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A Study on Extraction, Characterization and Anti Microbial Activity of Anthocyanin of Musa Paradisiaca (Banana) Flower Bract

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ABSTRACT: The most important class of water-soluble pigments in plants and the largest class of phenolic compounds, anthocyanins constitute the reason for the distinctive red, purple, and blue hues of many fruits, vegetables and flowers. Plant anthocyanins have been extensively researched for their therapeutic potential. Anthocyanins have anti-diabetic, anti-cancer, anti-obesity, anti-microbial, and anti-inflammatory properties and also help in preventing cardiovascular disorders. Banana (Musa paradisiaca) flower bract, edible waste of banana production, was investigated as best origin of anthocyanin and natural colorant. This experiment aims in the extraction of anthocyanin from Banana Bract and is done by using Solvent extraction Method (SEM) using different solvents such as Ethanol, Methanol and Water. The characterization of the extracts was carried out using UV-Visible Spectrophotometer, TLC, FITR and resulted in confirmation of the presence of anthocyanin in the extracts. The total anthocyanin content resulted as 30.72, 17.86 and 33.64mg respectively, UV- Visible Spec resulted in the presence of Anthocyanin content and the reading were detected at 540, 535 and 430nm confirming the presence. The Anti-Oxidant study is carried out for the extracted anthocyanin and resulted in the Total Phenolic compounds is 76, 82 and 79mg; the total anti-oxidant activity is 310, 278 and 319mg respectively. The anti-bacterial and anti-fungal activity were examined using S.typhi, E.coli, S.aureus, B.cereus, A.niger and A.flavus and results shown that the Water Extract has shown higher bacterial activity than Ethanol and Methanol extracts. And there is no Anti-fungal activity of Water extract whereas Methanol extract has shown higher Anti-fungal activity than Ethanol Extract.

1. INTRODUCTION:

The production of bananas accounts for over 16% of the total fruits produced worldwide, ranking in second place to citrus. India is the world's leading banana producer, accounting for 27% of global banana production (Kumar, M. K et al., 2013). Bananas are grown in 20 different varieties throughout numerous Indian states (Joyita M et al., 2020). The most underutilized byproduct that can be consumed by people is banana flower. The male banana bud, also known as a banana flower or blossom, is a maroon-colored leafy cone with layers of cream-colored florets near the end of the banana bunch. Blossom is nutritious and high in antioxidants, with numerous health benefits, yet often discarded when cultivating bananas (Damini Soni and Gargi Saxena., 2021). The outer surfaces and extreme tip of the convoluted bracts, which are typically purple or red in color (ranging from bright red to dark violet), may occasionally be yellow. The bracts are a type of modified leaf that are usually small but can occasionally be larger and brightly colored. Since it contains fiber, potassium, vitamin E, and unsaturated fatty acids, bract is

one of the best foods with numerous health benefits. Anthocyanins such delphinidin, pelaragonidin, peonidin, and malvidin were reportedly found in the Musa paradisiaca bract. (Ramakrishna.S & Yashwanth M.B., 2022)

Anthocyanins, the largest class of phenolic compounds and the most significant class of water-soluble pigments in plants, give many fruits, vegetables, grains of paradise, and flowers their characteristic red, purple, and blue colors (Martín et al., 2017). Plant anthocyanins have been extensively researched for their therapeutic potential. Anthocyanins have anti-cancer, anti-diabetic, anti-microbial, anti-obesity, and anti-inflammatory properties and also help in preventing cardiovascular disorders (Khoo et al., 2017).

The extraction of anthocyanin can be done by using Solvent extraction Method (SEM), Ultrasound Assisted Extraction, Microwave Assisted Extraction, Supercritical carbon dioxide extraction, combined extraction methods (Tan et al., 2022). The present research includes the extraction of the anthocyanin from the bracts of Banana using SEM and the characterization of Anthocyanin is carried out by using UV-Vis spectrophotometer, FTIR, TLC, the anti-oxidant activity of the extract, and the Anti-microbial activity of the Anthocyanin extract.

