# ULTRASONIC EXTRACTION OF PHENOILCS FROM SEASONAL FRUITS AND INVESTIGATION OF THE ADSORPTION CAPACITY OF ULTRASONICATED FRUIT RESIDUES

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## ABSTRACT

*Seasonal fruits have potential to contribute greatly to human nutrition because of their richness in phenolic contents, fibre and antioxidants. Even the non-edible portions of the fruits such as skin and fibre which are generally discarded are found to have decent amounts of phenolic content. Ultrasound extraction proved to be a promising technique in the extraction of phenolic compounds from plant derived products with number of advantages compared to other conventional extraction procedures. The present study focuses on extraction of phenolic compounds from seasonal fruits and their wastes obtained using ultrasound extraction procedure. TPC of fruits of Jambhul, Jackfruit and Alubhukara was 91.33, 40.84, 80.16 mg (GAE) /g DW respectively. Highest TPC of 175.79, 89.16, 85.97 mg Gallic Acid Equivalents (GAE) /g DW were observed for Jambhul skin, Jack fruit fibre and Alubhukara skin respectively. The residues of extraction process were further investigated for adsorption capacity on synthetic dyes for which the ddetermination coefficient $R^2$ of both Langmuir and Freundlich equation, was found near to unity, indicating good agreement data with both isotherm models mainly for Jambhul skin.This process would there by support extraction of phenolic compounds from non-edible fruit portions and give new dimension in using fruit wastes for phenolic extraction and the residue can be reused as an adsorbent.*

**Keywords: Adsorption, Gallic Acid, Total Phenolic Content, Ultra Sonication, Isotherms**

## I. INTRODUCTION

Seasonal fruits have potential to contribute greatly to human nutrition because of their richness in fibre and antioxidants [1]. Neochlorogenic and chlorogenic acid, two dominant phenolic compounds in prunes, were antioxidants toward isolated human LDL [2]. Consuming peaches, plums and nectarines is positively associated with nutrient intake, improves anthropometric measurements and reduced risk of hypertension [3]. Despite reports of plum benefits to human health, consumption remains low, which has been attributed to a lack of fruit ripening before consumption [4]. Alu bukhara contains high amounts of secondary plant metabolites mainly polyphenols, featuring a high antioxidant capacity. The benefit of fruits and berries in the human diet has received considerable attention in the recent years due to their rich amount of phenolic compounds with different biological activities [5-7].For extraction of phenolic compounds from foods, the commonly used

organic solvents are aqueous mixtures of ethanol, methanol, water, and acetone, sometimes in combination with weak or strong acids [5]. The yield and composition of phenolic compounds in extracts depend on the extraction conditions and are, in addition to the polarity of the solvent, influenced by, for example, temperature, duration of extraction, number of steps, solvent-to-sample ratio, and use of enzymes[8]. Sample preparation and removal of unwanted substances for accurate quantification of phenolics is important, but the extraction procedure is the primary determinant for the separation and recovery of phenolics. As mentioned earlier, extraction is generally influenced by the sample nature, particle size, solvent type as well as extraction techniques employed. Soxhlet, heated reflux extraction and maceration are conventional procedures frequently used to recover phenolics from solid samples. Microwave assisted extraction is an advanced processe but often suffer from disadvantages of short extraction times and fear of working with alcoholic and more volatile solvents. The Soxhlet and heated reflux methods are normally performed at 90 °C for several hours while maceration is performed over days at ambient temperature [9]. While there are many positive aspects of this method, there are substantial disadvantages, including: (1) the need to use large volumes of hazardous organic solvents, which are environmental pollutants and health hazards; (2) long extraction times and (3) interference with, and degradation of, targeted components due to both internal and external factors such as light, air, high temperatures and enzymatic reactions [10–12]. Ultrasonic radiation, which has frequencies higher than 20 kHz, facilitates the extraction of organic and inorganic compounds from solid matrices using liquid solvents. Sonication is the production of sound waves that create cavitation bubbles near the sample tissue, which break down to disrupt cell walls, thereby releasing cell contents [13-14]. An appropriate solvent is mixed with a sample and sonicated under controlled temperature for a specified time. Extract recovery is influenced not only by sonication time, temperature and solvent selection, but also by wave frequency and ultrasonic wave distribution [15]. Ultrasound has been used in both static and dynamic modes to extract phenolics from plant materials [16]. A static system is a closed-vessel extraction for which no continuous transfer of solvent occurs. In dynamic extraction, fresh solvent is supplied continuously, which allows efficient adsorption of analytes and their effective transfer from the extraction vessel. Continuous transfer of extracted analytes prevents degradation of any thermo-labile compounds by the heat associated with sonication [17-18]. Probe and bath systems are the two most common ways of applying ultrasound waves to the sample. Probe sonicators are constantly in contact with the sample and make reproducibility and repeatability difficult. In addition, the risk of sample contamination and foam production is higher. Bath sonicators can act on a range of samples simultaneously and allow for higher reproducibility [19]. Compared to conventional methods, UAE is one of the most simple, inexpensive extraction systems and can be operated rapidly in a broad range of solvents for large-scale preparations suited for industrial purposes [20]. As a method to extract phenolic compounds from Potentilla atrosanguinea and Pinus radiata, UAE has been shown to be more effective than maceration but less effective than heated reflux, MAE and UMAE methods [21] The fruit juice industry produces a large amount of byproducts that have low economic value viz. fruit seeds; skin, over ripened fruits, fiber etc. A large quantity remains improperly utilized. A strategy is required to convert this waste into value added products. The process for this conversion should be technologically efficient, economical and with ease of operation [22]. Many studies are devoted on phenolic extraction from edible portion of the fruit but little work has been done on phenolic extraction from non-edible portions which are generally discarded as a waste. Fruit wastes such as skin, seeds and the fibre content also have a good value of phenolic content present in them. The present study focuses on extraction of phenolic

