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A Review on Extraction, Isolation and Separation Technique Studies Anthocyanin

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Abstract: *Natural anthocyanin has reached its peak utilization as a biocolor in industry in recent years, and there is a growing demand to improve the extraction processes. This study examined three different extraction methods (traditional, microwave-enhanced, and ultrasonic) methods for extracting anthocyanin from Hibiscus rosa-sinensis flower petals using a combination of ethanol, citric acid, and water as extraction solvents. This study estimates the following: pH, titrable acidity, the overall content of anthocyanins, antioxidants, phenolic compounds, flavonoids, and soluble solids. According to the data, ultrasound assisted extraction yielded more anthocyanin (179.32 mg/l) than microwave assisted extraction (155.45mg/l) or conventional extraction (100.88 mg/l). Furthermore, this study found that in ultrasound-assisted extraction, extraction time is more important in aqueous solvents than in other solvents.*

Keywords: Anthocyanin; Hibiscus Extract; Differential ph, Microwave assisted extraction, Ultrasound assisted extraction

I. INTRODUCTION

The term anthocyanin comes from the Greek words νθός (anthos), meaning flower, and μανός (kyanos), meaning blue. Anthocyanins are plant-derived flavonoid pigments that give colour to fruits, flowers, and leaves. Anthocyanins come in many varieties of colour from orange-red to vivid red, purple, and blue. Anthocyanins, which are water-soluble flavyl salts, are structurally formed by coupling a sugar unit to an anthocyanidin. Their source is anthocyanidins, which are devoid of glycated groups. Flowers and fruits are shielded from UV light by anthocyanins in plants, which also stop harmful free radicals from being produced [14,22]. The antioxidant qualities of anthocyanins are therefore not surprising.(Aline B. Santamarina, 2023). Anthocyanins come in over 600 different varieties, with the main differences being the number as well as the location of hydroxyl groups, the degree of methylation of the hydroxyl groups, the type quantity of sugar molecules, the acids attached to the sugars. Despite the large number of anthocyanin molecules already present in the food matrix, only six anthocyanin types—pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin—have been thoroughly studied [23]. Differences in molecular structures one of the most studied anthocyanins. Anthocyanins are primarily found in fresh fruits (blackberry, raspberry, blueberry, cherry, strawberry), plums, figs, grapes, apples,and pomegranates, such like well vegetables red cabbage, purple sweet potato, eggplant, black/purple carrot, and others that may be relevant to local diets.(Aline B. Santamarina, 2023)

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The well-known flowering plant Hibiscus rosa-sinensis is a member of the Mallow family and genus Malvaceae. A great source of anthocyanin, hibiscus flowers are commonly available in India. The primary anthocyanins present in Hibiscus flowers are 3, 7-diglucoside, kaempferol-3-xylosylglucoside, quercetin-3-diglucoside, cyanidin-3 , and cyanidin-3-sophoroside-5-glucoside.(Atreyee Bal, 2024)
Pharmacological actions of Hibiscus Rosa-sinensis plant (EzzaIzzatiZulkurnain, 2023)

1.1. Antioxidant Activity

As a result, its ability to either scavenge free radicals or promote their breakdown, antioxidants work to prevent cell harm.

1.2. Anti- inflammatory Activity

Different pathways were used by flavonoids, saponins, and steroids to achieve their anti-inflammatory effects. Reactive oxygen species (ROS) production is inhibited by flavonoids and saponins, In furthermore, the signaling cascade reduces a number of inflammatory mediators, including nuclear factor-kappa B (NF-kB) and signal transducers and activators of transcription (STAT).

