Progress in Engineering Application and Technology Vol. 3 No. 2 (2022) 070–079

PEAT

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/peat
e-ISSN : 2773-5303

Evaluation of Antioxidant Property of Curcumin-loaded Nanoemulsion

Nurul Aini Adnan[1], Angzzas Sari Mohd Kassim[1]*, Aliff Hisyam A. Razak[1]

[1]Department of Chemical Engineering Technology, Faculty of Engineering Technology,
Universiti Tun Hussein Onn Malaysia, 84600 Pagoh, Johor, MALAYSIA

*Corresponding Author Designation

DOI: https://doi.org/10.30880/peat.2022.03.02.007
Received 27 January 2022; Accepted 20 July 2022; Available online 10 December 2022

Abstract: Antioxidants are substances that scavenge free radicals. Sources of antioxidants can be produced either from natural or synthetic. However, for the past few decades, the health concern towards the carcinogenicity of synthetic antioxidants has grown among skincare consumers, resulting in the increasing demand for natural antioxidants. Therefore, the study proposed to produce a curcumin-loaded nanoemulsion and evaluate its antioxidant property. The formulation of nanoemulsion involves the oil in water (o/w) emulsion type through the phase inversion temperature (PIT) method, that is used to produce nano-structured curcumin with high solubility, high bioavailability, high total phenolic contents and antioxidant activity. Nanoemulsion composed of black pepper oil and non-ionic surfactant Tween 20, with a mean droplet size ranging from 310 to 720 nm, was formulated for various concentrations of the oil and surfactant. Nanocurcumin (Nano-Cur) has obtained a particle size of 320 nm when the following optimal conditions were adopted as the oil-surfactant ratio (0.5:9.5). Inverter Microscope with Fluorescence has been used to determine the particle size distribution. Antioxidant activity tests were applied to the pure extracts, Nano-CUR, and the mixture of Tween 20 + BP oil. Results demonstrated that Nano-CUR has maximum antioxidant activity (94.6 ± 0.37 % RSA) with the highest reading of total phenolic content (2.58 ± 0.01 mg GAE/g). The correlation between total phenolic content and antioxidant activity also showed a positive correlation where both analyses are directly proportional to each other. Overall, the study provides helpful information on physical properties and antioxidant activity of curcumin-loaded nanoemulsion, which may benefit more natural-based products in the future.

Keywords: Curcumin, Nanoemulsion, Antioxidants

**Corresponding author: angzzas@uthm.edu.my*

1. Introduction

Plant-based products and herbal medicines have attracted great interest among researchers due to their application versatility. Medicinal plants are generally rich in bioactive compounds used in traditional or modern medicine [1]. Generally, turmeric constituents include curcumin, dimethoxy curcumin and bisdemethoxy curcumin as the main components [1]. Curcumin is a natural bioactive phenolic compound that exhibits a wide spectrum in therapeutic activity [2]. Furthermore, curcumin is discovered to exhibit high antioxidant activity that can be a useful tool in treating wounds [3].

During the last decades, interest in extracting curcumin with admirable properties has led to discovering new technology in extraction. The extraction method can easily affect curcumin's structural characteristics and properties [4]. Hence, the extraction process plays a significant role in exploring curcumin properties. Aqueous extraction by using subcritical water as the solvent is one of the traditional extraction methods used for the extraction of the bioactive compound from the plant [5].

For the past decades, many studies have reported the various health benefits of curcumin. However, the bioavailability of curcumin remains a concern among researchers due to its solubility in water [6]. With the aid of technology, the bioaccessibility of curcumin can be optimised. Nano-structured curcumin is discovered to be more efficient in extracting the bioactive compounds, enhancing the bioavailability of the lipophilic compounds in aqueous media [7]. Therefore, the oil in water (O/W) technique is proposed to formulate curcumin-loaded nanoemulsion and optimise the antioxidants properties in curcumin [8].