compounds from seasonal fruits and their wastes obtained using ultrasound extraction procedure. The final residue after the extraction was then subjected to adsorption studies on synthetic dyes. This process would there by support in the extraction of phenolic compounds from non-edible fruit portions and also will give a new dimension in using the fruit wastes for phenolic extraction and the residue of the entire process can be reused as an adsorbent. The Objectives of the present study are 1.To utilize three seasonal fruit varieties viz. Jackfruit, Alubhukhara and Jambhul and their wastes like skin, seeds, and fibre in extraction of phenols using the advanced technique of Ultra sonication. 2. To determine the total phenolic content in all the samples in terms of Gallic acid content. 3. To find out the best solvent used for extraction of phenolics. 4. To find out optimum concentration of the solvent for phenol extraction. 5. To reuse the residue of extraction process for adsorption studies. 7. To study the static capacity of adsorption of synthetic dyes by the fruit sample residue obtained after extraction procedure. 8. To study the effect of contact time, mass dosage on the static adsorption of the dye onto the fruit sample residues.

## II. MATERIALS AND METHODS

Jackfruit, Jambhul and Alubhukar were purchased in large quantities from a local market in Pune. All the fruits were separated into individual portions viz. skin, pulp, seeds and fiber and stored at $-4^0$C until their final usage to prevent it from damage and spoilage and to maintain uniformity in the quality throughout the entire project.

**Fig. 1:Fruit Samples After Pretreatment**

## III. CHEMICALS

Folin-Ciocalteau reagent, Sodium carbonate anhydrous was purchased from Fisher Scientific Mumbai, India. Methanol, Isopropanol, Ethyl acetate, Acetone Merk Mumbai, India. Gallic acid was kindly provided by as a test sample. Safranin 0.5%W/V, Coamassie Brialliant Blue G 250 from HIMEDIA, Mumbai, India. All other chemicals and reagents used in the present study were of analytical grade.

## IV. EXTRACTION OF PHENOLICS

The extraction procedure was adopted for different extracting solvents like Acetone, Methanol, Ethyl acetate and Iso propanol at varying concentrations ranging from 0- 100% for the Jackfruit skin, pulp and fibre, Jambhul skin and pulp, Alubhukara skin and pulp. The ultrasound-assisted extraction (UAE) was carried out in an ultrasonic device (BIO – TECHNICS INDIA, ISO 9001: 2000) with an ultrasound power of 150 W, 230 Volts, Amplitude of 15 and a capacity of 5.5Ltr and frequency of 40 kHz, equipped with a temperature controller and a digital timer. Fruit samples of (1.0 g) were accurately weighed and placed in a capped tube, then mixed with a 10ml of extraction solvent. After wetting the fruit samples, the tube with the suspension was immersed into water in the ultrasonic device, and irradiated under the predetermined conditions for 30min. After ultrasonic extraction, the sample was centrifuged at 10,000 rpm for 10 min and the supernatant was collected. The precipitation was taken back and extracted again under the same conditions.

