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Supercritical fluid extraction of torch ginger: encapsulation, metabolite profiling, and antioxidant activity

Abstract

Objectives

The objective of this study was first to perform the supercritical fluid extraction (SFE) and encapsulation of torch ginger (*Etilingera elatior*) inflorescences into a functional powder. Second objective was to evaluate the powder's characteristics, metabolite profiles, and antioxidant activity.

Methods

Torch ginger inflorescences were extracted via SFE technique, and the obtained extract was encapsulated by a spray-drying process with maltodextrin as an encapsulating agent. Subsequently, the powder was evaluated for its physical characteristics, determination of metabolite profiles by using a Fourier Transform Infrared Spectrophotometer (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS), and antioxidant activity.

Results

Spray drying encapsulation process managed to yield around 59.8% of torch ginger extract powder (TGEP) by using 10% of extract, which the obtained yield was twice higher than in another study. TGEP showed inconsistent agglomeration behaviour in particle size examination with distinct sizes concentrating at 2.2 μm and 17.4 μm , respectively. Brunauer-Emmett-Teller (BET) analysis of TGEP unveiled a considerably high surface area (1.13 m^2/g), pore volume (0.218 cm^3/g), and pore size (384.6 nm). The metabolites profile of TGEP was studied and characterized using two spectroscopic analyses. Analysis by the FTIR showed the presence of O-H, C-H, C=C, C=O, CO-O-CO, C-N, and C-O functional groups in the sample. Subsequently, the result of the GC-MS characterization revealed about 59 metabolites that predominantly fatty acids (30.5%), terpenes and derivatives (20.3%), fatty acid esters (16.9%), and alcohols (8.47%) were present in TGEP. The powder also demonstrated a high antioxidant activity based on the evaluation of its total phenolic content (23.3 ± 0.662), 2,2-diphenyl-

1-picrylhydrazyl radical scavenging capacity (1.31 ± 0.002), and ferric reducing antioxidant power (2919.5 ± 19.9), which were better than previous studies.

Conclusion

Therefore, this study unveiled TGEP as a functional powder with a high content of bioactive compounds with excellent bioactivity.

Keywords: *Etilingera elatior*, Encapsulated torch ginger extract powder, Supercritical fluid extraction, Spray drying, Encapsulation, Metabolite

1. Introduction

1 Torch ginger or scientifically known as *Etingera elatior* is a perennial herbaceous plant belong in the
2 *Zingiberaceae* family which is endemic to the Southeast Asian region. It grows in a large colonies and
3 has a pink, ovoid-shaped, inflorescence with a unique fragrance (NParks, 2019). In the Southeast Asian
4 gastronomy, torch ginger is synonymous in various cuisine such as *Asam Laksa* in Malaysia, *Pecel* in
5 Indonesia, and added to traditional Thai meat dishes in Thailand (Rachkeeree et al., 2018).

6 Torch ginger has been receiving major traction among researchers and various studies have been
7 conducted to unveil its promising medicinal value. Torch ginger has been reported to contains various
8 phytochemical constituents specifically secondary metabolites that serve a multitudes of biological
9 functions. Secondary metabolites in the class of lipids, phenolics, and terpenes are often found in torch
10 ginger's extract and its metabolites profile was well documented in literature (Ghasemzadeh et al., 2015;
11 Marzlan et al., 2020; Wijekoon et al., 2011; Wijekoon et al., 2013). As mentioned earlier, these classes
12 of secondary metabolites have been scientifically proven to render various biological functions namely,
13 as antioxidant, antimicrobial, and antibiotic (Hussein & El-Anssary, 2018; Lachumy et al., 2010).

14 Furthermore, torch ginger also distinctively known as an aromatic flower and this aroma is fundamental
15 in the Southeast Asian gastronomy (Oh et al., 2019; Raji et al., 2017). Hence, apart from elevating
16 the sensory experience of food, the inclusion of torch ginger in daily diet is presumed to elicit
17 the functional importance of bioactive compounds, which are vital for optimal human health
18 (Abdelwahab et al., 2010; Lachumy et al., 2010).

19 In recent decade, supercritical fluid extraction (SFE) has been well reported as a safe and efficient
20 extraction technique for various plant-based natural products in broad array of application. SFE utilise
21 supercritical carbon dioxide (SCO₂) as a solvent in which this unique state delivers a gas-like diffusivity
22 and liquid-like solubility throughout the extraction process (Sunol et al., 2019). These properties are
23 important to induce the penetration of solvent deep into the plant matrices which it will increase the
24 rate of mass transfer between the extracted material and the solvent used (Arumugham et al., 2021).

25 Additionally, the use of CO₂ also provides an added advantage to this technique as the Food and Drug
26 Administration (FDA) has classified the substance as Generally Recognized as Safe (GRAS) (FDA,

27 2020). Hence, this unique mechanism of SFE promotes the extraction, isolation, and retention of plant's
28 beneficial compounds, such as terpenes, flavonoids and phenolics in the obtained extract (Caballero et
29 al., 2020).

30 The encapsulation technique has ushered in a multifaceted benefit to the plant extract. This technique
31 has been claimed to protect bioactive compounds against oxidation, creates a thermal barrier, preserve
32 the physical structure of the compounds, and conserve the organoleptic attributes of the compound
33 (Mooranian et al., 2014). The aforementioned benefits are made possible via this technique as the
34 bioactive compounds are trapped in a capsule-like structure with a shell made up of biomaterials or
35 synthetic polymer that serves as a protective layer (Onsaard & Onsaard, 2019). Therefore, researchers
36 and industry players have preferred the encapsulation technique to produce bioactive compound-rich
37 extract suitable for various applications.

38 Several studies have been conducted on developing plant-extract-based powder from various herbal
39 plant species in literature (Rajabi et al., 2015; Simon-Brown et al., 2016). However, to the authors'
40 knowledge, only one study on developing torch ginger extract powder using the encapsulation
41 technique exists in the literature, which has been published by Anuar et al. (2021). Consequently, due
42 to insufficient studies on torch ginger-extract powder, information about its phytochemical composition
43 and bioactivity remain scarce in the literature. In this study, the torch ginger extract powder (TGEP)
44 was developed, the retention of bioactive compounds in the powder was evaluated, and its bioactivity
45 was tested. Therefore, the objective of this study was first to perform the supercritical fluid extraction
46 (SFE) and encapsulation of torch ginger (*Etilingera elatior*) inflorescences into a functional powder.
47 Meanwhile, the second objective was to evaluate the powder's characteristics, metabolite profiles, and
48 antioxidant activity.