1.3. Anti-Diabetic

According to a study by Moqbel et al.43, the Non-Obese Diabetic (NOD) mice that spontaneously developed type 1 diabetes or Insulin-Dependent Diabetes Mellitus (IDDM) shown a notable anti-diabetic impact on the ethanolic extract of Hibiscus rosa-sinensis leaves' chloroform fraction. Before receiving the hibiscus extract, the mice had fasting blood glucose levels of 278.6 mg/dl and 290 mg/dl. When the mice were administered 100 and 200 mg/kg of the chloroform fraction of Hibiscus rosa-sinensis per body weight, their blood glucose levels plummeted drastically to 94.5 and 90.2 mg/dl, respectively, both of which were within normal limits.

Material and Methods (Sri Raghavi R, 2022)

Material collected:-The hibiscus petals used as raw materials were gathered in 2024–2025 from the Botanical Garden's Department of pharmacognosy at Maharashtra. The collected flowers' petals were taken off, cleaned with distilled water, and allowed to dry in the shade for three daysThey ground the dried petals into powder. Additionally, powdered shade-dried petals were used for extraction.

Extraction :- The anthocyanin extraction approach involved the use of a variety of solvents and procedures. The solvents used included water, ethanol, and citric acid. Ultrasound-assisted extraction, microwave-assisted extraction, and conventional extraction are three types of extraction procedures. There are nine treatments total, each with three replications. The investigation was conducted with a completely randomized statistical design. The collected data was statistically analyzed.

Treatment details:

T1 Conventional Extraction with Distilled Water
T2 Conventional Extraction with 2% Citric acid
T3 Conventional Extraction with 85% Ethanol
T4 Microwave Assisted Extraction with Distilled Water
T5 Microwave Assisted Extraction with 2% Citric acid
T6 Microwave Assisted Extraction with 85% Ethanol
T7 Ultrasound Assisted Extraction with Distilled water
T8 Ultrasound Assisted Extraction with 2% Citric acid
T9 Ultrasound Assisted Extraction with 85% Ethanol

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Conventional extraction (CE)

The Du and Francis, 1973 method was used in conventional extraction with very minor adjustments. In a conical flask, 50 g of hibiscus petal powder that had been shade-dried was collected. Following the addition of 500 milliliters of a solvent (distilled water, 2% citric acid, and 85% ethanol), the mixture is kept in a hot water bath at 60°C for 60 minutes. A muslin cloth are used to squeeze and filter the extract and petals, and if necessary, Whatman filter paper is added for additional filtering. The 400 ml of extracted liquid was reduced to 90 ml of concentrated extract by using a rotary evaporator (SPAN Automation, SARE29G) set to 60°C and 40 rpm for the 30 minutes. This concentrated extract is utilized for the additional examinations.

Treatments	Anthocyanin (mg/mL)	pH
T1	45.71	7.2
T2	62.32	4.4
T3	100.88	6.1
T4	96.54	7.3
T5	130.12	4.3
T6	15.45	6.4
T7	179.32	7.1
T8	170.22	4.1
T9	162.14	6.3
S.Ed	2.56	0.17
CD at 5%	5.38	0.36

Table 1: Effect of extraction methods on different parameters – Anthocyanin, pH,

Microwave assisted extraction (MAE)

In the Ashitha et al., 2020 [3] study, the exposure time (s), microwave power (W), and sample/solvent ratio (g/ml) have been specified for microwave-aided extraction. In a beaker, 25 ml of solvent (distilled water, 2% citric acid, and 85%

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ethanol) was mixed with 1g of sample powder at a 1:25 ratio. This mixture was given 400 W of microwave radiation to the extractor cells (ETHOS X, Microwave Assisted Extraction System). For 85% ethanol, the exposure periods were 180 seconds with water and 120 seconds with 2% citric acid.

