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COMPARATIVE EXPERIMENTAL STUDY OF NATURAL, NON-TOXIC SOLVENTS FOR SUSTAINABLE GALLIC ACID SEPARATION

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Gallic acid, a polyphenolic compound with strong antioxidant activity, exhibits notable anti-inflammatory, anticancer, antibacterial and antioxidant properties, making it valuable across the food, packaging, cosmetics and pharmaceutical industries. For its commercial utilization, efficient and sustainable extraction from natural sources is essential. This study investigates the feasibility of liquid–liquid extraction (LLE) for the separation of gallic acid from aqueous solutions using natural solvents. Four vegetable oils - mustard, sesame, sunflower and soybean oil - were evaluated as extractants at various gallic acid concentrations. Equilibrium data collected at 298.15 ± 1 K were used to calculate distribution coefficients (K_D) and extraction efficiencies (*%E*). The results revealed the following average trends in K_D and *%E*: soybean oil (0.4019, 28.69%) > sunflower oil (0.3372, 25.23%) > sesame oil (0.2982, 22.99%) > mustard oil (0.2676, 21.15%), respectively. The highest efficiency was achieved with soybean oil at 0.05 mol·L^{-1} gallic acid, yielding 30.40% separation. Multiple separation units in series may be applied to achieve even higher separations. These findings highlight the potential of natural solvents for environmentally safe gallic acid separation.

Keywords: gallic acid, natural solvents, extraction efficiency, distribution coefficient, sustainable separation

1. Introduction

Gallic acid (3,4,5-trihydroxybenzoic acid) is a naturally occurring polyphenolic compound widely distributed in the plant kingdom (*Figure 1*). It is commonly found in tea leaves, oak bark, gallnuts, berries, nuts and other plant-derived foods, making it a frequent component of the human diet. Due to its structural features, particularly the presence of multiple hydroxyl groups, gallic acid demonstrates strong antioxidant activity, which underpins many of its biological and industrial applications [1]. From a biological standpoint, gallic acid exhibits diverse pharmacological properties, including antioxidant, anti-inflammatory, antibacterial and anticancer activity [2]-[4]. As an antioxidant, it helps mitigate oxidative stress by neutralizing reactive oxygen species, thereby reducing cellular damage and lowering the risk of chronic diseases such as cardiovascular disorders, neurodegenerative conditions and certain cancers. The anti-inflammatory properties of gallic acid are particularly relevant in modulating immune responses and alleviating inflammation-related pathologies, while its antibacterial activity supports applications in both health and food preservation. Together, these multifunctional attributes highlight gallic acid as a bioactive molecule of significant therapeutic interest [1]-[6].

In addition to its pharmacological relevance, gallic acid is extensively used in multiple industries. In the food sector, it serves as a natural preservative and antioxidant, enhancing product stability and extending shelf life, while also contributing to the flavour and colour profiles of various foods as well as beverages. Within the pharmaceutical industry, gallic acid is incorporated into formulations targeting inflammatory diseases, cancer and oxidative stress-related disorders. Similarly, in

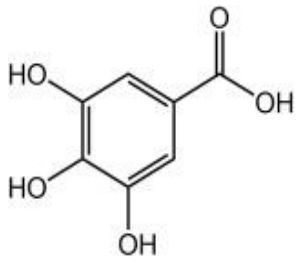

Figure 1: Chemical structure of gallic acid

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Table 1: Physicochemical properties of the compounds used in the experiment

Compound	IUPAC name	Molecular structure	Manufacturer/ supplier
Gallic acid	3,4,5-Trihydroxy-benzoic acid	$C_7H_6O_5$ (Benzoic acid derivative)	Sigma-Aldrich Ltd
Mustard oil	Allyl isothiocyanate	$C_3H_5N{=}C{=}S$	Patanjali Ayurved Ltd, India
Sesame oil	-	-	
Sunflower oil	-	$C_{57}H_{104}O_6$	
Soybean oil	-	$C_{57}H_{104}O_6$	