49

50 **2. Materials and methods**

51 **2.1. Torch ginger sample and chemicals**

52 Torch ginger (*Etilingera elatior*) inflorescences were procured from a farm in Kuala Pilah, Negeri
53 Sembilan, Malaysia. The sample was harvested and delivered to the authors' laboratory within the same
54 day. Subsequently, the sample was stored in the laboratory's refrigerator for storage under refrigerated

55 conditions (± 4 °C). Analytical grade organic solvents and chemicals were used in the experiments for
56 an optimum precision. The ²² N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) used for the
57 analysis was bought from Thermo Fisher Scientific (Waltham, MA, United States of America).
58 Denatured ethanol (99% purity) and acetic acid (glacial, $\geq 99\%$ assay) were obtained from HmbG
59 Chemicals (Hamburg, Germany). Additionally, hydrochloric acid (fuming 37%), ⁹ gallic acid, Folin-
60 Ciocateu phenol reagent, and ferric chloride hexahydrate were purchased from Merck (Darmstadt,
61 Germany). Meanwhile, anhydrous pyridine (99.8%), methoxyamine hydrochloride (98%), sodium
62 acetate (anhydrous), sodium carbonate (anhydrous), gallic acid (TraceCERT[®]), ¹⁰ 2,2-Diphenyl-1-
63 picrylhydrazyl radical (DPPH), and (\pm)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
64 (Trolox) were procured from Sigma-Aldrich (St. Louis, MO, United States of America).

65

66 2.2. Preparation

67 2.2.1. Pre-processing of torch ginger

68 The inflorescences were separated from its stalk and later, they were cut into smaller pieces.
69 Subsequently, the sample was lyophilized by using the Labconco FreeZone Benchtop freeze dryer
70 (Missouri, United States of America) that was operated at -40 °C and the vacuum level was set at 133
71 $\times 10^{-3}$ mBAR. The drying process was conducted until the sample was sufficiently dried ($\pm 10\%$
72 moisture content) which it took approximately four days to complete. Using a RT-CR30S 3HP cutting
73 mill with a cyclone powder collector (Rong Tsong Precision Technology Co., Dawei Rd., Taichung,
74 Taiwan), the dried sample was pulverized into powder with particles approximately 0.22 mm in size.
75 Following that, the remaining debris were removed from the powder by sieving it using a 200 x 50 mm
76 sieve with the aperture size of 0.220 mm and the powder was stored in an airtight container.

77

78 ¹³ 2.2.2. Supercritical fluid extraction of torch ginger

79 ¹⁹ Supercritical fluid extraction (SFE) technique was performed to extract and isolate essential oil from
80 torch ginger by using a laboratory scale extraction plant (Deven Supercritical Pvt. Ltd., Phatak Baug,
81 Navi Peth, Pune, India). The extraction process was carried out according to method used in our

82 previous study with some modifications (Naziruddin et al., 2022). In brief, a filter bag with a pore size
83 of 45.0 μm was filled with approximately 200 g of torch ginger powder and subsequently positioned
84 within the high-pressure extraction vessel of the SFE unit. Stream of liquid CO_2 (99.8% purity, 1.2 kg/h)
85 was flowed into the chiller for it to be cooled at 5 °C and it was later pressurised by a high-pressure
86 pump before entering the extraction vessel. Inside the extraction vessel, liquid CO_2 was converted to a
87 supercritical state (SCO_2) upon being pressurised to 28 MPa at 50 °C. Consequently, the SCO_2
88 penetrated the sample's microporous matrix to induce the extraction and isolation of the desired
89 compounds. The entire extraction process took about six hours to complete, by which the yield was
90 collected every consecutive hour and dispensed into an amber glass bottle. The bottle was tightly capped
91 and hermetically sealed by wrapping it with a sheet of parafilm for storage at 4 °C.

93 2.2.3. Spray drying encapsulation of torch ginger extract

94 Prior to the process, a mixture made up of torch ginger extract (10%), water (80%), maltodextrin as an
95 encapsulating agent (7%), and emulsifiers (glyceryl monostearate and sodium stearyl lactylate) each
96 at 1.5% was prepared. Subsequently, the prepared mixture was evenly mixed and homogenised for 15
97 minutes at 6000 rpm by using a Silverson L5M-A laboratory mixer (Silverson Machines, Inc., East
98 Longmeadow, Massachusetts, United States of America). The aforementioned machine was operated
99 with a short stop at every consecutive 5-minute to allow the cool down of the rotor blades. The mixture
100 was further homogenised for 30 minutes by applying speed at 5800 rpm using a GEA Lab Homogeniser
101 PandaPLUS 2000 (GEA Group Aktiengesellschaft, Düsseldorf, Germany). The formed emulsion was
102 dried using a Büchi B-290 spray dryer (Büchi Labortechnik AG, Flawil, Switzerland) which equipped
103 with an atomiser nozzle (0.5 mm in diameter) at 15 MPa. The spray drying process was conducted
104 under the following conditions: inlet air temperature at 130 °C, outlet air temperature at 50 ± 1 °C, flow
105 rate fixed at 150 L/h, and feed suspension rate set at 180 mL/h. Upon completion, the obtained powder
106 was transferred into an amber glass bottle and tightly sealed. The bottle was stored in a refrigerator for
107 storage at a refrigerated condition (± 4 °C). Schematic diagram of the supercritical fluid extraction
108 (SFE) process and the encapsulation of torch ginger extract by spray drying is showed in Fig. 1.

109

110 [Fig. 1 about here]

111

112 Yield of the encapsulated torch ginger extract powder (TGEP) obtained from the process was
113 determined according to the equation used by Navarro-Flores et al. (2020) which given as follows:

114

$$115 \text{Yield}_{\text{TGEP}} (\%) = \text{Powder obtained (g)} / \text{Solids in the feed solution (g)} \times 100 \% \quad (1)$$

116

117 2.3. Analysis of Powder Characteristics

118 2.3.1. Particle size analysis and BET analysis

119 The particle size distribution of TGEP was studied using a Mastersizer 2000 particle size analyser
120 equipped with Scirocco 2000 sample dispersion unit (Malvern Instruments Ltd., Malvern, United
121 Kingdom). Prior to analysis, the refractive index of TGEP was determined at 1.52 by using a PAL-RI
122 refractometer (ATAGO Co., Ltd., Tokyo, Japan). TGEP was precisely weighed at 2.0 g, and it was
123 loaded into the hopper attached to the Scirocco 2000 sample dispersion unit, and the pressure was set
124 at 4 bar. In addition, the specific surface area and porosity distribution of TGEP were investigated with
125 Brunauer-Emmett-Teller (BET) analysis through a Micromeritics ASAP 2000 equipment. Before
126 analysis, the sample was degassed for 30 min at 60 °C under a continuous nitrogen gas flow.

