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Infusion variables for maximum bioactive compounds yield in dry scent leaf, *Ocimum gratissimum* Linn, tea

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Abstract

Despite its traditional and culinary use, the industrial application of scent leaf, *Ocimum gratissimum* Linn, as a tea is low, especially in Nigeria. To support its industrial and wider use as a tea, this study examines the infusion variables capable of yielding the maximum bioactive compounds in dry scent leaf as well as its nutritional value. Study finding revealed that a maximum bioactive compounds yield was realised at 0.3 mm (particle size), water temperature of 70 ºC and soaking duration of 7 minutes. The infusion was rich in carbohydrates (9.95 %), protein (4.18 %), potassium (120.5 ppm), phytate (2.32 mg/100g) and vitamin C (12.94 mg/100g), but low in crude fibre (0.07 %), magnesium (8.67 ppm), saponin (0.42 mg/100g), and vitamin A (205.92 µg/100g**)**. This indicates that scent leaf tea could provide a modest contribution to dietary energy and amino acids, capable of serving as a natural electrolyte and mineral source**.** In the analysis, this study used 2 grammes (g) of dry, milled scent leaf of average moisture content below 10 per cent (%) (wet basis) and 25 millilitres (mL) of water.

Keywords: *Scent leaf tea; Bioactive compounds yield; Infusion variable; Nutritional value*

Introduction

Scent leaf, *Ocimum gratissimum* Linn, a medicinal and aromatic herb widely used in traditional medicine and culinary applications, has gained increasing attention due to its rich bioactive compounds (Orafidiya, *et al.*, 2001; Prakash and Gupta, 2005). These compounds, including essential oils, flavonoids, saponins, tannins, and other phytochemicals, have contributed to its various health benefits, such as antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic properties (Osei *et al*., 2021; Okigbo *et al.*, 2009). Owing to this, there has been a growing interest in enhancing the extraction yield of these compounds for use in food, beverages, and medicinal preparations (Nwankwo et al., 2020). However, the release of these compounds in water depends heavily on the interplay between soaking duration, water temperature, and surface area, each playing a vital role in the release of phytochemicals and dissolved solids into the solvent medium (Chemat *et al.*, 2017; Adeyemo and Olagoke, 2020).

There is no acceptable single technique for extracting bioactive compounds from plant matters (Ghenabzia *et al*., 2023). However, one of the most common methods of extracting bioactive compounds from plant materials is infusion, where plant matters are soaked in hot or warm water to release their soluble compounds (Bello *et al.*, 2019; Adeyemo and Olagoke, 2020) (see the study of Ghenabzia *et al*. (2023) for a review of the different methods of extraction of bioactive compounds and their effects on biological activity). The extraction performance is influenced by several factors, including temperature, soaking duration and particle size of the plant material. These significantly impact the quantity and quality of the dissolved favours and phytochemicals in the final infusion (Okoro *et al*., 2021; Oladipo and Fagbohun, 2022). Temperature plays a vital role in the extraction process by enhancing the solubility of water-soluble compounds and increasing the diffusion rate of phytochemicals from the plant tissue into the water (Chemat et al., 2017). The studies of Okoro *et al*. (2021) and Bello *et al*., (2019) have shown that higher infusion temperatures significantly increase the dissolved solids in herbal infusions due to enhanced solubility of compounds such as flavonoids, saponins, and tannins. However, excessive heating may lead to the degradation of thermolabile compounds, including certain vitamins and polyphenols, and antinutrients (Adesina *et al.*, 2021; Sudirman *et al*., 2024), thereby necessitating careful studies to balance solubility and thermal stability. Soaking duration is another key factor influencing extraction performance. Longer soaking duration generally allows more time for the plant material to release its soluble compounds into the water, thus promoting a higher concentration of extractable bioactive compounds (Adeyemo and Olagoke, 2020). While the literature is thin on the optimal soaking duration, Nwankwo et al. (2020) observed that extending soaking duration from 3 to 10 minutes led to significant increases in antioxidant and polyphenol contents yield in herbal teas. Notwithstanding this, Wang and Weller (2006) had cautioned that prolonged exposure may also result in the loss of certain volatile compounds due to evaporation or degradation. Therefore, determining an appropriate soaking duration is central to maximizing nutrients release without jeopardising the stability of beneficial phytochemicals (Adeyemo and Olagoke, 2020). Particle size is equally important, as it directly affects the surface area of the plant material exposed to the solvent. Smaller particle sizes result in a greater surface area, enhancing the extraction performance by allowing more water to come into contact with the exposed plant matter, thus leading to more effective leaching of soluble compounds (Bello *et al.*, 2019). However, larger particle sizes may hinder the extraction process, limiting the amount of bioactive compounds that can be released into the water. Studies, including those of Adeyemo and Olagoke (2020) and Okoro *et al.* (2021), have demonstrated that pulverizing plant materials into fine particles significantly improves extraction yield compared to coarser sizes. Where tea bags are not used, extremely fine particles may also lead to filtration difficulties and increased turbidity in infusions, suggesting that while finer particles enhance extraction, they must be balanced with product clarity. Overall, the literature is thin on the optimal particle size, water temperature, and soaking duration beyond which further reduction/increase may not significantly improve extraction or can even negatively impact the process.

