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Phytochemical Compositions, Microencapsulation, and Antioxidant Activity of Aqueous Extract from *Tithonia diversifolia* Leaves

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Abstract

Tithonia diversifolia is a flowering plant from the Asteraceae family widely used in traditional medicine in Indonesia. This study aimed to evaluate the phytochemical composition of aqueous extract from *T. diversifolia* leaves and enhance the stability of its bioactive compounds through microencapsulation technology using chitosan as wall materials. Microencapsulation is a technique used to coat core materials with micro-sized protective layers to enhance stability, control release, protect from environmental factors, and improve handling properties. Based on LC-MS/MS analysis, aqueous extract from *T. diversifolia* leaves contains phytochemicals such as gilluone B, tagitinine A, thyrotundin, tadeonal, and stigmasterol glucoside. The microencapsulation of aqueous extract from *T. diversifolia* leaves plant resulted in encapsulation efficiency of 76.03 % with the optimum state of manufacture at a chitosan concentration of 0.05 % (w/v) and a stirring time of 90 min. The antioxidant activity of the aqueous extract from *T. diversifolia* leaves (70.43 µg/mL) and microcapsules (139.24 µg/mL). The bioactive compound releases were tested at pH 2.2 and 7.4 over 30 to 120 min. The findings showed a release of 53.36 % at pH 2.2 and 80.32 % at pH 7.4 at 120 min. After the release assay, the FTIR characterization showed changes in the structure of the functional groups, such as O-H, C-H alkanes, aromatic C=C, and N-H amines. The SEM shows differences in the surface morphology of the microcapsules before and after the release test. The microcapsules were initially spherical, but after the release test, their surface became uneven. This study confirms that microencapsulation can protect and release active ingredients in a controlled manner according to pH and release time.

Keywords: *Tithonia diversifolia*, Phytochemical, Chitosan, Microencapsulation, Antioxidant, Release

Introduction

Tithonia diversifolia (locally known in Indonesia by various names such as daun insulin, paitan, rondosemoyo, kembang bulan, kayu paik, kipait and harsaga) is a flowering plant from the *Asteraceae* family that is known to have a wide range of health benefits [1] and is traditionally used as a herbal remedy in various tropical countries, including Indonesia [2]. This plant contains bioactive compounds such as tannins, saponins, flavonoids, and terpenoids [3], which demonstrate antibacterial, anti-proliferation, anti-inflammatory, analgesic, antioxidant, and antidiabetic activities [4]. However, a detailed identification of these bioactive compounds is crucial to confirm their pharmacological potential. LC-MS/MS is a very sensitive analysis

method to identify phytochemical profiles in plant extracts [5]. In this study, LC-MS/MS was used to identify specific compounds in aqueous extract from *T. diversifolia* leaves.

Bioactive compounds in plant extracts are known to be susceptible to degradation in sub-ideal environmental conditions. This challenge directly affects the effectiveness of lant extracts in their utilization as medicinal plants [6,7]. To overcome this issue, stabilization techniques like nano-emulsions and encapsulation help extend shelf life and maintain compound efficacy. Microencapsulation is a technique that functions to protect core compounds with a coating layer in the form of small particles [8]. Microencapsulation with drying techniques such as spray drying is fast, simple, and feasible to scale [9].

The selection of polymers as coating materials for microcapsules greatly affects the release characteristic, stability, and toxicity of the resulting microcapsules [10]. Chitosan is a polysaccharide obtained from crab and shrimp shell waste. Chitosan is widely used in the microencapsulation process because of its biodegradability, non-toxicity, and high biocompatibility [11]. The structure of chitosan is formed from 2 types of units, namely the acetylated unit (β(1-4)-N-acetyl-D-glucosamine) and the deacetylated unit (D-glucosamine). The process of chitosan deacetylation takes place in 2 stages, using sodium hydroxide and water as auxiliary ingredients [12].

The microcapsules that have been made are further analyzed to determine the ability of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent which is considered more effective, has a simple, fast, and sensitive method. Antioxidant activity is expressed in IC_{50} values that can inhibit 50 % of free radicals from DPPH. A low IC_{50} value indicates higher antioxidant activity [13]. Antioxidants are necessary to combat oxidative stress, therefore, they are able to prevent and repair damage caused by free radicals [14]. The release profile was evaluated at pH 2.2 and 7.4 to simulate physiological conditions in the human body, representing the stomach and intestinal or bloodstream environments, respectively. These pH levels were chosen to assess how effectively the microcapsules protect and release active compounds in conditions mimicking the gastrointestinal tract [15,16].

This study developed a microencapsulation method for a aqueous extract from *T. diversifolia* leaves using chitosan with spray drying technique. The biological activity of the optimal microcapsule was carried out by conducting an antioxidant activity test. *In vitro* testing aims to evaluate the effect of microencapsulation on the release and stability of bioactive materials and their interaction with blood sugar regulatory cells. Characterization of microcapsules using SEM and FTIR was carried out to determine the surface morphology of microcapsules and identify the functional groups of microcapsules.

While numerous studies have investigated the bioactive potential of *T. diversifolia* research on the stability and release mechanism of its active compounds remains limited. Most prior studies have primarily focused on identifying bioactive compounds without further exploring formulations that could enhance their stability and efficacy. Therefore, this study employs the spray drying method to microencapsulation aqueous extract from *T. diversifolia* leaves, aiming to enhance its stability, prolong shelf life, and regulate the release of active compounds. This approach has been scarcely explored in previous research and holds significant potential for expanding the pharmaceutical applications of aqueous extract from *T. diversifolia* leaves.

Materials and methods

Chemicals and instrumentation

The research materials used were purchased from Merck: Chitosan (low molecular weight, 50,000 - 190,000 Da), sodium tripolyphosphate (Na-TPP, technical grade, 85 %), quercetin (95 % HPLC, solid), ascorbic acid (pharmaceutical secondary standard), 2-2-diphenyl-1-picrylhydrazyl (DPPH), aluminum chloride (anhydrous, powder, 99.99 % trace metals basis), and phosphate buffered saline (PBS). The leaves of *Tithonia diversifolia* were collected by UPT Materia Medica in Batu, East Java, Indonesia, and their identity was confirmed through a species determination letter with the voucher number 074/794/102.20-A/2022. The instruments used in this study were Scanning Electron Microscope (SEM with TM 3000 Hitachi), Fourier Transform Infrared Spectrometer (FTIR with type Shimadzu Prestige 21), and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS with Thermo Fisher Scientific).

Extract preparation

The extraction of *T. diversifolia* leaves was performed using the cold extraction method, specifically maceration with aqueous solvents. The leaves powder was weighed to 100 g and 1,000 mL of aqueous solvent was added in a 1:10 ratio. The maceration process was conducted for 24 h. The aqueous extract was filtered using Whatman No. 1 filter paper (11 cm diameter, ± 11 µm pore size) prior to further analysis. Evaporation of the extract was carried out using a rotary evaporator at 80 - 90 °C and 70 rpm until a thick extract of *T. diversifolia* leaves was produced. Finally, the extract was stored at 4 °C for further analysis [17].