2. MATERIALS AND METHODS:

2.1 Collection of the sample

Banana flower was procured from the local farm of Coimbatore, Tamilnadu, India. Which was brought to the laboratory and the bract was separated and cleaned before drying. Under shade of sunlight the sample was dried to avoid the loss of sensitive components. Followed by the dried sample was stored in an air tight container and used for further study.

2.2 Preparation of extract

Different solvents of Water, Methanol and Ethanol with 0.5% HCl were used in order to identify the applicable solvent for the extraction of anthocyanin. 10 g of the dried banana bract was dissolved in 100 ml of solvent and kept on shaker for 5 h at room temperature. After incubation 1ml of acetic acid was added as a co solvent to extract the anthocyanin content and incubated for 24hrs in an orbital shaker. After incubation the solution mixture was filtered using Buchner funnel and the remaining solids were washed. (Sujithra, S., & Manikkandan, T. R., 2019)

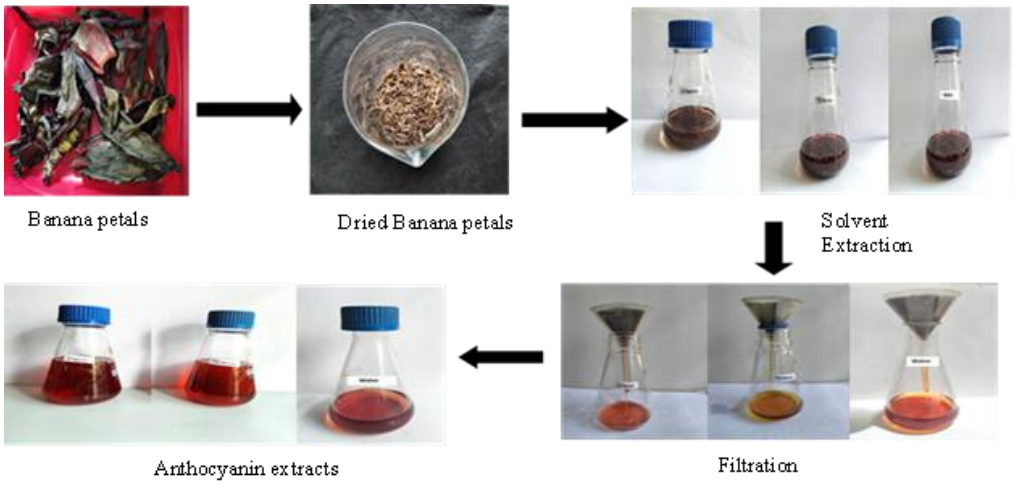


Fig 1: Extraction of Anthocyanin from Banana Petals using solvent extraction method

2.3 Estimation of anthocyanin content

UV- Vis Spectrophotometer LT 291 was used to estimate the total Anthocyanin content of the three extracts prepared, the extracts were diluted to pH 1.0 and the wavelengths of 510nm an 700 nm were used to calculate the absorbance values of the extracts using the formula A = [(A510 - A70) pH 1.0 – (A510- A700) pH 4.5] (Shehata et al., 2020)

2.4 Identification and characterization of anthocyanin content

2.4.1 UV-Visible study

The extracted anthocyanin content was examined under UV Visible spectrophotometer – Labtronics LT291. The samples were scanned in the wavelength ranging from 200-800 nm and the characteristic peaks were detected by keeping the respective solvent as blank. (H Munawaroh et al., 2016)

2.4.2 FTIR

FTIR study was done for the identification of the functional groups present in the anthocyanin sample. Using shimadzu instrument from 4000^{cm-1} to 400^{cm-1} with $1cm^{-1}$ as resolution. (Bhushan et al., 2023)