The study of Edo et al. (2023) has offered an excellent review of the phytochemistry and pharmacological compounds present in scent leaf. Although not exhaustive, Onyebuchi and Kavaz (2020) explored the effect of extraction temperature and solvent type on the phytochemical and bioactive properties of *O. gratissimum* extracts Assiéné, *et al.* (2021) examined the combined effect of particle size, temperatures and soaking times on some Nigerian herbs including *Moringa oleifera*, *Vernonia amygdalina*, and *Hibiscus sabdariffa*,, while that of Builders, et al., (2020) looked at the effect of these influencing factors on some herbal tea blends such as lemon grass (*Cymbopogon citratus*), hibiscus (*Hibiscus sabdariffa*, commonly known as Zobo), and ginger (*Zingiber officinale*) infusions. The review of these literature strongly supports the influence of temperature, soaking duration, and particle size on the extraction of bioactive compounds from herbal plants. However, studies investigating the combined effect of, and suggesting the, soaking duration, water temperature and particle size that offers a higher extraction yield of bioactive compounds from dry scent leaf are rather scarce. This is the motivation and justification for this study. It fills this knowledge gap by evaluating the interactive effect of particle size, water temperature and soaking duration on dry scent leaf tea, suggesting the treatment that offers the highest bioactive compounds yield and its nutritional value. The outcome of this study contributes to scent leaf tea and therapeutic product development and use.

Materials and Methods

Sample sourcing, collection and pre-treatment

Fresh scent leaves, *Ocimum gratissimum* Linn, were sourced from a garden recognized for its adherence to sustainable agricultural practices in Ado Ekiti in November 2024. Harvesting was carried out in the early hours of the morning (around 6:00 am) to minimize postharvest wilting and quality degradation, transported to the laboratory in ventilated, sealed containers to maintain its freshness and prevent microbial contamination, and manually sorted to remove any damaged, insect infested, and discoloured leaves. The selected leaves were subjected to ambient air drying at room temperature under the shade. Leaves moisture content was monitored using the gravimetric method (modified after ISO 662) until it fell below 10 per cent (%) (wet basis), considered suitable for storage and further processing.