Table 2: Physicochemical properties of the various solvents

Solvent	M.W. (g/mol)	B.P. (K)	Density (g/cm^3)	Viscosity (cP)	Dielectric constant (ε)
Mustard oil	99.15	424-443	0.81	93.9	4.0-4.5
Sesame oil	138.12	611-639	0.92	65	3.0-3.5
Sunflower oil	114.17	380-390	0.92	39.55	2.8-3.2
Soybean oil	891.00	525-551	0.92	36	2.5-3.0

cosmetics, gallic acid functions as a UV-protective and skin-lightening agent, further expanding its utility. Its broad spectrum of activities has therefore positioned gallic acid as a promising natural compound for developing functional foods, nutraceuticals, pharmaceuticals and cosmeceuticals [1]-[6].

The commercial application of gallic acid requires efficient and sustainable extraction from natural sources, e.g. techniques including conventional solvent extraction, distillation, adsorption and crystallization [7],[8]. Among these, liquid-liquid extraction (LLE) has emerged as a sustainable approach for separating and purifying compounds like gallic acid from complex mixtures. This method has proven effective for the extraction of several high-value acids , including benzene-acetic acid [9], lactic acid [10],[11], protocatechuic acid [12],[13], acrylic acid [14]-[16], caproic acid [17],[18], propionic acid [19]-[23], phenylacetic acid [24], levulinic acid [25], picolinic acid [26], citric acid [27], itaconic acid [28],[29], nicotinic acid [30], hydroxybenzoic acid [31] and tartaric acid [32], using a variety of extractants. In this method, gallic acid, the solute, is transferred from an aqueous into an organic phase. Considering the widespread use of traditional solvents such as dichloromethane, ethyl acetate and chloroform, concerns about their environmental impact have prompted research into natural solvents [33]. Natural solvents provide environmentally beneficial and sustainable substitutes

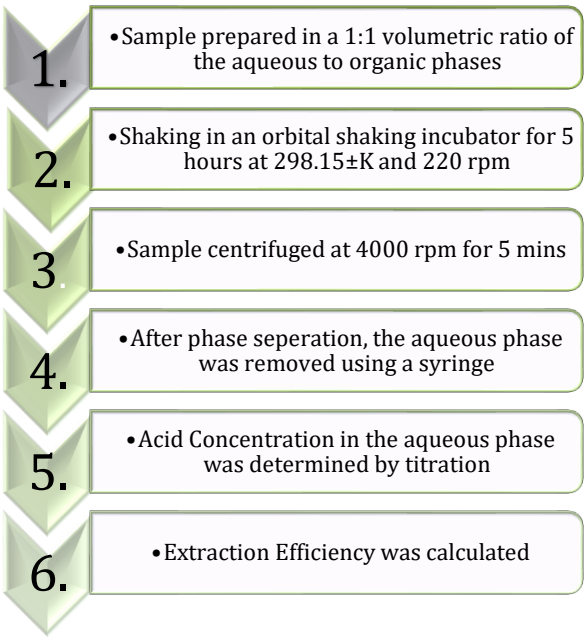

Figure 2: Experimental procedure for gallic acid separation

since they are made from renewable resources like biomass or plants [34]. Compared to their traditional equivalents, they are biodegradable, non-toxic and have smaller environmental impacts. Examples include ethanol, ethyl lactate and terpenes like limonene [35]. By analyzing factors such as extraction efficiency, yield, selectivity and environmental considerations, this study aims to compare the effectiveness of various natural non-toxic solvents in the extraction of gallic acid.

2. Experimental

2.1. Samples and reagents

Gallic acid was purchased from Sigma-Aldrich Ltd and natural solvents like mustard, sesame, sunflower and groundnut oil were procured from Patanjali Ayurved Ltd (*Table 1*). Physicochemical properties of the chemicals used are shown in *Table 2*.

