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ANTIOXIDATIVE PROPERTIES OF SELECTED MICRO-ENCAPSULATED PLANTS POWDER PREPARED USING ULTRASONIC SPRAY-DRYING TECHNIQUE

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ABSTRACT

Ultrasonic spray drier technology has been practice because of its numerous advantages, including providing more uniform droplets and reducing bioactive compounds damages. This study was aimed to measure the antioxidant properties of several microencapsulated plants powder (MPP) prepared using ultrasonic spray dying technique. The plant samples were treated using ultrasonic spray drier at 80°C inlet temperatures and 10% of gum Arabic relative to solid content. The collected MPP were analysed for their antioxidant activities. The plants use in this study were *C. ternatea, M. indica, S. rebaudiana, P. macrocarpa, K. salvarezii* and *R. apiculata,* All MPP showed somewhat very promising high antioxidative activities with *C. ternatea* significantly exhibited the highest ($P<0.05$) antioxidant power in nearly all antioxidative test analysis performed. On the other hand, *M. indica* showed the least ability in antioxidant power and the content of bioactive compounds. Results of the study point that the production of MPP and microcapsules is feasible as a functional ingredient in food industry as it can retain the antioxidative properties, which could lead to a more sustainable usage of natural resources.

Key words: Ultrasonic spray drying, antioxidative activities, microencapsulated plants powder (MPP)

INTRODUCTION

Plants researches have generated special interest because of their bioactive properties, as they are rich in bioactive compounds such as flavonoids, anthocyanins and proanthocyanins. Various extraction and preservation techniques have been used for extracting these bioactive compounds from plant extraction ultrasonic extraction method (Ferrari *et al*., 2012). Anthocyanins are one of the most interesting groups of plant compounds and they were found to reduce cancer and heart disease risks with properties of holding oxygen (Duthie *et al*., 2006). Therefore, apart from existing methods, alternative production methods that could retain anthocyanins have to be explore. However, to the best of our knowledge the ultrasonic low temperature extraction and gum Arabic encapsulation has not yet been fully explored. Ultrasonic extraction is inexpensive and very simple yet permits higher surface area contact between sample and solvent (Benita, 2006). Thus, the present study is conducted to investigate the influence of temperature used and gum Arabic used.

Ultrasonic spray-drying of plants pulp is an alternative technology for better plants conservation and has economic potential. The reduced contact time as well as the low temperatures makes ultrasonic spray-drying suitable for highly sensitive food components (Ferrari *et al*., 2013). The liquid plants juice is converted into a solid form via this

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method, which could increase storage stability, protects phenolic compounds, and provides high antioxidant power. However, there are some disadvantages of this type of drying such as stickiness and hygroscopicity of products influenced by the presence of low molecular weight sugars and acids (Phisut, 2012). This problem could be avoided by the addition of microencapsulated carrier to the ultrasonic sprayed dried product. Micro-encapsulation, which has been widely used to protect food ingredients, offers greater effectiveness. Therefore, this study was aimed to evaluate the antioxidants properties of several microencapsulated plants powder (MPP) prepared using ultrasonic spraying technique.

MATERIALS AND METHODS

All plants sample (*Clitoria ternatea*, *Stevia rebaudiana*, and *Phaleria macrocarpa*) were collected in Kuala Nerus, Terengganu, Malaysia except for *Kappaphycu salvarezii, Mangifera indica*, and *Rhizophora apiculata* from Sabah, Perlis and Besut, Malaysia, respectively. The plants were stored at –18°C until used. Gum arabic (Merck, Darmstadt, Germany) were used as the encapsulation material. All the other reagents used for analyses were of analytical grade.

Microencapsulation by ultrasonic spray drying

The plant sample (150 g) was blended with 1500 mL distilled water, filtered using muslin cloth and microencapsulated with gum Arabic at concentration of 10% relative to solid content. The emulsions were dried using an ultrasonic spray dryer (YKNTECH, Kulim, Malaysia) equipped with a nozzle atomizer. Outlet temperature (80°C) and feed flow rate was kept at 8 mL/min. The micro-encapsulated plant powder were collected and stored in opaque, heat sealed PP plastic at 4°C prior to further analysis.

Powder bioactive compounds extraction

Ten grams of plant sample of all the MPP samples were transferred to dark-coloured flasks and mixed with 200 mL of 60% methanol (MERCK, Germany) and kept at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. The process was repeated after 48 h. The supernatants were combined and evaporated to dryness under vacuum at 40°C. The extracts were stored in amber sterile tubes and stored in at 4°C prior to further analysis.

