry, both at high school as well as undergraduate levels.

Keywords: *Artocarpus heterophyllus*, Freehand cutting, GC-MS

INTRODUCTION

The Artocarpus heterophyllus is a species of tree of the mulberry family(Moraceae) is known by other names jackfruit (Eng.), Kathal, Panas (Hindi), Kanthal (Beng.), Palaa (Tamil), Phanas (Guj & Mar) and Chakka (Malayalam). It is native to Western Ghats of India, Malaysia and also found in central and eastern Africa, south-eastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific Islands.

The Artocarpus heterophyllus contains various chemical constituents as several flavones colouring matters, morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone and artocarpanone [1]. Morin (3,5,7,2′,4′-pentahydroxyflavone) is a natural bioflavonoid that was originally isolated from the member of the moraceae family and is a constituent of many herbs, fruits, and wine.[2]

Dyes are substances of natural or synthetic origin, soluble in a medium which is usually used to impart a desired colour to a non-food material like paper, leather, wood, textiles and even cosmetics in a process known as dying [3]. Dyes are also referred to as stains and can be used to add colour to tissues, blood cells or organelles within individual cells as well as microorganisms such as bacteria, fungi and yeast to make them optically distinct. Stains are generally used to add colour to animal tissues, plant tissues, microbes and spores to make them optically distinct and the technique is known as Staining. Recent studies have given useful result in which such abundant dye plants were used as histological stains for some tissue components .These dyes are found in the root, root bark, leaves, flowers, stem, stem bark, fruit skins and nut shell. The efficiency of some local natural herbal dyes for use in staining plant materials was found to be non-toxic and eco-friendly. They are also a source of cheap stains for use in plant histology [4]. Most stains in current use are chemically synthesized from cheap petroleum by-product, they show superior fastness property and wide variety of colours [5] and they are found to be hazardous to man's health [6,7]. Some synthetic dye components are carcinogenic or at least strongly allergenic resulting in their withdrawal as their hazard becomes recognized [8]. The usage of vast amount of synthetic dyes has resulted in water, land and air pollution which has greatly disturbed the earth ecological balance and cause health hazards [9]. As a result of this, manufacturing of synthetic dyes has been banned in some countries like India, Netherland and Germany which were the first producers of such dyes [6,10]. This has stimulated the search for alternative dyes for staining microbial cells, food samples, tissues and other materials which are relatively cheaper, eco-friendly and biodegradable. One of the ways to providing an alternative to synthetic dye is the provision of natural dye from plants and animal origin which has become an important topic due to the increase in environmental awareness targeted at reducing the deleterious effects of hazardous synthetic dyes to living things.Therefore, there is a need for alternative source of dye which is easily available from plants which are eco-friendly, biodegradable, non-toxic to man and easy to produce.

METHODOLOGY

Extraction of dye from various solvents

Yellow coloured substances are present inside the bark of the Jackfruit tree, Peel the bark of the Jackfruit tree then the yellow-coloured substance was scraped from it. Collect about 50gm of yellow substance from the tree then air dried and finally powder it by using a grinder. 1g of sample were soaked for 24hrs in 100ml of solvents (Ethanol, Acetone, Hexane, Butanol, Water). Then the mixture was filtered, after filtration the residue was placed in the oven for 5 minutes and dried. In order to check in which solvent has more extraction, then the weight of the filter paper before filtration and residue after filtration should be taken.

Quanlitative phytochemical analysis

About 5g of powdered sample was dissolved in 50ml of ethanol and kept it in a shaker for 24 hours.The extracts were filtered through Whatmann No.1 filter paper and residue was collected. The filter was concentrated using a rotator vacuum evaporator to get ethanol extract of the dried powdered sample. Each time before extracting with the next solvent the residue was air dried thoroughly to remove the traces or solvent used. The preliminary qualitative phytochemicals studies were performed for testing the different chemical groups present in different extracts

Botanical microtechnique

Botanical micro technique is an aggregate of methods providing micro visualization of gene and gene product in an entire plant. Plant micro technique is also a study providing valuable experimental information. Plant micro technique involves classical methods developed over a hundred years ago and new methods developed to expand our research scope and depth in botanical micro studies. Both traditional and new micro technique is useful for experimental research, and some will have a significant influence on further study. Different methods are used to prepare plant specimens, including direct macroscopic examinations, freehand sections, clearing, maceration, embedding, and staining.