Phytochemical screening

A confirmatory qualitative analysis of plant extracts was conducted to determine the major classes of compounds, including flavonoids, saponins, alkaloids, tannins, steroids, and terpenoids, using standardized procedures [18-20].

Test for flavonoids

A total of 0.01 g of aqueous extract from *T. diversifolia* leaves was heated in 10 mL of water. The resulting solution was then filtered. After filtration, 3 drops of HCl and 0.1 g of magnesium ribbon were added. Any changes in color or appearance were then observed.

Test for saponins

A 0.01 g of the aqueous extract from *T. diversifolia* leaves was taken and mixed with hot water. The mixture was then shaken for 10 s, resulting in the formation of stable foam (1 - 10 cm) for 30 min. Afterward, 1 drop of 2N HCl was added and observations were made.

Test for alkaloids

A total of 0.01 g of the aqueous extract from *T. diversifolia* leaves was dissolved in 5 mL of chloroform and then filtered to obtain the filtrate. Next, 1 mL of 2 M HCl was added to the filtrate, and the top layer was mixed with the Mayer-Wagner reagent. The formation of a cream or brown-red precipitate indicated the presence of alkaloids. Any changes were then observed.

Test for tannins

A 0.01 g of the aqueous extract from *T. diversifolia* leaves was placed in a test tube and boiled with 10 mL of distilled water. Then, 1 % ferric chloride ($FeCl_3$) was added and mixed until homogeneous. The appearance of a blue-black coloration indicated the presence of tannins.

Test for steroids

A total of 0.01 g of the aqueous extract from *T. diversifolia* leaves was dissolved in 2 mL of chloroform. Then, 3 mL of sulfuric acid (H_2SO_4) was slowly added until distinct layers formed. The appearance of a red top layer and a greenish bottom layer indicated the presence of steroids. Any changes were then observed.

Test for terpenoids

The presence of terpenoids was identified by the formation of a reddish-brown color. In this test, 0.01 g of the extract was dissolved in methanol, followed by the addition of 1 mL of anhydrous acetic acid. Then, 3 mL of sulfuric acid (H_2SO_4) was added. Any resulting changes were observed.

Microencapsulation procedures

The process began with 0.5 g of aqueous extract from *T. diversifolia* leaves being weighed and then dissolved in 17.5 mL of distilled water. Then, 50 mL of chitosan solution with varying concentrations of 0.05, 0.1 and 0.2 % (w/v) in 2 % (v/v) acetic acid (pH 4) was gradually added while stirring with a magnetic stirrer at 50 rpm for 60 min. Afterward, 200 mL of Na-TPP solution was gradually introduced, and the mixture was stirred for 90 min. The resulting colloidal chitosan microcapsules containing the aqueous extract from *T. diversifolia* leaves and Na-TPP were then dried using a Buchi spray dryer at an inlet temperature of 105 °C, an outlet temperature of 85 °C, and an air pressure of 1 bar to produce microcapsule powder [21].

The microcapsule preparation was repeated under modified conditions, in which 0.5 g of aqueous extract from *T. diversifolia* leaves was dissolved in 17.5 mL of distilled water. This time, 50 mL of chitosan solution, at the optimal concentration determined in the previous preparation, was gradually introduced into the pH 4 acetic acid (2 % (v/v) solution while stirring at 500 rpm for 60 min. The mixture was then combined with 200

mL of Na-TPP solution, which was added incrementally, with stirring times varied at 60, 90 and 120 min. The resulting colloidal chitosan microcapsules and Na-TPP encapsulated leaves extract were dried under the same Buchi spray dryer conditions, producing microcapsule powder [21].

Total flavonoid content determination

The total flavonoid content was determined using a colorimetric method [22,23], with quercetin used as the standard to create a calibration curve for flavonoid quantification. A 0.05 g sample of the extract and microcapsules was weighed and dissolved in 5 mL of methanol. The solution was then incubated at 40 °C for 45 min and centrifuged at 1,000 rpm for 10 min. From the supernatant, 0.6 mL was taken and mixed with 0.6 mL of a 2 % aluminium chloride solution. The mixture was incubated at room temperature for 23 min. The absorbance of the resulting solution was measured at 420 nm, corresponding to the maximum wavelength of quercetin. The total flavonoid content was then calculated using the quercetin standard curve and expressed as mg QE/g (milligrams of quercetin equivalent per gram) [21].

$$\%\ \text{Efficiency Encapsulation} = \frac{\text{Total flavonoid content microcapsules}}{\text{Total flavonoid content extracts}} \times 100\ \%$$

Antioxidant activity assay using DPPH

Antioxidant activity was analysed for 3 samples: Microcapsules, extracts, and ascorbic acid. Multiple concentrations were prepared for each: Microcapsules (120, 140, 160, 180 and 200 µg/mL), extracts (20, 40, 60, 80 and 100 µg/mL), and ascorbic acid (2, 4, 6, 8 and 10 µg/mL). From each concentration, 3 mL of solution was transferred into a dark vial, followed by the addition of 2 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution. A control solution was prepared by mixing 3 mL of ethanol with 2 mL of DPPH in a dark vial. All mixtures were homogenized and incubated for 20 min in the dark to minimize light interference with the DPPH method. The absorbance was measured at 516 nm, the maximum wavelength of DPPH. Antioxidant activity was calculated using the formula [24]:

$$\%\ \text{Antioxidant Activity} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{(\text{Absorbance control})} \times 100\ \%$$

The IC_{50} value, representing the concentration required to inhibit 50 % of free radical activity, was used to determine the best antioxidant activity. IC_{50} values were also calculated for comparator antioxidants to assess the potency of the extract compared to proven standards. IC_{50} was determined using a linear regression equation, with sample concentration on the x-axis and antioxidant activity percentage on the y-axis. By substituting 50 for y, the corresponding x value (IC_{50}) was obtained.

Controlled release assay

The release test for active ingredients was conducted using Simulated Gastric Fluid (SGF) with PBS at pH 2.2 and Simulated Intestinal Fluid (SIF) with PBS at pH 7.4. The pH adjustment was carried out using 0.1 M HCl and 0.1 M NaOH. A total of 0.025 g of microcapsules were weighed and mixed with 5 mL of PBS solution. The mixture was incubated at 37 °C with continuous stirring at 100 rpm, and samples were collected at intervals of 30, 60, 90 and 120 min. After each interval, the mixture was centrifuged for 10 min to separate the supernatant from the precipitate for further analysis. A 1 mL sample of the supernatant was transferred to a test tube, and 1 mL of 2 % $AlCl_3$ solution (w/v) was added. The mixture was shaken until homogeneous and incubated for 20 min at room temperature. Absorbance was measured using a Thermo Scientific UV-Vis spectrophotometer (Genesys 150, Thermo Fisher Scientific, USA) at a wavelength of 420 nm. Sampling was conducted discontinuously, with a separate beaker used for each time interval. The concentration of active ingredients in the SGF and SIF media was determined using a linear equation derived from the quercetin standard curve created earlier [25-27].

$$\%\ \text{Release} = \frac{\text{Total flavonoid content release from microcapsules}}{\text{Total flavonoid content in microcapsules}} \times 100\ \%$$

FTIR, SEM and LC-MS/MS analysis

The characterization of microcapsules was performed using FTIR and SEM. Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu-Type IR Prestige-21) was employed to identify functional groups in the extracts, chitosan, Na-TPP, and microcapsules. Samples were first dried and compressed into KBr pellets before measurement within the wavenumber range of 4,000 - 400 cm^{-1}. Scanning Electron Microscopy (SEM) using a Hitachi TM 3000 was utilized to observe the morphology of the microcapsules, with magnifications ranging from 500× to 15,000×. Additionally, liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Thermo Fisher Scientific) was applied to identify unknown compounds by analyzing their mass-to-charge (m/z) ratios.