2.4.3 TLC

The anthocyanin content of the extracted samples were analyzed via TLC by calculating the Rf value. The TLC plate was set with 5cm width and 8cm length and spotted the sample 1cm above from the bottom using capillary tubes (about 50 µl). TLC silica gel 60 F254 plates were used for the evaluation of the pigment profile was from Merck KGaA, Germany. After loading the sample on a plate, the eluent mixture of N-Butanol: acetic acid: formic acid: water (2:2:2:1v/v) was utilized and the Rf value was calculated based upon the bands formed, it was then calculated and compared. (Ho et al., 2024)

$$\text{Rf value} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

2.5 Antioxidant study

2.5.1 Total phenolic content

Total phenolic content of the extract was studied with the Folin–Ciocalteau reagent method with modifications. To 0.5 ml of each extract, 2.5 ml of 0.2 N Folin–Ciocalteau's reagent was added, mixed by gentle shaking, and kept for 10 minutes in room temperature. To the mixture, 2 ml of Na2CO3 (20%, w/v) was added and incubated at 30ºC for 20 min. Three replicates were maintained per each experimental procedure. The absorbance of the sample was recorded at 765 nm using a UV/vis spectrophotometer (Labtronics LT291, Microprocessor). The phenolic content of the extract was estimated from the standard curve of Gallic acid and the results were expressed in Gallic acid equivalent (GAE)/g (gallic acid standard was prepared in methanol at the concentration of 20, 40, 60, 80, and 100 µg ml-1) of extract. (Zaahin et al., 2017).

2.5.2 Total antioxidant activity

Using phospho molybdenum method total antioxidant activity was confirmed by the protocol with slight modification. 0.5ml of the sample was mixed with the 0.5ml of reaction mixture contain 0.6 M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate reagent. The solutions were incubated at 50^0C for 90minutes with blank. After incubation the tubes were normalized into room temperature and the absorbance was read at 695nm using spectrophotometer (LT 291 labtronics microprocessor). Ascorbic acid was used a standard to calculate the mg/ml of the total antioxidant activity. (Phatak et al., 2013)

2.6 Anti-microbial study

Antibacterial activity of the anthocyanin extracts (Water, Ethanol and Methanol) were tested with well diffusion method against the bacteria of *S.typhi, E.coli*, *S.aureus* and *B.cereus*. Mueller hinton agar was prepared, and poured to petriplate for solidification, after solidification, 70µl of the bacterial culture was swabbed using sterile cotton swab and wells were made with corck borer followed by the sample was added (20µl). Antibiotic disc (Ciprofloxacin -CIP 5mcg) was placed as a positive control, aqueous, methanol and ethanol extracts were used as negative control respectively and the plate was incubated at 37^0C for 24 hrs. After incubation anti-bacterial activity of the sample was measured based on the zone of inhibition in mm using Antibiotic zone scale, Himedia.

For the antifungal study malt extract agar was prepared (39gm in 1000ml of distilled water, followed by autoclaving) and poured to petri plate. After solidification 80 µl of fungal culture of *A.niger* and *A.flavus*was were added and spread, using above said method sample also added and incubated 3-5 days at 30^0C. 5 µl of Fluconazole was used as a standard (10mg/ml), After incubation zone of inhibition was measured in mm. (Jesteena et al., 2018)

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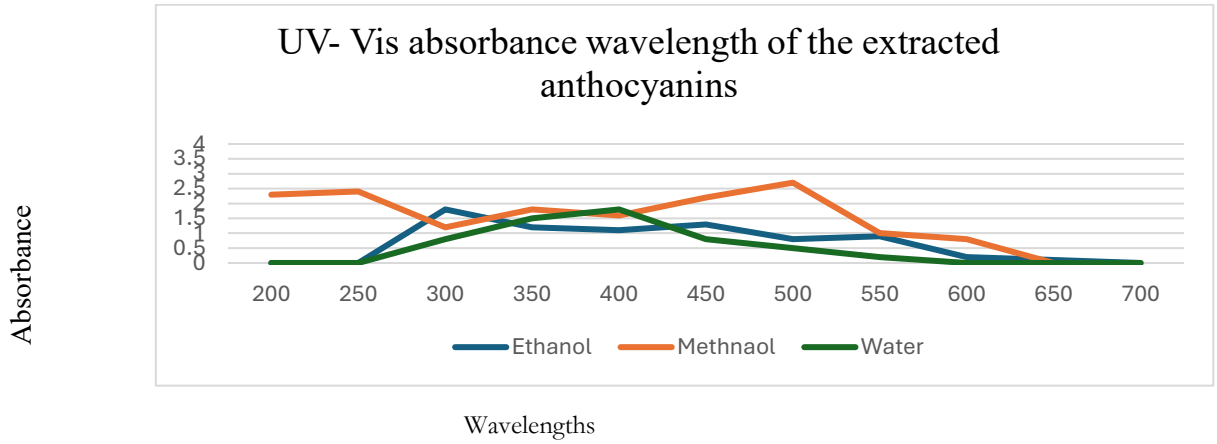
3. RESULTS & DISCUSSION