2. Materials and Methods

2.1 Materials

Curcuma longa (Pahang), Black pepper oil (Plant Therapy, India), Tween 20 (Merck Darmstadt, Germany), Folin-Ciocalteu reagent (Merck Darmstadt, Germany), Sodium carbonate (Na_2CO_3) solution (Merck Darmstadt, Germany), Gallic acid solution (Sigma Steinheim, Germany), DPPH reagent (Sigma Steinheim, Germany).

2.2 Experimental Methods

2.2.1 Collecting, processing and extracting *Curcuma longa* sample

The sub-critical water extraction (SWE) is built from the prototype on a laboratory scale into a 70-L sub-critical water extractor industry scale (AM Zaideen Sdn. Bhd.). The SWE apparatus is as shown in Figure 1. The sample after processing was weighed and embedded in the extraction cell, and the ratio of sample to water as a solvent is 1:3. The water then was filled into the tank and purged with N_2 for 1 hour to remove the dissolved oxygen. The water was delivered through the system at a steady flow rate using a high-pressure pump [9]. The pressure and temperature were set at 10 bar (1Mpa) and 120 ˚C, respectively. After 15 minutes of retention time, the extracts exit the cell and let it cool down before being stored in the freezer at 4˚C until further analysis.

Figure 1: The 70-L sub-critical water extractor

2.1.2 Preparation of Nanocurcumin

The preparation of nano-curcumin via an oil in water (o/w) method [8], the black pepper oil and surfactant, Tween 20 was mixed together by a ratio of 1:9 w/w and continuously stirred for 30 minutes 1000 rpm to form an oily phase. Accurately 100 mg of extracted curcumin was added to the mixture. The mixture was agitated under continuously stirred using a magnetic stirrer for 1 hour at room temperature with a speed of 500 rpm [8]. The final nano-emulsion was formed by adding deionized water to the oily phase by the ratio of 1:5 and was heated at 70 ˚C and 75 ˚C for 30 minutes with a speed of 500 rpm. The mixture then undergone rapid cooling at 5 ˚C for 15 minutes. The sample was stored at 4 ˚C until further analysis. The sample extract mixture was prepared with different ratios of oil and surfactant as in Table 1.

Table 1: Different oil and surfactant composition in o/w emulsion

Solution	Oil (g)	Surfactant (g)
Curcumin 1	0.5	9.5
Curcumin 2	1.0	9.0
Curcumin 3	1.5	8.5
Curcumin 4	2.0	8.0

2.1.3 Characterisation of Nanocurcumin Emulsion

The nanocurcumin was characterised for particle size, pH and colour of the emulsion.

2.1.3.1 Particle Size Dispersion

Inverter Microscope with Fluorescence (Olympus IX-HOS, Japan) was used to analyse the size dispersion of curcumin-loaded nanoemulsion. A small amount of emulsion sample was first fixed on the glass slide and the cover slip was put onto it. The sample then was analysed under magnification of 4x and the diameter size of particles was determined. This procedure was repeated for magnification of 10x, 20x and 40x before microscopic imaging.

2.1.3.2 pH of Formulated Nanoemulsion

The nanoemulsion's pH was checked using pH meter (Model HI 3220, China) at 20 ± 1 ˚C. A small amount of nanoemulsion was first put into the beaker and the pH meter was dipped into the solution to determine the pH.

2.1.3.3 Color of the Emulsion

Emulsion stability has an impact on the appearance of products, and emulsion instability may often be seen with the naked eye. In this regard, without expensive analytical instruments, visual observation is perhaps the easiest, cheapest, and quickest way to determine the gravitational separation of the emulsion [10].