127

128 2.3.2. FTIR spectroscopy analysis

129 The metabolite screening of TGEP was performed using a Spectrum 100 Fourier Transform Infrared
130 Spectrophotometer (FTIR) (PerkinElmer Inc., Waltham, United States of America) by using method
131 used in our previous study (Naziruddin et al., 2021). The scanning range of the FTIR was set from 4000
132 – 600 cm⁻¹ with a scanning resolution of 4 cm⁻¹. By grinding, potassium bromide (KBr) was combined
133 with TGEP and the powder mixture was pelletised before analysis.

134

135 2.4. Metabolites Profiling by GC-MS

136 Prior to Gas Chromatography-Mass Spectrometry (GC-MS) analysis, TGEP was derivatized according
137 to the method used by Robinson et al. (2005). Briefly, 25.0 mg of TGEP was placed inside a 2.0 mL

138 centrifuge tube, and about 50.0 μL of anhydrous pyridine was then added into it. The mixture was
139 subjected to sonication for a duration of 10 minutes at 30 °C by using an Elmasonic S 30 (H) ultrasonic
140 device (Elma Schmidbauer GmbH, Singen, Germany). Subsequently, about 100 μL of methoxyamine
141 HCl (20 mg/mL in pyridine) was pipetted into the solution and it was vortexed for one minute. Two
142 consecutive incubation was conducted by which the solution was initially incubated for 2 hours at 60
143 °C and it was again incubated for 30 minutes at 60 °C upon the addition of 300 μL of MSTFA. Lastly,
144 the solution was filtered using a 0.22 μm nylon syringe filter, and the filtered liquid was then transferred
145 to an amber vial to be left at room temperature overnight.

146 The GC-MS method used in our previous study was followed for the identification of metabolites in
147 TGEP and some modifications were made to improve the detection (Naziruddin et al., 2022). The
148 derivatized fraction of TGEP was analysed by using a TSQ Quantum XLS GC-MS system (Thermo
149 Scientific, United States of America). TGEP's aliquot was injected (1 μL injection volume) into an
150 Agilent J&W DB-5MS column (length: 30 m, inner diameter: 0.25 mm, and film thickness: 0.25 mm)
151 (Agilent Technologies, California, United States of America) in split-less mode and the carrier gas used
152 was helium at 1.0 mL/min. The column was first held at 80 °C for 5 min and afterwards increased at 8
153 °C/min to 200 °C. Subsequently, the temperature of the oven was gradually raised to 280 °C at a ramp
154 rate of 4 °C/min, and maintained at that level for 15 minutes. The temperature of the ion source and
155 interface were regulated at 280 °C and 250 °C, respectively. The GC-MS analysis was performed in a
156 total ion chromatography (TIC) mode and the full scan data was collected within a mass scan range of
157 40 to 600 m/z. To identify the compounds present, the acquired mass spectra for each chromatographic
158 peak were compared with a retention time index and mass spectral libraries for GC-MS that were
159 created by the National Institute of Standards and Technology (NIST). The data version used for this
160 analysis was NIST17 (NIST, 2017).

161

162 **2.5. Analysis of Antioxidant Activity**

163 **2.5.1. Quantification of the Total Phenolic Content**

164 Total Phenolic Content (TPC) in TGEP was quantified using the Folin-Ciocalteu method (Xiao et al.,
165 2020). TGEP (precisely weighed at 0.5 mg) was dissolved in 1 mL ethanol and shaken for 1 minutes.

166 Meanwhile, ethanolic gallic acid (GA) calibration solutions were prepared at five concentration levels
167 which ranging from 6.25×10^{-3} mg/mL – 0.1 mg/mL. Briefly, approximately 0.1 mL of the extract was
168 pipetted into a test tube and mixed with 0.5 mL of 50% Folin-Ciocalteu reagent. Subsequently, the
169 solution was mixed using a vortex mixer (OHAUS Corporation, New Jersey, United States of America)
170 for 3 minutes and about 7.9 mL of distilled water was added to the tube. The solution was allowed to
171 set at room temperature for 5 minutes. Following that, 7.5% sodium carbonate solution was added to
172 made the final volume of 10 mL and later it was incubated in a dark room (± 28 °C) for 2 hours.
173 Throughout the incubation, the tube was periodically shaken at every 30 minutes to ensure it was fully
174 reacted. Both sample and calibration standard solutions were determined for its absorbance at the
175 wavelength of 765 nm by using a ultraviolet-visible (UV-Vis) spectrophotometer of the GENESYS™
176 10S model (Thermo Fisher Scientific, Waltham, United States of America). The TPC's result was
177 expressed as milligram (mg) of gallic acid equivalents (GAE) per gram (g) of TGEP (mg GAE/g
178 TGEP). Calibration curve of the gallic acid standard was constructed and its linear equation was used
179 to estimate the TPC value of TGEP.

180

181 **2.5.2. DPPH radical scavenging capacity assay**

182 The DPPH assay was performed based on previously reported methods with minor alterations (Trucillo
183 et al., 2018). Sample was prepared by dissolving TGEP in ethanol at five different concentrations (0.1
184 mg/mL, 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL, and 6.25×10^{-3} mg/mL in ethanol). Meanwhile,
185 all five levels of GA calibration solutions were also prepared in ethanol which ranging from 0.05 mg/mL
186 to 3.125×10^{-3} mg/mL in concentration. Precisely weighed DPPH at 0.0197 g was dissolved in 500 mL
187 ethanol to make a 10^{-4} M solution. Briefly, about 1 mL of the diluted TGEP was mixed with 3.0 mL
188 DPPH solution in a test tube and intensely shaken for 1 minutes. The test tube was allowed to incubate
189 for 30 minutes in a dark environment at ambient temperature. A UV-Vis spectrophotometer (similar
190 model used for TPC assay) was used to measure the absorbance at a wavelength of 517 nm for the
191 sample, standard, and control (diluted DPPH in ethanol absolute). Scavenging capacity (%) of the
192 sample and standards were estimated by solving the equation (2) as follows:

193

194 Scavenging capacity (%) = $[1 - (abs. A / abs. B)] \times 100 \%$ (2)

195

196 ³ Where *abs. A* is the absorbance of sample or standards and *abs. B* is the control's absorbance. Reduction
197 of the initial DPPH radical concentration by 50% based on its respective TGEP's concentration was
198 represented by EC₅₀ value.