2.2. Experimental approach

By dissolving appropriate amounts of gallic acid in distilled water, aqueous solutions of various concentrations ranging from 0.01 to 0.05 mol/L were prepared [36]. As illustrated in *Figure 2*, liquid-liquid extraction was carried out in 100 ml Erlenmeyer flasks at a constant temperature of 298.15 ± 1 K. The solutions were mixed in a 1:1 ratio, namely 10 mL of aqueous solution mixed with 10 mL of extractant. The flasks were placed in an orbital shaking incubator (Model: S-24 BL, REMI) at 298.15±K and 220 rpm for 5 hours.

Several natural solvents, including soybean, sunflower, sesame and mustard oils, were used separately

as the organic phase. To achieve unambiguous phase separation, the samples were centrifuged in a REMI R-4C centrifuge for 5 minutes at 4000 rpm after equilibration had been achieved. The samples were titrated using a freshly made 0.01 M NaOH solution to assess the gallic acid concentration in the aqueous phase, moreover, the pH of the aqueous phase upon separation was also measured. A mass balance equation based on the amount extracted in the aqueous phase was then used to calculate the concentration of gallic acid in the organic phase. To ensure accurate results were recorded, the experiments were repeated under identical conditions.

2.3. Experimental uncertainty

Uncertainty in the experimental data may have resulted from instrumental errors or random fluctuations. Deviations from the mean or average values were regarded as experimental errors. The calculations were based on the mean values of the parameters. Experiments that were duplicated exhibited errors of less than ±2% of the initial value with a confidence interval of 95%. The following equation was used to estimate the standard experimental uncertainty, which was approximately x ± 0.001:

$$A(x) = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \quad (1),$$

where n denotes the number of experimental observations, x_i represents an experimental observation and $\bar{x}$ stands for the mean experimental value.

3. Extraction mechanism

Three essential phases in the extraction process of gallic acid from aqueous solutions control its transfer to a natural solvent without the formation of solvates. Due to the strong interaction between their functional groups and the generated complexes, a variety of natural solvents were used, including mustard oil, soybean oil, sunflower oil and sesame oil. According to the Law of Mass Action, the extraction steps were as follows: (1) gallic acid dissociation in the aqueous phase; (2) undissociated acid partitioning between the aqueous and organic phases; and (3) glutaric acid dimerization in the organic phase.

3.1. Ionization in aqueous media

The reaction mechanism for the ionization of gallic acid molecules in the aqueous phase is:

$$\mathrm{HGlA} \overset{K_{HGA}}{\longleftrightarrow} \mathrm{H^+ + GlA^-} \quad (2),$$

$$K_{HGlA} = \frac{[H^+][GlA^-]}{[HGlA]} \quad (3),$$

where K_{HGlA} denotes the equilibrium constant.

3.2. Partitioning of undissociated gallic acid molecules

The undissociated gallic acid molecules were dispersed between the organic and aqueous phases:

$$\mathrm{HGlA_{aq}} \overset{P}{\leftrightarrow} \mathrm{HGlA_{org}} \quad (4),$$

$$P = \frac{[HGlA]_{org}}{[HGlA]_{aq}} \quad (5),$$

where P denotes the partition coefficient.

3.3. Dimerization of gallic acid in the organic phase

If the solute-solute hydrogen interaction is stronger than the solute-solvent hydrogen bond, undissociated gallic acid molecules in the organic phase may dimerize according to:

$$2\mathrm{HGlA_{org}} \overset{D}{\leftrightarrow} \mathrm{HGlA_{2,org}} \quad (6),$$

$$D = \frac{[HGlA]_{2,org}}{[HGlA]^2_{org}} \quad (7),$$

where D denotes the dimerization constant of gallic acid in the organic phase.

