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Effects of blanching and drying treatment on extraction of soluble components from dried *Centella asiatica*

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Abstract
Centella asiatica (L.) is a perennial, creeper, faintly aromatic and a valuable medicinal herb. Fresh leaves of *Centella asiatica* can be blanched and dried for purpose of making dry product that can be preserved long time to make product for healthy as: dry-leave tea or soluble powder drinks from extracting of dry product. We have realized blanching and drying processes to acquire high quality of dried product. Fresh *Centella asiatica* leaves are blanched in water at temperature of 85 °C in 30s to obtain green chlorophyll color and to minimize losing soluble component. And optimized drying conditions of blanched leaves are: temperature of 75.5 °C and time of 3.47h, with highest content of soluble component of 34.27% (absolule dry component) by extracting in water at 90 °C; and dry product contained with content of vitamin C 0.91mg% and asiaticose of 0.684%.

Keywords: *Centella asiatica, asiaticoside, blanching, drying, soluble component*

1. Introduction
Centella asiatica, commonly centella, grows in tropical swampy areas. The stems are slender, creeping stolons, green to reddish-green in color, connecting plants to each other. It has long-stalked, green, reniform leaves with rounded apices which have smooth texture with palmately netted veins. The leaves are borne on pericladial petioles, around 2 cm. The rootstock consists of rhizomes, growing vertically down. They are creamish in color and covered with root hairs. The flowers are pinkish to red in color, born in small, rounded bunches (umbels) near the surface of the soil. Each flower is partly enclosed in two green bracts. The hermaphrodite flowers are minute in size (less than 3 mm), with 5-6 corolla lobes per flower. Each flower bears five stamens and two styles. The fruit are densely reticulate, distinguishing it from species of Hydrocotyle which have smooth, ribbed or warty fruit. The crop matures in three months, and the whole plant, including the roots, is harvested manually [5, 6].

Fig 1: *Centella asiatica*

Table 1: Scientific classification of *Centella asiatica*

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Apiales
Family:	Mackinlayaceae
Genus:	*Centella*
Species:	*C. asiatica*

Over the years, several reviews have reported the studies on Centella:

Mahanom, H. (1999) evaluated the effect of oven drying at 50 °C ±1 °C for 9 hour, 70 °C ±1 °C for 5 hour and freeze drying on retention of chlorophyll, riboflavin, niacin, ascorbic acid and carotenoids in herbal preparation consisting of 8 medicinal plants including *Centella asiatica*. Results revealed that both type and conditions of the drying treatments affected retention of all phytochemicals analysed. Herbal preparation developed using oven drying was found to have inferior phytochemicals content compared to that obtained by freeze dryer. Nevertheless, the herbal preparation developed using all treatments still retain appreciable amount of phytochemicals studied, especially carotenoids, ascorbic acid, niacin and riboflavin and thus have potential for commercial purposes [3].

Mohd Zainol *et al*. (2009) evaluated the effect of different drying methods on the degradation of flavonoids in *Centella asiatica*. *C. asiatica* leaf, root and petiole were dried using air-oven, vacuum oven and freeze drier. Flavonoid was determined utilizing reverse-phase high performance liquid chromatography (RP-HPLC). Results of the study revealed the presence of high concentration of flavonoids in *C. asiatica* leaf, root and petiole, which include, naringin (4688.8 ± 69 μg/100 g, 3561.3 ± 205 μg/ 100 g, and 978.3 ± 96 μg/ 100 g), rutin (905.6 ± 123 μg/ 100 g, 756.07 ± 95 μg/ 100 g, and 557.25 ± 58 μg/ 100 g), quercetin (3501.1 ± 107 μg/ 100 g, 1086.31 ± 90 μg/ 100 g, and 947.63 ± 83 μg/ 100 g) and catechin (915.87 ± 6.01 μg/ 100 g, 400.6 ± 67 μg/ 100 g, and 250.56 ± 18 μg/ 100g). Luteolin, kaempferol and apigenin on the other hand, were inconsistently present in some parts of *C. asiatica*. Air-oven treatment resulted in the highest total flavonoids degradation followed by vacuum oven and freeze dried with percent degradation of 97%, 87.6% and 73%, respectively. Catechin and rutin were found to be the most stable flavonoids with percent degradation up to 35%, 66% and 76% for freeze dried, vacuum oven and air oven, respectively [4].