GC-MS analysis of bioactive compounds

A mixture of analyte separated into individual components when heated. The heated gases are Carried through a column with inert gas, which is a carrier gas (Helium). The separated Substance flow into MS, compounds are identified separately. Gas Chromatography-Mass Spectrometry (GCMS), is a combination of gas Chromatography and mass spectrometry used to identify unknown samples and separate Volatile and semi-volatile compounds. Ethanol extract of *Artocarpus heterophyllus* used for this technique.

Usually, x-axis of GC-MS chromatogram shows amount of time taken for analytes pass Through column and reach mass spectrometer detector. The peaks that are shown corresponds to the time at which each of components reached the detector. The y-axis or area of peaks is a Reflection of amount of analyte. When looking at GC-MS chromatogram, area will be based on the number of counts taken by Mass spectrometer detector at retention point. Unknown compounds are identified based on Retention times of known standards with other detectors. The mass spectrometer then allows Identification of compound by mass spectrum obtained at time of testing.

Results and Discussion

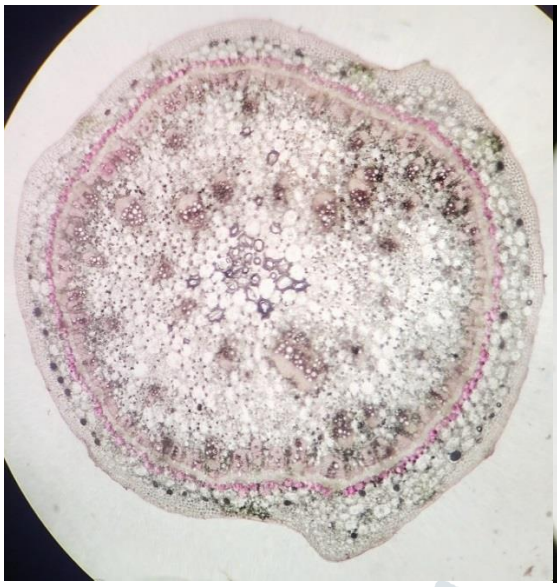
Out of 5 different solvents, Ethanolic,Aqueous,Hexane,Acetonic and Butanolic extract of the bark ,Ethanolic extract showed the maximum extraction when compared to other solvents.The difference in the weight of the residue in ethanolic extract was found to be 0.670 gms. Hence, phytochemical analysis was done using ethanolic extract.

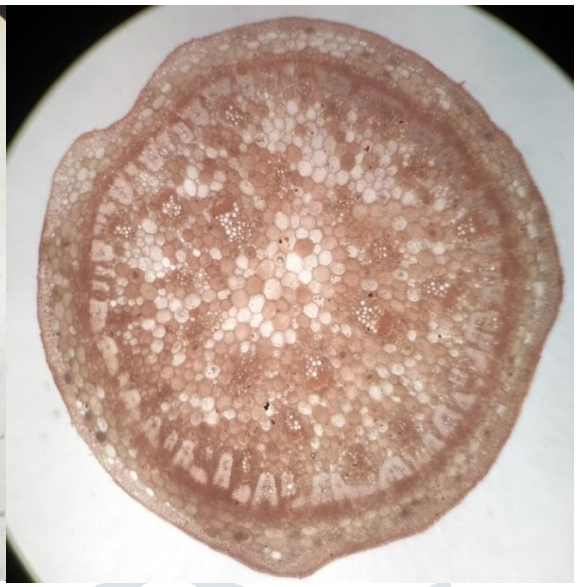
The phytochemical analysis of the *Artocarpus heterophyllus* tree bark was carried out and experiment shows the presence of phytochemicals in ethanol extract contain glycoside, phytosterol, phenol,flavonoid and absence of alkaloid, carbohydrate, saponin, tannin, protein, and diterpenes.

Simple Staining:

Cells typically have negatively charged cell walls, the positive chromophores in basic dyes tend to stick to the cell walls, making them positive stains. Thus, commonly used basic dyes such as basic fuchsin, crystal violet, malachite green, methylene blue, and safranin typically serve as positive stains. So the newly prepared morin stain also shown the similar property.