Tea preparation and total dissolved solids (TDS) measurement

600 grammes (g) of air-dried scent leaves were milled and sieved into three pre-selected particle size ranges using the literature and similar tea in the market as a guide: 0.30 mm, 0.60 mm, and 1.18 mm. 2 g each was weighed and placed in labelled tea bags. For tea preparation, 25 millilitres (mL) of distilled water were measured and poured into a glass beaker. The initial electrical conductivity (EC) and total dissolved solids (TDS) of the distilled water were measured prior to infusion. The beaker was then heated to the pre-selected temperatures of 30, 50, and 70 °C. Cost of water heating (or energy cost) and possible phytochemical degradation or loss favoured limiting the maximum water temperature to 70 °C. The bagged scent leaf samples (based on particle size) were immersed in the heated water and allowed to infuse for the pre-selected soaking durations of 3, 5, and 7 minutes (see Figure 1 for the tea preparation process). The pre-selected soaking duration drew on real life situation and documentary evidence. At the end of each soaking duration, the bags were removed. Post-infusion measurements of TDS and EC were carried out using the same calibrated digital EC/TDS meter) to determine the amount of solids extracted from the measured 2 g dry scent leaf samples. The TDS of each tea sample were obtained by subtracting the pre- from the post- infusion measurements.

The extraction rate, a measure of how much bioactive compounds (in this case, using the TDS as an indicator) extracted per unit time, was calculated using Equation 1, while Equation 2 was used to evaluate the extraction efficiency. As shown in Equation 2, the theoretical infusion yield represents the maximum amount of bioactive compounds that can potentially be extracted from a given amount of herbs.

$$\text{Extraction rate (mg/min)} = \frac{\text{TDS}\left(\frac{\text{mg}}{\text{L}}\right) \text{ x Volume of infusion water (L)}}{\text{Soaking time (min)}} \quad \text{Eq. 1}$$

$$\text{Extraction efficiency (\%)} = \left(\frac{\text{Extracted TDS}}{\text{Theoretical infusion yield of scent leaf}}\right) \text{ x } 100 \quad \text{Eq. 2}$$

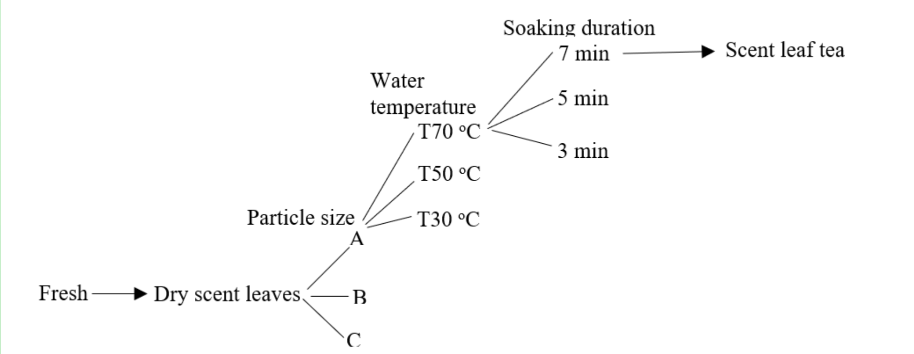


Fig. 1: The schematic diagram illustrating the tea preparation process
Note: A indicates 0.3 mm, B 0.6 mm and C 1.18 mm particle size

Nutrients evaluation

The proximate contents - protein, fat, ash, and carbohydrate - of the scent leaf tea were assessed following standard procedures outlined in the AOAC (2009). The anti-nutritional elements - tannins, phytates and oxalates - were quantified using spectrophotometric techniques described in Adebiyi et al. (2019). Minerals – Na, Ca, K, and Mg – and vitamins – A and C (selected based on their health impacts) – were determined based on the methods described in AOAC (2005), Onwuka, 2005). Samples for analyses were obtained from the 25 mL. Overall, costs of laboratory analyses served to limit the numbers of treatments and nutritional elements considered.