The ratio of the total acid concentration in the organic phase, which includes dimers and free molecules, to the total acid concentration in the aqueous phase, which includes dissociated and undissociated forms, is known as the overall distribution coefficient of gallic acid. This coefficient is affected by parameters like the concentration of H^+ ions and the ionic strength of the solution. The effect of acid dissociation is limited in extremely dilute acid solutions with pH values below the pK_a of the acid, simplifying the K_D equation:

$$K_D = P + 2PD[HGlA]_{aq} \quad (8).$$

When the experimental data is plotted as K_D vs. $[HGlA]_{aq}$, this equation can be used to calculate the values of P using the intercept and gradient D. The extraction efficiency *(E%)* of gallic acid can be computed as follows:

$$E\% = \frac{K_D}{1+K_D} \times 100 \quad (9).$$

4. Result and discussion

The extraction equilibrium of gallic acid using various diluents was investigated. Data on the extraction efficiency and distribution coefficient are summarized in *Table 3*. Equilibrium isotherms for acid extraction are illustrated in *Figure 3*, revealing a linear relationship between the acid concentrations in the aqueous and organic phases over a broad range, consistent with Henry's law. However, at higher acid concentrations, non-linear behavior is observed, indicating deviations from ideality and Henry's law. Since a low concentration

Table 3: Separation of gallic acid using conventional and natural diluents at 298±1K

Diluents	$[HGlA]_{in}$ (mol/L)	$[HGlA]_{aq}$ (mol/L)	$[HGlA]_{org}$ (mol/L)	K_D	Avg K_D	E%	Avg E%	P	D
Mustard oil	0.01	0.008	0.001	0.234	0.267	19.07	21.14	0.077	1.264
	0.02	0.015	0.004	0.257		20.51			
	0.03	0.023	0.006	0.276		21.77			
	0.04	0.031	0.008	0.282		21.99			
	0.05	0.038	0.011	0.288		22.40			
Sesame oil	0.01	0.007	0.002	0.265	0.298	21.20	22.99	0.084	1.374
	0.02	0.015	0.004	0.290		22.50			
	0.03	0.023	0.007	0.304		23.33			
	0.04	0.030	0.009	0.311		23.75			
	0.05	0.037	0.012	0.319		24.20			
Sunflower oil	0.01	0.007	0.002	0.317	0.337	24.10	25.23	0.009	1.568
	0.02	0.015	0.004	0.324		24.50			
	0.03	0.022	0.007	0.333		25.01			
	0.04	0.029	0.010	0.342		25.53			
	0.05	0.036	0.013	0.369		27.02			
Soybean oil	0.01	0.007	0.002	0.369	0.401	27.12	28.69	0.105	1.715
	0.02	0.014	0.005	0.379		27.50			
	0.03	0.021	0.008	0.395		28.33			
	0.04	0.028	0.012	0.428		30.12			
	0.05	0.034	0.015	0.436		30.40			

$[HGlA]_{in}$ denotes the initial pentanedioic acid concentration,
$[HGlA]_{aq}$ stands for the pentanedioic acid concentration in the aqueous phase and
$[HGlA]_{org}$ represents the pentanedioic acid concentration in the organic phase.