Data analysis

All methods were performed in triplicates to ensure the reliability and reproducibility of the results. Statistical analysis was conducted using SPSS software version 26, with data analyzed and presented through descriptive narratives and tables. The microencapsulation results, which involved variations in coating concentration and stirring time, were assessed for normality using the Kolmogorov-Smirnov test, followed by a 1-way analysis of variance (ANOVA) at a 95 % confidence level ($\alpha = 0.05$) to determine statistical significance. These tests were conducted under the assumptions of normally distributed data and homogeneous variance.

Results and discussion

Phytochemical screening of aqueous extract from *T. diversifolia* leaves

The aqueous extract from *T. diversifolia* leaves obtained was subjected to further analysis through phytochemical testing. While modern techniques have advanced the determination of phytochemical content, conventional qualitative tests remain commonly used for initial screening of plant phytochemicals. **Table 1** summarizes the phytochemical test results for aqueous extract from *T. diversifolia* leaves.

Table 1 Phytochemical of Aqueous Extract from *T. diversifolia*.

No	Phytochemical	Plant Parts			
		Leaves	Leaves [28]	Stems [28]	Roots [28]
1.	Flavonoids	+	+	+	+
2.	Saponins	+	+	+	+
3.	Alkaloids	+	+	+	+
4.	Tannins	+	+	+	+
5.	Steroids	+	-	+	-
6.	Terpenoids	-	+	-	-

Phytochemicals are bioactive compounds found in plants that have been shown to possess therapeutic properties. These compounds are considered to have medicinal value and can be used as natural medicines or drugs due to their potential health benefits [29]. Medicinal plants have been traditionally used to treat various diseases due to the presence of bioactive compounds like alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, and terpenes. These compounds offer pharmacological effects such as antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and other therapeutic properties, which contribute to the medicinal value of the plant [30]. Based on the phytochemical tests conducted aqueous extract from *T. diversifolia* leaves., **Table 1** shows the presence of active compounds such as flavonoids, saponins, alkaloids, tannins, and steroids. Flavonoids are known for their potent antioxidant and anticancer properties. Saponins help inhibit inflammation, while alkaloids exhibit analgesic, antispasmodic, and antibacterial

effects. Tannins possess antimicrobial, anti-inflammatory, antifungal, antiparasitic, and antioxidant properties. Steroids are recognized for their antibacterial activity [31,32].

The LC-MS/MS test was employed on the aqueous extract of *T. diversifolia* leaves to identify, quantify, and characterize chemical compounds, particularly secondary metabolites and bioactive compounds. This method is highly beneficial due to its ability to detect even trace amounts of compounds with exceptional sensitivity and selectivity.

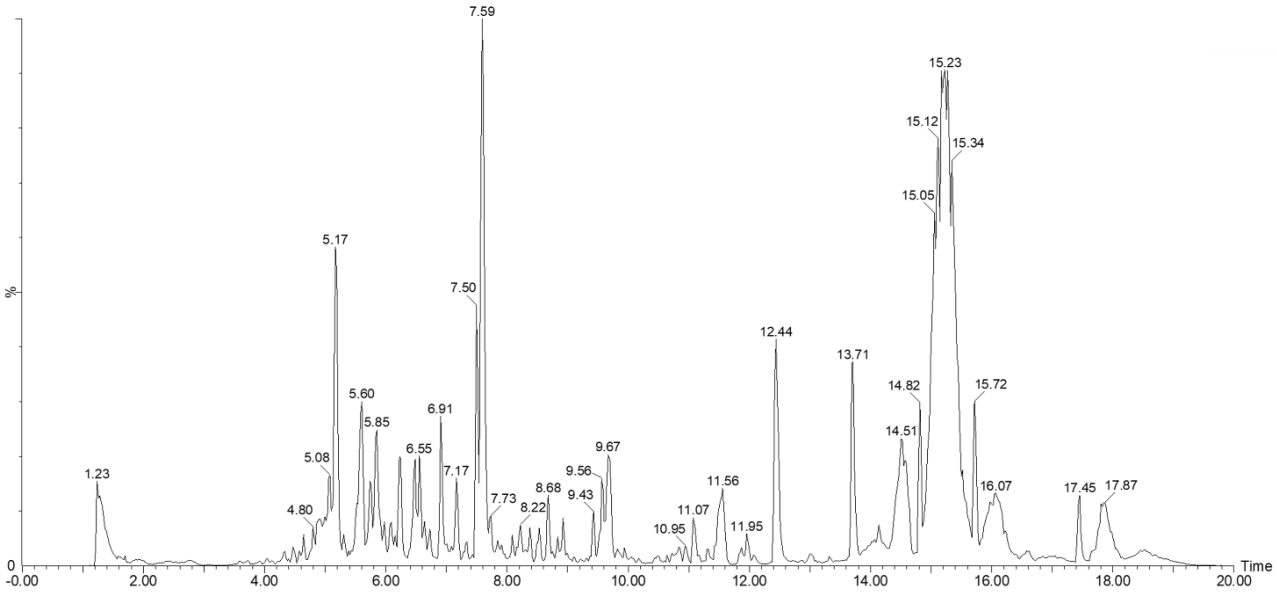
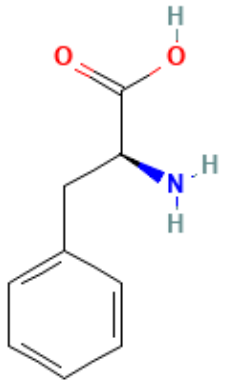


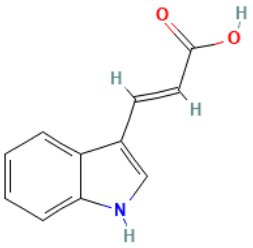
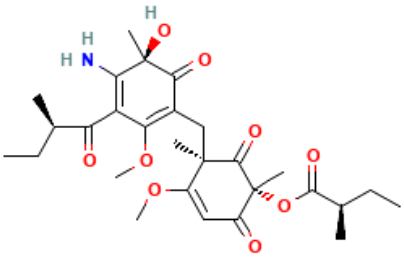
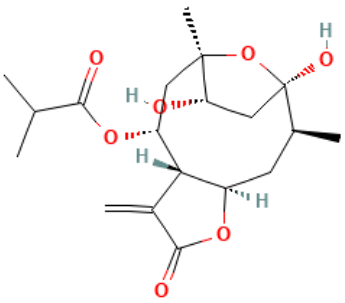
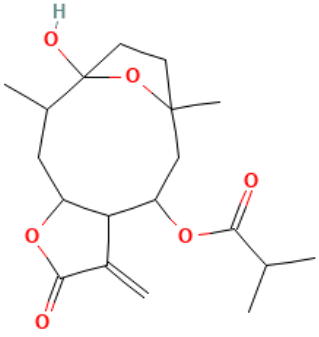
Figure 1 Chromatogram LC-MS/MS of aqueous extract from *T. diversifolia* leaves.

