Research Article

Microencapsulation of *Thunbergia laurifolia* Crude Extract and Its Antioxidant Properties

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Abstract
In this study, the antioxidant activity and total phenolic content of *Thunbergia laurifolia* Lindl. or Rang Chuet (RC) extracts from leaf, stem and rhizome were evaluated by using ferric reducing antioxidant power assay (FRAP) and the folin ciocalteu method for total phenolic compounds (TPC). The extracts were prepared by infusion using different amount of plant powder (2.5, 5.0, and 7.5 g) at different concentrations of ethanol as 0, 25, 50, and 75% and extraction time of 24, 48, and 72 h. The crude extract of 7.5 g leaf powder extracted for 72 h using water as the extraction solvent showed the highest antioxidant properties and total phenolic content. This extraction condition produced a FRAP content of 2.62 ± 0.01 mmol Fe^{2+}/g that was significantly differed from those of the stem and rhizome and the highest TPC content of 877.36±18.75 (mg GAE/g). The crude extract from the leaf was subsequently encapsulated by using β-cyclodextrin (BCD) and maltodextrin 20DE (MD) as coating materials using freeze-drying method. The encapsulated powder was investigated for its antioxidant activity. The highest encapsulation efficiency (EE) was obtained when only maltodextrin 20DE was used. The storage stability of encapsulated *T. luarifolia* leaf crude extract was then studied by storing the encapsulated powder at 35, 45, and 55°C for 5 weeks. The storage temperature had no effect on the stability of the encapsulated powder when TPC was used as the criteria unlike that of FRAP which was inconsistent during storage.

Keywords: *Thunbergia laurifolia* Lindl, Antioxidant activity, β-Cylcodextrin, Maltodextrin, Encapsulation

1 Introduction

In Thailand, *T. luarifolia* is widely known as Rang Chuet (RC) [1]. The leaves and roots are commonly used as an antidote for poisoning caused by insecticides and in treating drug addiction. It can be found in herbal and nutraceutical markets as herbal teas, capsules, and powders. The leaf extracts are a source of natural antioxidants because of the high radical scavenging and ferric reducing properties. *T. laurifolia* has been reported to have several biological activities related to antioxidants such as antimutagenic, neuroprotective and hepatoprotective properties. It has also been reported to have anti-inflammatory, antipyretic, and antidiabetic properties [2]. Currently, there are a few studies about its bioactive constituents. So far, 8-epi-grandifloric acid, 3'O-β-glucopyranosyl stilbericoside and phenolic acids of caffeic, gallic, and protocatechuic acid have been isolated from the aerial part of *T. laurifolia* [3].

A distinct amount of materials has been encapsulated in the food industry like antioxidants, sweeteners, enzymes and vitamins. Microencapsulation is carried out to protect these antioxidants against degradation, controlling their release, as well as masking their taste and flavor. Due to the liquid nature of most extracts that contain the bioactive compounds, most encapsulation techniques are often drying processes. These include freeze-drying, spray-drying, and fluid-bed coating. Among these technologies, the most used one for food

industry is spray-drying [4] because of its flexibility and low cost, together with freeze-drying, especially for heat-sensitive compounds because it conserves almost intact the initial functional properties of these compounds [5].

The drying technique and the coating material often affect the retention capacity of compounds within the matrix. Coating materials can be natural or synthetic film-forming polymers. The chemical and physical properties of the core material, processes used to form microcapsules, and the absolute properties desired in microcapsules determine the choice of coating to be used [6]. Therefore, it is important to properly select both the coating material and the encapsulation technique to maximize the incorporation and retention of the functional compounds within the encapsulation matrix [7].

β-cyclodextrin (BCD), is a cyclic oligosaccharide. It has demonstrated the inclusion of major component molecules in the wall materials and has fast release kinetics. Maltodextrin (MD) has been widely used as a coating material because of its relatively low-cost, neutral taste, and aroma. It is also water-soluble and has low viscosity at high solid ratios. It is available in different molecular weights enabling it to provide different wall densities around the core material [8]. Therefore, to achieve the best possible combination of features, a mixture of wall materials may be an appropriate solution. The results of [9] showed that the best results from the encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds were achieved when phenolic compounds were encapsulated by freeze-drying using maltodextrin as wall material. At these conditions, the amounts of phenolic compounds and flavonoids retained in the encapsulated sample corresponded to 62 and 73%, respectively, while 73–86% of the antioxidant activity present in the original extract was preserved. These studies showed the potential of freeze-drying technique.

Studies of antioxidant properties of *T. laurifolia* have been investigated. However, there is no publication on microencapsulation of *T. laurifolia* crude extract and its antioxidant properties so far. Therefore, this research was aimed to study the extraction condition of *T. laurifolia* from different parts of the plant as leaf, stem, and rhizome. As well as the suitable condition of encapsulation of *T. laurifolia* crude extract by using BCD and MD. Moreover, the effect of temperature on the antioxidant property of encapsulated powder during storage was also investigated.

2 Materials and Methods

2.1 *Plant materials*

The plant materials used in this project were the dried leaves, stems, and the rhizome of RC, which were purchased from local suppliers. They were sorted and grounded in a blender to fine powder approximately 80–100 mesh in diameter. The powder was then stored in airtight containers at room temperature until further use.