Frederico Pittella *et al*. (2009) studied antioxidant and cytotoxic activities of *Centella asiatica*. The phenolic (Folin-Dennis) and flavonoid (colorimetric assay) constituents, antioxidant [2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay] and cytotoxic activities of an aqueous extract (AE) of *Centella asiatica* leaves were investigated. The aqueous extract (50 g/L) was obtained by infusion followed by cold maceration for 24 h. The levels of phenolic and flavonoid compounds were 2.86 g/100 g and 0.361 g/100 g, respectively. The AE showed elevated DPPH scavenging activity, with an IC_{50} value of 31.25 μg/mL. The AE had a promising activity against mouse melanoma (B16F1), human breast cancer (MDA MB-231) and rat glioma (C6) cell lines, with IC_{50} values of 698.0, 648.0 and 1000.0 μg/mL; respectively. A positive correlation was established between the level of flavonoids, antioxidant and antitumor activities [1].

Sakshi Singh *et al*. (2010) provided its immense importance as economic plant with medicinal value as well as brief information of its products in the market launched, showing its dependability [8].

Puziah Hashim *et al*. (2011) investigated triterpene composition and bioactivities of *Centella asiatica*. Leaves of *Centella asiatica* (Centella) were analysed for their triterpene composition and bioactivity such as collagen enhancement, antioxidant, anticellulite and UV protection capacity properties. Triterpenes of Centella were measured using HPLCPAD on an Excil ODS 5 μm (C18) column for the simultaneous determination of asiatic acid, madecassic acid, asiaticoside and madecassoside. Centella was found to contain significant amounts of madecassoside (3.10 ± 4.58 mg/mL) and asiaticoside (1.97 ± 2.65 mg/mL), but was low in asiatic and madecassic acid. The highest collagen synthesis was found at 50 mg/mL of Centella extracts. The antioxidant activity of Centella (84%) was compared to grape seed extract (83%) and Vitamin C (88%). Its lipolytic activity was observed by the release of glycerol (115.9 μmol/L) at 0.02% concentration. Centella extracts exhibited similar UV protection effect to OMC at 10% concentration [7].

Hashim, P. (2011) covered the biological activity of the plant's active constituents in relation to its food and beverage applications. The plant cultivation and biotechnological approaches to improve the production of desired bioactive constituents by cultured cells will also be reviewed. In addition, the range of chemical compositions found in Centella and safety aspects are also included [2].

W. Trirattanapikul, S. Phoungchandang (2012) investigated the appropriate stage of maturity of *Centella asiatica* (L.) Urban leaves. Mature leaves with large diameter contained high total phenolics and % inhibition. Microwave blanching for 30 s retained the highest total phenolics and the microwave blanching for 30 s and 45 s retained the highest % inhibition. Modified Henderson and Modified Chung-Pfost models showed the best fit to both fresh and blanched leaves for equilibrium moisture content, $X_e = f(RH_e, T)$ and equilibrium relative humidity, $RH_e = f(X_e, T)$, respectively. The Modified Page model was the most effective model in describing the leaf drying. All drying was in the falling rate period. The drying constant was related to drying air temperature using the Arrhenius model. Effective moisture diffusivities increased with increasing temperature and blanching treatments as well as dehumidification by heat pump-assisted dehumidified dryer. The heat pump-assited dehumidified drying incorporated by the microwave blanching could reduce the drying time at 40 °C by 31.2 % and increase % inhibition by 6.1 %. Quality evaluation by total phenolics, % inhibition and rehydration ratio showed the best quality for *C. asiatica* leaves pretreated by microwave blanching and dried at 40 °C in heat pump-assisted dehumidified dryer [9].

In our research, we investigate and optimize the blanching and drying process to preserve chlorophyll and maintain the highest soluble content by water extraction at 90 ºC from the dried *Centella asiatica* leaf. Its product can be consumed as tea or functional food having asiaticoside which is very benificial to human health.