Statistical analyses

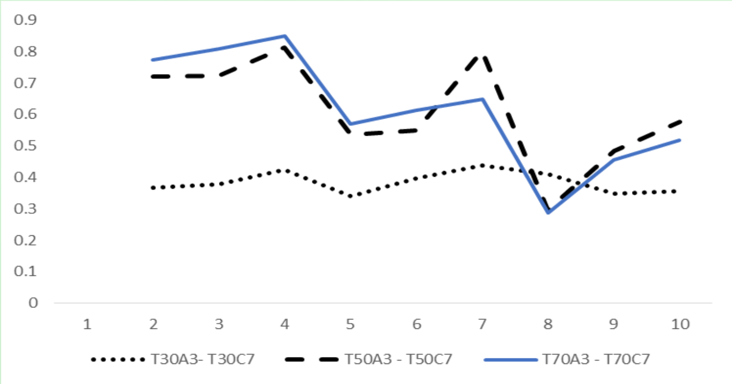
The obtained TDS data were subjected to both descriptive (mean, median, standard deviation, etc.) and inferential (one-way between-groups analysis of variance (ANOVA) and independent-samples t-test) statistical analyses. For the ANOVA, data were checked for significant outliers (using the boxplot), normality (using the Shapiro-Wilk test), and homogeneity of error variances (using the Levene's test). A p-value ≤ 0.001 suggests a violation of normality and homogeneity tests, while data points located outside the boxplot whiskers suggest the presence of significant outliers. The one-way ANOVA was used to explore any significant difference in grouped (by temperature) treatment TDS, while the independent-samples t-test was used to compare the means between the treatments with the highest and the lowest nutritional values, using IBM SPSS Statistics version 25, alpha (α) value of 0.001 for the test of significance. In the case of the one-way between-groups ANOVA, where differences exist, post-hoc comparisons were carried out using the Tukey Honestly Significant Difference (HSD) test.

Results and discussion

TDS value

The average moisture content of the fresh scent leaves at harvest was 82.5 % (wet basis), while that of the dry scent leaves was 2.96 % (wet basis). Dry scent leaves were considered suitable for further analyses once their moisture contents fell below 10 % (wet basis). The 10 % moisture content (wet basis) was adopted to prevent microbial attack in storage, decolouration and loss of nutrients. Twenty seven experimental treatments (or Runs) were carried out to measure the TDS values (Figure 1). The X-axis represents coded combinations of temperature, particle size and soaking duration, while the Y-axis represents the mean TDS values. The summary of measurements for the top 5 and bottom 5 TDS treatments, including their extraction rate and efficiency values is illustrated in Table 1.

The distribution of mean TDS values under various experimental treatments revealed a general increase in mean TDS values, with increase in water temperature. Except for T30A3 – T30C7 that had a fairly stable mean TDS yield, others T50A3 – T50C7 and T70A3 – T70C7 showed a decreasing mean TDS value, impacted by particle size. At lower temperatures, the combine effect of particle size and soaking duration is slight. It becomes more pronounced at higher temperatures. Soaking duration had an increasing yield and bioavailability of active compounds impact under varying particle size holding temperature constant (see the spikes at 4, 7 and 10, Figure 1). This is supported by Table 1. It lists the five treatments with the highest mean TDS values, all of which involved finely ground particles (0.30 mm) and elevated temperatures (50–70°C), with longer soaking durations (5–7 minutes) compared with the bottom lowest five. The peak mean TDS value of 0.87 mg was observed in treatment T70A7 (70 °C, 0.30 mm, 7 min), underscoring the importance of combined thermal and particle size effects on bioactive compounds yield. This finding aligns with existing literature which suggests that smaller particles increase surface area for solute transfer, while higher temperatures accelerate cell wall softening and solute diffusion (Yusoff *et al.*, 2022; Rudrapal *et al.*, 2023).