2.1.4 Preparation of Standard Gallic Acid for Calibration Curve

The standard gallic acid solution was prepared by dissolving 0.5 mg of it in 50 mL of methanol and 50 mL of distilled water Several concentrations of gallic acid solutions in methanol (50, 100, 150, 250, and 500 mg/mL) were prepared from the stock solution. 1 mL of each concentration was pipetted into different test tubes. 5 mL of 10.00 % Folin–Ciocalteu reagent (FCR) and 4 mL of 7.50 % sodium carbonate (Na_2CO_3) were added to each concentration, resulting in a final volume of 10 mL. The resulting, blue-colored mixture was then thoroughly shaken and incubated at room temperature for 30

minutes. The absorbance was then measured against a blank at 760 nm (methanol). The FCR reagent oxidises phenols in plant extracts and changes into the dark blue, measured by a UV-visible spectrophotometer. All of the tests were done in triplicate, and the calibration curve was plotted using the average absorbance values obtained at various gallic acid concentrations.

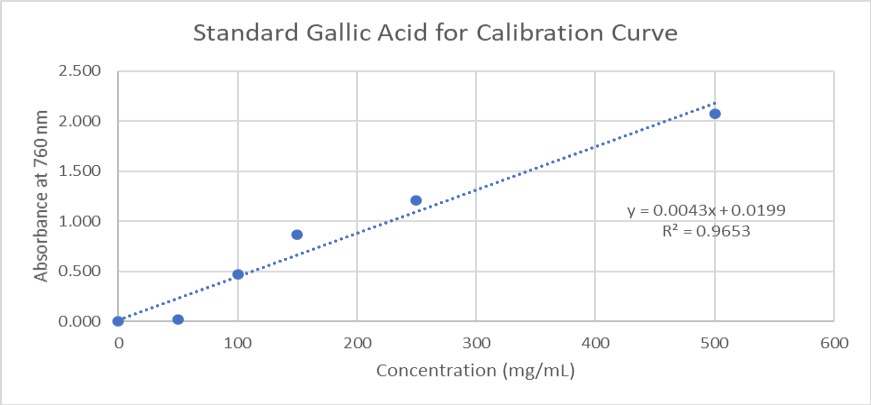

Figure 2: Standard curve of gallic acid

2.1.5 Determination of Total Phenolic Content

The Folin-Ciocalteu technique was used to evaluate the total phenolic content (TPC) of turmeric extract [11]. Briefly, 2 mL of extracts were inserted into different test tubes and was mixed thoroughly with 2.5 mL of 10.00 % Folin-Ciocalteu reagent (Merck Darmstadt, Germany). After 5 minutes, 2 mL of 7.50 % sodium carbonate (Na_2CO_3) solution was added, and the final mixture was allowed to incubate for 1 hour at room temperature in the dark. The intensity of the blue-coloured complex was measured at 760 nm using a UV-vis spectrophotometer. The samples were measured in three replicates. The standard curve of the gallic acid solution was prepared by varying the values of 50, 100, 150, 250 and 500 mg/L [11]. The TPC was expressed in terms of equivalent (g GAE/g of turmeric).

2.1.6 Determination of Free Radical-Scavenging Activity using DPPH assay

The antioxidant activity of all extracted samples was determined through the free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [12]. Approximately, 0.1 mM DPPH methanolic solution was prepared by mixing 4 mg of DPPH (Sigma Steinheim, Germany) and 100 mL of methanol. Then, 1.5 mL of sample extracts was added with 2 mL of 0.1 mM DPPH methanolic solution into the mixture. The mixture was thoroughly mixed and incubated for 1 hour in the dark. The absorbance was measured at 517 nm using a UV-vis spectrophotometer (T80 series, Germany). The samples were measured in triplicates. Percentage of DPPH scavenging activity was calculated as % inhibition of DPPH = [Abs control –Abs sample / Abs control] x 100.

2.1.7 Statistical Analysis

All analysis were performed duplicate, and the data are reported as the mean ± standard deviation (SD). Data were analysed using Minitab (version 19.0) and Microsoft Excel 2016. Statistical analyses of the biochemical data were conducted using Tukey's test through one-way ANOVA analysis. $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Characterisation of Formulated Nanoemulsion