2. Material and methods
2.1 Raw material
Fresh *Centella asiatica* leaf is provided from one vegetable garden in Cu Chi district, HCM City, Vietnam. It should be transferred to laboratory within 2 hours, natural green of chlorophyll, uniform, intact and kept in 15÷20 ºC within 1÷2 days.

2.2 Research protocol

2.2.1 Processing protocol

Fresh *Centella asiatica* leaf → Separation → Washing → Blanching → Cooling → Drying → Cooling → Packing → Dried *Centella asiatica* leaf.

2.2.2 Blanching

Centella asiatica leaf is heated by blanching in fresh water and NaCl solution before drying. Effect of blanching temperature and time to product color and soluble content is investigated under the fixed drying parameters: temperature 70^{o}C in 4.5 hours to moisture content 5÷6%.

2.2.3 Drying

Blanched *Centella asiatica* leaf is introduced to drying to get the highest yield of soluble content in water at 90^{o}C, using the response surface methodology (RSM) based on two-variable central composite design (CCD); target parameters are soluble content (%). Two experimental parameters are drying temperature (Z_1) and drying duration (Z_2). We conduct 11 tests including 3 tests at master matrix (Z_0), 8 tests at star matrix. After receiving the regression equation, we continue apply the experimental plan by climbing method. To facilitate the optimization, we modify varians so that each independent varian change to three levels -1, 0, 1 by following formula:

$$x_i = \frac{(z_i - z_0)}{\Delta z_i}$$

Where:

Xi: coded varian

Zi: uncoded varian

Zo: value at core of varian

ΔZ: jump step

Regression equation:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

2.2.4 Soluble extraction

Soluble extraction is carried out with 1 gram of dried *Centella asiatica* leaf in 100mL of distilled water at 85÷90^{o}C, within 30 minutes. The extract is analysed the soluble content, mineral and asiaticoside. The control sample is prepared from dried green tea (in Lam Dong province, Vietnam) to compare the extractable metal from both two kinds of dried leaf.

2.3 Analysing methods

Several physic-chemical compositions are executed: moisture content (TCVN 5613-91), saponin (Namba method), protein (AOAC 960.52), lipid (TCVN 6508:1999), glucide (TCVN 4594-88), minerals (AOAC 923.03), vitamin C (EN 14130:2003), Ca (TCVN 6626:2000), K (AOAC 985.35), Mg (AOAC 985.35), Mn (AOAC 986.15), P (AOAC 985.35), asiaticoside (HPLC).

Sensory evaluations for texture, color, flavor, taste, aroma of blanched *Centella asiatica* leaf are conducted by comparative approach by specialists. All experiments are triplicated and statistically handled by ANOVA under software Stagraphic plus 5.1; optimize the drying process by Mode 5.

3. Results and discussion

3.1 Nutrient compositions of fresh *Centella asiatica* leaf

Table 2: Nutrient compositions of fresh *Centella asiatica* leaf

Composition	Unit	Leaf	Stem	Element	Unit	Leaf	Stem
Moisture	%	82.98	92.97	Vitamin C	mg%	3.46	-
Protein	%	3.3	1.00	K	%	0.12	0.24
Glucid	%	0.68	0.85	Mg	mg/kg	525.0	241.6
Lipid	%	0.1	0.08	Mn	mg/kg	54.0	14.7
Cellulose	%	1.39	1.84	Ca	mg/kg	867.7	316.5
Ash	%	1.22	1.00	P	mg/kg	289.3	157.0

Fresh *Centella asiatica* leaf is easily deteriorate owing to high moisture content of its leaf and stem so it must be blanched and dried to moisture content 5÷6% so that we have a stable source of raw material. Moreover, fresh *Centella asiatica* leaf has good nutrient owing to abundant protein and minerals such as K, Mg, Mn, and Ca.

3.2 Effect of blanching:

Blanching has a very important effect to product quality by temperature and time: glue status of protoplasm will be swelled, condensed and separated to out off cell membrane, create high permeability of cell membrane. So water vapor exists freely, drying more easily. Blanching also inactivates oxidative-reductive enzyme, activate chlorophyllase enzyme, increase organic acidity inside tissue, and better maintain green color of chlorophyll.