Antioxidative activities

2,2-diphenyl-2-picrylydrazyl (DPPH) radical scavenging activity assay

2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) reacts with an antioxidant compound and is reduced, donating hydrogen, which causes a colour change that can be measured using a spectrophotometer (Singh *et al*., 2002). Absorbance values were measured at 517 nm after 30 min.

Ferric thiocyanate (FTC) method

FTC method was used to measure peroxide level at the initial stage of lipid peroxidation. This method was carried out as described by Kikuzaki & Nakatani (1993). A mixture of 4.0 mg of plant sample in 4 mL absolute ethanol (99.5%), 4.1 mL of 2.52% linoleic acid in 99.5% absolute ethanol, 8.0 mL of 0.05 M phosphate buffer (pH 7.0), and 3.9 mL water container in a screw-cap vial will be placed in an oven at dark or amber bottle at 37–40°C. 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% ammonium thiocyanate were added to 0.1 mL of this mixture. Three min after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm at every 24 h interval until 1 day after absorbance of the control reached its maximum value. In this assay, butylated hydroxy toluene (BHT) and α-tocopherol were used as positive controls while mixture without sample or blank was used as the negative control.

Thiobarbituric acid (TBA) test

The test was conducted according to the modified method of Kikuzaki & Nakatani (1993). To 2.0 mL of the sample solution, 1.0 mL of 20% aqueous trichloroacetic acid (TCA) and 2.0 mL of 0.67% aqueous thiobarbituric acid (TBA) solution were added. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 mins. After cooling to 25°C, it was then centrifuged at 3000 rpm for 20 mins. Absorbance of the supernatant was measured at 531 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay.

Total phenolic content

The total phenolic content of sample powder was evaluated using a method described by Zainol *et al*. (2003). The sample with a concentration of 1 mg/mL methanol was added to 4.5 mL of deionized distilled water and 0.5 mL of Folin-Ciocalteu's reagent was then added to the solution. After mixing the tubes, the samples were maintained at room temperature for 5 min followed by the addition of

5 mL of 7% sodium carbonate and 2 mL of deionized distilled water. Next, the samples were incubated for 90 min at 23°C with intermittent shaking. The absorbance was measured by spectrophotometer at 750 nm. The total phenolic content was expressed as mg of gallic acid equivalents (GAEs) per gram of dried sample.

Total flavonoid content

Total flavonoid content assay were adapted from Chang *et al*. (1997). Briefly, 50mg/10mL methanol of sample was mixed with 1.5 mL of methanol and then, 0.1 mL of 10% aluminum chloride was added, followed by 0.1 mL of potassium acetate and 2.8 mL of distilled water. The mixtures were incubated at room temperature for 30 mins. The absorbance was measured by a spectrophotometer at 415 nm. The result was expressed as mg rutin equivalents (RE) per gram of dried sample.

Surface morphology analysis

The spray dried microencapsulated plants powder (MPP) powder was examined using a Hitachi TM1000 operating under vacuum (ca.10–7 mbar) at 1 kV and 10 μA. The samples were mounted on sample chamber and kept extremely still. Then, the magnification, focus, contrasts and brightness were adjusted according to resolution (Yousefi *et al*., 2014).

Colour profile analysis

The colour analysis was based on the method described in Perkins-Veazie *et al*. (2001). Together with the transparent PP plastic which used to store the microencapsulated plants powder, it was flashed directly using Minolta CT-310 Colorimeter (Konica Minolta, Japan). The L*, a* and b* colour values represented whiteness or brightness/darkness (L), redness/greenness (a) and yellowness/blueness (b).

Statistical analysis

The results were analysed for statistical significance (p=0.05) using one-way ANOVA by SPSS statistical software (SPSS 16.0 for Windows) and Tukey's test was used for pairwise comparison of the mean values.

RESULTS AND DISCUSSION

2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay

DPPH free radical scavenging action is generally utilized as a part of different sorts of test particularly in plant sort (Iwashima *et al*., 2005). All specimens and measures had significant DPPH searching action. Figure 1 demonstrates the consequences of DPPH searching movement in MPP and chose models. The plant MPP tests indicate broadening consequences of searching action where they surpassed 90%. *C. ternatea* and *P. macrocarpa* indicated to some degree great DPPH hindrance 65% and 60%, respectively. *M. indica*, extract was the least hindrance which was 45%.