Table 2 LC-MS/MS interpretation data of aqueous extract from *T. diversifolia* leaves.

No.	Compound	RT	Molecular Weight (w/z)	Formula	% Fit Conf	Classification
1.	Phenylalanine	2.58	166.0855	$C_9H_{12}NO_2$	99.32	Amino Acids
	Structure					



(Source: PubChem)

No.	Compound	RT	Molecular Weight (w/z)	Formula	% Fit Conf	Classification
2.	3-Indoleakrilic Acid	3.72	188.0712	$C_{11}H_{10}NO_2$	84.22	Amino Acids
	Structure			 (Source: PubChem)		
3.	Gilluone B	6.91	534.2703	$C_{28}H_{40}NO_9$	99.32	Terpenoid
	Structure			 (Source: PubChem)		
4.	Tagitinin A	7.50	369.1913	$C_{19}H_{29}O_7$	99.60	Seskuiterpen Lacton
	Structure			 (Source: PubChem)		
5.	Thyrotundin	8.69	353.1964	$C_{19}H_{29}O_6$	99.94	Seskuiterpen Lacton
	Structure			 (Source: PubChem)		

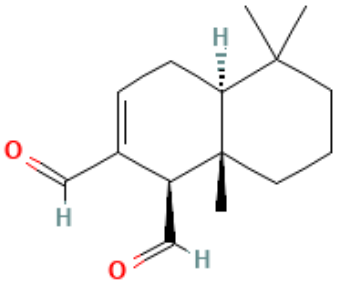
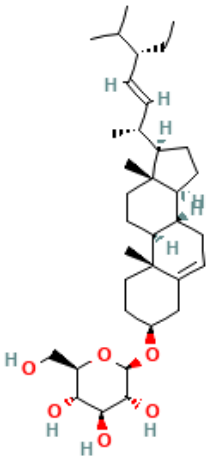
No.	Compound	RT	Molecular Weight (w/z)	Formula	% Fit Conf	Classification
6.	Polygodial	9.31	235.1698	$C_{15}H_{23}O_2$	99.51	Sesquiterpenoid
	Structure					
				(Source: PubChem)		
7.	Stigmasterol Glucoside	17.87	575.4298	$C_{35}H_{59}O_6$	99.97	Sterol
	Structure					
				(Source: PubChem)		

Figure 1 shows the LC-MS/MS chromatogram of the aqueous extract from *T. diversifolia* leaves and **Table 2** presents the corresponding LC-MS/MS data. In addition to the phytochemical screening, the extract was analyzed using LC-MS/MS, revealing the presence of various bioactive compounds, including phenylalanine, indoleacrylic acid, gilluone B, tagitinin A, thyrotundin, tadeonal, and stigmasterol glucoside. Gilluone B is a terpenoid compound with anti-inflammatory and antioxidant properties, which help reduce inflammation and protect cells from oxidative damage [33]. Tagitinin A, identified in this study, has been shown to regulate blood glucose levels, suggesting its potential in diabetes treatment [34]. Thyrotundin has been reported to exhibit antidiabetic potential by decreasing insulin resistance through the upregulation of PPARγ (Peroxisome Proliferator-Activated Receptor Gamma) activity, indicating its role as a promising candidate for the treatment of diabetes mellitus and related metabolic disorders [35]. Polygodial, a sesquiterpene compound, is known for its wide range of therapeutic effects, including antifungal, antibacterial, anti-inflammatory, and anticancer activities [36,37]. Finally, stigmasterol glucoside demonstrates both anti-inflammatory and antimicrobial activities, further supporting its therapeutic potential [38,39].

Microencapsulation of aqueous extract from *T. diversifolia*

The microencapsulation of aqueous extract from *T. diversifolia* leaves is carried out using spray drying techniques. **Table 3** presents the data on total flavonoid content (TFC) and encapsulation efficiency (% EE) of the resulting microcapsules. The microcapsules are prepared by varying the concentration of the chitosan coating and the stirring duration, with the pH maintained at 4. Among the variations, the microcapsules produced with a chitosan concentration of 0.05 % (w/v) and a stirring time of 90 min at pH 4 demonstrated the highest encapsulation efficiency. As a result, these specific conditions were selected for further analysis.

Table 3 Total flavonoid content and encapsulation efficiency (% EE) of microcapsules containing aqueous extract from *T. diversifolia* leaves.

Sample	Total flavonoid content (mg QE/g)	% EE
Microcapsules prepared at 0.05 % chitosan (w/v)	0.378	75.36 % ± 0.002[a]
Microcapsules prepared at 0.1 % chitosan (w/v)	0.304	60.54 % ± 0.002[b]
Microcapsules prepared at 0.2 % chitosan (w/v)	0.296	58.94 % ± 0.002[c]
Microcapsules prepared at a stirring time of 60 min	0.154	30.62 % ± 0.007[b]
Microcapsules prepared at a stirring time of 90 min	0.377	75.14 % ± 0.002[a]
Microcapsules prepared at a stirring time of 120 min	0.136	27.07 % ± 0.002[c]

In this study, microencapsulation was conducted using chitosan as the coating material and sodium tripolyphosphate (Na-TPP) as the cross-linking agent, employing the spray drying method. Chitosan was selected not only due to its biocompatibility and biodegradability but also because of its inherent antioxidant properties. These antioxidant effects are attributed to the ability of the ammonium ion group (NH_3^+) in chitosan to react with hydroxyl radicals (OH•), thereby neutralizing oxidative stress [40]. Na-TPP serves as a vital cross-linking agent in the formation of chitosan-based nanoparticles. It facilitates the cross-linking process through ionic interactions, particularly with the positively charged amino groups of chitosan. This ionotropic gelation process leads to the formation of stable and compact nanoparticles, which can encapsulate active ingredients more efficiently. Moreover, chitosan's ability to enhance the penetration of large molecules across mucosal surfaces makes it especially advantageous for drug and bioactive compound delivery. Although chitosan is insoluble in water and most organic solvents, it becomes soluble in aqueous acetic acid due to the protonation of its glucosamine units. This characteristic is essential for preparing the chitosan solution used in the encapsulation process [29].