The phase inversion temperature (PIT) method relies on the changes of non-ionic surfactant solubility with temperature. The diameter size of the nanoemulsions fabricated at two different heating temperatures, 70 ˚C and 75 ˚C was shown in Table 2. The results showed at the heating temperature of 75 ˚C, the sample 1B shown the smallest particle size with the diameter of 320 nm while at the heating

temperature of 70 ˚C, the larger particle size was observed at sample 1A with the diameter of 720 nm. Similar result was obtained by study [13] where at higher phase inversion temperature, the fine smaller emulsion is produced. According to Vinh et. al, (2020), emulsification is promoted through PIT as the interfacial tension becomes extremely low. The system will produce a very small and narrow size distribution through rapid cooling. Hence, the result indicated that samples at 75 ˚C are experimental conditions that produced emulsions with a good average diameter size than samples at 70 ˚C. This suggests that the temperature of 75 ˚C is closer to the PIT and facilitates better spontaneous emulsification [13]. However, although the diameter size for the samples at 75 ˚C is better compared to the samples from 70 ˚C, the samples had high diameter size (the average size droplet >200 nm) than the theoretical value of nanoparticles which are between 20 to 200 nm. This high physical parameter could be related to the stability of the nanoemulsion during storage. The nanoemulsion could have much lower physical parameters if been observed after one-month storage [13].

Table 2: Characterisation of nanoemulsion formulations on different temperatures and ratios of oil and surfactant (OSR)

PIT Temperature (˚C)	Sample		OSR (BP oil: T20)	OSR (%)	Diameter size (nm)	pH	Colour
70	A	1	0.5:9.5	5.2	530	5.42	Yellow (clear)
		2	1.0:9.0	11.1	590	5.10	Yellow (clear)
		3	1.5:8.5	17.6	670	4.75	Yellow (cloudy)
		4	2.0:8.0	25.0	720	4.34	Yellow (cloudy)
75	B	1	0.5:9.5	5.2	320	5.76	Yellow (clear)
		2	1.0:9.0	11.1	410	5.24	Yellow (clear)
		3	1.5:8.5	17.6	460	4.82	Yellow (cloudy)
		4	2.0:8.0	25.0	550	4.55	Yellow (cloudy)

As shown in Table 2, the oil-surfactant ratio (OSR) contributed to the changes in the physicochemical properties. For both PIT, the ratios were varied from 0.5: 9.5, 1.0: 9.0, 1.5: 8.5 and 2.0: 8.0. The smallest droplet size is observed in sample 1B consisting of 0.5 g of oil and 9.5 g of surfactant with a diameter of 320 nm. The droplet diameter was observed to decrease with the increase of surfactant concentration (Table 2). According to Vinh et. al, (2020), the addition of surfactant to the nanoemulsion system caused the interfacial film to condense and stabilise, resulting in a small droplet size. This finding also supported by Moghaddasi et. al, (2018) who found that increasing the concentration of surfactant molecules can stabilise a bigger oil-water interface, resulting in smaller nanoemulsions. The mean particle diameter increased significantly when the amount of BP oil in a nanoemulsion was increased by more than 20.00 %, and no clear nanoemulsions could be generated (droplet size > 200 nm) [8]. At certain compositions, the large particle sizes observed at greater OSR or BP oil concentrations could be attributable to phase changes in the surfactant-oil-water system [8]. Hence, it can conclude that at the lowest OSR (5.20 %), smallest particle (d=320 nm) was obtained.

From the results obtained, it can be observed at sample 1B with the pH value of 5.76 is the highest reading and the lowest pH value is observed in sample 4A with the reading of 4.34. A similar result has been reported by Anjali et. al, (2012) which with the highest OSR (1:3), the pH of the sample shown the highest value of 5.60. From Table 4.2, it can be concluded that with the increase of surfactant concentration, the pH of the nanoemulsion sample also increased. In addition, it also can be observed at sample 1 and 2 for both PIT, the pH of samples was suitable for cream formulation as optimum, and the best pH for human skin ranged from 5 to 6 [15]. On the other hand, the pH value for sample for 3 and 4 for both PIT is not considered as it is not suitable for human skin due to its low pH (acidic).