3.1 Estimation of anthocyanin content

In the present experiment on extraction of anthocyanin from banana bracts was carried out by using solvents namely Ethanol, Methanol and Water. The polarity of the solvents influenced the extraction rate of the anthocyanin. Sujithra, S., & Manikkandan, T. R. (2019) has stated that the banana flower bract extracts around 14-32 mg/ 100g based on the type of solvent used and their polarity. In this experiment the Water extracted anthocyanin has the highest yield with 33.72mg followed by Ethanol extract with 30.72mg and the least was obtained by Methanol extract with 17.86mg repectively.

3.2 Identification of anthocyanin content

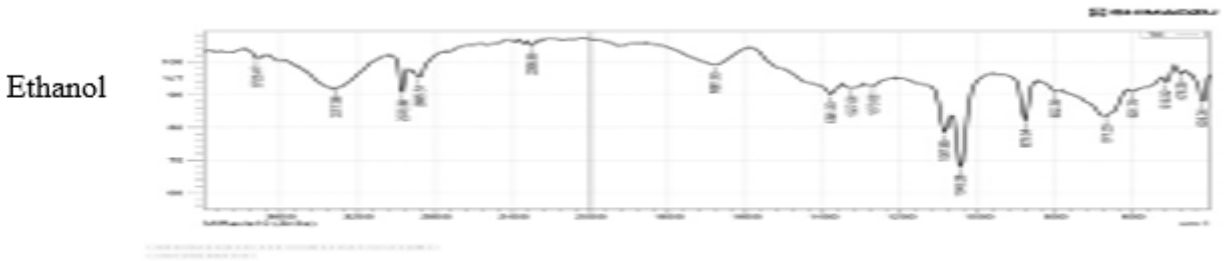
3.2.1 UV-Visible study



Graph 1: Represents the UV- Vis absorbance wavelength of the extracted anthocyanins

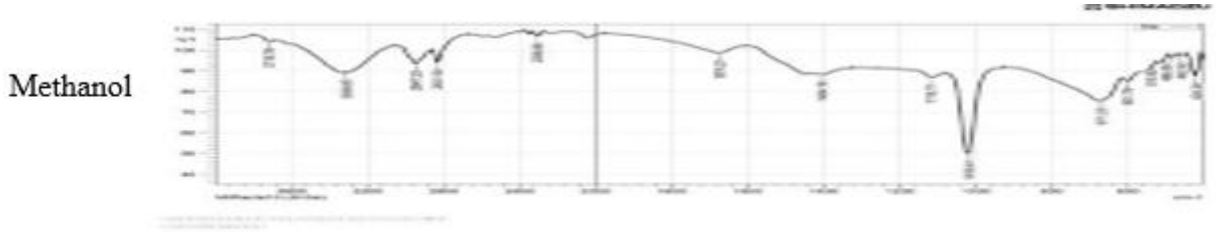
According to Filip, M et.al., (2012) stated that the anthocyanins from different plants were studied under UV-Vis at 260-550nm at pH 1.0 and resulted that, the anthocyanins were detected at the range of 400-550nm. And from the above graphs, the ethanol, methanol and water extracted anthocyanin at pH 1.0 have shown the peak at 540, 535 and 430nm respectively, suggesting the presence of Anthocyanin group in the extracts.