The information delineated that MPP of *C. ternatea* indicated fundamentally the most astounding antioxidative activity ($p < 0.05$) among every one of the samples tested. It demonstrated that *C. ternatea* had solid cell reinforcement rummaging action. It was trusted that, the compound in the concentrate tests can respond with DPPH arrangement by donating an electron or hydrogen to DPPH (Zahin *et al*., 2010). *C. ternatea* MPP displayed the best radical searching action among all which might be because of the great condition or non-coagulating condition of the micro-encapsulated powder permitted completely showing of its cancer prevention agent limit. Comparative outcomes were seen by Kamkaen and Wilkinson

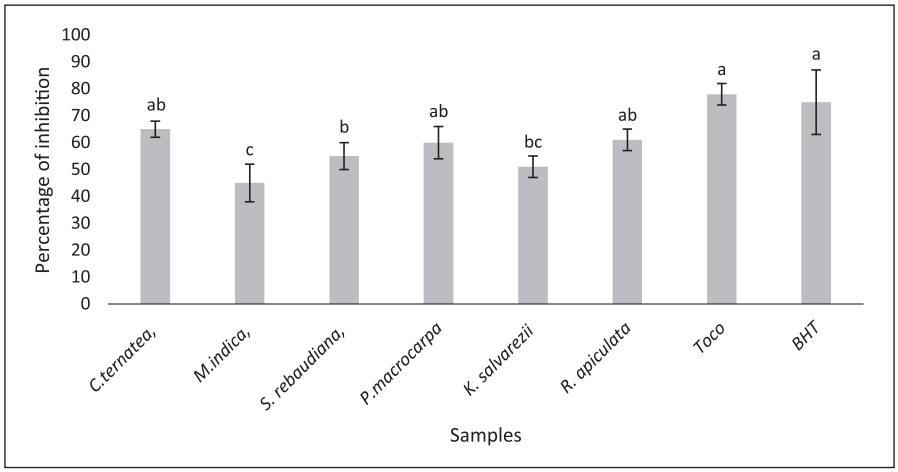

Fig. 1. Percentage of DPPH inhibition by each sample at 30 min.
Data are expressed as mean ± SD of triplicate experiments. Values with different letter are significantly different at $p<0.05$.

(2009), who specified that a water concentrate of *C. ternatea* has more grounded cell reinforcement action, as tested by the lessening of DPPH, than that of an ethanol extract. In addition, as *C. ternatea* is known to contain anthocyanin, warm debasement, oxidation, enzymatic response (particularly polyphenoloxidase, which is known to assume the primary part in corruption of anthocyanins colors) and different components can adjust anthocyanins content amid handling (Scibisz and Mitek, 2009) in this manner decreasing the cancer prevention agent limit.

Ferric thiocyanate (FTC) method

Figure 2 demonstrates that arrangement MPP removes displayed high antioxidative properties in FTC examination, whereby *C. ternatea* extricate delineated the best absorbance esteem (0.54 ± 0.12) of all samples. High absorbance esteems show high linoleic corrosive oxidation and add to the low levels of antioxidant activity (Elmastas *et al*., 2006). The most noteworthy antioxidant activity was measured at fifth day of incubation as it demonstrated the control had accomplished ideal level and step by step diminished on day six. This is because of the oxidation of linoleic corrosive that created linoleic corrosive hydroperoxides was deteriorated to numerous auxiliary items. The control test diminished due to non-accessibility of linoleic corrosive which appeared amid the sixth day of hatching (Chen *et al*., 1996).

The FTC method was utilized to determine the value of peroxide at the underlying phase of lipid peroxidation (Figure 3). In this technique, the peroxides formed amid the linoleic corrosive oxidation respond with Fe^{2+} to shape Fe^{3+}, which at that point responds with thiocyanate to shape an unpredictable which has most extreme absorbance