## V. DETERMINATION OF TOTAL PHENOLIC CONTENT

The total phenolic content was measured by Folin-ciocalteau method. For phenolics gallic acid was used as a standard. Methanol extracts of phenolics (1 mL) from fruit samples was added with 2.5 mL of Folin-ciocalteau reagent and was diluted with distilled water in a ratio of 1:10. Sodium bicarbonate (20%) 2mL was added to this mixture. This mixture was allowed to stand for 30 min at room temperature in dark and absorbance was measured at 760 nm. The standard curve was linear between 1 and 8 µg/mL gallic acid. Results were represented as mg of GAE/g DW of fruit.

## VII. ESTIMATION OF TOTAL PHENOLIC CONTENT

Total Phenol Content (TPC) was determined in comparison with standard gallic acid and the results are expressed in terms of mg GAE/g dry sample. From Table 1 it can be seen that Total Phenolic Content (TPC) of fruits of Jambhul, Jackfruit and Alubhukara was 91.33, 40.84, 80.16 mg (GAE) /g DW respectively. Further efforts were made to analyse the non edible portion of jambhul skin, alubhukara skin , alubhukara seeds, jack fruit skin, jackfruit seeds and jackfruit fiber. Highest total phenolics of 175.79 mg Gallic Acid Equivalents (GAE) /g DW were observed for jambhul skin in Acetone solvent whereas Jack fruit fibre showed TPC of 89.16 mg Gallic Acid Equivalents (GAE) /g DW in Isoproponol solvent and Alubhukara registered a value of 85.97 Gallic Acid Equivalents (GAE) /g DW in Ethyl acetate solvent. According to the findings of Arun et al. (2011) phenolic content in jambhul seed observed was 471.67 mg Gallic Acid Equivalents (GAE) /g. Thus the non edible portions can also be considered for utilization in value added nutraceutical foods.

## VIII. ADSORPTION STUDIES

Adsorption kinetic experiments were carried out in a shaker. Safrannin dye with a concentration of 5ml/1000ml was prepared and 40ml of this dye solution was transferred in each of five test tubes. Kinetic experiments were carried out by agitating these tubes at room temperature at a constant agitation speed and natural $P^H$. Agitation

was made for min. at a stirring speed of rpm. 2 ml of samples were drawn between time intervals of 30 min. The samples were then centrifuged for 15min at 5000rpm and the supernatant solutions were analysed using Calorimeter at a maximum wavelength of 620 nm for Safrannin samples. Each experiment was continued until 120 min assuming that the equilibrium conditions are reached when no further decrease in dye concentration was observed. Maximum dye removal for Jambhul pulp was observed to be 83.34% , Jambhul skin was 73.33%, Alubhukara skin was 58.88% , Alubhukara pulp was 34.44% and Jackfruit fibre was 74.44%.

