Influence of Ultrasound Parameters on Phenolic Yield and Antioxidant Activity in *Carissa carandas L.* Extracts

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Abstract- This investigation refined the extraction methodology of total phenolic content (TPC) from the nutritionally and medicinally significant *Carissa carandas L. L.* fruit, constituting an essential procedure for leveraging its therapeutic capabilities in accordance with established health benefits. Employing the Folin-Ciocalteu assay for the quantification of TPC, a comparative analysis of solvents revealed that ethanol emerged as the most effective solvent, producing a peak TPC of 9.42 ± 0.05 GAE (mg/g). This superior efficiency was attained utilizing a 50% ethanol concentration at a moderate temperature of 45°C for a duration of 25 minutes, markedly exceeding the maximum yields obtained with distilled water (5.30 ± 0.01 GAE (mg/g)) and acetone (4.61 ± 0.002 GAE (mg/g)). The endeavor seeks to apply the sustainable ultrasound-assisted extraction (UAE) technique to scale up this optimized procedure, thereby validating its use as an environmentally friendly alternative. The resultant high-quality, TPC-enriched extracts have been affirmed to possess significant potential for immediate utilization in anti-aging cosmeceuticals and the formulation of innovative, health-oriented food products.

Keywords- *Carissa carandas* L., Total phenolic content, Ultrasonic Assisted Extrcation.

I. INTRODUCTION

Karaunda (*Carissa carandas L.*) represents a plant of considerable significance, frequently overlooked in its application as both a medicinal and nutritional resource, predominantly cultivated in India, while its worldwide relevance is augmenting in response to the escalating demand for natural resources that contribute to an enhanced quality of life [1]. The therapeutic capabilities of this plant are deeply embedded in its diverse array of major bioactive constituents, which encompass alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds, and tannins [1]. Empirical studies examining various components of the plant (fruit, leaf, seed, and pulp) have illuminated its substantial promise as a natural antioxidant and an anti-aging agent [2]. Methodical extraction utilizing an array of solvents indicated that the leaf extract exhibited remarkable potency, generating the highest concentrations of ursolic acid, phenolics, and flavonoids, with ethyl acetate identified as the most efficacious solvent for ursolic acid and ethanol for total phenolics and flavonoids [2]. In addition to its conventional applications, research substantiates its effectiveness in the domains of medicine, health, cosmetics, as well as a natural preservative and coloring agent [3], possessing verified antioxidant, anti-inflammatory, antimicrobial, and anti-tyrosinase properties [4]. From a nutritional standpoint, Karaunda is exceptionally rich; a single serving ranging from 100g to 200g can fulfill 100% of the daily recommended intake for essential minerals including iron, zinc, manganese, copper, chromium, and vitamin C, rendering it a formidable ally against nutrient deficiencies [4]. Moreover, the fruit serves as a substantial source of carbohydrates (61% - 67%), reducing sugars (4.7% - 13%), fats (4.5% - 19%, particularly polyunsaturated fatty acids), and protein (1.6% - 3.2%) [4].

Given the high total phenolic content (TPC) of Karaunda, analysis is predominantly performed using the cost-effective Folin-Ciocalteu assay [5]. This TPC supports its cosmeceutical potential: fresh extracts are promising for anti-aging and skin-whitening, while dried ripe extracts are suitable for anti-acne products [6].

To maximize the extraction of these valuable compounds, methods such as Microwave-Assisted Extraction (MAE) have been optimized. MAE kinetics demonstrated that increased microwave power and

solvent volume significantly enhance efficiency [6], suggesting that high-quality extracts made under these conditions are viable for new food products and prompting interest in alternatives such as Ultrasound-Assisted Extraction (UAE) [6].

Table 1:Biologically active compounds in *Carissa carandas* L. [1,4]

Plant parts	Compounds Found	Function
Roots	Cardiac glycosides, salicylic acid, 2-acetyl phenol, carinol, lignan, sesquiterpenes, lupeol, β-hydroxybetulinic acid, β-sitosterol, β-sitosterol glycoside, α-amyrin, des-N methylnoracronycine (an acridone alkaloid)	Cardiac glycosides and salicylic acid cause a small decrease in blood pressure. Volatile compounds are also present.
Leaves	Carrissic acid (an isomer of ursolic acid)	Carrissic acid is a new compound reported to be present in the leaves.
Fruits	β-caryophyllene, carindone, linalool, lupeol, benzyl acetate, β-sitosterol, ascorbic acid, polyphenols (7-8%), crude proteins (12-15%), free acid (25-30%), hydrocarbons (50-60%)	This is a mixture of volatile compounds. GC-MS analysis is generally used to determine the chemical composition of the oil.