0.7730	0.8085	0.8510	0.5690	0.6120	0.6490	0.2860	0.4565	0.5170
	3	4	5	6	7	8	9	10
T30A3	T30A5	T30A7	T30B3	T30B5	T30B7	T30C3	T30C5	T30C7
0.3680	0.3775	0.4240	0.3390	0.3980	0.4375	0.4115	0.3495	0.3575
T50A3	T50A5	T50A7	T50B3	T50B5	T50B7	T50C3	T50C5	T50C7
0.7215	0.7235	0.8120	0.5360	0.5495	0.8030	0.2970	0.4835	0.5755
T70A3	T70A5	T70A7	T70B3	T70B5	T70B7	T70C3	T70C5	T70C7

Fig 1: The TDS means plots (n = 2) for the scent leaf tea under the various treatments

Note: T30A3 means: water temperature 30 ºC, A (particle size) = 0.30 mm (finely ground), and 3 = soaking duration (min); B = 0.60 mm (moderately ground), and C = 1.18 mm (coarsely ground). For example, T70C7 indicates: water temperature 70 ºC, particle size 1.18 mm, and 7 mins soaking duration. Table 1: The top 5 and bottom 5 mean TDS values, including their extraction rate and extraction efficiency

Table 1: The top 5 and bottom 5 mean TDS values, including their extraction rate and extraction efficiency

Rank	*Treatment*	*Temperature (°C)*	*Particle size (mm)*	*Soaking duration (min)*	*Mean TDS value* (mg/L)*	*Extraction rate (x 10^{-3} mg/min)*
Top 5						
Run 21	T70A7	70	0.30	7	0.87	3.11
Run 12	T50A7	50	0.30	7	0.82	2.93
Run 15	T50B7	50	0.60	7	0.81	2.89
Run 20	T70A5	70	0.30	5	0.81	4.05
Run 19	T70A3	70	0.30	3	0.78	6.50
Bottom 5						
Run 9	T30C7	30	1.18	7	0.36	1.29
Run 8	T30C5	30	1.18	5	0.35	1.75
Run 4	T30B3	30	0.60	3	0.34	2.83
Run 16	T50C3	50	1.18	3	0.30	2.50
Run 25	T70C3	70	1.18	3	0.29	2.42

*n = 2

Table 2: Results of the descriptive statistical tests

Statistic	*Mean (std. error)*	*Median*	*Variance*	*Std. deviation*	*Minimum*	*Maximum*	*Range*	*Interquartile range*	*Skewness (std. error)*	*Kurtosis (std. error)*
T30A3 - T30C7	0.39 (0.02)	0.37	0.004	0.065	0.283	0.525	0.242	0.124	0.37 (0.54)	-0.396 (1.04)
T50A3 - T50C7	0.61 (0.04)	0.60	0.029	0.170	0.269	0.890	0.621	0.261	-0.30 (0.54)	-0.412 (1.04)
T70A3 - T70C7	0.614 (0.05)	0.60	0.038	0.195	0.280	0.949	0.669	0.218	0.18 (0.54)	-0.311 (1.04)
Overall	0.54 (0.04)	0.51	0.035	0.186	0.269	0.949	0.680	0.297	0.55 (0.33)	-0.557 (0.64)

Statistic	*99 % confidence interval for mean:Lower bound*	*Upper bound*	*5 % trimmed mean*
T30A3 - T30C7	0.34	0.43	0.38
T50A3 - T50C7	0.50	0.73	0.62
T70A3 - T70C7	0.48	0.75	0.61
Overall	0.47	0.60	0.53