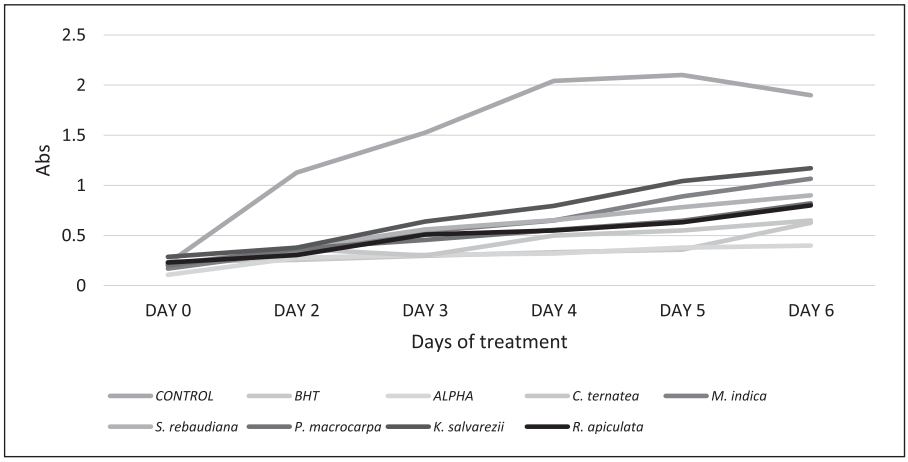

Fig. 2. Antioxidant activities as measured by FTC method.
Data are expressed as mean ± SD of triplicate experiments. Values with different letter are significantly different at $p<0.05$.

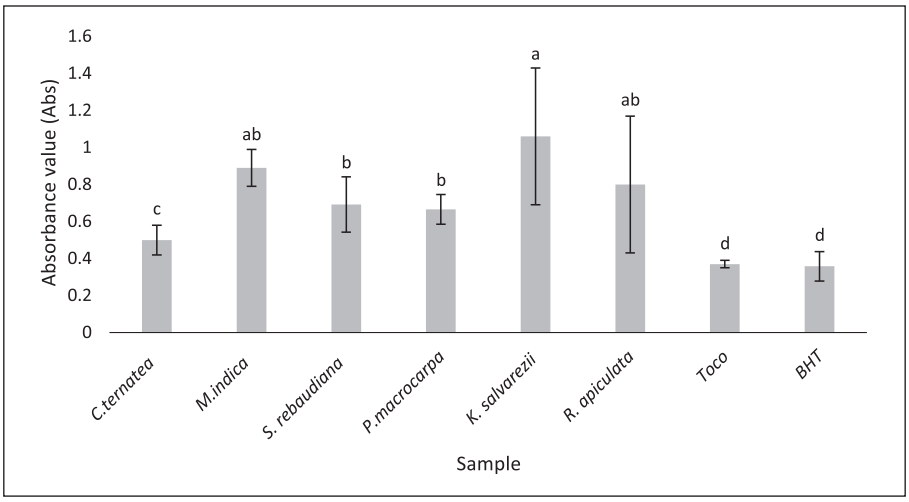

Fig. 3. Antioxidant activities of all the plant samples on 6th Day as measured by FTC assay.
Data are expressed as mean ± SD of triplicate experiments. Values with different letter are significantly different at $p<0.05$.

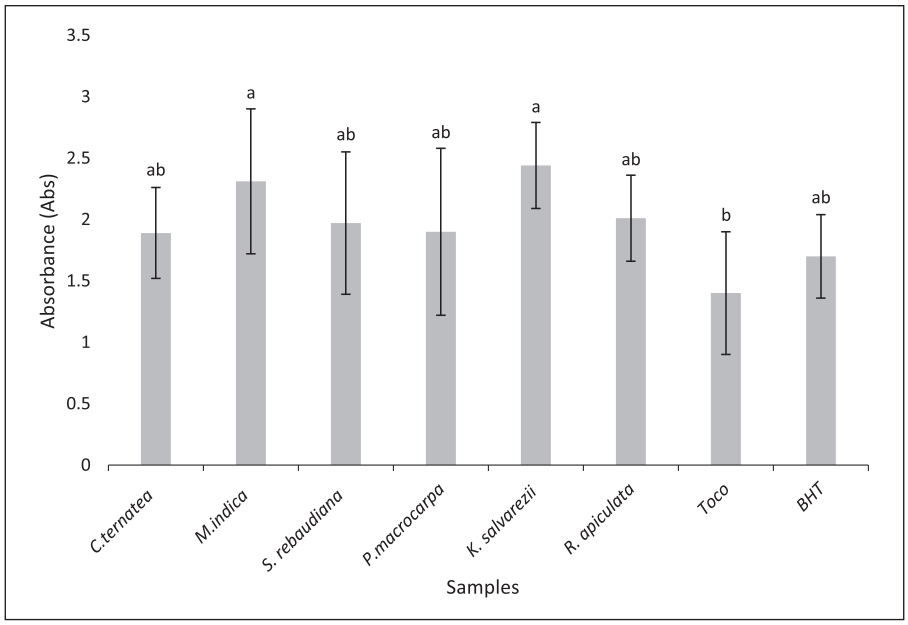

Fig. 4. Antioxidant activities on Day 6 as measured by TBA method in different MPP products. Data are expressed as mean ± SD of triplicate experiments. Values with different letter are significantly different at $p<0.05$.