Ultrasonic-assisted extraction (UAE) is a novel and effective method for extracting bioactive compounds from plant matrices, typically using ultrasonic waves in the 20-100kHz frequency range [7]. This technique relies on the propagation of sound waves, which create repeating cycles of high pressure (compression) and low pressure (rarefaction) [8]. During the low-pressure rarefaction stage, if the pressure drops below the vapor pressure of the liquid, acoustic cavitation occurs, which is the rapid formation and collapse of small bubbles [8]. These bubbles grow through a process called "rectified diffusion" [9]. The resulting physical forces, such as shockwaves and acoustic streaming, destroy cell structures, decrease particle size, and improve solvent contact with target compounds, thereby significantly enhancing the extraction efficiency [8]. UAE is widely favored for oil extraction because it is economically valuable and environmentally friendly [7, 8]. The true power of UAE manifests during the rapid and violent collapse of these cavitation bubbles during the compression cycle. This implosion generates extreme localized conditions, including transient hot spots with temperatures reaching approximately 5000 K and pressures soaring to about 2000 atmospheres[19]. Ultrasonic-Assisted Extraction (UAE) stands at the forefront of this paradigm shift, consistently demonstrating advantages over conventional and even some other advanced extraction techniques. MAE is another advanced technique that utilizes microwave energy to heat the solvent and matrix, accelerating extraction. While effective, UAE is often compared favorably or seen as complementary to MAE[20].

Optimizing the extraction efficiency of polyphenolic compounds from *Carissa carandas L.* was the main goal of this investigation. To determine the most advantageous conditions, a thorough analysis was carried out across a variety of experimental parameters (such as time, temperature, and solvent concentration) and solvent systems.

II. MATERIALS AND METHODS:

The sample was prepared and extracted using ripe *Carissa carandas L.* fruits, a digital ultrasonic extractor, petroleum ether, 99% ethanol, 99% acetone, and a Wattman No. 1 filter paper digital tray drier and for the purification and analysis are sodium hydroxide, distilled water, sodium carbonate, gallic acid, and Folin- ciocaltacue reagent.

I.1. Sample Preparation:

Fruits of *Carissa carandas L.*, a native fruit of the Salem district, Tamil Nadu, were purchased from a local market. The fruits were of oval shape and red-orange color. Potable water was used to wash them, and they were reduced to coarse powder in order to enhance their surface area for effective drying. The powder was dried for 3 hours at 65°C until the target moisture content was attained.

I.2. Defatting and Initial Extraction:

The dried *Carissa carandas L.* powder was then defatted by soaking it in petroleum ether overnight, with a solvent-to-solid ratio of 1:2[10]. The resulting

defatted powder was filtered using Whatman No. 1 filter paper. The extraction process was then initiated using the Ultrasonic-Assisted Extraction (UAE) method.

I.3. Bound Polyphenol Extraction:
After the initial extraction, the residue was filtered out. The bound polyphenols were then extracted from the residue using an alkaline hydrolysis method [11].

I.4. Polyphenol Quantification:
The total polyphenol content, including both the initial extract and the bound polyphenol extract, was determined using the Folin -Ciocalteu method [12]. To a 5 mL sample of distilled water, 0.5 mL of Folin- Ciocalteu's reagent was added. After 3 minutes, 1 mL of 7.5% sodium carbonate solution and 1 mL of the polyphenol extract were added to the mixture, which was then diluted to 10 mL with distilled water. The mixture was heated in a water bath at 50°C for 16 minutes.
The absorbance of the samples was measured at 765 nm using a UV-Vis Shimadzu spectrophotometer. A standard curve was generated by measuring the absorbance of different concentrations of gallic acid at the same wavelength. The total polyphenol content in the extracts from the leaves, stem bark, fruit pulp, and seeds was calculated based on this standard curve and expressed as Gallic Acid Equivalents (GAEs) per 100 g dry weight basis [13]

III. RESULTA AND DISCUSSIONS

III.1. Sample preparation:
To make extraction easier, the 200g whole fruit was first cleansed twice with water to get rid of any dirt, then let to dry in the sun for five days before being ground into a coarse powder. After grinding and drying, the yield was 157g.

III.2. Defatting and Initial Extraction:
Two separate trials were conducted: in the first, 2g of the defatted CCP was extracted with 40ml of ethanol and 40ml of acetone with each of 8 combinations using the parameters such as extraction time, extraction temperature and solvent concentration. And for the comparative study of polyphenol extraction distilled water is also used as a solvent for extraction using the same three parameters. A comparative analysis was performed to evaluate the effectiveness of these two solvent choices. Now the extracted polyphenol along with the solvent is centrifuged at 1000rpm for 5 times for 5 minutes each.