2.2 *To study the extraction condition of RC crude extract*

A combination of ethanol and water at various ratios is commonly used to investigate the extraction of antioxidants from plant materials [10]. A 3 × 3 × 3 factorial and a randomized block design with 3 replications were used in this section. Three parts of RC plants used were leaf, stem, and rhizome. The plant powder (2.5, 5, and 7.5 g) was mixed with 50 mL of different concentrations of ethanol (0, 25, 50, and 75%) and the mixtures were macerated at 24, 48, and 72 h with a constant shaking speed of 120 rpm at room temperature. Subsequently, each extract was filtered through Whatman No. 1 filter paper and stored in the brown bottle at –80°C for further analysis. Antioxidant activities and total phenolic content of RC extracts were evaluated using ferric reducing antioxidant power assay (FRAP) and the folin ciocalteu method for TPC.

2.2.1 Determination of ferric reducing antioxidant power (FRAP) in RC crude extract

The method of [11] was utilized. Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mm TPTZ solution (M&P Impex, Thailand) in 40 mm HCl (M&P Impex, Thailand), and 20 mm ferrous chloride solution (M&P Impex, Thailand) in the proportion of 10:1:1 (v/v), respectively. The FRAP reagent was freshly and daily prepared and was warmed to 37°C in a water bath prior to use. The extract of 20 μL was added to 1 mL of the FRAP reagent. The absorbance

of the reaction mixture was then recorded at 593 nm after 30 min incubation in the dark by using UV spectrophotometer (UNICO S1200, NJ, USA). The standard curve was constructed using ferric sulphate solution (100 to 2000 µm) and the results were expressed as µm equivalents of ferric per g dry weight of plant materials. All measurements were taken in triplicate and the mean values were calculated as Fe^{3+} to Fe^{2+}/mg.

2.2.2 Determination of the total phenolic compounds (TPC)

Gallic acid (M&P Impex, Thailand) was used as the standard and prepared at 25, 50, 75, and 100 mg/L. Samples (200 µL) were introduced into test tubes; 1.0 mL of folin ciocalteu's reagent (M&P Impex, Thailand) and 0.8 mL of sodium carbonate (M&P Impex, Thailand) (7.5%) were added, mixed, and left to stand for 30 min. Absorption at 765 nm was measured by using UV spectrophotometer (UNICO S1200, NJ, USA). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per grams [12].

2.3 *To study the suitable condition of encapsulation*

Encapsulation of each extract was investigated for the effects of three factors as crude leaf extract (%), BCD (%), and MD (%) by using a randomized block design and a mixture design with 3 replications. Encapsulation efficiency (EE) was analyzed. The percentage of each factor was varied from 5 to 15% for the crude leaf extract, 0 to 50% for β-cyclodextrin, and 35 to 95% for maltodextrin 20DE as shown in Table 1.

Table 1: Total formulas for *T. laurifolia* leaf extract encapsulation from mixture design

Formula	LE (%)	BCD (%)	MD (%)
P1	15	50	35
P2	5	50	45
P3	15	0	85
P4	5	0	95
P5	10	25	65

Note: P1 – P5: Formulation from mixture desi^{g}n; LE –leaf extract; BCD – β-cyclodextrin; MD – maltodextrin.

2.3.1 Microencapsulation

Carrier agents both BCD and MD were mixed with *T. laurifolia* extract. The mixture was then homogenized at 600 rpm for 15 min by using a shaker. After that, the mixture was desiccated under vacuum at a condenser temperature of –40°C for 24 h with a freeze dryer. Dried cakes were grounded into powder and stored in a closed container at –80°C.

2.3.2 Evaluation of microencapsulated T. laurifolia leaf extract

a) Microencapsulation yield (MY)

The microencapsulation yield was calculated using the following formula [Equation (1)]:

$$\frac{Weight\ of\ encapsulated\ powder}{Total\ weight\ of\ all\ ingredients} \times 100 \quad (1)$$

b) Encapsulation efficiency (EE)

The microencapsulation efficiency was calculated using the following formula [Equation (2)]:

$$\frac{Total\ antioxidant\ in\ powder}{Total\ antioxidant\ in\ extract} \times 100 \quad (2)$$

c) Moisture content (MC)

One gram of *T. luarifolia* leaf extract powder was placed in an oven at 103ºC for 8 h. The sample was cooled down in a desiccator and weighed. Moisture content was calculated using the following formula [Equation (3)]:

$$\frac{Weight\ of\ sample - Weight\ of\ dried\ sample}{Weight\ of\ sample} \times 100 \quad (3)$$

2.4 *To study the effect of storage temperature on the antioxidant properties of encapsulated T. laurifolia crude extract powder*

A 3 factorial design and a randomized block design with 3 replications were used in this section. Encapsulated *T. laurifolia* leaf extract powder (2 g) in aluminium foil was stored at 35, 45, and 55°C. The degradation of antioxidant activity was investigated for 5 weeks and the sampling was conducted weekly.

2.5 *Data analysis*

All experiments were conducted in three replications

and statistical analysis was done using ANOVA with Duncan's multiple range test ($p < 0.05$) by SAS software version 9.4.

3 Results and Discussion

3.1 *Extraction condition*

RC powder (2.5, 5, and 7.5 g) of leaf, stem, and rhizome was macerated with ethanol (0, 25, 50, and 75%) for 24, 48, and 72 h with shaking condition at 120 rpm at room temperature. The antioxidant properties of crude extract as FRAP and TPC were shown in Tables 2–4 for leaf, stem, and rhizome, respectively.