## IX. RESULTS AND DISCUSSIONS

Ultrasonication was carried out at different concentrations of the solvents viz. 20%, 40%,60% and 80% for all the samples. **Table1** shows the maximun total phenolic content of the samples at different solvent concentrations. Jackfruit pulp recorded maximum total phenolic content of 40.84 mg Gallic Acid Equivalents (GAE) /g DW in 100% isopropanol as an extracting solvent, jackfruit fibre has a TPC value of 89.16 mg Gallic Acid Equivalents (GAE) /g DW in 80% isopropanol as an extracting solvent and Jackfruit seed recorded maximum TPC of 69.41 mg Gallic Acid Equivalents (GAE) /g DW in 40% Acetone as an extracting solvent. Jamun pulp showed maximum TPC of 91.33 mg Gallic Acid Equivalents (GAE) /g DW in 40% Acetone as an extracting solvent, its skin recorded maximum TPC of 175.79 mg Gallic Acid Equivalents (GAE) /g DW in 80% Acetone as an extracting solvent whereas Alubhukara pulp showed maximum TPC of 80.16 mg Gallic Acid Equivalents (GAE) /g DW in 40% Acetone as an extracting solvent and its seed recorded maximum TPC of 85.87 mg Gallic Acid Equivalents (GAE) /g DW in 20% Ethyl acetate as an extracting solvent. Adsorption equilibrium data which express the relationship between mass of adsorbate adsorbed per unit weight of adsorbent and liquid-phase equilibrium concentration of adsorbate are represented by adsorption isotherms and provide important design data for adsorption system. The equilibrium data for removal of safrannin in the present investigations were analyzed using Langmuir adsorption model:

$C_e/q_e = 1/Q_0b\ C_e/Q_0$ --------- (1)

where, Ce (mg/L) is the equilibrium concentration of dye, $q_e$ is the amount of dye adsorbed at equilibrium time (mg/g) and $Q_0$ and b are Langmuir constants related to adsorption capacity and energy of adsorption, respectively. The linear plots of Ce/qe versus Ce (Fig. 5) suggest the applicability of the above model for the present system, showing formation of monolayer coverage of the adsorbate at the outer surface of adsorbent. The values of $Q_0$ and b were determined from the slope and intercept of the plots, respectively, and are given in **Table 2**. The essential characteristics of Langmuir isotherm can be expressed in terms of dimensionless constant separation factor for equilibrium parameter, $R_L$ [24] which is defined as given below:

$R_L = 1/(1+bC_0)$ ---------(2)

where $C_0$ is the initial dye concentration (mg/L) and b is the Langmuir constant (L/mg). The values of $R_L$ = 0.3634 for the studied system at different initial concentrations were found to be in between 0 and 1. **Table 2** which indicates favorable adsorption of dye onto the adsorbent. Freundlich isotherm was also applied for the adsorption of dye[25]

$Log_{10}(x/m) = \log_{10}K_f + (1/n)\log_{10} C_e$ ---------(3)

where, x is the amount of dye adsorbed (mg), m is the weight of adsorbent used (g), and Ce is the equilibrium concentration of dye in solution (mg/L). Kf and n are the constants incorporating all factors affecting the adsorption process (adsorption capacity and intensity). Linear plots of $\log_{10}$ (x/m) versus $\log_{10}$ Ce shows that adsorption also follows Freundlich isotherm well (Fig. 7). Values of $K_f$ and n were calculated from the intercept and slope of the plot and are given in Table 2. Mckay et al. [26] have reported that an 'n' value in the range 2-10 indicates favourable adsorption. Determination coefficient, $R^2$ of both Langmuir and Freundlich equation, is near to unity, indicating the good agreement data with both the isotherm models.

| Solvent | Jackfruit | | | Jamun | | Alu bhukhara | |
|---|---|---|---|---|---|---|---|
| | Pulp | Fibre | Seed | Pulp | Skin | Pulp | Skin |
| Methanol | 34.64 (40%) | 45.21(40%) | 66.12(20%) | 0.015(100%) | 0.032(0%) | 25.35(20%) | 45.21(40%) |
| Isopropanol | 40.84(100%) | 89.16(80%) | 46.67(80%) | 49.34(100%) | 35.92(80%) | 32.83(100%) | 75.34(60%) |
| Acetone | 28.90(80%) | 24.04(20%) | 69.41(40%) | 91.33(40%) | 175.79(80%) | 80.16(40%) | 57.39(100%) |
| Ethyl Acetate | 35.21(60%) | 81.89(80%) | 66.12(20%) | 43.23(60%) | 78.95(100%) | 46.21(60%) | 85.87(20%) |

**Table1: Content of total phenolics in Jackfruit, Jambhul and Alu bhukhara in (mgGAE/g), % values indicate extracting solvent%**

| Adsorbent | Safranin dye | | | | | |
|---|---|---|---|---|---|---|
| Jamun skin | Langmuir | | | Freundlich | | |
| | $Q_0$ | b | $R^2$ | Kf | n | $R^2$ |
| | 4.291 | 0.0701 | 0.991 | 26.73 | 8.22 | 0.9466 |