at 500nm. The absorbance readings in FTC test communicated the measure of ferric particle (Fe^{3+}). Linoleic corrosive is utilized as the peroxide source. The peroxide responds with ferrous chloride ($FeCl_2$) to give a ferric chloride color which is red (Tiwari *et al*., 2013). The inhibitory impact oxidation of ferrous chloride to ferric particle by cancer prevention agent is assessed by checking the development of ferric thiocyanate complex. All specimens demonstrated to some degree noteworthy ($p<0.05$) distinctive with α-tocopherol and BHT. In light of the outcomes, distinctive microencapsulated plant powder indicated diverse cell reinforcement action in FTC strategy which might be because of the lessening of hydroperoxides, inactivation of free radicals, chelation of metal particles or blend of them (Zainol, 2004). The outcomes demonstrates that the greater part of its specimens showed great impact in repressing linoleic corrosive oxidation contrasted with control. The hindrance of linoleic corrosive was beat seen in *C. ternatea*, and took after by *P. macrocarpa*, *S. rebaudiana, R. apiculata, M. indica*, lastly the slightest was seen in *K. salvarezii*.

Thiobarbituric acid (TBA) test

Figure 4 shows the best TBA value was seen in *P. macrocarpa*, trailed by *C. ternatea, M. indica, S. rebaudiana, R. apiculata, M. indica* and *K. salvarezii*, individually yet the vast majority of the examples demonstrated no huge ($p>0.05$) among each other. The most astounding cell reinforcement action tried utilizing TBA technique was found in *P. macrocarpa* (1.85 ± 0.08). *K. salvarezii* separate displayed the most minimal TBA esteem (2.44 ± 0.35) of all examples. This might be on the grounds that when the example had solid cell reinforcement properties, the odds to accomplish high measure of MDA at later phase of lipid peroxidation is lower as the underlying phase of lipid peroxidation has been defeated by the cancer prevention agent mixes. The TBA results were not in concurrence with the FTC results on the grounds that examples that contain lesser measure of peroxide at the underlying phase of lipid peroxidation does not really implies the cancer prevention agent action will be high at later stage.

It might be recommended that the measure of peroxide in the underlying phase of lipid peroxidation was higher than the measure of peroxide in the optional stage. Moreover, just certain lipid peroxidation items create MDA (constantly low yields), and MDA is neither the sole finished result of greasy peroxide arrangement and disintegration nor a substance produced only through lipid peroxidation. In this manner, many factors, for example, jolt for and states of peroxidation) regulate MDA development from lipid. As said prior, *C. ternatea* has unsaturated fat arrangement made up from oleic and linoleic corrosive (Sethiya *et al.*, 2009), consequently the lipid oxidation of these unsaturated fat might be represent the high measure of peroxide at the underlying stage.