As shown in Table 1, Run 21 exhibited the peak extraction performance, with a TDS of 0.875 mg**.** Run 21, which was characterized by fine particle size (0.30 mm), highest temperature (70°C), and longest soaking time (7 minutes), likely enhanced nutrients transfer due to increased surface area and thermal energy. Similar extraction performance was observed in Runs 12, 15 and 20, highlighting the significance of the combined effect of temperature, particle size and soaking duration. However, the lowest extraction performances were observed in Runs 25 and 16, both associated with coarser particles and potentially lower soaking durations. On the other hand, holding temperature and particle size constant, the extraction rate increased with decrease in soaking duration (see, for example, Runs 21, 20, and 19; and Runs 9 and 8 in Table 1). Although the water-to-leaf ratio in this study was held constant for all the treatments (12.5 to .0; (v/m)), the diffusion rate was expected to tail off after some few minutes of soaking due to saturation effects, water temperature cooling off, plant tissue resistance, and extractability limitation associated with use of water-infusion method. As revealed in Figure 1, increasing the soaking duration did contribute to a higher extraction yield. The only exception was T30C3 – T30C7 that resulted in a decrease. Particle size (1.18 mm) and the possibility of nutrients migrating back into the plant tissues may explain the observed phenomenon. With scent leaf having an average theoretical infusion yield, the Runs with the highest and lowest extraction efficiency were 21 and 25, respectively (Table 1). Overall, the extraction kinetics were more strongly influenced by the interplay of higher temperature, fine particle size**,** and increased soaking duration**.** This observation is consistent with the findings of Adeoye *et al*. (2023) and Chen *et al*. (2022) who emphasized that diffusion and solute migration are enhanced by higher temperatures and reduced particle sizes due to lower diffusion barriers and improved solubility.

Table 2 illustrates the results of the descriptive statistical tests. Looking at Table 2 and Figure 1 together, the T70 grouping had the lowest minimum mean TDS, 0.86 mg, facilitated by higher particle size (1.18 mm) and lower soaking duration (3 minutes). Overall, the T70 grouping had the highest median TDS (0.61 mg), followed by the T50 grouping (0.58 mg), further supporting the influencing role of water temperature in nutrients extraction. The T30 grouping had the lowest range (0.098 mg), while the T70 grouping had the highest range (0.565 mg). The difference in range between T70 and T50 was slight (Table 2). This suggests the need to check if there is any statistical significant difference between the mean TDS values of the three groupings.

Data checked for significant outliers, normality (W = 0.958 (T30), 0.964 (T50), 0.954 (T70)); $p > 0.001$) and test of homogeneity of variances ($p > 0.001$) revealed no statistically significant violation. As shown in Table 3, the results of the one-way between-groups ANOVA revealed a statistically significant difference at the $p < 0.001$ level in mean TDS for the different temperature levels: $F\ (2, 51) = 13.11$, $p = 0.000$; the effect size was large (partial eta squared = 0.34). Post-hoc comparisons using the Tukey HSD test indicated that the mean TDS values for T30 grouping (m = 0.39, SD = 0.07), T50 (m = 0.611, SD = 0.17) and T70 grouping (m = 0.614, SD = 0.20) were significantly different from each other. The T50 grouping (m = 0.611, SD = 0.17) and T70 grouping (m = 0.614, SD = 0.20) did not differ significantly from one another. This suggests that looking at water temperatures, T50 and T70 groupings did not differ in terms of their mean nutrients yield. However, by mean and median values (Table 2), The T70 grouping was slightly higher. Therefore, the effect of water temperature is crucial to nutrients yield holding other infusion variables constant.

Table 3: Results of the one-way ANOVA

	Sum of squares	*df*	*Mean square*	*F*	*Sig.*
Between groups	0.622	2	0.311	13.11	0.000
Within groups	1.210	51	0.24		
Total	1.832	53			

Nutrients value

As shown in Table 4, the treatments with the highest mean TDS value (Run 21) and with the lowest mean TDS value (Run 25) were analysed for their nutrients values. For Run 21, the analysis revealed that the tea was rich in carbohydrates (9,95 %), protein **(**4.18 %), potassium (120.5 ppm), phytate (2.32 mg/100g) and vitamin C (12.94 mg/100g), but low in crude fibre (0.07 %), magnesium (8.67 ppm), saponin (0.42 mg/100g), and vitamin A (205.92 µg/100g**).** This indicates that the tea could provide a modest contribution to dietary energy and amino acids, with scent leaf tea capable of serving as a natural electrolyte and mineral source**.** The anti-nutrients profile was low and within tolerable limits, posing minimal interference with mineral absorption, while the infusion retained a beneficial level of vitamin C (12.94 mg/100g)**,** which is vital for antioxidant function and immune health. This supports the studies of Afolayan and Jimoh (2009) and Gbashi *et al.* (2021) who emphasized that a careful balance of extraction variables not only increased extraction yield but also preserved the integrity of bioactive and nutritional compounds. However, Table 4 further revealed that extraction performance had some implications for tea nutritional value. The t-test results between Runs 21 and 25 were statistically significant at $p<0.01$ levels (Table 4).