As shown in **Table 3**, microcapsules prepared using a 0.05 % chitosan concentration exhibited the highest flavonoid content (0.378 mg QE/g) and an encapsulation efficiency of 75.36 % ± 0.002. These results suggest that the concentration of chitosan plays a significant role in the encapsulation efficiency. Higher efficiency indicates a more effective encapsulation of active compounds, providing better protection against degradation. At lower chitosan concentrations, the resulting particles tend to have a larger surface area, which may enhance the adsorption and retention of flavonoids on or within the particle matrix. Additionally, the stability of the microcapsules can be attributed to the electrostatic interactions between the protonated amine groups (NH_3^+) of chitosan and the negatively charged components of the plant extract, further reinforced by cross-link formation with Na-TPP [30]. These interactions contribute to the structural integrity and sustained release capabilities of the microcapsules, which are critical factors in controlled delivery systems.

Microcapsule characterization

The characterization of aqueous extract from *T. diversifolia* leaves microcapsules was conducted using Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM). FTIR analysis, as shown in **Figure 2** and interpreted in **Table 4**, was performed to identify the functional groups in the microcapsules. Meanwhile, SEM characterization, presented in **Figure 3** observed surface morphology of the microcapsules.

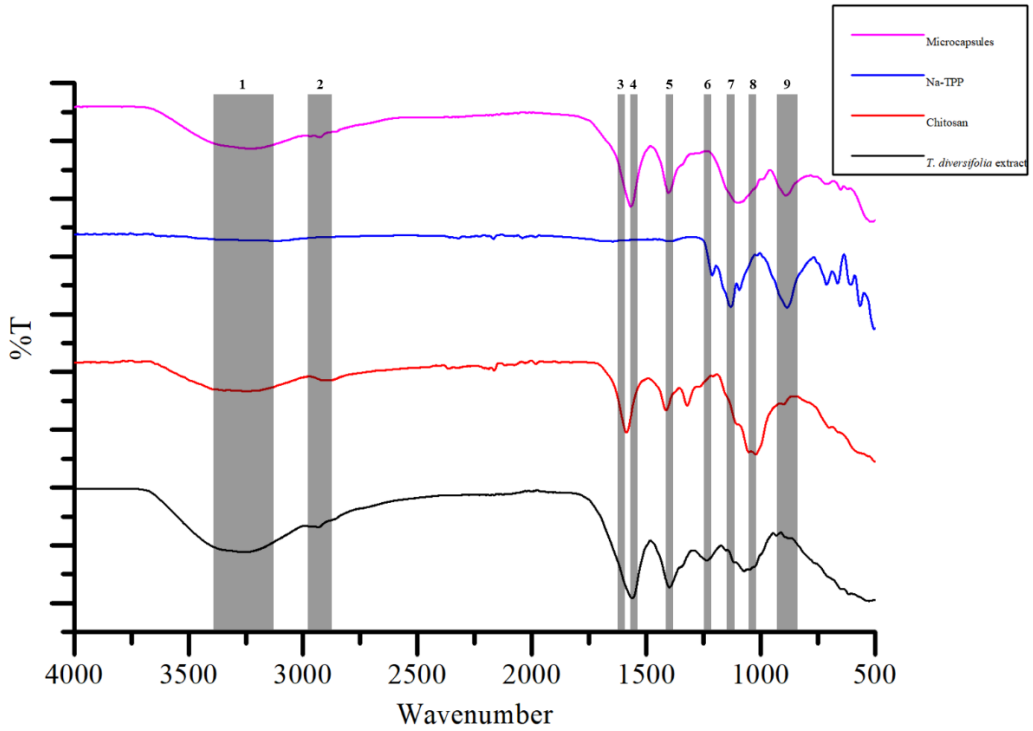


Figure 2 FTIR spectra of aqueous extract from *T. diversifolia* leaves, chitosan, Na-TPP, and microcapsules.

Table 4 FTIR spectra interpretation results.

Extract	Chitosan	Na-TPP	Microcapsules	Functional group	Compound identification
3,284.57	3,260.32	3,221.81	3,242.21	-O-H- Stretching	Alcohol
2,933.72	2,879.52	-	2,926.59	-C-H- Stretching	Alkane
-	1,585.95	-	-	N-H Bending	Amina
1,560.28	-	-	1,567.41	-C=C Stretching	Aromatic
1,397.69	1,413.38	-	1,401.97	-O-H- Bending	Alcohol
1,235.10	-	-	-	-C-N Stretching	Amina
-	-	1,129.56	1,098.18	-P-O-C Stretching	Phosphate
-	1,021.17	-	-	-C-N Stretching	Amina
-	-	884.25	891.38	C=C Bending	Alkane

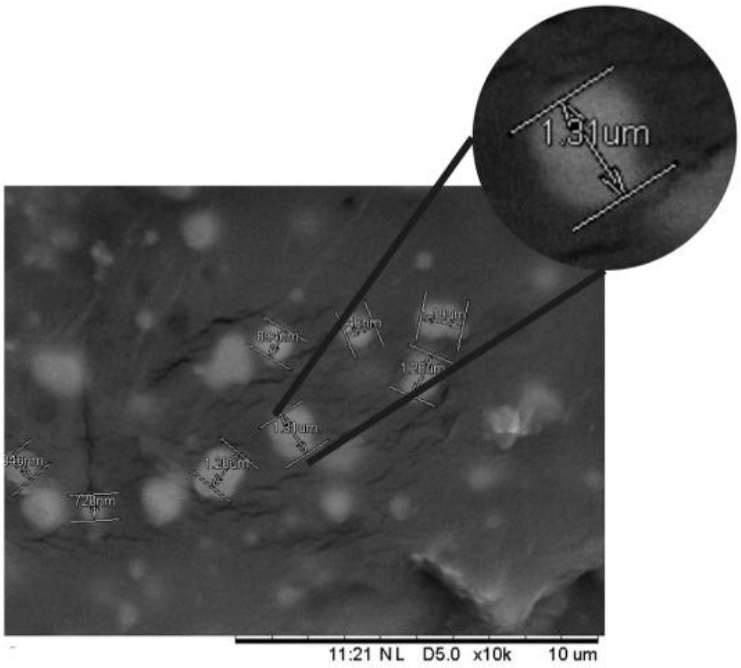


Figure 3 SEM of aqueous extract from *T. diversifolia* leaves microcapsules at 10,000× magnification.