Dicot stem-(Amaranthus plant)



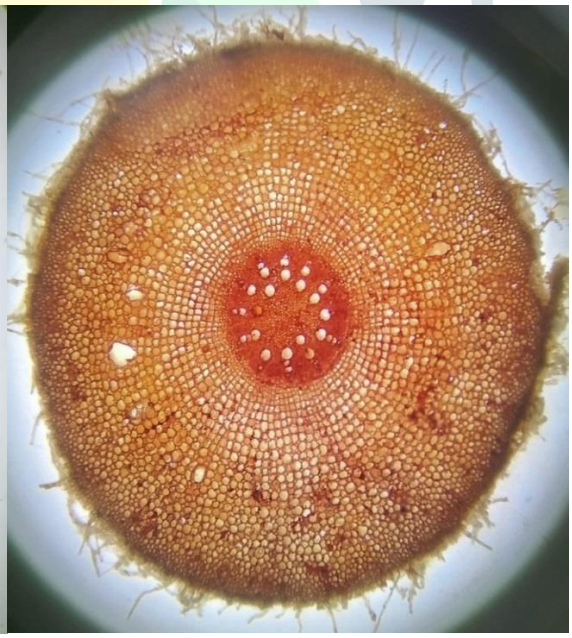


(Fig.1 Plant cell before staining) (fig.2 plant cell after staining with morin)

Double stain/ Counter stain:

The morin stain provide a contrast to the safranin and crystal violet stain, which allows easy and rapid detection of the plant's vascular tissue. While Safranin and morin have same staining property thus morin is convenient to stain plant cell.

MONOCOT ROOT



(fig.3 plant cell before staining) (fig.4 plant cell after staining with safranin and morin)

GC-MS chromatogram of the ethanolic extract of *Artocarpus heterophyllus* tree bark