3.2.2 FTIR

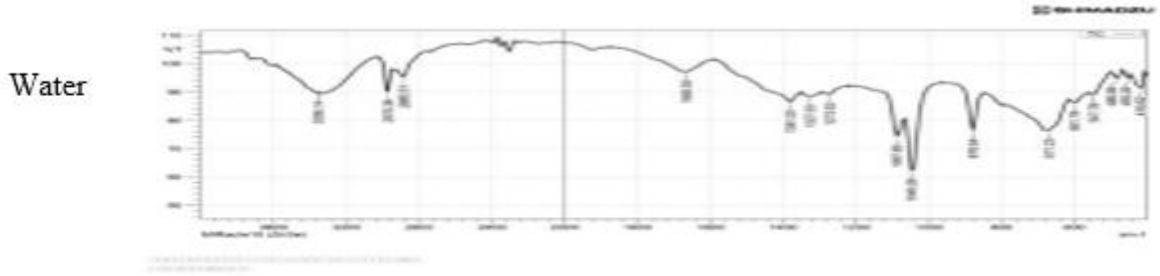


Graph 2

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Graph 3



Graph 4

Graph 2,3& 4: FTIR detection of the extracted anthocyanins.

According to Uzma Y et.al. (2021) Fourier transform infrared spectroscopy (FTIR) spectroscopy detects functional groups such as vibrational bands like N-H, O-H, C-H, C = O (ester, amine, ketone, aldehyde), C = C, C = N (vibrational modes of a tetrapyrrole ring) and simply C = N. FTIR spectra the bands around 3600 to 3200 cm^{-1} relate to the H bonds and around 3000 to 2800 cm^{-1} with C–H/OH/NH groups. And the above results show that at the reading peaked at 3317, 3309nm and 3356nm of each extract supporting the presence of O-H, H bonds, C-H stretching, C=O/C=C which are structural backbone in the Anthocyanin structure confirms the presence of Anthocyanin compounds in the extracts.

3.2.3 TLC:

Thin layer chromatography has been used to detect the presence of anthocyanin in the given extracts and resulted in the Rf value 0.59, 0.52 and 0.6 respectively for Ethanol, Methanol and water extracts. And according to Filip, M et.al., (2012) the anthocyanin extracts of different berries has confirmed the presence of anthocyanins and anthocynadins between the hRf of 36- 82 suggesting the presence of anthocyanin in the banana bract extract.

3.3 Antioxidant study

The below results show that methanol extracted anthocyanin has slightly more total phenolic content followed by water extract and ethanol extract. The total anti-oxidant activity shows that water extracted anthocyanin has more anti-oxidant activity than the other two extracts. The total phenolic content and anti-oxidant activity of the anthocyanin extracted were expressed in mg/gm. Senevirathna, N (2025) confirmed that the anthocyanin extracted from banana bracts has phenolic compounds and anti-oxidant compounds.

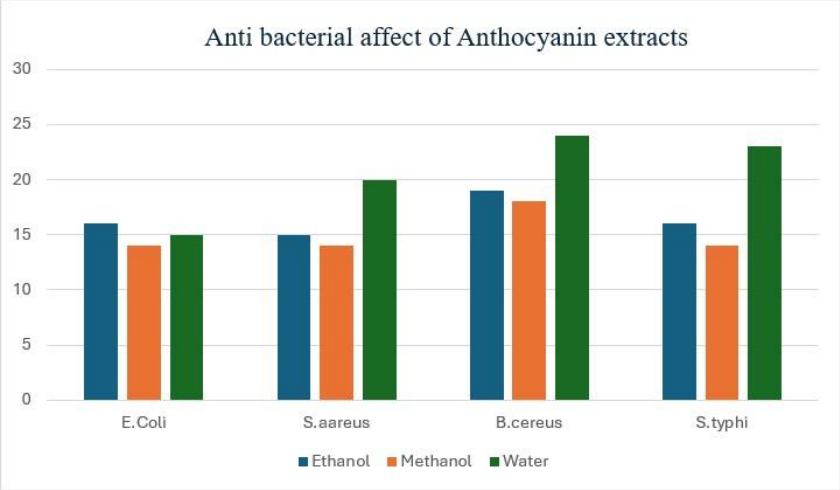
Extracts	mg/g of total phenol	mg/l of total antioxidant activity
Ethanol	76	310
Methanol	82	278
Water	79	319

Table 1: Total phenol and Anti-oxidant activity of Anthocyanin extracts.