As depicted in Figure 3, the oil and surfactant phases were separated due to the increases in OSR, and after water addition, it was observed that the prepared nanoemulsion seemed to be clear in low OSR. For both PIT, in lower OSR (Cur 1 and 2), the samples were observed to be a clear yellow solution, while at high OSR (Cur 3 and 4), the samples were noticeable to be the more whitty yellowish solution. The transparent nanoemulsions that contained the smallest droplets (d ≈ 320-420 nm) could only be produced if the BP oil was mixed with a certain amount of surfactant (Tween 20) at the 5.20 % and 11.10 % of OSR (Table 1). A study by Moghaddasi et. al, (2018) also reported the similar visual observation on the color of the formulated nanoemulsion. Moghaddasi et. al, (2018) found that at the lowest OSR, the sample was observed to be in clear yellow solution while at the highest OSR, the nanoemulsion was observed to be in cloudy yellowish solution. Meanwhile, according to Vinh et. al, (2020), nanoemulsion with optical transparency can interest the pharmaceutical and cosmetic industry which is favourable for the formulation of cream. Hence, this result indicated that the nanoemulsion could be produced in a clear yellow, homogenous oily solution at a high surfactant concentration.

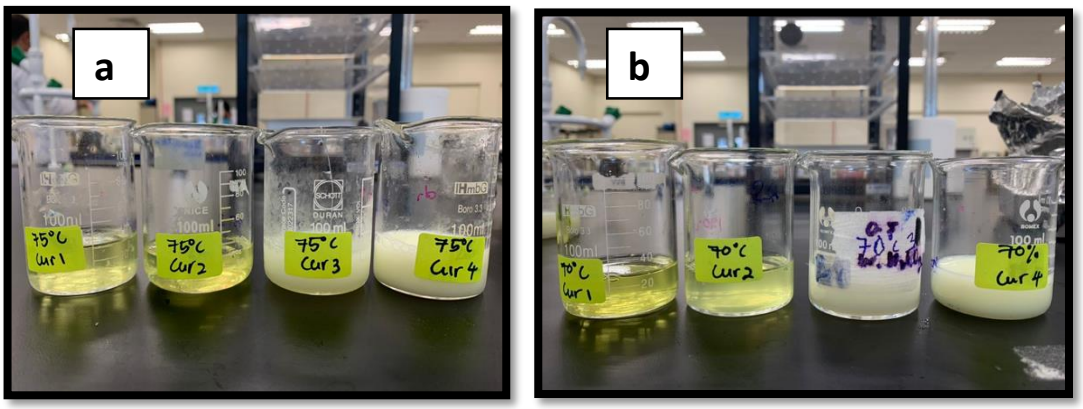

Figure 3: Colour observation on nanoemulsion produced at (a) 75 ˚C (b) 70 ˚C

3.2 Total Phenolic Content of Formulated Nanoemulsion

Due to their redox characteristics, phenolic compounds are significant plant components having antioxidant activity. The hydroxyl groups contained in plant extracts aid in free radical scavenging. Total phenolic contents of plants extract were tested using the diluted Folin-Ciocalteu reagent. The results for total phenolic content were derived from a calibration curve ($y = 0.0043x + 0.0199$, $R^2 = 0.9653$) of gallic acid (0 – 500 mg/mL) and expressed in gallic acid equivalents (GAE) per gram dry extract weight (Figure 2). Table 3 shows the results on the total phenolic content of pure turmeric extracts at different concentrations. At 100 mg/mL concentration, the pure extracted turmeric shown the value of 2.5 ± 0.012 mg GAE/g followed by 1.5 ± 0.01 mg GAE/g and 1.0 ± 0.05 mg GAE/g for concentration at 70 mg/mL and 50 mg/mL respectively. Hence, this conclude that pure extracted turmeric with higher concentration (100 mg/mL) exhibit the higher total phenolic content (2.5 ± 0.012 mg GAE/g).