Table 3: Effect of blanching temperature (^{o}C) to sensory quality of *Centella asiatica* leaf

Temperature	Discoloration (%)	Appearance of blanched leaf and stem
75	97.67±1.43	Completely oliu green
80	11.33±0.56	Partly oliu green
85	0	Dark-green
90	0	Dark-green, soft stem
95	0	Dark-green, soft leaf and stem

Blanching in 75 ºC and 80 ºC, leaf and stem turn into oliu green, ion Mg^{2+} of chlorophyll is replaced with 2 ions H^{+} of water, form a new substance pheophytin having oliu green color. Blanching in 85 ºC, leaf and stem turn into dark-green, chlorophyllase catalysis to form chlorophyllide and inactivate other enzymes such as polyphenolxydase oxidable phenol to dark color. Blanching in 90 ºC and 95 ºC, leaf and stem become dark-green, soft and breakable. So we choose blanching in 85 ºC for further experiments.

Table 4: Effect of blanching time (s) to color and soluble content of the blanched and dried *Centella asiatica* leaf

Blanching time (s)	Color and structure of *Centella asiatica* leaf	Soluble content from dried *Centella asiatica* leaf (%)
0	Dark-green	24.21[a]
30	Dark-green	33.49[b]
60	Dark-green, soft leaf	31.76[b]
90	Dark-green, soft-deteriorable leaf	28.61[c]
120	Dark-green, soft-deteriorable leaf & stem	25.57[d]
Blanching in 85 ºC; a, b, c, d are significant different indicators at error 5%.		

In blanching, vegetable tissue become soft-deteriorable, soluble particles are extracted more easily. However if we prolong the blanching time, these soluble particles will be released to blanching solution; leaf and stem become deteriorable. So we select 30s for blanching. We also demonstrate the blanching in NaCl solution.

Table 5: Effect of NaCl concentration to color and taste of *Centella asiatica* leaf during blanching at 85 ºC, 30s

No	Blanching solution	Color of leaf and stem	Taste of extract from dried *Centella asiatica* leaf
1	Clean water	Dark-green	Sweet, bitter
2	NaCl 0.1%	Dark-green	Sweet, bitter
3	NaCl 0.3%	Dark-green	Salty
4	NaCl 0.5%	Dark-green	Salty

Blanching in NaCl 0.3% and 0.5%, leaf has dark-green color, salty taste. Blanching in clean water and NaCl 0.1%, leaf and stem have dark-green color, bitter taste of specific *Centella asiatica* leaf. So we select blanching in clean water at 85 ºC within 30s.

3.3 Optimize the drying *Centella asiatica* leaf

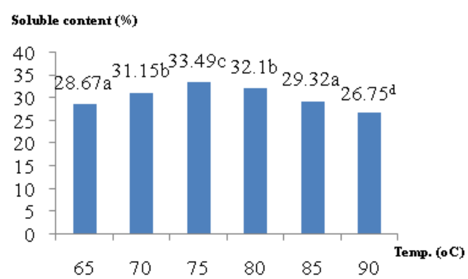


Fig 2: Soluble content in *Centella asiatica* leaf affected by different drying temperatures

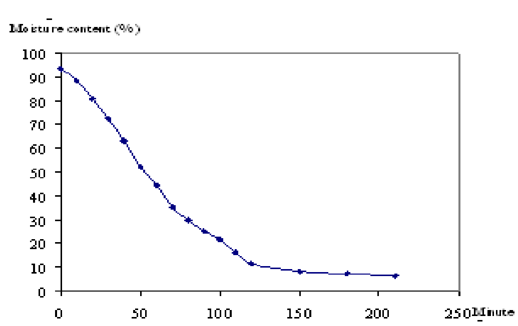


Fig 3: Drying curve of *Centella asiatica* leaf at 75 ºC

The higher drying temperature, the less drying time is noticed, especially in range 75-90 ⁰C. At high drying temperature, moisture vapors quickly, glue status of protoplasm becomes constricted. When soaking the dried *Centella asiatica* leaf into hot water, extractability will be improved. If drying temperature comes to 80 ⁰C, the extractable soluble content will be gradually decreased due to protein denaturation. So 70 ºC is adequate. Drying in 100 minutes, moisture content has been reduced significantly and came to 5÷6% at 200 minutes. Optimize the drying process by using the response surface methodology (RSM) based on two-variable central composite design (CCD); target parameters are soluble content (%). Two experimental parameters are drying temperature and drying duration. Regression equation.