3.4Anti-microbial activity:

3.4.1 Anti-bacterial activity:

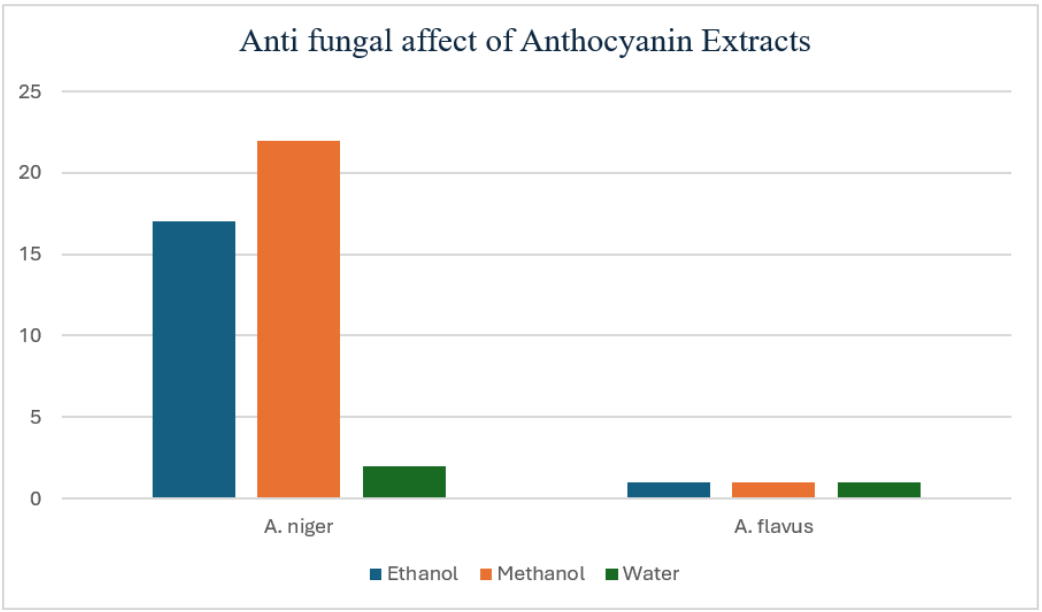
The anti-bacterial activity was carried out using 4 organisms which are pathogenic to human and resulted in possessing the activity against all the four organisms. The anti microbial activity was calculated using the inhibition zone in mm. All the three extractions were subjected to the test against all the organisms. The Water extract showed the highest inhibition against B. cereus and S. typhi followed by S. aareus and the least inhibition was shown against E.Coli. Similarly, the Methanol and water extracts showed the highest inhibition against B. cereus and similar inhibition rate on the other three organisms.



Graph 5: Anti bacterial activity of anthocyanin extracts

3.4.2 Anti - Fungal activity:

The anti-fungal activity was carried on 2 organisms in which only the anti- fungal activity was shown against A. niger (Ethanol, Methanol and Water extracts) where there is little to no anti- fungal activity shown in A. flavus.



Graph 6: Anti bacterial activity of anthocyanin extracts

4. CONCLUSION:

The present study demonstrated that anthocyanins extracted from banana bracts represent a valuable natural source of bioactive compounds with significant functional potential. The extraction process yielded pigments rich in antioxidant properties,

confirming that banana bracts, an underutilized agricultural by-product, can be effectively valorized for high-value applications. The characterized anthocyanin extract exhibited strong anti-oxidant activity, indicating its potential role as a natural antioxidant. And the anti-microbial activity of the extracts shows the potential for using this as a natural anti-microbial agent.

The findings highlight the feasibility of incorporating banana bract anthocyanins into food systems, particularly in the development of functional foods, natural colorants, smart and edible coatings. Their natural origin and bioactivity make them promising alternatives to synthetic additives, aligning with the increasing consumer demand for clean-label and sustainable food products. Furthermore, the utilization of banana bracts contributes to waste reduction and supports sustainable food processing practices. Overall, anthocyanin extraction from banana bracts offers both technological and environmental advantages.

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