Figure 2 presents a comparison of FTIR spectra between the aqueous extract of *T. diversifolia* leaves, chitosan, Na-TPP, and the resulting microcapsules. As shown in **Table 4**, the FTIR spectrum of the microcapsules exhibits several prominent absorption peaks. The peak at 3,243.21 cm^{-1} corresponds to -O-H stretching vibrations, indicative of hydroxyl groups commonly found in flavonoids. A peak at 2,926.59 cm^{-1} is associated with C-H_3 stretching, typical of alkane compounds, while the band at 1,567.41 cm^{-1} represents -C=C stretching vibrations, characteristic of aromatic ring structures. Additionally, a peak at 1,401.97 cm^{-1} indicates the presence of alcohol (-O-H) functional groups, and the peak at 1,098.18 cm^{-1} corresponds to -P-O-C stretching, suggesting the presence of phosphate groups. The FTIR spectrum of the microcapsules shows strong similarity to that of the extract, indicating the presence of active phytochemical constituents. Simultaneously, the spectrum also reflects the characteristic peaks of the chitosan-Na-TPP matrix used as the encapsulating agent. Notably, the peak at 1,098.18 cm^{-1} confirms the presence of phosphate groups derived from sodium tripolyphosphate, which acts as a cross-linker during the microencapsulation process. The interaction between phosphate ions from Na-TPP and the protonated amino groups (NH_3^+) of chitosan confirms the formation of a stable polymeric network, thereby supporting the successful encapsulation of the extract's bioactive components. These findings demonstrate that the encapsulation process using spray drying was effective in coating and protecting the active compounds [41]. Based on **Figure 3**, the morphology of the resulting microcapsules appears to be generally spherical with smooth surfaces and varied size distributions. This variation may be attributed to the spray drying process, which is influenced by factors such as temperature and feed viscosity. The smooth and rounded structure suggests that the drying temperature used was optimal, promoting rapid droplet solidification and minimizing surface collapse or shrinkage during particle formation [42].

Microcapsule antioxidant activity test

The biological activity of the microcapsules was assessed by testing for antioxidant activity. The in vitro antioxidant activity was evaluated using the DPPH free radical capture test, which is an effective method for analysing the antioxidant potential of an extract or compound. The results of the test are typically expressed in terms of IC_{50}, which refers to the concentration of the sample solution required to inhibit 50 % of free radical activity.

Table 5 Antioxidant activity test.

Sample	IC_{50} Value (µg/mL)
Ascorbic acid	8.41 ± 0.022
Extract	70.43 ± 0.096
Microcapsules	139.24 ± 0.022

Bioactive compounds found in medicinal plants offer substantial health benefits, especially as natural antioxidants capable of neutralizing free radicals and reactive oxygen species (ROS). Antioxidant assays are frequently employed to assess the antidiabetic potential of drug candidates, due to the well-established association between oxidative stress and the progression of diabetes mellitus [43,44]. In this study, antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. DPPH is an organic compound with an unstable nitrogen radical that produces a deep purple solution, which decolorizes upon reduction by an antioxidant [45]. The results showed that microcapsules produced under optimal conditions had an IC_{50} value of 139.24 µg/mL, which was higher than that of *T. diversifolia* leaf extract (70.43 µg/mL) and ascorbic acid (8.41 µg/mL). These findings indicate that the antioxidant activity of the microcapsules is comparatively lower. This difference can be attributed to the variation in bioactive compound concentrations among the 3 samples, as antioxidant activity generally increases with higher levels of flavonoids, phenolics, and other active constituents.

The moderate antioxidant activity of the microcapsules suggests that a higher concentration is required to scavenge 50 % of the DPPH radicals. Despite this, the microcapsules still exhibit antioxidant potential, supporting their candidacy for therapeutic applications [27]. One contributing factor to the reduced activity is the confinement of bioactive compounds within the microcapsules, which limits their immediate interaction with DPPH radicals [46]. It is important to note that the primary role of the coating material is not to enhance the antioxidant activity of the core substance but rather to shield it from environmental factors [26].

Moreover, the higher IC_{50} value of the microcapsules compared to the crude extract can be explained by 2 main factors: (1) Reduced bioavailability of active compounds due to the encapsulating matrix, which can hinder solubility and diffusion, and (2) limited interaction between antioxidants and DPPH radicals as the chitosan coating forms a physical barrier. Since microcapsules release their contents gradually, antioxidant compounds may not be present in sufficient concentration at the time of measurement. To better understand these observations, future studies are recommended to investigate the release kinetics of the encapsulated compounds during antioxidant testing, as well as to compare the concentration of flavonoids and phenolic compounds before and after encapsulation [21,25]. Such insights will be valuable in optimizing encapsulation techniques to balance both protection and bioactivity of plant-derived compounds.

Controlled release of microcapsule

In vitro release tests were conducted on microcapsules at pH 2.2 and pH 7.4, with release durations of 30, 60, 90 and 120 min. These variations in pH and release time were applied to evaluate their impact on the release of the microcapsules. **Figure 4** presents the microcapsule release profile, while **Table 6** summarizes the percentage of microcapsule release at both pH 2.2 and pH 7.4.

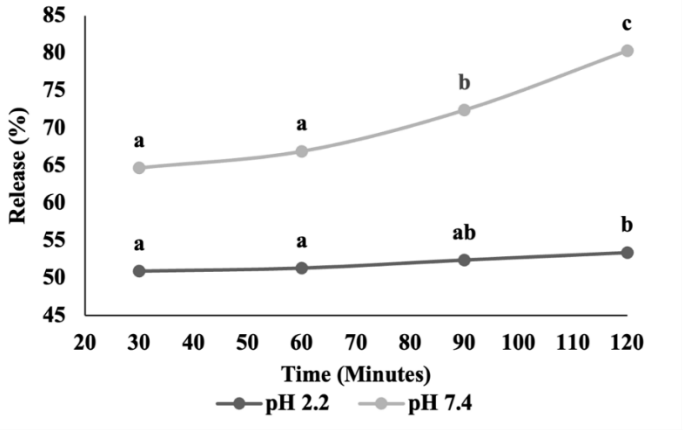
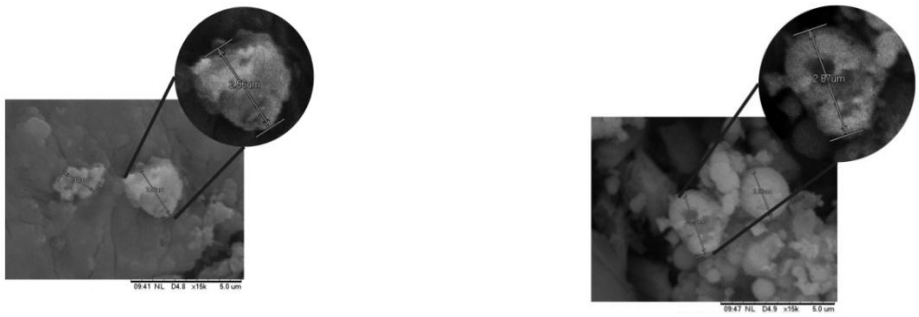


Figure 4 Release profile of bioactive from microcapsule at pH 2.2 and pH 7.4. Different letter notations indicate significant difference between treatments at a significance of $\alpha = 0.05$.

Table 6 Release profile of bioactive from microcapsule at pH 2.2 and pH 7.4.

Time (Min)	% Release at pH 2.2	% Release at pH 7.4
30	50.9 ± 0.68	64.7 ± 1.27
60	51.3 ± 0.95	66.9 ± 0.86
90	52.4 ± 0.99	72.4 ± 0.62
120	53.4 ± 0.66	80.3 ± 2.23



(a) **(b)**

Figure 5 SEM of microcapsule after release of bioactive ingredients at: (a) pH 2.2 magnification of 15,000× and (b) pH 7.4 magnification of 15,000×.