As seen from Table 2, it was observed that on average, the TPC content increased with increase in weight. Using 7.5 g powder provided the highest 877.36 ± 18.75 mg GAE/g followed by 5 g (740.72 ± 10.78 mg GAE/g) and 2.5g (596.85 ± 10.57 mg GAE/g), respectively. This was the same trend with FRAP values in which the highest (2.62 ± 0.01 mmol Fe^{2+}/g) was observed when 7.5 g was used for extraction of 72 h using 0% ethanol. Equally, the FRAP value increased with an increase in weight of powder. When 2.5 g was used, the highest FRAP (1.05 ± 0.03 mmol Fe^{2+}/g) was obtained, while for 5 g, the FRAP was 1.75 ± 0.09 mmol Fe^{2+}/g.

The concentration of the extracting solvent also had an impact on the antioxidant activity. It was observed that the highest antioxidant activity was obtained from 0% ethanol, which confirms previous studies like what was reported for the leaf extract from [13].

Table 2: Antioxidant activity of *T. laurifolia* leaf crude extract using different conditions

Weight of Sample (g)	Ethanol (%)	Time (h)	FRAP (mmol Fe^{2+}/g)	TPC (mg GAE/g)
2.5	0	24	0.15 ± 0.01 ^{gf*}	425.54 ± 15.66 ^{fed}
		48	0.10 ± 0.01 ^{gf}	557.42 ± 16.24 ^{edc}
		72	1.05 ± 0.03 ^{c}	596.85 ± 10.57 ^{bedc}
	25	24	0.04 ± 0.01 ^{g}	264.69 ± 17.04 ^{fihg}
		48	0.06 ± 0.01 ^{g}	262.59 ± 15.26 ^{fihg}
		72	0.05 ± 0.01 ^{g}	157.65 ± 12.19 ^{ih}
	50	24	0.03 ± 0.01 ^{g}	178.85 ± 14.22 ^{ihg}
		48	0.06 ± 0.01 ^{g}	151.33 ± 11.52 ^{ih}
		72	0.05 ± 0.01 ^{g}	286.52 ± 12.45 ^{fihg}
	75	24	0.05 ± 0.01 ^{g}	225.72 ± 11.34 ^{fihg}
		48	0.09 ± 0.01 ^{gf}	254.01 ± 8.55 ^{fihg}
		72	0.06 ± 0.01 ^{g}	435.38 ± 15.21 ^{fed}
5	0	24	0.16 ± 0.01 ^{gf}	405.24 ± 11.85 ^{fedg}
		48	0.13 ± 0.01 ^{gf}	740.72 ± 10.78 ^{bac}
		72	1.75 ± 0.09 ^{b}	614.59 ± 11.62 ^{bdc}
	25	24	0.08 ± 0.01 ^{gf}	241.29 ± 12.53 ^{fihg}
		48	0.12 ± 0.02 ^{gf}	247.26 ± 10.14 ^{fihg}
		72	0.09 ± 0.01 ^{gf}	291.23 ± 18.72 ^{fihg}
	50	24	0.08 ± 0.01 ^{gf}	200.78 ± 12.70 ^{fihg}
		48	0.13 ± 0.02^{gf}	111.89 ± 17.10 ^{i}
		72	0.07 ± 0.01 ^{g}	277.38 ± 17.70 ^{fihg}
	75	24	0.10 ± 0.01 ^{gf}	294.75 ± 19.19 ^{fihg}
		48	0.22 ± 0.02 ^{egf}	310.83 ± 18.94 ^{fihg}
		72	0.14 ± 0.01 ^{gf}	581.29 ± 19.37 ^{edc}
7.5	0	24	0.26 ± 0.02 ^{edf}	567.86 ± 13.61 ^{edc}
		48	0.37 ± 0.01 ^{ed}	719.54 ± 12.42 ^{bac}
		72	2.62 ± 0.01 ^{a}	877.36 ± 18.75 ^{a}
	25	24	0.12 ± 0.02 ^{gf}	212.10 ± 18.52 ^{fihg}
		48	0.18 ± 0.01 ^{gf}	186.44 ± 17.77 ^{ihg}
		72	0.11 ± 0.01 ^{gf}	241.71 ± 18.81 ^{fihg}
	50	24	0.13 ± 0.02 ^{gf}	199.46 ± 14.73 ^{fihg}
		48	0.20 ± 0.02 ^{egf}	235.41 ± 14.44 ^{fihg}
		72	0.16 ± 0.03 ^{gf}	373.76 ± 16.53 ^{fehg}
	75	24	0.17 ± 0.01 ^{gf}	290.16 ± 12.34 ^{fihg}
		48	0.41 ± 0.03 ^{d}	309.65 ± 12.67 ^{fihg}
		72	0.20 ± 0.02 ^{egf}	810.89 ± 10.34 ^{ba}

* Different letters represent a significant difference ($p < 0.05$)

Table 3: Antioxidant activity of *T. laurifolia* stem crude extract using different conditions