Table 3: Total phenolic content on pure curcumin extracts at different concentrations

Concentration (mg/mL)	Mean OD ± SD	OD (control)	TPC ± (mg GAE/g)
100	1.4170 ± 0.019	1.702	2.5 ± 0.012
75	1.1725 ± 0.120	1.702	1.5 ± 0.010
50	1.6260 ± 0.003	1.702	1.0 ± 0.052

On the other hand, Table 4 shows the total phenolic content of the formulated nanoemulsion samples. In the present study, the result clearly showed that nanoemulsion, sample 1B, with the OSR of 0.5: 9.5 at a heating temperature of 75 ˚C had the highest total phenolic content (2.58 ± 0.013 mg GAE/g extract). The nanoemulsion sample of 1B (2.58 ± 0.013 mg GAE/g extract) showed significantly increased ($p<0.05$) observed in total phenolic content compared to the pure turmeric extract at 100 mg/mL concentration (2.5 ± 0.012 mg GAE/g extract). Thus, the result indicated that nanoemulsion formulation had increased the total phenolic content in the plant extracts.

In addition, the extraction processes and solvents are in responsible of dissolving the plant's natural components. Plant components can either be polar or non-polar in nature. Since phenolic compounds have a hydroxyl group and are more soluble in polar organic solvents, the phenolic content estimates in this study deviated slightly from those previously published [16;12]. A study by Maizura et. al, (2011) reported that pure turmeric ethanolic extracts exhibit 6.79 ± 1.0 mg GAE/g extract. This could be due to the presence of different amounts of gallic acid and the duration of incubation, geographical variation, and extraction methods, all of which can affect the amount of phenolics present [16]. According to Tanvir et. al, (2017a), The phenolic content of ethanolic extracts was found to be significantly higher than that of aqueous extracts.

3.3 Total Antioxidant Activity by DPPH assay

Results of the activity of free radical scavenging of plants extracts are presented in Figure 4. The figure demonstrated the comparison of radical scavenging activity for pure curcumin, prepared Nano-CUR, BP oil, and Tween 20 and BP oil mixture. Results showed that, prepared Nano-Cur contained the highest DPPH radical scavenging activity (94.6 ± 0.37 %), followed by pure turmeric extract (87.6 ± 0.04%), a mixture of Tween 20 and BP oil (34.1 ± 0.08 %) and BP oil (13.9 ± 0.08 %). Nano-CUR has the highest antioxidant activity due to the synergistic effect from the addition of BP oil and surfactant, Tween 20 into the pure extracts. Authors have also reported similar results demonstrating that Nano-Cur inhibited the highest radical scavenging activity [8]. Hence, it can be proven that with the aid of BP oil and Tween 20, it can enhance the antioxidant content in the plant extracts.

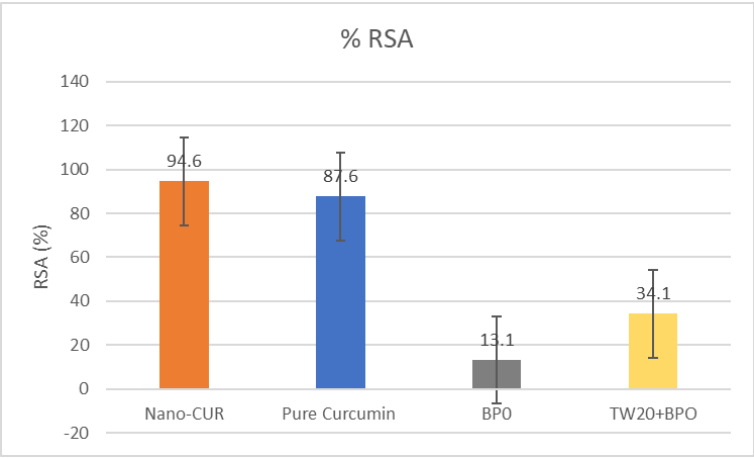

Figure 4: The comparison of % radical scavenging activity for pure extracts, Nano-Cur, BP oil and the mixture of BP oil and Tween 20