199

200 ¹ 2.5.3. Ferric reducing antioxidant power (FRAP) assay

201 ² FRAP assay was conducted by applying methods described by Benzie and Strain (1996) and Tomasina
202 ² et al. (2012) with slight modifications. Freshly prepared FRAP reagent solution was prepared by
203 combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM hydrochloric acid), and
204 20 mM ferric chloride hexahydrate (FeCl₃) with a volume (mL) ratio of 25: 2.5: 2.5. Ethanolic TGEP's
205 extract was prepared by dissolving the sample (1 mg) in 1 mL of ethanol. Meanwhile, Trolox calibration
206 solutions in the concentration of 19.977 μM, 9.988 μM, 4.994 μM, 2.497 μM, and 1.251 μM were also
207 prepared in ethanol. ³³ The assay was performed by mixing 8.7 mL of FRAP reagent with 0.3 mL TGEP's
208 ethanolic extract and it was put aside to incubate at 50 °C for 1 hour. ⁵¹ Using a UV-vis spectrophotometer,
209 ²⁵ the absorbance at 593 nm was measured to monitor the reduction of ferric iron (Fe³⁺) to ferrous iron
210 ³⁹ (Fe²⁺) by the antioxidants present in both TGEP and the standard. Results were expressed as micromolar
211 (μM) of Trolox equivalents (TE) per g of TGEP (μM TE/g TGEP). Calibration curve based on the
212 Trolox standards was constructed and the obtained equation was used to calculate the FRAP value.

213

214 ⁴² 3. Results and Discussion

215 3.1. Yield of the encapsulated torch ginger-extract powder

216 In the process, about 400 g of the prepared emulsion with a total solid mass of 80.0 g (50:50 torch
217 ginger's extract and encapsulating agent) was fed into the spray dryer, which produced 47.8 g of TGEP.
218 Hence, based on the determination by equation (1), the process managed to yield around 59.8% of
219 TGEP. The obtained yield in this study was found to be substantially ⁴ higher than the yields reported by
220 Anuar et al. (2021) which were in the range of 15 % – 36 % based on various formulations tested.

221

222 **3.2. Particle size analysis and BET analysis**

223 Fig. 2 displays the distribution of particle size for encapsulated torch ginger-extract powder. The median
224 diameter, $D_{0.5}$ for TGEP was measured at $6.188 \pm 0.771 \mu\text{m}$. Meanwhile, the sample revealed two
225 distinct particle sizes concentrating at $2.2 \mu\text{m}$ and $17.4 \mu\text{m}$, respectively. This non-uniform size
226 distribution was likely due to the formation of large agglomerates as a result of spray drying at low inlet
227 air temperature ($\leq 140 \text{ }^\circ\text{C}$) (Both et al., 2020). Additionally, Siccama et al. (2021) also mentioned that
228 the presence of high residual moisture content and low glass transition temperature of the spray dried
229 powder might increase its stickiness which leads to agglomeration. Nonetheless, the produced TGEP
230 showed relatively small size of particles which was less than $50 \mu\text{m}$.

231

[Fig. 2 about here]

232

233

234 In BET analysis (Table 1), TGEP was examined with the presence of considerably high surface area,
235 pore volume, and pore size features. Surface area purportedly affected the functionality of powders:
236 solubility, flowability, rehydration, and wetting characteristics. Furthermore, high surface area of
237 functional powder also contributed to a high degree of solvation which is paramount for the absorption
238 of retained compounds (Burgain et al., 2017; Koç & Kaymak-Ertekin, 2014). The outcomes' trend of
239 BET analysis were in agreement with results reported by Zhang et al. (2018), as the spray-dried powder
240 obtained from their research exhibited higher surface area (ranging from 1.54 to $2.18 \text{ m}^2/\text{g}$) with greater
241 porosity. The authors also reported that the physical characteristics of spray-dried powder often affected
242 by the spray drying inlet air temperature and type of atomiser. Zhang et al. (2018) also reported that the
243 inlet air temperature in between $120 \text{ }^\circ\text{C}$ to $160 \text{ }^\circ\text{C}$ can induced the increased of powder's surface area
244 due to formation of particles with dryer and harder coating. The aforementioned inlet air temperature
245 reflected to this study as the spray drying process was conducted at $130 \text{ }^\circ\text{C}$. Hence, similar powder
246 characteristics reported by Zhang et al. (2018) were expected

247

[Table 1 about here]

248

250 3.3. FTIR analysis

251 The FTIR spectrum of TGEP is displayed in Fig. 3, and the peaks in the fingerprint region were assigned
252 based on their corresponding functional groups in Table 2. A wide band was observed extending from
253 3000 cm^{-1} to 3600 cm^{-1} , which corresponded to the O-H stretching vibration of alcohols and carboxylic
254 acids in the sample. Several researchers have been reported the present of alcohols, such as 1-
255 Dodecanol, Tetradecanol, and 1-Undecanol in the torch ginger extract. Meanwhile, carboxylic acid that
256 predominantly fatty acids such as Hexacosanoic acid, Decanoic acid, and Hexadecanoic acid also has
257 been reported to present in torch ginger extract (Al-Mansoub et al., 2021; Anzian et al., 2020; Marzlan
258 et al., 2020). The spectra showed two intense sharp peaks at 2853 cm^{-1} and 2925 cm^{-1} which were in
259 relation to the C-H stretching vibration of alkyl group. Additionally, the presence of alkene in TGEP
260 can be identified by a medium peak formed at 1463 cm^{-1} which corresponded to C-H bending of
261 methylene group and a 1652 cm^{-1} shoulder band which representing the double bonds (C=C) vibrational
262 stretching of alkenyl group. Alkane and alkene which belong to the class of hydrocarbons also has been
263 reported by Al-Mansoub et al. (2021), as the author managed to identify the presence of Cyclododecane,
264 Heptadecane, 1-Decene, and 1-Tetradecene in the ethanolic torch ginger extract. The sample also
265 exhibited a sharp peak at 1727 cm^{-1} which indicating the C=O stretching of carbonyl group such
266 aldehyde, ester, and carboxylic acid. This finding was in agreement with results reported by Marzlan
267 et al. (2020), as the authors identified esters such as Lauryl acetate, Myristyl myristate, and (E)-9-
268 Tetradecen-1-ol acetate in torch ginger oil extracted by supercritical fluid extraction. Anhydride group
269 also potentially present in TGEP as indicated by the intense peak formed at 1025 cm^{-1} which attributing
270 to the CO-O-CO stretching of the functional group. Subsequently, a sharp peak present at 1200 cm^{-1}
271 signalled the C-N stretching of amine, while a broad peak formed at 1250 cm^{-1} to 1310 cm^{-1} might be
272 corresponded to the C-O stretching of aromatic ester. Lastly, a noticeable peak formed at 750 cm^{-1} could
273 be associated to C-H bending of 1,2-disubstituted and monosubstituted compounds that present in
274 TGEP. Hence, the results proved that the presence of chemically-diverse compounds in TGEP was
275 valid.