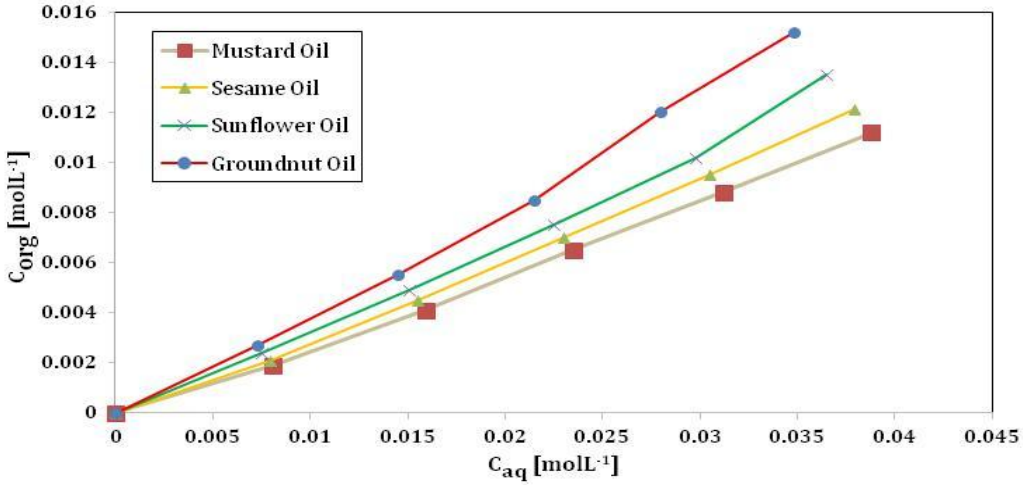

Figure 3: Extraction equilibrium for the separation of glutaric acid using different solvents (mustard oil, sesame oil, sunflower oil, soybean oil) at 298±1K

Table 4: Comparison of the extraction results of gallic acid

Solvents	Range of K_D	Average K_D	Range of E%	Average E%
Mustard oil	0.2340-0.2886	0.2676	19.07-22.40	21.15
Sesame oil	0.2658-0.3192	0.2982	21.20-24.20	22.99
Sunflower oil	0.3333-0.3690	0.3372	24.10-27.02	25.23
Soybean oil	0.3698-0.4367	0.4019	27.12-30.40	28.69

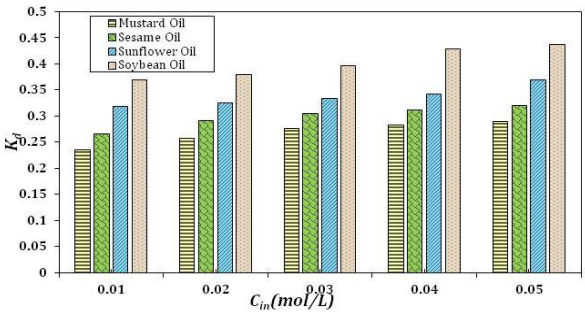

Figure 4: Variation in the initial acid concentrations against the distribution coefficients for different solvents

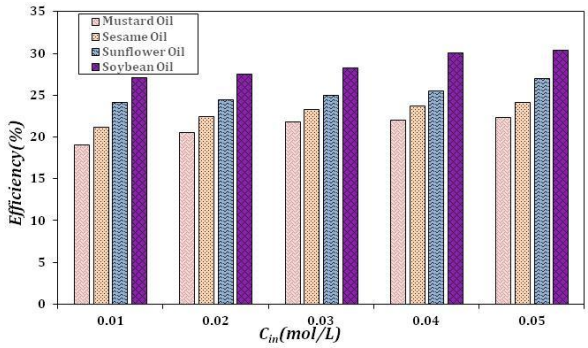

Figure 5: Variation in the initial acid concentrations against the extraction efficiencies for different solvents

range of gallic acid was used, the aqueous and organic phases generally exhibited a linear relationship.

4.1. Effect of diluents

It is essential for diluents to dissolve acid complexes and prevent them from interacting with one another in order for solvent extraction to be efficient, ensuring that the activity coefficient of the organic phase is constant. On the other hand, polar acid complexes tend to group together and are less amenable to non-solvating solvents, which might lead to issues like precipitation, phase separation or coacervate formation [30]. Important properties such as the dielectric constant, the ability to donate or accept electrons, the dipole moment as well as partitioning between the water and oil phases control the behaviour of the solvent in these systems. No chemical reaction takes place during the extraction process, rather acids simply a partition between the organic and aqueous layers based on the physical equilibrium. Acid is still contained in a sphere of water molecules in oxygen-donor solvents and several solvent molecules must be present at the interface to compete with water for those hydration bonds.