Ultrasound assisted extraction (UAE)

In ultrasound-assisted extraction, 15 ml of solvent (distilled water, 2% citric acid, and 85% ethanol) was mixed with 1 g of powdered material at a 1:15 ratio. The combination was put into the ultrasoundassisted extraction system at a 40 kHz frequency for 30 minutes (Aryanti et al., 2019). The extraction was finished using the aforementioned methods, and observations were made through additional analysis. For every treatment, the following parameters were monitored: titrable acidity, pH, total phenols, total flavonoids, total soluble solids, anthocyanin content, and antioxidant analysis. The visual titration method described by Ranganna (1986) was used to determine the total titrable acidity. The pH was measured with a digital pH meter (AOAC, 2000). To determine the total phenolic content, we employed the Singleton and Rossi (1965) [21] approach. Total flavonoids were discovered in agreement with Liu et al.'s2008study [13]. A hand refractive meter was used to express the total soluble solids in ^{0}Brix.The crude extract's total monomeric anthocyanin content was determined using spectrophotometry and the pH differential technique. In summary, the buffer solutions used are 0.4 M sodium acetate (pH 4.5) and 0.025 M potassium chloride (pH 1.0). Samples were diluted using a 1:10 buffer solution, and absorbance was measured at 520 and 700 nm wavelengths. The total amount of anthocyanins in the sample was determined using the following formula: canidin-3-O-glucoside (mg) similar per 100g mass. Baghya Nisha Radhakrishnan (2023).

Using the Folin-Ciocalteu test, the total phenolic content (TPC) of crude hibiscus anthocyanin extracts was determined. To initiate the reaction, 200µL of sample extract was mixed with 750µL of 7% sodium carbonate (w/v) and 0.25 mL of Folin-Ciocalteu reagent (1:9 with water). After 30 minutes of dark incubation at 37 ° c at (room temperature), at the tubes' absorbance at 765 nm was measured with a UV/Vis spectrophotometer and compared to the blank sample. A standard calibration plot was created with known gallic acid concentrations (10 µm/mL to 500 µm/mL). The total phenolic content was calculated using the calibration plot and expressed as mg gallic acid equivalents (mg GAE) of phenol per gram of extract. Baghya Nisha Radhakrishnan, 2023 .

Isolation and Separation: - (Baghya Nisha Radhakrishnan, 2022)

Thin layer chromatography (TLC)

For thin layer chromatography (TLC), 20 cm by 20 cm silica gel plates were used. After being spotted on the plate, the sample was allowed to run in a solvent mixture including water, acetic acid, and butanol in a 4:1:2 ratio. Visual observations of the pigment patches were made.

Paper Chromatography

When utilizing paper chromatography (PC), the hibiscus ethanolic extract was spotted on a Whatman Num-1 filter and allowed to flow in a 9:1 solvent mixture of acetone and petroleum ether. The chromatography paper was left undisturbed for half an hour, and the pigment spots were visually inspected in accordance with Muchuweti and Chikwambi's protocol [15]. The formula below was used to designate the pigment spots and calculate the Rf values

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Six trials were used for each experiment. Using the VETSTAT program, the t-test and analysis of variance were conducted. The findings were provided as mean ± SE. Duncan's multiple range test was performed to discover significant changes in the effects of solvents, duration, temperature, and pH on anthocyanin extraction ($P<0.05$). Using 3D surface graphs, the treatments' notable differences were displayed.

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II. RESULT AND CONCLUSION

After over all view of review study understand that, This article contrasted three methods of extraction for anthocyanin standardization: traditional extraction, microwave-assisted extraction, and ultrasound assisted extraction. Anthocyanin, total antioxidant and phenolic content activity were all evaluated in order to determine the optimal extraction method. Comparing ultrasound assisted extraction to microwave assisted extraction and the traditional extraction, the latter proved to be the most effective method for obtaining anthocyanin. According to another research, employing water as a solvent improved the extraction effectiveness of ultrasound-assisted extraction. Nevertheless, more investigation is needed to evaluate stability of the standardized anthocyanin extract utilized in this study. It found that methanol with HCl produced a higher yield than other test solvents. According to the results, the ideal conditions for extracting anthocyanins were 60 °C for two hours at pH 3–3.5 using ethanol that had been acidified with 1% citric acid.

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