276

277

[Fig. 3 about here]

278

279

[Table 2 about here]

280

281 **3.4. Metabolite profiling and identification by GC-MS**

282 GC-MS chromatogram of the detected metabolites in TGEP was showed in Fig. 4. Broad array of
283 masses was acquired (scan range 40 – 600m/z) within the scan time of 5.0 to 55.0 minutes and presence
284 of metabolites were monitored at various retention times (RTs). The metabolites' identities were
285 confirmed by comparing the generated spectral pattern with those of established ⁵³ spectral library
286 developed by National Institute of Standards and Technology (NIST), data version NIST17 (NIST,
287 2017). Additionally, the detected metabolites were classified by referring to the comprehensive
288 database of FooDB which was developed by the renounce research teams specialising in metabolomics
289 (TMIC, 2021).

290

291

[Fig. 4 about here]

292

293 Altogether, about 59 metabolites belong to the 19 different classes of compounds ⁷⁴ were identified in
294 TGEP as listed in Table 3. The number of metabolites able to be detected in this study is substantially
295 higher and more diversified than other reported torch ginger's metabolite studies. In literature, ⁵⁶ studies
296 conducted by Marzlan et al. (2020) and Anzian et al. (2020) reported the numbers of metabolite found
297 in torch ginger's essential oil at 20 and 33 respectively. In brief, most of the detected metabolites were
298 belong to four major classes which were fatty acids (30.5%), terpenes and derivatives (20.3%), fatty
299 acid esters (16.9%), and alcohols (8.47%).

300

301

[Table 3 about here]

302

303 Among these major classes of metabolites, alcohols particularly 1-Dodecanol was reported to render a
304 potent antibacterial activity against several bacterial species (Marzlan et al., 2022; Marzlan et al., 2020).

305 Additionally, the pharmacological activities of terpenoids were also well-known and heavily studied in

306 the literature. Uvaol, a triterpene, was reported by Agra et al. (2016) to be effective as an active
307 ingredient for the treatment of inflammation caused by allergic reaction.

308

309 Torch ginger notoriously known for its distinctive aroma which is a vital characteristic of Southeast
310 Asian's cuisine. Expert described that torch ginger embodies a sweet, tangy, and lemongrass-like aroma
311 profile (Khor et al., 2017). Although the key aromatic compounds that responsible for torch ginger's
312 aroma have never been reported in literature, such study was already being conducted for ginger
313 (*Zingiber officinale* Roscoe) and galangal (*Kaempferia galanga* L.) which also belong in the
314 *Zingiberaceae* family. The researchers identified the presence of metabolite composition that consist of
315 aldehyde, alcohol, hydrocarbon, ketone, terpene, and ester to be accountable for the distinctive aroma
316 of both herbs (Hasegawa et al., 2016; Pang et al., 2017). In spite of the differences in species, similar
317 composition as previously mentioned was also found in TGEP and the aroma profiles exude by the
318 compounds could be assumed similar to torch ginger. Pang et al. (2017) mentioned that the presence of
319 primary odorants in ginger namely, monoterpenes and sesquiterpenes could be associated to the woody,
320 minty, citrusy, and herbal-like aroma. The researchers also attributed the sweet notes (balsamic and
321 floral) of ginger to the presence of metabolites in the class of alcohol, aldehyde, terpene and terpene
322 derivative. Additionally, humulene, a sesquiterpene found in TGEP was also reportedly presence in
323 galangal and it was presumed to emits the unique galangal-like aroma (Hasegawa et al., 2016). The
324 formerly defined aroma profile of ginger and galangal are noticeably similar to the general description
325 of torch ginger's aroma. Therefore, it is presumed that the key aromatic compounds of torch ginger
326 were retained in the TGEP, which would be beneficial for the application in food.

327

328 **3.5. Antioxidant activity**

329 The antioxidant capacity of TGEP was evaluated based on the performance of TPC, DPPH, and FRAP
330 as showed in Table 4. The quantified value of TPC for TGEP was found to be higher than other reported
331 values in literature which were in the range of 2.12 – 19.4 mg GAE/g (Anzian et al., 2017; Yan &
332 Asmah, 2010). The TPC values of the aforementioned studies were based on the quantification in the
333 fresh and dried forms of torch ginger. Therefore, it indicates that the phenolic compounds present in

334 torch ginger retained at an exceptional level in TGEP. Additionally, this claimed also supported by the
335 presence of Hexadecyl-(E)-p-coumarate which is a phenolic acid identified in TGEP by the GC-MS.

336

337 [Table 4 about here]

338

339 ¹ The free radical scavenging capacity of TGEP was predicted based on its antioxidants ability to reduce
340 ⁸⁶ DPPH radical. The activity of scavenging DPPH radicals was measured by the EC₅₀ value in which it
341 indicates the effective TGEP's concentration needed to reduce the DPPH radical's absorbance by 50%.
342 ⁵⁸ The value of EC₅₀ for TGEP was determined at 1.31 ± 0.002 mg/mL, which was substantially lower
343 than such value reported by Nurain et al. (2013) at 3.47 ± 0.420 mg/mL based on the determination in
344 ethanolic torch ginger's extract. This low value of EC₅₀ for TGEP signified its high antioxidant activity
345 which could potentially linked to its high TPC value. Additionally, the quantified FRAP value of TGEP
346 also was found to be substantially higher as compared to such values reported by Bunleu and Buavaroon
347 (2018) and Wijekoon et al. (2011) which were in the range of 9.0 – 130 µM Fe(II)/g. The excellent
348 performance of TGEP's antioxidants in the assay demonstrated its high reactivity against the Fe³⁺ -
349 TPTZ and effectively reduced it to Fe²⁺ - TPTZ. Hydroxyl and carbonyl-rich compounds in plants have
350 been associated with excellent reducing capabilities and stabilisers (Mohamad et al., 2014; Pradeep et
351 al., 2022). Based on Table 3, TGEP possessed abundant compounds with sufficient hydroxyl and
352 carbonyl groups present, such as dodecanal (aldehyde), xanthophyll, and β-Sitosterol (terpene)
353 (Mahavy et al., 2022; Tovey, 2019). The oxidation-reduction abilities of these compounds allow the
354 binding of metals and inactivate them via chelation (Azri et al., 2019).