Weight of Sample (g)	Ethanol (%)	Time (h)	FRAP (mmol Fe^{2+}/g)	TPC (mg GAE/g)
2.5	0	24	0.05 ± 0.01 kji*	182.08 ± 10.72 ghefij
		48	0.05 ± 0.01 kji	128.93 ± 12.05 hlmkij
		72	0.06 ± 0.01 kji	126.73 ± 19.85 hlmkij
	25	24	0.04 ± 0.01 kj	176.23 ± 13.58 ghefkij
		48	0.04 ± 0.02 kj	114.30 ± 10.32 hnlmkij
		72	0.05 ± 0.01 kji	222.79 ± 16.75 gcefd
	50	24	0.04 ± 0.01 kj	100.23 ± 8.33 onlmkij
		48	0.04 ± 0.01 kj	137.03 ± 15.57 ghlmkij
		72	0.05 ± 0.01 kji	159.63 ± 15.28 ghlfkij
	75	24	0.04 ± 0.01 kj	76.87 ± 14.28 onlm
		48	0.03 ± 0.01 k	19.60 ± 4.03 o
		72	0.03 ± 0.01 k	86.78 ± 11.90 onlmk
5	0	24	0.16 ± 0.01 edf	192.61 ± 18.77 ghefi
		48	0.22 ± 0.02 cb	230.13 ± 11.10 cefd
		72	0.17 ± 0.02 ed	296.15 ± 13.76 cb
	25	24	0.08 ± 0.02 khjgi	158.22 ± 18.14 ghlfkij
		48	0.12 ± 0.01 egf	164.72 ± 16.13 ghelfkij
		72	0.11 ± 0.01 hg	322.79 ± 10.42 b
	50	24	0.06 ± 0.01 khji	45.82 ± 5.43 onm
		48	0.08 ± 0.02 khjgi	139.25 ± 6.34 ghlkij
		72	0.09 ± 0.01 hjgi	134.25 ± 7.22 ghlmkij
	75	24	0.06 ± 0.01 khji	87.13 ± 1.40 onlmk
		48	0.07 ± 0.01 khji	19.60 ± 2.03 o
		72	0.08 ± 0.02 khjgi	109.66 ± 14.40 onlmkij
7.5	0	24	0.19 ± 0.02 ed	175.87 ± 13.62 ghefkij
		48	0.25 ± 0.01 b	406.61 ± 14.73 a
		72	0.35 ± 0.02 a	252.88 ± 11.09 cebd
	25	24	0.11 ± 0.01 hgf	135.05 ± 11.96 ghlmkij
		48	0.16 ± 0.01 ed	202.58 ± 13.12 ghefd
		72	0.20 ± 0.01 cd	283.55 ± 10.68 cbd
	50	24	0.11 ± 0.01 hg	91.35 ± 8.37 onlmkj
		48	0.12 ± 0.01 egf	134.30 ± 15.08 ghlmkij
		72	0.12 ± 0.02 egf	138.76 ± 12.64 ghlkij
	75	24	0.10 ± 0.01 hgi	103.49 ± 8.51 onlmkij
		48	0.10 ± 0.02 hgi	24.54 ± 1.05 on
		72	0.05 ± 0.01 kji	105.16 ± 8.50 onlmkij

* Different letters represent a significant difference ($p < 0.05$)

However, 75% showed some antioxidant activity, suggesting that it could be used for extraction. Overall, the highest TPC (877.36 ± 18.75 mg GAE/g) and FRAP (2.62 ± 0.01 mmol Fe^{2+}/g) was obtained from 7.5 g of leaf powder, using 0% ethanol as the extraction solvent for 72 h.

From Table 3, the FRAP value increased with an increase in the weight. The highest FRAP value (0.35 ± 0.02 mmol Fe^{2+}/g) was obtained from 7.5 g of sample. On average, the highest FRAP value was obtained from 0% ethanol and kept reducing with an increase in the ethanol concentration. The ideal extraction time was 72 h. The longer the extraction time, the higher the FRAP content. However, when considering TPC content, the trend was the same as for FRAP in terms of weight of powder with 7.5 g producing the highest TPC content (406.61 ± 14.73 mg GAE/g), followed by 5 g (322.79 ± 10.42 mg GAE/g). The extraction concentration of 0% and 25% ethanol was more ideal for extraction, whereas 48 h produced the highest TPC. When using 7.5 g, longer time of 72 h was required to produce an amount equivalent to that when using 5 g and 2.5 g, respectively. These results were similar to [14] where the antioxidant activity was well correlated with the increase in concentration of aqueous stem bark extract of *Schotia latifolia* Jacq.

Overall, the highest antioxidant activity TPC (406.61±14.73 mg GAE/g) and FRAP (0.35 ± 0.02 mmol Fe^{2+}/g) was obtained from 7.5 g of stem powder extracted by using 0% ethanol for 48 h and 72 h, respectively.

From Table 4, it was noticed that the FRAP content increased with an increase in weight of the powder and concentration of ethanol, in which the highest FRAP content 1.04 ± 0.03 mmol Fe^{2+}/g was obtained from 7.5 g of sample, using 75% ethanol and extraction time of 48 h. It was observed that 48 h extraction time was the most ideal for rhizome extraction followed by 24 h. When observing the TPC content, the higher the weight of the powder, the more TPC content. The highest TPC was 1112.9 ± 18.93 mg GAE/g obtained by using 7.5 g of powder and extraction time of 72 h with 75% ethanol followed by 887.54 ± 19.91 mg GAE/g at the same amount of powder and 48 h of extraction with 75% ethanol. Like FRAP content, the higher the concentration of ethanol, the higher the TPC content. Extraction of the rhizome needed longer time to achieve reasonably higher TPC. These results are like those reported by [15] where the highest antioxidant activity of rhizome powder from *coleus forskohlii* briq was observed from the ethanol extract.