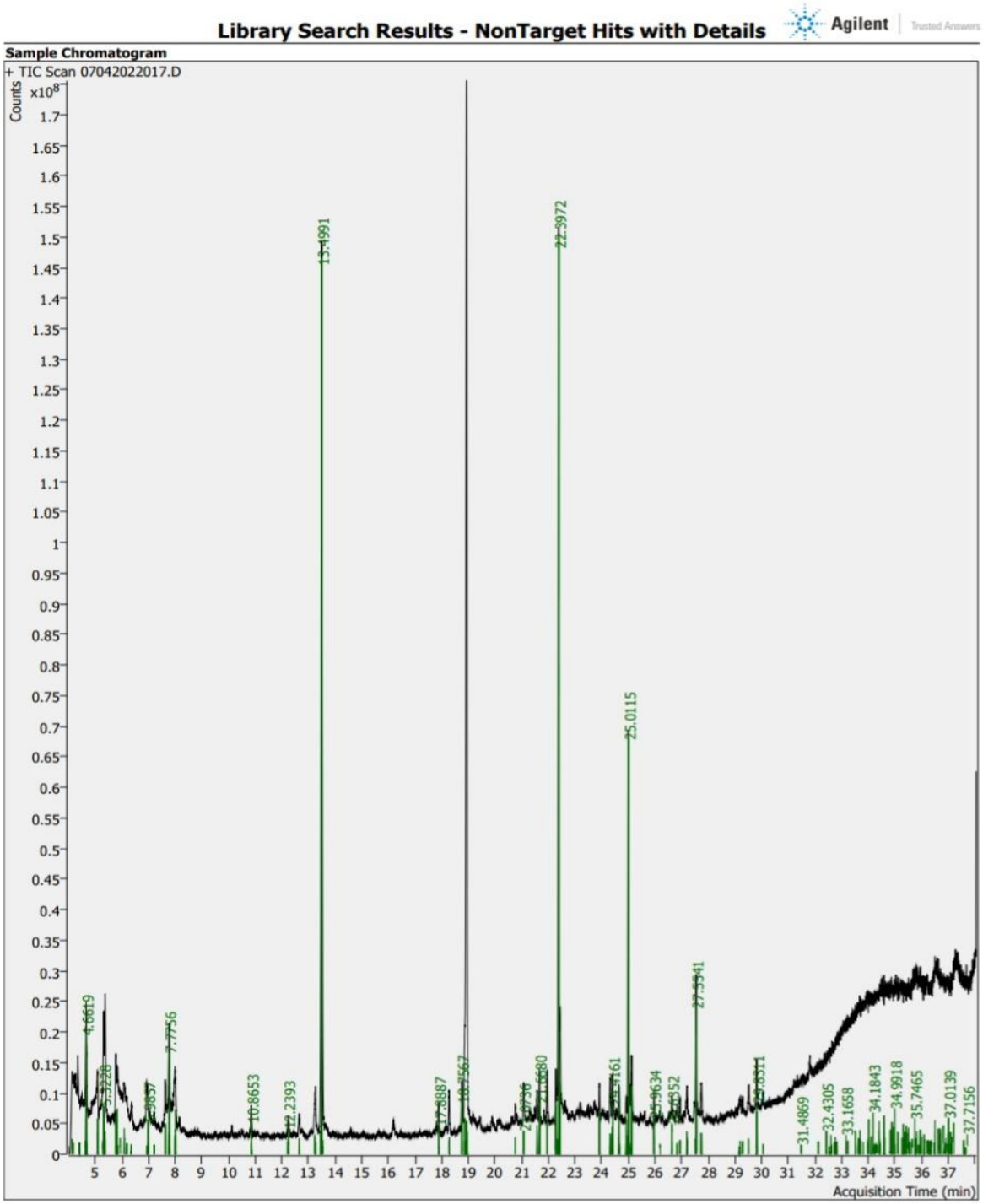


Figure 1: GC-MS chromatogram of the ethanolic extract of *Artocarpus heterophyllus* tree bark

Identification of the Components

The interpretation of mass spectroscopy (GC-MS) was conducted using data base of Library: NIST having more patterns. The spectrum of the unknown component was compared with the spectrum of the known component stored in the Library: NIST. The retention time, molecular weight, molecular formula and composition percentage of the sample material was recorded .The results pertaining to GC-MS analysis lead to the identification of a number of compounds from gas chromatography fractions of the ethanolic extract of *Artocarpus heterophyllus*. They were identified through mass spectrum attached with GC (figure 1). The GC-MS analysis of the ethanolic extract of *Artocarpus heterophyllus* was reported and

presented in table1.The identified compounds of *Artocarpus heterophyllus*, their retention time, percentage composition are given in Table 1. The results revealed that the presence of different chemical components.

The results showed the presence of major compound identified as Hexadecane and Dodecane, thus the present study shows that the majority of the compounds were Hexadecane (22.3972) and it was used for radiolabelling exosomes and hydrogels, and for positron emission tomography.

. Conclusion:

Based on the results of research that has been done can be concluded that the Synthetic dyes traditionally used in anatomical studies might be expensive and produced by specific companies. In contrast, the use of alternative dyes can both represent an inexpensive substitute as well as an environmentally friendly option for conducting plant anatomy studies. Hence, the study on the utilisation of dye isolated from *Artocarpus heterophyllus* tree bark for botanical microtechnique come out of four phases and they are Phase-I: collection of plant material, Phase-II: solvent extraction, Phase-III:phytochemical analysis, Phase-IV:Staining of plant cell, Phase-V :identification of compound by GC-MS.

Thus the findings suggest that the developed method can be useful in mixed practical classes of plant anatomy, chemistry, and/or biochemistry, both at high school as well as undergraduate levels

Reference

1. www.traditionaltree.org
2. R. Dayal and T.R. Seshadri. Colourless compounds of the roots of Artocarpus heterophyllus. Isolation of new compound artoflavone. Indian J Chem. 12: 895-896 (1974).
3. K. Shinomiya, M. Aida, Y. Hano and T. Nomura. A diels-alder-type adducts from Artocarpus heterophyllus. Phytochemistry. 40(4): 1317-1319 (1995).
4. Braide W, Akobundu C, Nwaoguikpe RN, Njiribarko LC. The use of extracts from four local Nigerian plants for the staining of selected bacteria and moulds. African Journal of Microbiology Research. 2011;5(1):79-86
5. Chukwu O, Odu C, Chukwu D, Hafiz N, Chidozie V, Onyimba L. Application of extracts of henna (Lawsonia inamis) leaves as a counter stain. African Journal of Microbiology Research. 2011;5(21): 3351-3356
6. Tiford, M. The long history of hematoxylin. Journal of Biotechnic and Histochemistry. 2005;80(2):73-78. Available:www.denverpost.com assesed05/06/2017
7. Prescot M, Harley J, Klein P. Microbiology. Microbial Staining Techniques. Published by Willey, Joanne M; Sherwood, Linda; Woolverton, Christopher J; Prescott, Lansing M. McGraw Hill Publishers, New York. 2009;122-156.
8. Okolie NJ. Staining of Ova of intestinal parasites with extracts of Hibiscus sabdariffa and Azadirachta indica. International Science Research Journal. 2008;1(2):116-119.
9. Akinloye AJ, Illoh HC, Olagoke AO. Screening of some indigenous herbal dyes for use in plant histological Staining. Journal of Forestry Research. 2010;21(1):81-84
10. Ihuma J, Asenge G, Abioye K, Dick S. Application of meth-anolic extracts from Hibiscus sabdariffa line as a biological staining agent for some fungal species. International Journal of plant, Animal and Environmental Science. 2012;2(3):543-49.