355

356 The outstanding performance of TGEP in the aforementioned antioxidant assay could be linked to the
357 existence of various metabolites in the class of terpenoids as profiled in Table 3. Terpenoid has been
358 vastly studied in literature and the evidences for its potency as an antioxidant were well recorded.
359 Terpenoids, namely humulene and uvaol found in TGEP have been reported in multiple studies able to
360 induce the reduction of oxidative stress by effectively control the autoxidation reaction (Allouche et al.,
361 2010; Gunawan et al., 2016). Additionally, the abundance of TGEP's metabolites with a hydroxyl,

362 methoxy, and carboxylic acid groups could potentially contribute to the high antioxidant capacity as
363 these functional group were reported to render a vital effect on the antioxidant ability (Chen et al.,
364 2020).

365

366 **3.6. Potential future as a plant-based functional food additive**

367 The development of functional food additives derived from plants has aroused the interest of consumers
368 for a much healthier alternative and reduced the dependency on its artificial counterparts (Domínguez
369 et al., 2021). However, various shortcomings in applying plant extract at the industrial scale might
370 induce complexity in the manufacturing process and not be economically sound. Plant extract must be
371 handled with the utmost care as it is highly volatile, hydrophobic, and prone to stability issues when
372 exposed to environmental stressors (e.g., extreme temperature, light) (Kfoury et al., 2016; Rezaei &
373 Nasirpour, 2019). The encapsulation technique has been known to offset these problems as it can trap
374 the plant's bioactive compounds in an encapsulating agent and makes it more stable (Muñoz-Shugulí
375 et al., 2021). Based on the evaluations, TGEP has demonstrated the retention of various metabolites
376 with different bioactive functions and maintained its antioxidant capability. This technique also captures
377 the key aromatic compounds of torch ginger, which would be vital for its application as a food additive.

378

379 **4. Conclusion**

380 Present study revealed the extraction, encapsulation and comprehensive metabolite profiling of torch
381 ginger-extract powder. Spray drying encapsulation process managed to yield around 59.8% of TGEP
382 by incorporating 10% supercritical fluid-torch ginger extract into encapsulating agent mixture, which
383 the obtained yield was twice higher than other study. Based on the performed analyses, the developed
384 powder showed the present of varying valuable bioactive compounds. From the particle size analysis,
385 TGEP revealed aggregated feature, which shown by two distinct particle sizes concentrating at 2.2 μm
386 and 17.4 μm , respectively. BET analysis of TGEP unveiled a considerably high surface area (1.13 m^2/g),
387 pore volume (0.218 cm^3/g), and pore size (384.6 nm) which were purportedly affected by the spray
388 drying inlet air temperature. The FTIR analysis revealed the presence of O-H, C-H, C=C, C=O, CO-O-
389 CO, C-N, and C-O functional groups in the sample. Meanwhile, based on the GC-MS analysis, about

390 59 metabolites that predominantly fatty acids (30.5%), terpenes and derivatives (20.3%), fatty acid
391 esters (16.9%), and alcohols (8.47%) were identified in TGEP. TGEP also demonstrated an excellent
392 antioxidant capacity based on its high activity in the DPPH radical scavenging capacity (1.31 ± 0.002),
393 ferric reducing antioxidant power assay (2919.5 ± 19.9), and high value of total phenolic content (23.3
394 ± 0.662)⁸³ in comparison to the previous studies. Therefore, this study has indicated that the developed
395 encapsulated torch ginger extract powder able to retain the beneficial bioactive compounds which
396 makes it a promising functional powder.

397

398 **Conflict of Interest**

399 Authors declared no conflict of interest¹⁸

400

401 **Data Availability**

402 All data generated or analysed during this study are included in this published article.

403

404 **Tables and figures**405 **Table 1.** BET analysis data of TGEP

Sample	Surface Area (m ² /g)	Pore Volume (cm ³ /g)	Pore Size (nm)
TGEP	1.131	0.218	384.6

406

407 **Table 2.** Assignment of FTIR spectra peaks and corresponding functional groups

Wavenumbers (cm ⁻¹)	Band assignment
3000 cm ⁻¹ to 3600 cm ⁻¹	O-H stretching vibration of alcohols and carboxylic acids
2853 cm ⁻¹ and 2925 cm ⁻¹	C-H stretching vibration of alkyl group
1463 cm ⁻¹	C-H bending of methylene group
1652 cm ⁻¹	Double bonds (C=C) vibrational stretching of alkenyl group
1727 cm ⁻¹	C=O stretching of carbonyl group
1025 cm ⁻¹	CO-O-CO stretching of anhydride group
1200 cm ⁻¹	C-N stretching of amine
1250 cm ⁻¹ to 1310 cm ⁻¹	C-O stretching of aromatic ester
750 cm ⁻¹	C-H bending of 1,2-disubstituted and monosubstituted compounds

408

409 **Table 3.** Metabolites composition in TGEP as identified by GC-MS.

Nr.	Metabolite	Retention time (min)	Molecular formula	Molecular weight (g/mol)	Probability (%)
<i>Acetate Esters</i>					
1.	1-Tetradecyl acetate	20.14	C ₁₆ H ₃₂ O ₂	256	23.72
2.	Lauryl acetate	17.39	C ₁₄ H ₂₈ O ₂	228	36.04
<i>Acid anhydride</i>					
3.	2,5-Furandione, 3-dodecyl-	32.45	C ₁₆ H ₂₆ O ₃	266	10.42
<i>Alcohols</i>					
4.	1-Dodecanol	15.35	C ₁₂ H ₂₆ O	186	13.35
5.	11-Tetradecen-1-ol, (E)-	18.22	C ₁₄ H ₂₈ O	212	11.34
6.	cis-9-Tetradecen-1-ol	18.22	C ₁₄ H ₂₈ O	212	10.46
7.	cis-11-Tetradecen-1-ol	18.22	C ₁₄ H ₂₈ O	212	10.06
8.	1-Heptatriacotanol	29.96	C ₃₇ H ₇₆ O	536	24.73
<i>Aldehyde</i>					
9.	Dodecanal	10.09	C ₁₂ H ₂₄ O	184	44.48
<i>Amine</i>					
10.	2,6-Octadien-1-amine, 3,7-dimethyl-	11.76	C ₁₀ H ₁₉ N	153	12.92
<i>Carboxylic esters</i>					
11.	4-Azido-2-nitrobutyric acid, 2,6-di-t-butyl-4-methoxyph	34.96	C ₁₉ H ₂₈ N ₄ O ₅	392	21.06