**Table 2: Values Of Langmuir And Freundlich Parameters Obtained For The Studied Systems**

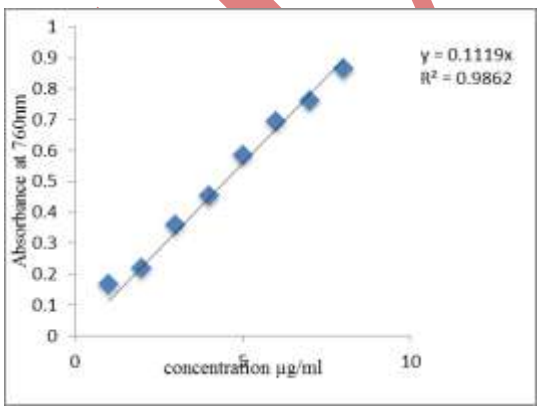


**Fig.2: Standard Curve Of Gallic Acid In Acetone**

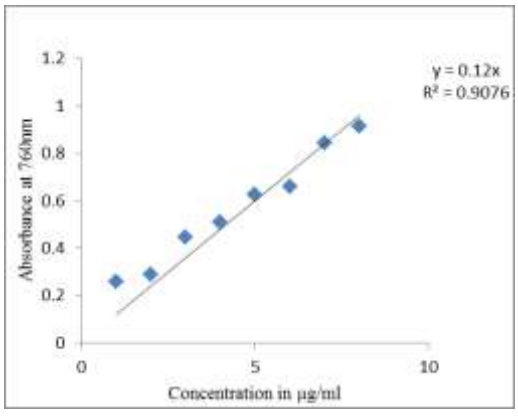


**Fig.3: Standard Curve Of Gallic Acid In Isoproponol**

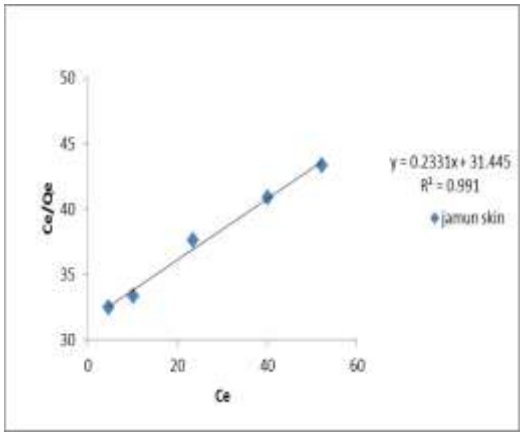


**Fig.4: Standard Curve Of Gallic Acid In Ethylacetate As Adsorbent**

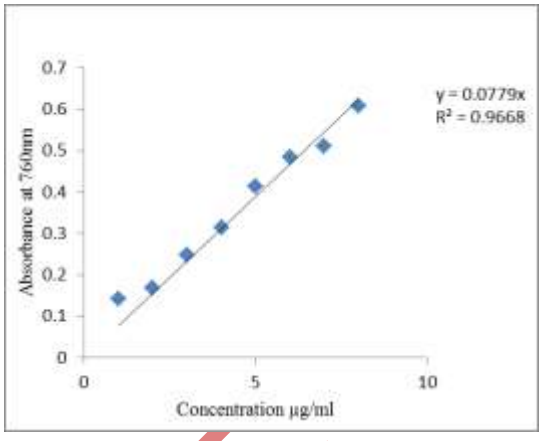


**Fig.5: Langmuir Isotherm Of Jambhul Skin**

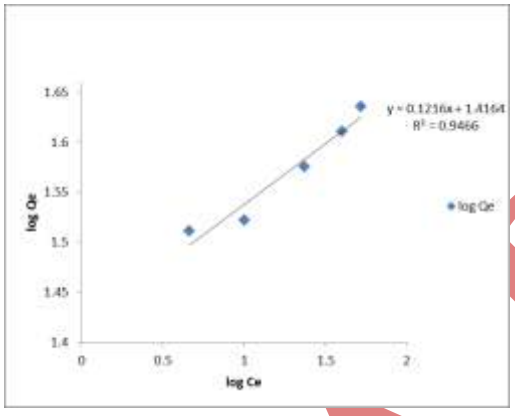


**Fig.7: Freundlich Isotherm Of Jambhul Skin**

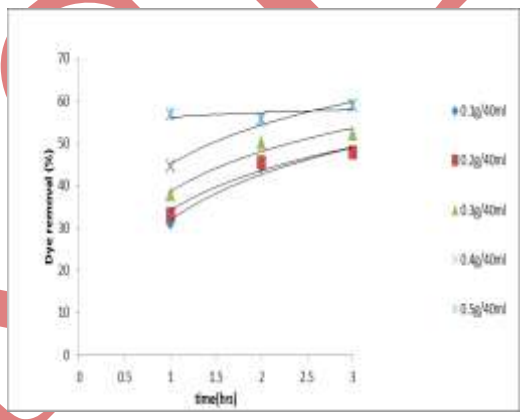


**Fig. 6: Graph Showing % Dye Removal On Alubhukhara Skin**

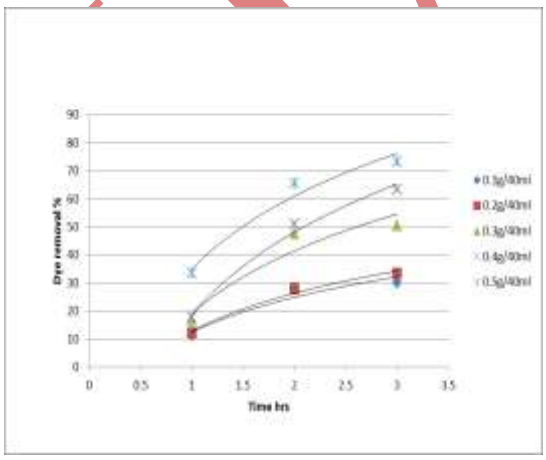


**Fig. 6: Graph showing % Dye removal on Jambhul skin**

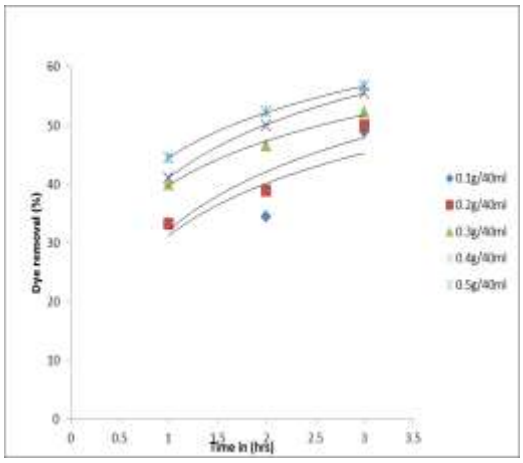


**Fig. 6: Graph showing % Dye removal on Jackfruit fibre**

## X. CONCLUSIONS

It can be concluded from the study that Acetone and Ethyl acetate are found to be best extracting solvents for TPC from the studied fruit samples. From the TPC values of different fruit samples, it was observed that Acetone as an extracting solvent works best for extracting hard samples such as seeds and Ethyl acetate has better extraction efficiency for softer samples such as skin and pulp. Besides the edible portion of fruits, the non-edible portion such as skin, seeds, fibre also contain appreciable values of TPC. The non-edible portions of the fruit can also be further used as adsorbent materials for synthetic dye removal. Future studies can explore adsorption studies on other synthetic dyes and parameters such as effect of pH, temperature, dye concentration etc can be assessed. The removal of Safrannin using ultrasonic extraction residues has been investigated under different experimental conditions in batch mode. The adsorption of Safrannin was dependent on adsorbent dose and Safrannin concentration.Initial pH of solution had no marked effect on the adsorption for the dye. Among all the non edible fruit varieties used for the study Jambhul skin showed the best fit for both Langmuir and Freundlich isotherms. The present study concludes that non edible portions of the fruits such as skin and fibre residues even after ultrasonic extraction showed good adsorption characteristics on synthetic dyes such as safrannin. The study can further be extended on adsorption of different dyes and parameters such as effect of $P^H$, temperature, initial concentration of dye etc. can be explored.

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