Overall, the highest antioxidant activity TPC (1112.93 ± 18.93) mg GAE/g and FRAP (1.04 ± 0.03 mmol Fe^{2+}/g) were obtained from 7.5 g of dry rhizome powder extracted by using 75% ethanol for 72 and 48 h, respectively.

Table 4: Antioxidant activity of *T. laurifolia* rhizome crude extract using different conditions

Weight of Sample (g)	Ethanol (%)	Time (h)	FRAP (mmol Fe^{2+}/g)	TPC (mg GAE/g)
2.5	0	24	0.03 ± 0.01 ^{mn*}	27.85 ± 3.23 ^{khji}
		48	0.04 ± 0.01 ^{mn}	4.13 ± 0.15 ^{kj}
		72	0.02 ± 0.01 ^{n}	20.26 ± 1.19 ^{k}
	25	24	0.02 ± 0.01 ^{n}	1.65 ± 0.30 ^{kj}
		48	0.04 ± 0.01 ^{mn}	16.35 ± 2.25 ^{kji}
		72	0.02 ± 0.01 ^{n}	30.88 ± 7.38 ^{khji}
	50	24	0.05 ± 0.01 ^{mnl}	40.78 ± 2.29 ^{khji}
		48	0.05 ± 0.01 ^{kmnl}	53.33 ± 2.32 ^{khjgi}
		72	0.03 ± 0.01 ^{mn}	57.49 ± 3.36 ^{khjgi}
	75	24	0.16 ± 0.02 ^{figh}	278.31 ± 11.84 ^{f}
		48	0.20 ± 0.01 ^{fe}	440.69 ± 5.94 ^{ed}
		72	0.03 ± 0.01 ^{mn}	386.43 ± 18.65 ^{e}
5	0	24	0.06 ± 0.01 ^{kmnl}	1.42 ± 0.01 ^{kj}
		48	0.11 ± 0.01 ^{jkihl}	29.53 ± 1.94 ^{khji}
		72	0.08 ± 0.01 ^{jkmnl}	16.94 ± 1.38 ^{k}
	25	24	0.04 ± 0.01 ^{mn}	7.15 ± 2.79 ^{kj}
		48	0.14 ± 0.01 ^{jfigh}	39.97 ± 1.78 ^{khji}
		72	0.05 ± 0.01 ^{kmnl}	21.78 ± 1.45 ^{khji}
	50	24	0.12 ± 0.01 ^{jkigh}	73.90 ± 9.23 ^{khjgi}
		48	0.17 ± 0.01 ^{fgh}	94.89 ± 10.05 ^{hjgi}
		72	0.11 ± 0.01 ^{jkighl}	120.53 ± 14.46 ^{hgi}
	75	24	0.39 ± 0.01 ^{d}	512.71 ± 16.29 ^{d}
		48	0.61 ± 0.02 ^{c}	740.00 ± 18.38 ^{c}
		72	0.09 ± 0.01 ^{jkiml}	780.09 ± 13.29 ^{c}
7.5	0	24	0.18 ± 0.01 ^{fg}	3.36 ± 0.58 ^{kj}
		48	0.15 ± 0.01 ^{figh}	60.42 ± 7.94 ^{khjgi}
		72	0.15 ± 0.01 ^{figh}	17.46 ± 2.91 ^{k}
	25	24	0.09 ± 0.01 ^{jkimnl}	4.02 ± 0.14 ^{kj}
		48	0.34 ± 0.01 ^{d}	64.79 ± 2.22 ^{khjgi}
		72	0.10 ± 0.02 ^{jkiml}	33.77 ± 3.06 ^{khji}
	50	24	0.25 ± 0.01 ^{e}	107.20 ± 7.58 ^{hjgi}
		48	0.34 ± 0.02 ^{d}	125.92 ± 6.60 ^{hg}
		72	0.21 ± 0.01 ^{fe}	156.32 ± 17.49 ^{g}
	75	24	0.85 ± 0.01 ^{b}	699.46 ± 18.33 ^{c}
		48	1.04 ± 0.03 ^{a}	887.54 ± 19.91 ^{b}
		72	0.25 ± 0.02 ^{c}	1112.93 ± 18.93 ^{a}

* Different letters represent a significant difference ($p < 0.05$)

Table 5: Antioxidant properties of the crude extract from the three parts of *T. laurolia*

Part	Weight (g)	Ethanol (%)	Time (h)	FRAP (mmol Fe^{2+}/g)	TPC (mg GAE/g)
Leaf	7.5	0	72	2.62 ± 0.01^{a}	877.36 ± 18.75^{a}
Stem	7.5	0	48	0.25 ± 0.01^{c}	406.61 ± 14.73^{b}
			72	0.35 ± 0.02^{c}	252.88 ± 11.09^{b}
Rhizome	7.5	75	48	1.04 ± 0.03^{b}	887.54 ± 19.91^{a}
			72	0.25 ± 0.02^{c}	1112.93 ± 18.93^{a}

* Different letters represent significant difference ($p < 0.05$)

Table 5 demonstrates the suitable extraction conditions of each part of RC plant. It was observed that 7.5 g dry powder showed the highest antioxidant activity for all parts of the plant. The use of 0% ethanol produced the highest antioxidant activity of the crude extracts when leaf and stem powders were used, whereas the rhizome required 75% concentration for its extraction. However, the extraction time varies for each RC part. The leaf needed 72 h to obtain the highest antioxidant activity for both FRAP and TPC, whereas for the stem, it needed 48 h to obtain the highest TPC content of 406.61 ± 14.73 mg GAE/g and 72 h to obtain the highest FRAP content of 0.35 ± 0.02 mmol Fe^{2+}/g. This was quite opposite for the rhizome, 48 h produced the highest FRAP content of 1.04 ± 0.03 mmol Fe^{2+}/g, whereas the highest TPC content 1112.93±18.93 mg GAE/g was obtained from 72 h.