III.3. Bound polyphenol extraction:
Specifically, 1.0 g of the residue was treated with 10 mL of 2 M NaOH in 60% methanol or 95% ethanol for 4 hours at 40°C. Following this, 3 mL of 6 M HCl and 3 mL of 1 M formic acid (in methanol) were added. The mixture was then centrifuged at 5,000×g, and the supernatant was filtered[11].

III.4. Polyphenol Quantification:
The quantified polyphenols was found using Folin – Ciocaltue assay which is a spectroscopic method for the quantification of extracted polyphenol. The quantification result was obtained from a standard curve plotted using gallic acid and the polyphenol was expressed as Gallic acid equivalent. The final results are discussed in Figure

Table 2: Total Phenolic Content (TPC) across various extraction conditions for *Carissa carandas* L.

Test set	Solvent concentration (%)	Extraction temperature (°C)	Extraction time (minutes)	TPC (GAE (mg/g)) Ethanol	TPC (GAE (mg/g)) Acetone	TPC (GAE (mg/g)) Water
T1	50	30	25	8.12 ± 0.007	2.41 ± 0.001	3.86 ± 0.05
T2	50	45	25	9.42± 0.05	3.32 ± 0.001	3.82 ± 0.02
T3	50	30	40	7.46± 0.15	3.14 ± 0.002	5.30 ± 0.01
T4	50	45	40	6.82± 0.01	4.61 ± 0.002	4.86 ± 0.61
T5	80	30	25	4.58 ± 0.005	4.05 ± 0.002	-
T6	80	45	25	4.56± 0.001	2.89 ± 0.005	-
T7	80	30	40	4.04± 0.001	4.16 ± 0.004	-
T8	80	45	40	4.60± 0.003	4.33 ± 0.002	-

The extraction of Total Phenolic Content (TPC) from *Carissa carandas L.* was analyzed using three solvents. The three highest concentrations of total polyphenol content were consistently obtained using ethanol as the solvent across three distinct experimental conditions. This observation is primarily attributed to the significant polarity difference between

the three solvents employed in the study. Specifically, the polarity of the solvent is known to have a substantial effect on the extract yield.

Ethanol was the most effective solvent, achieving a maximum TPC of 9.42 ± 0.05 GAE (mg/g) at 50% concentration, 45°C, and 25 minutes [Figure 1]. Lower 50% solvent concentrations generally provided higher yields than 80% for both ethanol and acetone[15].

Distilled water was less efficient than ethanol, yielding a maximum TPC of 5.30 ± 0.01 GAE (mg/g) at 30°C and 40 minutes [Figure 1]. Acetone was the least effective solvent, peaking at 4.61pm 0.002 GAE (mg/g) [Figure 1].

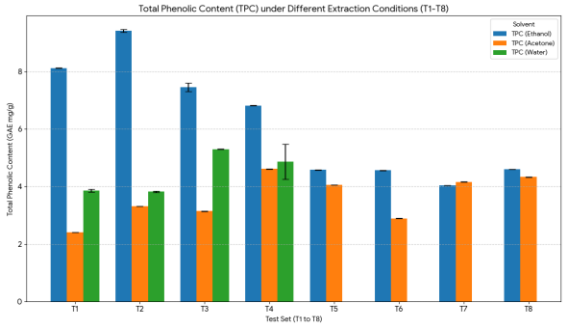

Figure 1. Graphical representation of Total Phenolic Content (TPC) across various extraction conditions for *Carissa carandas* L.

Overall, the data clearly establishes ethanol as the optimal solvent for maximizing TPC extraction under the tested experimental conditions. Acetone can also serve as a good solvent for polyphenol extraction; however, its effectiveness diminishes when attempting to extract the more polar components of the polyphenol mixture[17][18].

IV. CONCLUSION

This study effectively demonstrated that, under the studied experimental conditions, ethanol is the best solvent for maximizing the extraction of Total Phenolic Content (TPC) from *Carissa carandas L.* fruit. The maximum yield of 9.42 ±0.05 GAE (mg/g) was obtained with a 50% ethanol concentration for 25 minutes at 45 °C. The chosen conditions were validated since this much outperformed distilled water and acetone extractions. Going ahead, the suggested use of Ultrasound-Assisted Extraction (UAE) is supported as a cost-effective and environmentally beneficial method to expand this procedure. The fruit's enormous potential for use in the development of functional foods and the cosmeceutical sector is confirmed by the high-quality, TPC-rich extracts that are produced, which also fit with the plant's formidable traditional medicinal profile.

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