Encapsulation using chitosan-Na-TPP is a promising strategy for controlled drug delivery, especially for bioactive compounds that are sensitive to pH changes in the gastrointestinal tract. In acidic conditions (pH 2.2), such as in the stomach, chitosan becomes protonated to NH_3^+, increasing its positive charge. This strengthens its electrostatic interaction with the negatively charged Na-TPP, creating a denser and more compact microcapsule matrix. As a result, the diffusion of the active compound is restricted, leading to a slower release rate. This protective effect is crucial for preventing the premature degradation of bioactive in gastric fluids. In this study, only 53.4 % of the compound was released at 120 min at pH 2.2, demonstrating a sustained and gradual release profile under acidic conditions [42,43].

In contrast, under neutral to slightly alkaline conditions (pH 7.4), mimicking the intestinal environment, the amine groups in chitosan are deprotonated, decreasing their positive charge. This

weakens the interaction between chitosan and Na-TPP and increases electrostatic repulsion from ionized carboxyl groups (COO^-) within the microcapsule. The resulting structural expansion of the matrix facilitates a faster and more extensive release of the active compound. The data support this mechanism, with 80.3 % of the extract released at pH 7.4 after 120 min. The controlled disintegration of the microcapsule matrix in intestinal conditions is highly desirable for enhancing the bioavailability of active ingredients at the site of absorption [26,47].

Overall, the encapsulation technique using chitosan-Na-TPP not only stabilizes the active compounds through the gastric phase but also provides a pH-responsive mechanism for their targeted release in the intestines. This dual functionality significantly improves drug delivery efficiency by combining protection and controlled release. It also highlights the potential of such systems for pharmaceutical and nutraceutical applications where precise release kinetics are essential for therapeutic success [21].

Conclusions

The microencapsulation of aqueous extract from *T. diversifolia* leaves using chitosan improved encapsulation efficiency, antioxidant activity, and controlled release behavior at different pH levels. These results indicate its potential for developing targeted and stable delivery systems for bioactive compounds.

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References

[1] GR Santos, M Muñoz and G Franco. Fruit heteromorphism and germination success in the perennial shrub *Tithonia diversifolia* (Asteraceae). *Flora* 2020; **271**, 151686.

[2] E Sulistyowati. Structure elucidation of the leaf of *Tithonia diversifolia* (Hemsl) gray. *Jurnal Sains dan Matematika* 2015; **23(4)**, 101-106.

[3] TO Omolola. Phytochemical, proximate and elemental composition of *Tithonia diversifolia* (Hemsley) A. gray leaves. *International Annals of Science* 2019; **8(1)**, 54-61.

[4] K Douglas and J Jeruto. Phytochemistry and antimicrobial activity of extracts from medicinal plants *Tithonia diversifolia* and *Olea africana*. *British Journal of Pharmaceutical Research* 2016; **12(3)**, 1-7.

[5] YM Reddy, SPJ Kumar, KV Saritha, P Gopal, TM Reddy and GJ Simal. Phytochemical profiling of methanolic fruit extract of *Gardenia latifolia* Ait. by LC-MS/MS analysis and evaluation of its antioxidant and antimicrobial activity. *Plants* 2021; **10(3)**, 545.

[6] PL Kowalczewski and J Zembrzuska. Advances in biological activities and application of plant extracts. *Applied Sciences* 2023; **13(16)**, 9324.

[7] A Plaskova and J Mlcek. New insights into the application of water or ethanol-water plant extracts rich in active compounds in food. *Frontiers in Nutrition* 2023; **10**, 1118761.

[8] N Almayda, A Roosdiana, M Masruri and A Safitri. Evaluation of bioactive compounds, release profiles, and toxicity test from

microcapsules containing *Ruellia tuberosa* L. extracts utilizing gum arabic. *Journal of Pure & Applied Chemistry Research* 2024; **13(1)**, 52.

[9] BCI Piñón, PC Leyva, FY Terán, SV Espinosa, SC Álvarez and LMZ Saavedra. Encapsulation of active ingredients in the food industry by spray-drying and nano spray-drying technologies. *Processes* 2020; **8(8)**, 889.

[10] JF Parente, VI Sousa, JF Marques, MA Forte and CJ Tavares. Biodegradable polymers for microencapsulation systems. *Advances in Polymer Technology* 2022; **2022(1)**, 4640379.

[11] F Călinoiu, BE Ştefănescu, ID Pop, L Muntean and DC Vodnar. Chitosan coating applications in probiotic microencapsulation. *Coatings* 2019; **9(3)**, 194.

[12] SA Hussain, H Abdelkader, N Abdullah and S Kmaruddin. Review on micro-encapsulation with chitosan for pharmaceutical applications. *MOJ Current Research & Reviews* 2018; **1(2)**, 770-784.

[13] S Baliyan. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules* 2022; **27(4)**, 1326.

[14] K Amalia, A Dalimunthe, D Satria, Sitorus and SB Waruwu. Antioxidant activity of ethanol extract of Puguntano herb (*Picria fel-terrae* Lour.) and effect on superoxidase dismutase and lactate dehydrogenase levels in doxorubicin-induced rats. *Trends in Science* 2024; **21(10)**, 8199-8199.

[15] C Annisa, S Prasetyawan and A Safitri. Co-microencapsulation of *Ruellia tuberosa* L. and *Cosmos caudatus* K. extracts for pharmaceutical applications. *Makara Journal of Science* 2022; **26(2)**, 12.

[16] AK Pertiwi, C Annisa, Z Ningsih and A Safitri. Microencapsulation of *Ruellia tuberosa* L. extracts using alginate: Preparation, biological activities, and release. *Indonesian Journal of Chemistry* 2023; **23(2)**, 321.

[17] AR Sari, TR Saraswati and EY Yuniwarti. Antihyperglycemic activity of aqueous extract of insulin leaves (*Tithonia diversifolia*) on hyperglycemic rats (*Rattus norvegicus*). *Biosaintifika Journal of Biology and Biological Education* 2018; **10(3)**, 636-641.

[18] AM Lawal, R Abdullahi, MS Ibrahim, MY Kurfi, A Khalid and M Nuhu. Phytochemical analysis and thin-layer chromatography profiling of crude extracts from *Senna occidentalis* (leaves). *Journal of Biotechnology and Biomedical Science* 2019; **2(1)**, 12-21.

[19] S Dubale, D Kebebe, A Zeynudin and N Abdissa. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology* 2023; **15**, 51-62.

[20] SL Solanki, CM Modi, HB Patel, UD Patel and DH Bhadarka. Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh, Gujarat, India. *Journal of Pharmacognosy and Phytochemistry* 2019; **8(4)**, 3122-3126.