	enyl ester				
	<i>Coumaric acid ester</i>				
12.	Hexadecyl-(E)-p-coumarate, trimethylsilyl ether	38.38	C ₂₈ H ₄₈ O ₃ Si	460	36.63
	<i>Dithiane</i>				
13.	2-[3-(1-Ethoxyethoxy)propyl][1,3]dithiane	24.29	C ₁₁ H ₂₂ O ₂ S ₂	250	28.96
	<i>Fatty acids</i>				
14.	Dodecanoic acid, 3-hydroxy-	10.43	C ₁₂ H ₂₄ O ₃	216	12.20
15.	Dodecanoic acid	16.04	C ₁₂ H ₂₄ O ₂	200	63.76
16.	Dodecanoic acid, TMS derivative	17.59	C ₁₅ H ₃₂ O ₂ Si	272	56.20
17.	Undecanoic acid, TMS derivative	18.06	C ₁₄ H ₃₀ O ₂ Si	258	10.35
18.	Tetradecanoic acid	19.55	C ₁₄ H ₂₈ O ₂	228	39.02
19.	Myristic acid, TMS derivative	20.71	C ₁₇ H ₃₆ O ₂ Si	300	68.45
20.	Tridecanoic acid	22.34	C ₁₃ H ₂₆ O ₂	214	30.30
21.	Pentadecanoic acid	22.34	C ₁₅ H ₃₀ O ₂	242	18.22
22.	Palmitelaidic acid, TMS derivative	23.22	C ₁₉ H ₃₈ O ₂ Si	326	58.40
23.	Petroselinic acid, TMS derivative	23.22	C ₂₁ H ₄₂ O ₂ Si	354	11.55
24.	Palmitic Acid, TMS derivative	23.68	C ₁₉ H ₄₀ O ₂ Si	328	45.52
25.	Tridecanoic acid, TMS derivative	11.42	C ₁₆ H ₃₄ O ₂ Si	286	11.42
26.	Pentadecanoic acid, TMS derivative	10.98	C ₁₈ H ₃₈ O ₂ Si	314	10.98
27.	Octadecanoic acid	25.54	C ₁₈ H ₃₆ O ₂	284	11.19
28.	9,12-Octadecadienoic acid (Z,Z)-, TMS derivative	26.42	C ₂₁ H ₄₀ O ₂ Si	352	22.10
29.	Stearic acid, TMS derivative	26.90	C ₂₁ H ₄₄ O ₂ Si	356	37.56
30.	Heptadecanoic acid, TMS derivative	26.90	C ₂₀ H ₄₂ O ₂ Si	342	25.76
31.	2-Oleoylglycerol, 2TMS derivative	35.50	C ₂₇ H ₅₆ O ₄ Si ₂	500	16.18
	<i>Fatty acid esters</i>				
32.	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	25.54	C ₂₁ H ₃₈ O ₃	338	12.67
33.	9(E),11(E)-Conjugated linoleic acid, trimethylsilyl ester	26.42	C ₂₁ H ₄₀ O ₂ Si	352	36.47
34.	Butanoic acid, 4-cyano-2-nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxyphenyl ester	34.96	C ₂₀ H ₂₈ N ₂ O ₅	376	13.18
35.	cis-9-Tetradecenoic acid,	43.13	C ₂₁ H ₄₀ O ₂	324	43.13

	heptyl ester				
36.	cis-9-Tetradecenoic acid, isobutyl ester	35.88	C ₁₈ H ₃₄ O ₂	282	10.33
37.	Tetradecanoic acid, 2-oxo-, ethyl ester	37.61	C ₁₆ H ₃₀ O ₃	270	51.47
38.	Hexadecanoic acid, octadecyl ester	39.29	C ₃₄ H ₆₈ O ₂	508	23.56
39.	Hexadecanoic acid, tetradecyl ester	39.29	C ₃₀ H ₆₀ O ₂	452	16.16
40.	Hexadecanoic acid, hexadecyl ester	39.29	C ₃₂ H ₆₄ O ₂	480	11.41
41.	Oleic acid, eicosyl ester	46.10	C ₃₈ H ₇₄ O ₂	562	14.32
	<i>Hydrocarbon</i>				
42.	17-Pentatriacontene	41.55	C ₃₅ H ₇₀	490	14.77
	<i>Ketones</i>				
43.	Cyclododecanol	10.09	C ₁₂ H ₂₄ O	184	16.24
44.	(Z)-18-Octadec-9-enolide	25.16	C ₁₈ H ₃₂ O ₂	280	14.66
45.	15-Isopropenyl-3-(trimethylsilyl)oxacyclopentadecan-2-one	26.42	C ₂₀ H ₃₈ O ₂ Si	338	26.42
	<i>Phytoestrogen and derivative</i>				
46.	Estra-1,3,5(10)-trien-17β-ol	25.54	C ₁₈ H ₂₄ O	256	14.99
	<i>Xanthophyll</i>				
47.	.psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	45.88	C ₄₂ H ₆₄ O ₂	600	7.79
	<i>Terpenes and derivatives</i>				
	<i>Monoterpenes</i>				
48.	3-Cyclohexene-1-methanol, 5-hydroxy-α,α,4-trimethyl-	13.79	C ₁₀ H ₁₈ O ₂	170	14.18
49.	trans-3(10)-Caren-2-ol	13.79	C ₁₀ H ₁₆ O	152	13.79
	<i>Monoterpene derivative</i>				
50.	Sobrerol 8-acetate	13.79	C ₁₂ H ₂₀ O ₃	212	14.18
	<i>Sesquiterpenes</i>				
51.	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	15.17	C ₁₅ H ₂₄	204	33.15
52.	Humulene	15.17	C ₁₅ H ₂₄	204	24.07
53.	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	15.68	C ₁₆ H ₂₆ O ₂	250	11.47
	<i>Triterpenes</i>				
54.	ς-Sitosterol	46.47	C ₂₉ H ₅₀ O	414	15.68
55.	β-Sitosterol	46.67	C ₂₉ H ₅₀ O	414	30.42
56.	Uvaol, 2O-TMS	47.19	C ₃₆ H ₆₆ O ₂ Si ₂	586	10.48
57.	β-Sitosterol, TMS derivative	47.51	C ₃₂ H ₅₈ OSi	486	10.54

58.	Stigmast-5-ene, 3 β -(trimethylsiloxy)-, (24S)-	47.51	C ₃₂ H ₅₈ OSi	486	52.32
59.	<i>Terpene alcohol</i> 7,8-Epoxyloganostan-11-ol, 3-acetoxy-	41.08	C ₃₂ H ₅₄ O ₄	502	17.48

410

411 **Table 4.** Antioxidant activity of the encapsulated torch ginger-extract powder.

TPC (mg GAE/g TGEP)	EC ₅₀ of DPPH radical scavenging ability (mg/mL)	FRAP (μ M TE/g TGEP)
23.3 \pm 0.662	1.31 \pm 0.002	2919.5 \pm 19.9

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Values are means of triplicate determination \pm SD.

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434

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437 **3. Fig. 3.** FTIR spectrum of TGEP.