Overall, the best condition of extraction from the three parts of RC was that of the leaf with a FRAP content of 2.62 ± 0.01 mmol Fe^{2+}/g that was significantly different from that of the stem and rhizome at ($p > 0.05$) and TPC content of (877.36 ± 18.75 mg GAE/g). This same extraction condition was then used for encapsulation.

An antioxidant is a substance in foods that when present at low concentrations compared to those of an oxidizable substrate, can significantly decrease or prevent adverse effects of reactive species on normal physiological functions in humans [16], [17]. Antioxidant capacity is associated with those compounds that can protect a biological system against potentially harmful effects of processes or reactions of reactive species.

Antioxidant actions take up mechanisms like acting as physical barriers to prevent the generation or access of reactive species to important biological sites e.g., cell membranes, they can be in form of chemical traps that absorb energy and electrons from reactive species such as anthocyanidins and carotenoids, they can be catalytic systems that neutralize or divert reactive oxygen species such as superoxide dismutase [16] they can bind or inactivate metal ions to prevent generation of reactive species for example ferritin and they can act as chain breakers which destroy and scavenge reactive oxygen species such as flavonoids, uric acid, and ascorbic acid [18].Prevention of oxidative damage is achieved by primary antioxidants directly scavenging free radicals. The need for simple, reliable, and convenient antioxidant capacity determination methods is on the rise due to the protective effects exhibited by antioxidants within medical, nutritional, biological, and agrochemical fields.

The antioxidant capacity determination methods used in this project were FRAP and TPC. The total phenol assay by folin ciocalteu reagent is convenient, simple, and reproducible despite its undefined chemical nature [19]. The FCR-based assay is widely known and used as the total phenol assay. Many plants during their normal development or as a response to environmental stress conditions normally synthesize phenolic compounds in the form of secondary metabolites [20]. Phenolic compounds have important functional properties which have made them to be of great interest for food, pharmaceutical, and chemical industries. Phenolic compounds in RC have been mainly identified as chlorogenic acid. Diverse health benefits have been reported as a result of ingestion of phenolic compounds present in RC [21] mainly those from chlorogenic acid including antioxidant activity, antiobesity [22], anti-inflammatory [23], anti-diabetic [24], and anticancerous effects [25].

FRAP assay can reduce yellow ferric tripyridyltriazine complex (Fe (III)-TPTZ) to blue ferrous complex (Fe (II)-TPTZ) by the action of electron-donating antioxidants of phenolics [26]. The resulting blue color is then measured by the spectrophotometer at 593 nm and is taken to be linearly correlated to the total reducing capacity of electron-donating antioxidants. A decrease of 1 mol of Fe (III) to Fe (II) is interpreted

as 1 FRAP unit. The power of RC extracts to reduce the TPTZ Fe (III) complex to TPTZ Fe (II) complex (FRAP assay) was used to estimate its antioxidant potential. This method is simple, reproducible, robust, rapid, and inexpensive. FRAP can be readily applied to both aqueous and alcohol extracts of different plants because of its versatile nature. When measuring FRAP, the results are expressed as mol ferrous equivalents per gram of the sample.

Any substance with reducing ability in a reaction medium can be measured by FRAP assay. In this project, the antioxidant activity of RC was determined based on its ability to reduce ferric to ferrous. Moreover, there is a relationship between antioxidant activities and total phenolic contents in the RC extracts. It may be concluded that polyphenol compounds contribute to the antioxidant activity in RC extracts. The method used for extraction plays a critical role to the recovery of antioxidant phytochemicals. To achieve good extraction efficiency, the nature of both plant materials and the bioactive components should be considered. Lipophilicity or hydrophilicity affects solubility of a phytochemical in extracting solvent and, conversely, polarity of a solvent also has an impact on the extraction efficiency. There are many different extraction methods for antioxidant phytochemicals, most of them rely on solvent extraction using water, organic solvent or liquefied gas, or combinations of these at different temperature and pressure [27]. Water, alcohol, or a mixture of both has been mostly used in the extraction of polar antioxidants such as glycosides and phenolic acids.

In this project, water and the mixture of water and ethanol were used as 0, 25, 50, and 75% of ethanol for crude extraction of RC. It was found that the water extract showed the highest TPC content and antioxidant activity for both leaf and stem crude extracts, which was like the result reported for the leaf extract from [13]. The water extract showed the highest antioxidant activity. This might be because the main constituents are phenolic acids, flavonoids glucosides and flavonoids. Previous researcher reported that the source of antioxidant activity in plants were phenolic acids and flavonoids [28]. The RC leaf and stem samples that were extracted using ethanol, showed less antioxidant activity. They may have been composed of chlorophylls, chlorophyll derivatives and lutein. It could be explained that chlorophylls and chlorophyll derivatives exhibit low antioxidant activity. The efficiency of water as an extraction solvent produces higher antioxidant activity compared to that of ethanol extracts and this could explain why Rang Cheut is commonly taken as Tea.