Table 4: Run 21 (top highest) and Run 25 (bottom lowest) nutritional values

Parameter	*Value (mean*)* *Run 21*	*Run 25*	*% difference*	*t-tests*
Proximate (%):				p = 0.000, significant
Ash	0.21	0.17	+ 19.05	
Fat	0.19	0.24	- 26.30	
Crude fibre	0.07	0.06	+ 14.29	
Protein	4.18	3.18	+ 23.92	
Carbohydrate	9.95	8.72	+ 12.36	
Mineral (ppm):				p = 0.000, significant
Na	15.5	10.13	+ 34.65	
Ca	72.83	50.63	+ 30.48	
K	120.5	110.53	+ 8.27	
Mg	8.67	7.16	+ 17.42	
Anti-nutrient (mg/100g):				p = 0.000, significant
Tannin	2.20	2.65	- 20.46	
Phytate	2.32	3.18	- 37.07	
Saponin	0.42	0.53	- 26.19	
Oxalate	2.21	1.72	+ 22.17	
Vitamin:				p = 0.000, significant
A (µg/100g)	205.92	250.62	- 21.91	
C (mg/100g)	12.94	12.74	+ 1.55	

*n = 3; t-test (p < 0.001, Sig. (2-tailed)) suggests a significant difference between Runs 21 and 25, otherwise not. + indicates an increase, while – indicates a decrease. Data checked for significant outliers, normality and Levene's test for homogeneity of variances revealed no significant violation.

Conclusion

This study explored the combined effect of particle size, soaking duration, and water temperature on the extraction performance and nutritional composition of scent leaf, *Ocimum gratissimum* Linn, tea. The highest mean extraction yield – mean TDS of 0.88 mg/L - occurred at 0.3 mm (particle size), 70 ^oC (water temperature) and 7 minutes (soaking duration). This highlights the importance of combining fine particle size, longer soaking duration, with higher temperatures to maximize solute extraction in herbal teas. The tea obtained at mean TDS of 0.88 mg/L contained favourable nutritional characteristics, including appreciable amounts of protein (4.18 %), carbohydrates (9.95 %), potassium (120.5 ppm), and vitamin C (12.94 mg/100g), with low anti-nutrient levels. This outcome indicates that scent leaf tea holds promise as a nutritious and health-supporting herbal drink, capable of delivering essential nutrients and antioxidant benefits. To support its household use and industrial applications, the initial conclusion of this study is that dry scent leaf should be ground to 0.30 mm particle size, steeped in water at 70 ^oC, for 7 minutes. This is capable of ensuring maximum bioactive compounds extraction and nutritional value.

Notwithstanding the above, this study has some limitations worth mentioning. Firstly, the study assumes increase in mean TDS value to be associated with increase in nutritional value. This claim requires further investigations as some volatile nutrients may be evaporated or degraded at higher temperatures. Secondly, the boundary conditions, for example, for particle size, were purposively selected with some literature as a guide. However, future studies should look at 0.1 and 0.2 dry scent leaf particle sizes and compare the effects on bioactive compounds yield holding soaking durations (3, 5, 7 minutes) and water temperatures (30, 50 and 70 ^oC) constant, and thirdly, both the effect of changes in the volume of the dissolving solvent, water, and the interactions between water volume and solute mass, or water-to-leaf ratio, were not investigated. The present study had kept the solute mass constant at 2.0 g and solvent volume at 25 mL. To explain the possibility of any significant interactions between solvent volume and solute mass may benefit from future studies.

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