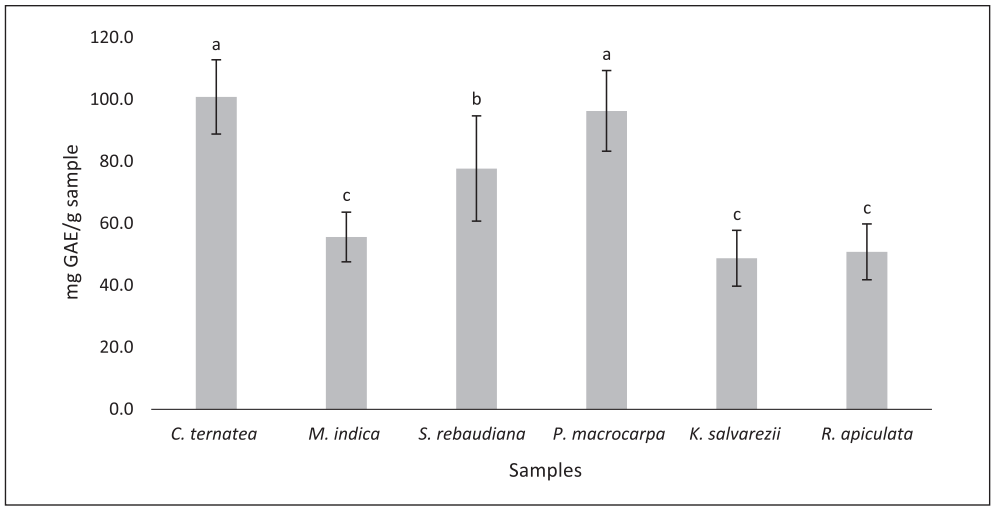

Fig. 5. Total Phenolic Content in different MPP products (mg GAE/g sample).
Data are expressed as mean ± SD of triplicate experiments. Values with different letter are significantly different at $p<0.05$.

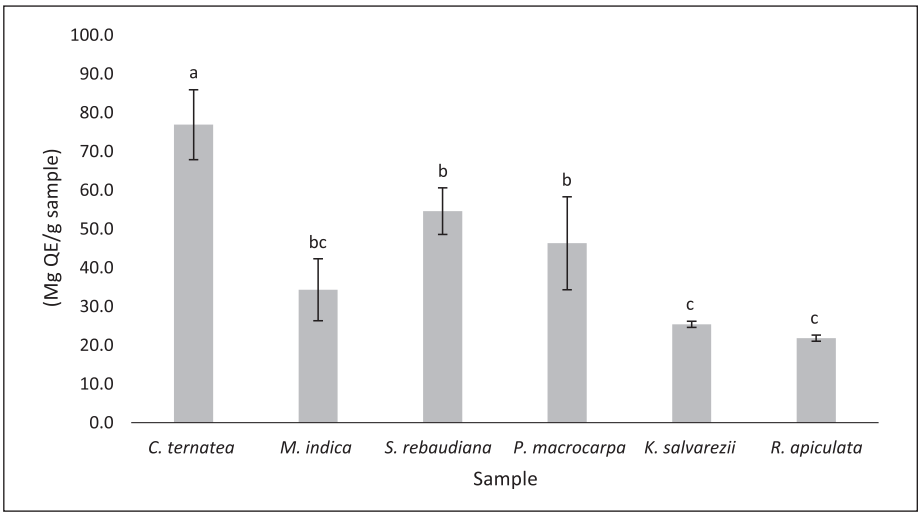

Fig. 6. Total Flavonoid Content in different MPP products (mg RE/g sample).
Means are reported from replica analysis. Means within the same column showing the different superscript letter are significantly different ($p<0.05$).

Total phenolic content

The total phenolic substance in the inspected separates ranged from 48 to 100 mg GA/g (Figure 5). The most elevated centralization of phenols was measured in *C. ternatea* and *P. macrocarpa* removes. High measure of aggregate phenolic content was clarified by Rabeta and An-Nabil (2013) who specified that phenolic mixes are water solvent regular cancer prevention agents which ordinarily a fragrant ring bearing at least one hydroxyl substituent. Moreover, in a similar research by Mohsen and Ammar, 2008, when comparing Total phenolic content (TPC) based on the parts of plant used, the flower exhibited higher TPC content compared with the leaves part in water extract sample. High solubility of phenols in polar solvents gives high convergence of these mixes in the concentrates got utilizing polar solvents for the extraction (Zhou & Yu, 2004). The solid impact of antioxidative exercises could be high because of the high substance of phenolic compound, for example, anthocyanin in *C. ternatea* and *P. macrocarpa*. Anthocyanin is a non-poisonous shade, phenolic compound exhibits in the frame orange to blue shading in the normal world. This phenol structure ability of catching free radicals, to assume a part as cancer prevention agent are accounted for to be higher than vitamin C and E (Padma and Vankar, 2010).