According to *Table 4*, when compared to mustard, sesame and sunflower oils, soybean oil exhibited the highest extraction yield ($E\%$ = 28.69%) and partition coefficient (K_D = 0.4019). Its high proportion of saturated and monounsaturated fatty acids such as oleic acid may contribute to its sustained fluidity and solute retention based on strong van der Waals forces. In contrast, mustard oil exhibited the lowest K_D (0.2676) and $E\%$ (21.15%) of all the oils tested, despite being strongly polar, that is, a dielectric constant of about 4.5, and having a higher capacity to take extra electrons from the allyl isothiocyanate group. In addition to preferring to react with sulphur and reactivity based on sulfone production, the higher polarity of mustard oil cannot have been influential [14]. It is also volatile and incompatible with nonpolar solutes due to its polarity. The yields of extraction efficiencies for sesame and sunflower oils were moderate with sunflower oil (K_D = 0.3372, $E\%$ = 25.23%) performing slightly better than sesame oil (K_D = 0.2982, $E\%$ = 22.99%). This could be because sunflower oil has a higher linoleic acid content and lower viscosity between oil solutions, which promotes solute diffusion, which is a specific property of solutes in nonpolar liquids. As a result, the best solvent for extracting separated nonpolar compounds is soybean oil, however, mustard oil is better as a polar extraction solvent [12].

4.2. Significance of the initial acid concentration

As the initial acid concentration increases in various solvents at 298 K, the distribution coefficient also increases as illustrated in *Figures 4* and *5*. The same trend may be observed in terms of the extraction efficiency, which rises as the acid concentration increases, because solvation shells, composed of both solvent and water molecules, form around the functional groups of the acid. Solute solubility is increased by this heterogeneous solvation environment, especially when inert diluents are present [37],[38].

4.3. Impact of properties of carboxylic acid

Efficiency in the extraction of carboxylic acids is mostly determined by two factors: (1) the level of water hydration the acid is subjected to and (2) the energy required to form a bond with water molecules [39]. It should be noted that the acid molecules retrieved from the aqueous phase are undissociated, namely have not broken apart. However, the acid molecules are more likely to form pairs, that is, dimers, in the organic phase [40]. The strength of an acid or how acidic it is in addition to the number of carboxylic groups, what functional groups are connected to the molecule and its size as well as the amount of water around the acid ions are some of the variables that affect its extractability. Studies have shown that more easily extracted acids are those that ionize more easily. Gallic acid, for example, is a stronger acid and can be extracted more easily than other acids due to its *pKa* of 4.4.

4.4. Discussion

Gallic acid was tested at different concentrations (from 0.01 to 0.05 mol/L) and its distribution coefficient (K_D) varied depending on the solvent used. The values ranged from 0.2340 to 0.4367 for mustard oil, 0.2340 to 0.2886 for sesame oil, 0.3175 to 0.3690 for sunflower oil and 0.3698 to 0.4367 for soybean oil. The overall trend was: soybean oil > sunflower oil > sesame oil > mustard oil. The same pattern was seen in terms of extraction efficiency with soybean oil performing the best (28.69%) and mustard oil the worst (21.15%). As the acid concentration increased, so did the distribution coefficient because stronger acids move more easily into the organic phase and at higher concentrations, fewer acid molecules remain bonded to water, making extraction easier [41]. Solvents with higher distribution coefficients usually exhibited certain properties: higher dielectric constants and levels of polarity as well as lower densities and dipole moments [42]. These factors, along with the toxicity of the solvents and environmental sustainability, play an important role in how well they extract acids [43].

5. Conclusions

The recovery of gallic acid from aqueous solutions by natural non-toxic solvents through liquid-liquid extraction was studied. The efficiency of the extraction process was evaluated based on the extraction efficiency and distribution coefficient, which were found to be related to the physical and chemical properties of the solvents. The effects of various diluents, the properties of the acid and their physicochemical constants on parameters such as the partition coefficient, distribution coefficient, dimerization constant and extraction efficiency were also studied. Physical extraction data revealed that soybean oil yielded the highest efficiency, i.e. 28.69% over the range of acid concentrations. Natural extractants produced lower extraction efficiencies but are preferred when the toxicity and sustainability of chemical solvents is a concern. Reactive extraction could be a potential alternative for achieving higher extraction efficiencies.

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