Numerous polyphenols have been identified in food with the two main types being flavonoids and phenolics. Polyphenols can reduce the risk of various diseases associated with oxidative stress by protecting cell constituents against oxidative damage. The phenolic groups in polyphenols form relatively stable phenoxyl radicals by accepting an electron, hence prevent chain oxidation reactions in cellular components [29]. Flavonoids have numerous properties which include free radical scavengers, potent antioxidants, metal chelators and lipid peroxidation inhibitors. The hydroxyl carbon in position three, double bonds between position two and three, a carbonyl group in carbon position four and polyhydroxlation of the A and B aromatic rings gives flavonoids their antioxidant and free radical scavenging function [28]. The radical scavenging activities of flavonoids are highly determined by the number and configuration of phenolic hydroxyl groups in the molecules and influenced by glycosylation and configuration of other substituents.

From this result, it could be concluded that RC extracts can be a natural source for antioxidants in food, although the amount required to produce antioxidant activity similar to standard use of antioxidants could be large and this can be reflected in this project as well because the highest antioxidant activity was obtained from the largest amount which was 7.5 g of the powder.

3.2 *Optimization of encapsulation*

β-cylcodextrin and maltodextrin 20DE were used as wall material for encapsulation and freeze drying was used as the encapsulation technique. The percentage of each factor was varied from 5 to 15% for the extract, 0 to 50% for β cylcodextrins, and 35 to 95% for maltodextrins. Microencapsulation yield (MY), moisture content (MC) were investigated. Encapsulation efficiency (EE) was measured by using TPC and FRAP as the criteria.

From the result in Table 6, there was no significant difference in MY among all samples ($p < 0.05$). The encapsulated powder from P2 had the highest MC 6.81%, whereas that from P1 and P3, P4 and P5 were not significantly different ($p < 0.05$).

Table 6: Encapsulation of *T. laurifolia* crude leaf extract at different conditions

Formula	LE (%)	BCD (%)	MD (%)	MY (%)	MC (%)	EETPC (%)	EEFRAP (%)
P1	15	50	35	$42.76 \pm 2.44^{a*}$	5.81 ± 0.72^{ab}	35.29 ± 2.68^{c}	16.16 ± 0.81^{a}
P2	5	50	45	39.80 ± 3.41^{a}	6.81 ± 0.50^{a}	41.54 ± 3.77^{bc}	14.28 ± 0.76^{bc}
P3	15	0	85	41.90 ± 2.25^{a}	6.29 ± 0.56^{ab}	60.57 ± 2.04^{a}	15.60 ± 1.32^{ab}
P4	5	0	95	41.15 ± 2.35^{a}	4.99 ± 0.83^{b}	54.40 ± 2.15^{ab}	13.77 ± 0.53^{c}
P5	10	25	65	41.03 ± 1.17^{a}	4.74 ± 0.55^{b}	53.12 ± 3.18^{ab}	15.01 ± 0.92^{abc}

Note: P1–P5: Formulation from mixture design; LE –leaf extract; BCD – βcyclodextrin; MD – maltodextrin; MY – microencapsulation yield; EE – encapsulation efficiency. *Different letters represent significant difference ($p < 0.05$)

When TPC content was used as criteria for EE, P3 provided the highest EETPC of 60.57%. That of P4 and P5 were not significantly different ($p < 0.05$) and P1 had the lowest EETPC of 35.29 ± 2.68%. When comparing FRAP of all the powders, P1 had the highest followed by P3, P5, P2, and P4. Phenolic compounds are very susceptible to oxidation when exposed to light, oxygen, and moisture due to the existence of unsaturated bonds in the molecular structures. So, there is need for them to be encapsulated to enhance their storage stability, make them safer as food ingredients to provide more benefits to the consumers [30], [31]. Moreover, P3 and P5 were not significantly different ($p < 0.05$) and due to lower cost of MD compared to BCD, P3 condition was chosen for the next experiment. This condition was 15% leaf extract and 85% maltodextrin.

In addition, the results revealed that the coating material used for encapsulation had an important role in the retention of antioxidant phenolic compounds within the matrix. The best results were achieved when using 100% maltodextrin as wall material. Under this condition, the amounts of phenolic compounds and flavonoids retained in the encapsulated sample corresponded to 60.57 ± 2.04% and 15.60 ± 1.32%, respectively.

These results were parallel with those reported by [32], where the highest content of phenolic compounds was attained when the compounds were subjected to freeze-drying using 100% maltodextrin as the wall material. This behavior may be because encapsulation efficiency highly relies on the nature of encapsulated compounds and the coating material used [33]. The antioxidant activity was expected to be lower when compared to the initial antioxidant activity of the *T. laurifolia* extract, due to the lower amounts of phenolic compounds and flavonoids present in the encapsulated sample.

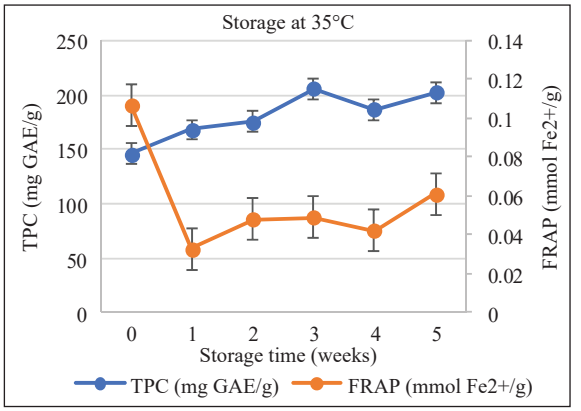
3.3 *Effect of storage temperature on the antioxidant properties of encapsulated T. laurifolia crude extract powder*

Encapsulated *T. laurifolia* leaf powder from the crude extract of the leaf was kept in aluminium foil and stored at 35, 45, and 55°C for 5 weeks. The antioxidant quality was analyzed weekly by measuring FRAP and TPC as shown in Figure 1.