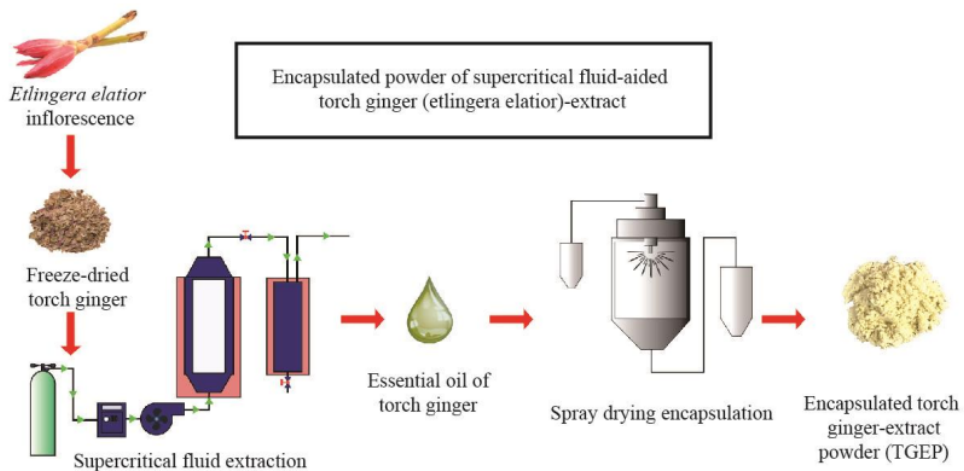
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439 **4. Fig. 4.** The chromatogram obtained by GC-MS (TIC) displays the metabolites that have been

440 identified in TGEP.

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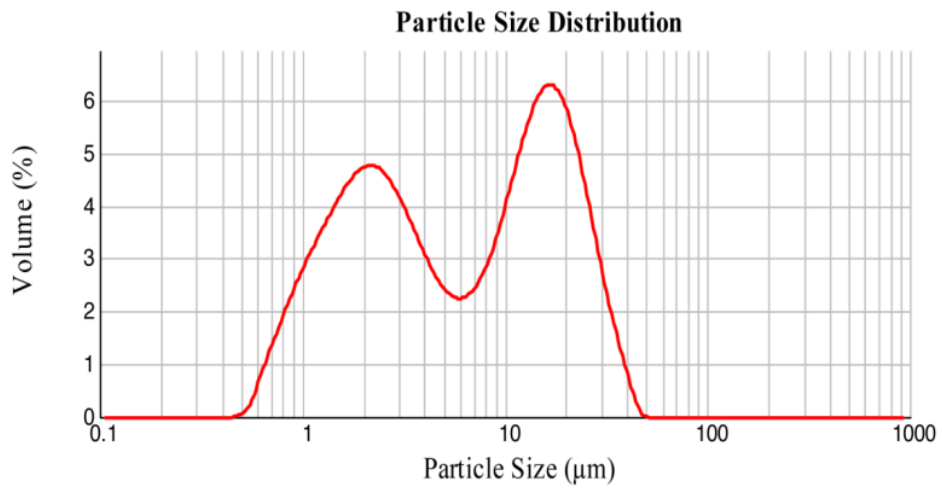
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468 **Fig. 2.** Particle size distribution of TGEP.

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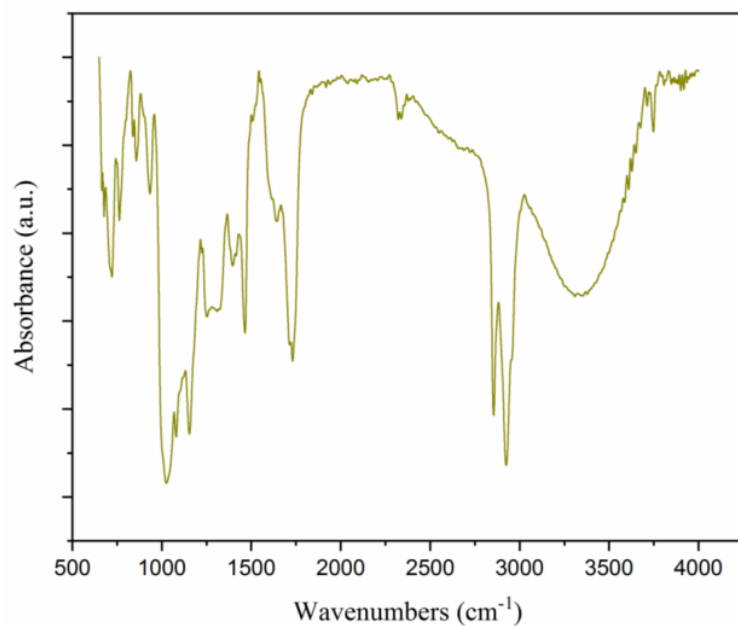
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482 **Fig. 3.** FTIR spectrum of TGEP.

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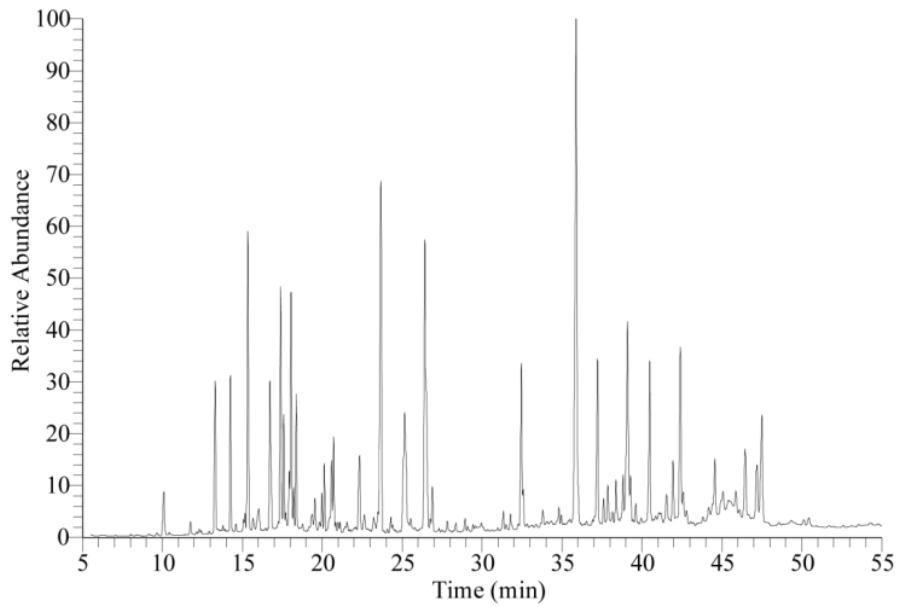
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