Total flavonoids content

The concentration of flavonoids in plant extracts extended from 25.4 to 76.9 mg/g (Figure 6). The convergence of flavonoids in *C. ternatea* separate

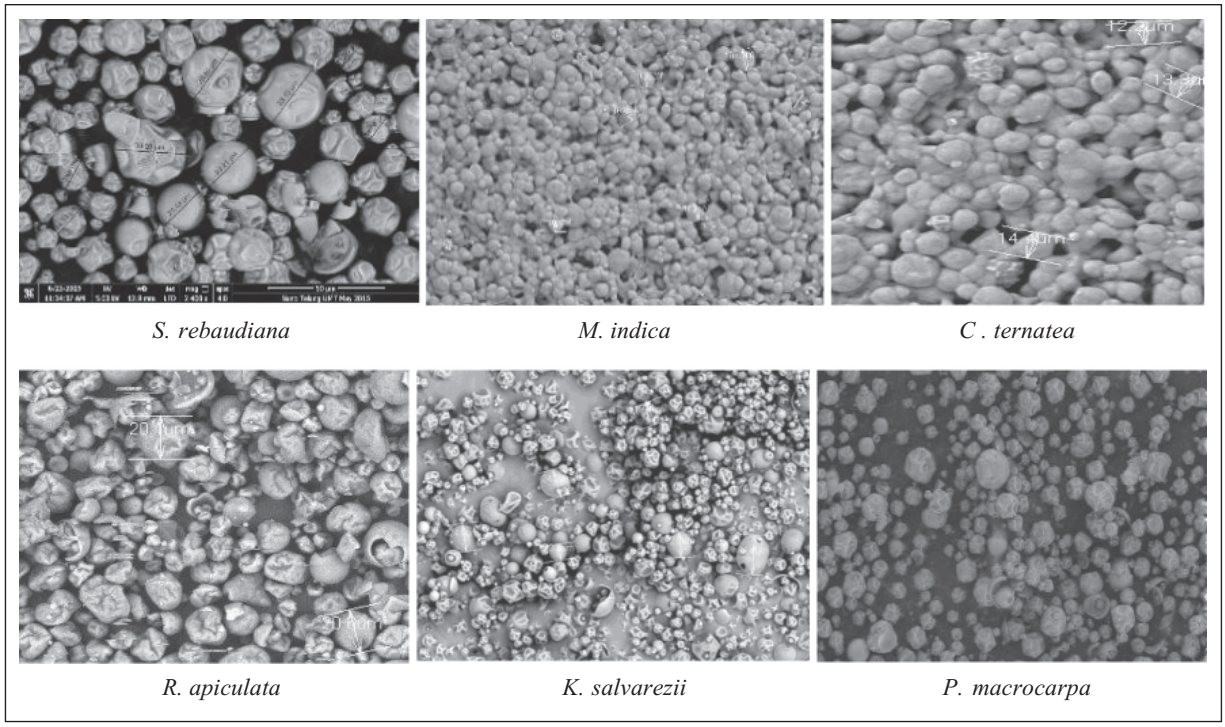

Fig. 7. Scanning electron microscopy (SEM) was used for surface morphology analysis (x500, 200μm). (*C. ternatea, M. indica, S. rebaudiana, P. macrocarpa, K. salvarezii* and *R. apiculata,* from left to right).

was 76.9 mg RE/g, which was fundamentally the same as the estimation of CH3)2CO remove focus. The most minimal aggregate flavonoids fixation was measured in petroleum ether and water extract. The concentration of flavonoids in plant extracts relies upon the extremity of solvents utilized as a part of the concentrate planning (Min and Chun-Zhao, 2005).

Surface morphology analysis

Figure 7 illustrates the SEM image the distinct difference between the MPP plants powder treated using ultrasonic spray drying technique. The resulting powders had a particle size in a range of 9.52 μm to 33μm. It was found that all MPP powders produced had a spherical structure and smooth surface but some dented spots. According to Wu *et al*. (2014) the formation of the dented surfaces are due to the shrinkage of the particles induced by high temperature during spray drying process. These properties are attributed to high solubility and good bulk density. Meanwhile, the MPP also depicted somewhat irregular size, unsmooth surfaces and dented surfaces. Some MPP powder have smaller size particles and more dents than others. In addition, more dented surfaces produced on encapsulated powder with combination wall material while few dented surfaces produced on encapsulated powder with single wall material (Nawi *et al*., 2015). This shape and surface characteristics could be one of the reason for a strong antioxidative activity.

CONCLUSION

Results of our study suggest that *C. ternatea,* and *P. macrocarpa* are good antioxidants sources after the ultrasonic spray drier technique. This current study provides basic principles and information for developing specific preservation techniques on sustaining the bioavaibility of antioxidants compounds and its activities. This study is also important to picture the current techniques of extracting natural antioxidants and to make sure their activities last. Therefore, the crucial results from this research might invoke further research on understanding the mechanisms in ultrasonic spray drying.

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