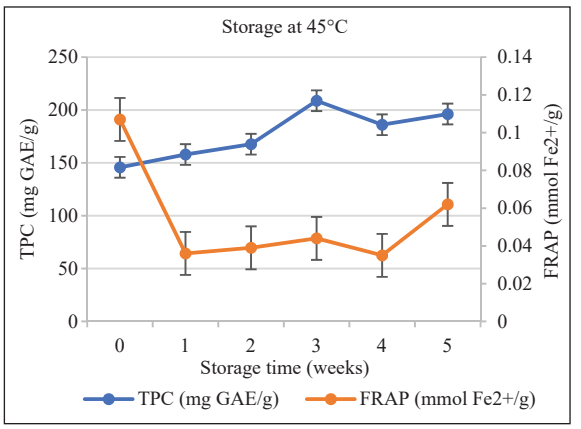
From Figure 1, it was noticed that the changes in the FRAP levels during storage at all 3 temperatures had similar patterns. The FRAP value decreased rapidly in the first week and then slightly increased at the end of the storage. These fluctuations may be explained by some phenolic compounds being dissociated and some being formed during storage while their antioxidant activities are probably still maintained [34].

The FRAP value was in the range of 0.058 to 0.062 mmol Fe^{2+}/g. On the other hand, the changes in TPC values during storage at 35, 45, and 55°C had a similar pattern throughout all the temperatures. The level of TPC increased until it reached the highest in the 3rd week for 35 and 45°C, which was in the range of 205.93 to 208.77 mg GAE/g, whereas that at 55°C, it increased gradually to a peak of 193.38 mg GAE/g in the 5th week.

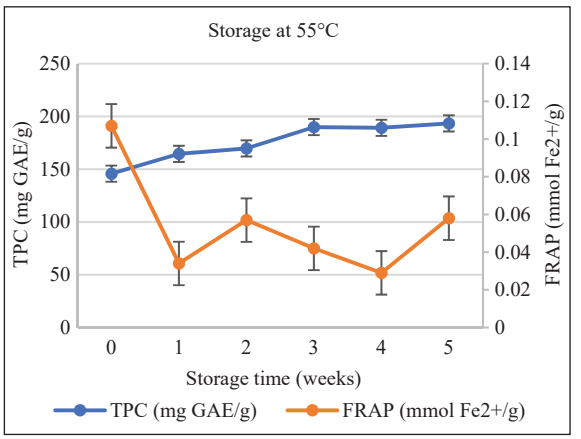
From Figure 2, it was observed that the storage temperature did not have statistically significant effect on the stability of the TPC in the encapsulated powder. This implied that TPC was well protected against temperature degradation. Moreover, the level of TPC noticeably increased during storage which may be a result of polyphenols formation during storage. The current findings are agreed with those reported by [34], [35] who found recoveries and formation of polyphenols because of the hydrolysis of cactus pear and blackcurrant. To our knowledge, there is



(a)



(b)



(c)

Figure 1: Antioxidant properties of encapsulated RC leaf powder stored at different temperatures (a) 35°C, (b) 45°C and (c) 55°C.

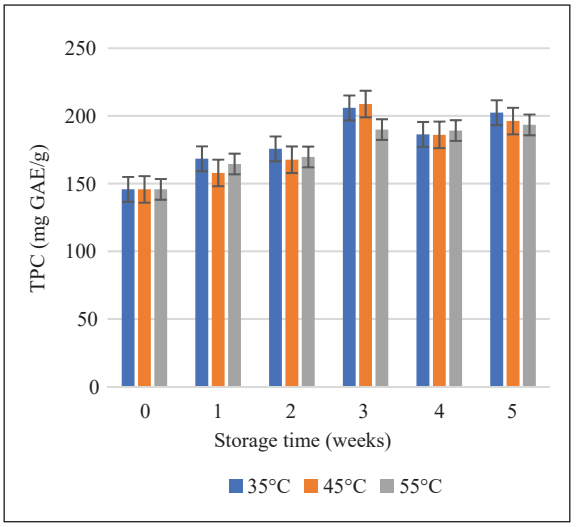


Figure 2: The stability of TPC in the encapsulated RC powder stored at different temperatures.

no previous report on the stability of extracts from *T. laurifolia*; thus, this information could provide the basis for further applications in different industries, such as those of the food and pharmaceutical sectors. Finally, *T. laurifolia* could comprise a good alternative of use as natural antioxidant in foods.

5 Conclusions

From this study, RC leaf crude extract was obtained by using water extraction for 72 h. The crude extract contained FRAP and TPC content as 2.62 ± 0.01 mmol Fe^{2+}/g and 877.36 ± 18.75 mg GAE/g, respectively. It was successfully encapsulated using 15% crude extract, 85% maltodextrin 20DE, freeze-drying technique, and showed high encapsulation efficiency values as well as satisfactory antioxidant storage stability. The highest EETPC and EEFRAP were 60.57 ± 2.04% and 15.60 ± 1.32%, respectively. Further studies should be focused on encapsulation technology and storage stability of *T. laurifolia* aiming at its future application in food products.

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