In addition, in Table 4, the result demonstrated effect of oil/surfactant ratio on the antioxidant activity of nanoemulsion by DPPH at different heating temperatures of 70 ˚C and 75 ˚C. The result shows that sample 1B with the ratio of 0.5 g of BP oil and 9.5 g of surfactant exhibits the highest radical scavenging activity (RSA) with 94.6 ± 0.40 % while the lowest was observed in sample 3A with % RSA of 4.0 ± 0.6%. This finding suggested that a larger surfactant content could aid curcumin breakdown in the oil phase, resulting in increased antioxidant activity. Tween 20 was discovered to improve oil dispersion into the water phase, hence boosting the antioxidant activity of the o/w emulsion in another study [17]. According to the study, the surfactant can affect the physical position of antioxidants in the O/W emulsion by solubilizing lipid soluble antioxidants into the aqueous phase. As a result, the amount of solubilized curcumin acting as an antioxidant is influenced by oil and surfactant concentrations, affecting the radical scavenging activity of curcumin-loaded nanoemulsions.

3.4 The Correlation Between Total Phenolic Content and Antioxidant Activity

Several studies by Maizura et. al, (2011) and Tanvir et. al, (2017a), reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant properties. Sample 1B has the highest total phenolic content (2.58 ± 0.013 mg GAE/g) and the highest radical scavenging activity (94.6 ± 0.37 %). According to the findings, the total phenolic content and the DPPH assay of plant extracts exhibit a highly significant positive correlation coefficient. The phenolic content of extracts was directly proportionate to their radical scavenging abilities (Table 4). A significant and linear relationship existed between the antioxidant activity and phenolic content of sample thus demonstrating that phenolic compounds are major contributors to antioxidant activity.

Table 4: The total phenolic content and % radical scavenging activity of different sample

Sample	TPC ± SD (mg GAE/g)	RSA ± SD (%)
1A	2.52 ± 0.002	94.0 ± 0.13
2A	2.42 ± 0.008	87.6 ± 0.04
3A	0.51 ± 0.000	4.0 ± 0.62
4A	0.45 ± 0.002	5.9 ± 0.17
1B	2.58 ± 0.013	94.6 ± 0.37
2B	2.56 ± 0.002	77.8 ± 0.04
3B	0.47 ± 0.000	12.9 ± 1.25
4B	0.44 ± 0.164	14.1 ± 3.20

4. Conclusion

The oil-in-water nanoemulsion formulation containing curcumin, black pepper oil, Tween 20 and deionized water was successfully produced by the phase inversion method. A smallest droplet size of 320 nm was measured. The results obtained demonstrated that Nano-CUR had the highest total phenolic content and antioxidant activity compared to pure extracted turmeric. The antioxidant activity of a combination of black pepper oil and Tween 20 was found to be synergistic. Total phenol content and antioxidant activity (DPPH assay) were shown to be highly correlated, supporting the idea of phenols as contributor of the antioxidant power of plants extracts.

Acknowledgement

The authors would like to thank the Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia and AM Zaideen Sdn. Bhd. for their supports.

References

[1] Amarowicz, R., & Pegg, R. B. (2019). Natural antioxidants of plant origin. In *Advances in Food and Nutrition Research* (1st ed., Vol. 90). Elsevier Inc. https://doi.org/10.1016/bs.afnr.2019.02.011

[2] Hewlings, S., & Kalman, D. (2017). Curcumin: A Review of Its Effects on Human Health. *Foods*, *6*(10), 92. https://doi.org/10.3390/foods6100092

[3] Sivamani, R. K., Ma, B. R., Wehrli, L. N., & Maverakis, E. (2012). Phytochemicals and Naturally Derived Substances for Wound Healing. *Advances in Wound Care*, *1*(5), 213–217. https://doi.org/10.1089/wound.2011.0330

[4] Nurhadi, B., Saputra, R. A., Setiawati, T. A., Husein, S. N., Faressi, F. R., Utari, C. D., Sukri, N., Kayaputri, I. L., & Setiasih, I. S. (2020). Comparison of Curcuma domestica and Curcuma xanthorrhiza oleoresins extracted using maceration, Soxhlet, and ultrasound-assisted extraction (UAE). *IOP Conference Series: Earth and Environmental Science*, *443*(1). https://doi.org/10.1088/1755-1315/443/1/012074