[21] A Safitri, A Roosdiana, N Kurnianingsih, F Fatchiyah, E Mayasari and R Rachmawati. Microencapsulation of *Ruellia tuberosa* L. aqueous root extracts using chitosan-sodium tripolyphosphate and their *in vitro* biological activities. *Scientifica* 2022; **2022(1)**, 9522463.

[22] N Phuyal, PK Jha, PP Raturi and S Rajbhandary. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Scientific World Journal* 2020; **2020(1)**, 8780704.

[23] REK Dibacto, BRT Tchuente, MW Nguedjo, YMT Tientcheu, EC Nyobe, FLE Edoun, MFG Kamini, RF Dibanda and GN Medoua. Total polyphenol and flavonoid content and antioxidant capacity of some varieties of *Persea americana* peels consumed in Cameroon. *Scientific World Journal* 2021; **2021(1)**, 8882594.

[24] SSN Fadlila, OEP Puspita, Z Ningsih and A Safitri. Microencapsulation of *Cosmos caudatus* Kunth extract using sodium alginate and *in vitro* antioxidant activity test. *Makara Journal of Science* 2024; **28(2)**, 1.

[25] N Almayda, M Masruri and A Safitri. Effectiveness of using gum arabic for co-microencapsulation of *Ruellia tuberosa* L. and *Tithonia diversifolia* extracts as encapsulating agent and release studies. *Scientifica* 2024; **2024(1)**, 9097238.

[26] IA Zahara, SM Ulfa and A Safitri. Comparative analysis of different natural polymers as coating agents for freeze-dried microencapsulation of *Cosmos caudatus* Kunth compounds. *Scientific World Journal* 2024; **2024(1)**, 6833341.

[27] FR Gunawan, SM Ulfa and A Safitri. Freeze-drying microencapsulation of *Ruellia tuberosa* L. extracts: A comparative study using different polymers as encapsulants. *Indonesian Journal of Chemistry* 2025; **25(1)**, 37.

[28] M Oloo and D Menge. Phytochemical screening and antimicrobial activity of crude extract of *Tithonia diversifolia*. *Open Journal of Biological Sciences* 2020; **5(1)**, 030-033.

[29] J Patil, R Rajput, P Patil, A Mujumdar and J Naik. Generation of sustained release chitosan nanoparticles for delivery of ketorolac tromethamine: A tubular microreactor approach. *International Journal of Polymer Materials and Polymer Biomaterials* 2020; **69(8)**, 516-524.

[30] A Sukmawati, IV Risdiyanti and CC Marthadilla. Release profile of betanin from chitosan microparticle containing beetroot (*Beta vulgaris* Linn) extract. *In*: Proceedings of the 4th International Conference Current Breakthrough in Pharmacy (ICB-Pharma 2022), Sukoharjo, Indonesia. 2022.

[31] R Yadav and M Agarwala. Phytochemical analysis of some medicinal plants. *Journal of Phytology* 2011; **3(12)**, 10-14.

[32] L Maheshwaran, L Nadarajah, SPNN Senadeera, CB Ranaweera, AK Chandana and RN Pathirana. Phytochemical testing methodologies and principles for preliminary screening/qualitative testing. *Asian Plant Research Journal* 2024; **12(5)**, 11-38.

[33] AK Block, MM Vaughan, EA Schmelz and SA Christensen. Biosynthesis and function of terpenoid defense compounds in maize (*Zea mays*). *Planta* 2019; **249(1)**, 21-30.

[34] S Sut. Identification of tagitinin C from *Tithonia diversifolia* as antitrypanosomal compound using bioactivity-guided fractionation. *Fitoterapia* 2018; **124**, 145-151.

[35] R Gang, M Matsabisa, D Okello and Y Kang. Ethnomedicine and ethnopharmacology of medicinal plants used in the treatment of diabetes mellitus in Uganda. *Applied Biological Chemistry* 2023; **66(1)**, 39.

[36] R Venkatesan. Polygodial, a sesquiterpene dialdehyde, activates apoptotic signaling in castration-resistant prostate cancer cell lines by inducing oxidative stress. *Cancers* 2022; **14(21)**, 5260.

[37] R Dasari. Synthetic and biological studies of sesquiterpene polygodial: Activity of 9-epipolygodial against drug-resistant cancer cells. *ChemMedChem* 2015; **10(12)**, 2014-2026.

[38] M Roopa, T Rhetso, R Shubharani and V Sivaram. nfluence of solvents on phytochemical constituent profile and antioxidant activities of various extracts of *Tithonia diversifolia* (Hemsl.) A. Gray flower. *Plant Cell Biotechnology and Molecular Biology* 2021; **22(37&38)**, 211-228.

[39] SM Gitahi, MP Ngugi, DN Mburu and AK Machocho. Contact toxicity effects of selected organic leaf extracts of *Tithonia diversifolia* (Hemsl.) A. Gray and *Vernonia lasiopus* (O. Hoffman) against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *International Journal of Zoology* 2021; **2021(1)**, 8814504.

[40] E Krishnamoorthy, ZA Aliyamveetil and RCN Rao. Antioxidant properties of chitooligosaccharides produced by enzymatic degradation of chitosan. *Fishery Technology* 2023; **60(3)**, 2582-2632.

[41] A Safitri, A Roosdiana, E Hitdatania and SA Damayanti. *In vitro* alpha-amylase inhibitory activity of microencapsulated *Cosmos caudatus* Kunth extracts. *Indonesian Journal of Chemistry* 2021; **22(1)**, 212-222.

[42] Buanasari, W Sugiyo and H Rustaman. Preparation and evaluation of plant extract microcapsules using chitosan. *IOP Conference Series: Earth and Environmental Science* 2021; **755(1)**, 012063.

[43] FS Buosi, A Alaimo, MC Di Santo, F Elias, GG Liñares, SL Acebedo, MAC Cataña, CC Spagnuolo, L Lizarraga, KD Martínez and OE Pérez. Resveratrol encapsulation in high molecular weight chitosan-based nanogels for applications in ocular treatments: Impact on human ARPE-19 culture cells. *International*

Journal of Biological Macromolecules 2020; **165**, 804-821.

[44] K Ratananikom and K Premprayoon. Ultrasonic-assisted extraction of phenolic compounds, flavonoids, and antioxidants from dill (*Anethum graveolens* L.). *Scientifica* 2022; **2022(1)**, 3848261.

[45] İ Gulcin and SH Alwasel. DPPH radical scavenging assay. *Processes* 2023; **11(8)**, 2248.

[46] A Safitri, F Fatchiyah, DRT Sari and A Roosdiana. Phytochemical screening, *in vitro* anti-oxidant activity, and *in silico* anti-diabetic activity of aqueous extracts of *Ruellia tuberosa* L. *Journal of Applied Pharmaceutical Science* 2020; **10(3)**, 101-108.

[47] C Li, K Fang, W He, K Li, Y Jiang and J Li. Evaluation of chitosan-ferulic acid microcapsules for sustained drug delivery: Synthesis, characterizations, and release kinetics *in vitro*. *Journal of Molecular Structure* 2021; **1227**, 129353.