[5] T Gopal, J., Muthu, M., & Chun, S. C. (2016). Water soluble nanocurcumin extracted from turmeric challenging the microflora from human oral cavity. *Food Chemistry*, *211*, 903–909. https://doi.org/10.1016/j.foodchem.2016.05.140

[6] Stankovic, I. (2004). Curcumin - Chemical and Technical Assessment. *Assessment*, *1*(8), 1–8.

[7] Ahmad, N., Ahmad, R., Al-Qudaihi, A., Alaseel, S. E., Fita, I. Z., Khalid, M. S., & Pottoo, F. H. (2019). Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. *RSC Advances*, *9*(35), 20192–20206. https://doi.org/10.1039/c9ra03102b

[8] Moghaddasi, F., Housaindokht, M. R., Darroudi, M., Bozorgmehr, M. R., & Sadeghi, A. (2018). Synthesis of nano curcumin using black pepper oil by O/W Nanoemulsion Technique and investigation of their biological activities. *Lwt*, *92*(January), 92–100. https://doi.org/10.1016/j.lwt.2018.02.023

[9] Valizadeh Kiamahalleh, M., Najafpour-Darzi, G., Rahimnejad, M., Moghadamnia, A. A., & Valizadeh Kiamahalleh, M. (2016). High performance curcumin subcritical water extraction from turmeric (Curcuma longa L.). *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1022*, 191–198. https://doi.org/10.1016/j.jchromb.2016.04.021

[10] Hu, Y. T., Ting, Y., Hu, J. Y., Hsieh, S. C., (2017). Techniques and methods to study functional characteristics of emulsion systems. *Journal of Food and Drug Analysis, 25(1), 16–26.* doi:10.1016/j.jfda.2016.10.021

[11] Chen, M. X., Alexander, K. S., & Baki, G. (2016). Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. *Journal of Pharmaceutics*, *2016*, 1–10. https://doi.org/10.1155/2016/5754349

[12] Tanvir, E. M., Hossen, M. S., Hossain, M. F., Afroz, R., Gan, S. H., Khalil, M. I., & Karim, N. (2017b). Antioxidant properties of popular turmeric (Curcuma longa) varieties from Bangladesh. *Journal of Food Quality*, *2017*. https://doi.org/10.1155/2017/8471785

[13] Vinh, T. D. T., Hien, L. T. M., & Dao, D. T. A. (2020). Formulation of black pepper (Piper nigrum L.) essential oil nano-emulsion via phase inversion temperature method. In *Food Science and Nutrition* (Vol. 8, Issue 4, pp. 1741–1752). https://doi.org/10.1002/fsn3.1422

[14] Anjali, C., Sharma, Y., Mukherjee, A., & Chandrasekaran, N. (2012). Neem oil (Azadirachta indica) nanoemulsion-a potent larvicidal agent against Culex quinquefasciatus. *Pest Management Science*, *68*(2), 158–163. https://doi.org/10.1002/ps.2233

[15] Charoenkul, K., & Phromyothin, D. (2017). Development and characterization of nano-cream preparation containing natural extract using nanoemulsion techniques. *Materials Today: Proceedings, 4(5), 6105–6110.* doi:10.1016/j.matpr.2017.06.101

[16] Maizura, M., Aminah, A., & Aida, W. M. W. (2011). Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (Zingiber officinale) and turmeric (Curcuma longa) extract. *International Food Research Journal*, *18*(2).

[17] Joung, H. J., Choi, M. J., Kim, J. T., Park, S. H., Park, H. J., Shin, G. H. (2016). Development of Food-Grade Curcumin Nanoemulsion and its Potential Application to Food Beverage System: Antioxidant Property and In Vitro Digestion. *Journal of Food Science,* 81(3), N745–N753. doi:10.1111/1750-3841.13224