$$Y = 34.25 + 0.35X_1 - 0.08X_2 - 2.01X_1^2 - 0.96X_2^2 - 0.69X_1X_2$$

With R^2 = 0.982 and Q^2 = 0.885 to express the credibility of this experiment. Build the regression equation on 3-D scale, optimize two-variable central composite design, we get the extractable soluble content Y = 34.27 (%) at optimal drying temperature X_1 = 75.5 (ºC) and drying time X_2 = 3.47 (h).

Table 6: Core value and jump step in the drying optimization

Factor	Drying temperature Z_1 (ºC)	Drying duration Z_2 (h)
Core value	75	3.5
Jump step	5	0.5

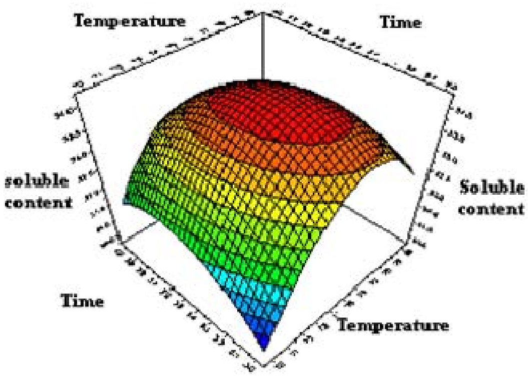


Fig 4: Response surface of the drying regression equation for *Centella asiatica* leaf on 3-D scale.

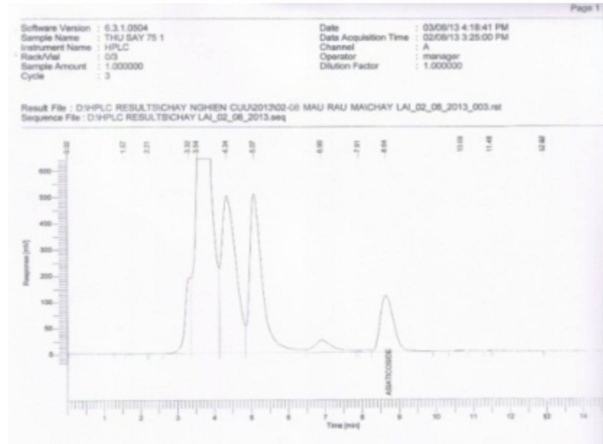
3.4 Nutrient composition in dried *Centella asiatica* leaf

Nutrient composition in the dried *Centella asiatica* leaf is shown in the table 7. It has a high content of minerals, soluble loss compared the fresh *Centella asiatica* leaf is minor, with exception of vitamin C 0.91 mg%. Soluble extract from the dried *Centella asiatica* leaf has abundant minerals such as K, Mg, Mn, Ca, P. Moreover, asiaticoside content in the dried *Centella asiatica* leaf is 0.684% with extraction yield 68.27%.

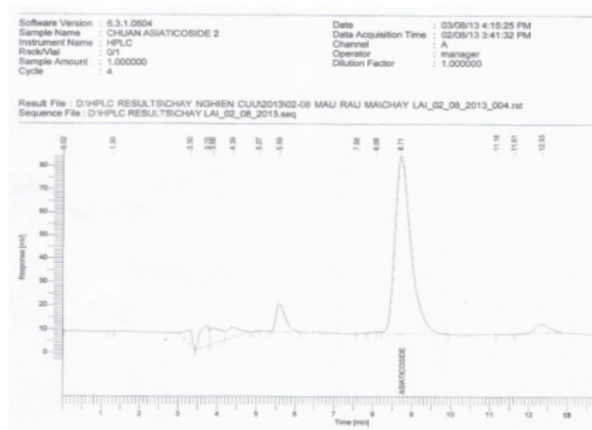
Table 7: Nutrient composition in dried *Centella asiatica* leaf and its extract

Composition	Dried *Centella asiatica* leaf	Extract of dried *Centella asiatica* leaf
	Content	Content (mg/L)[a]
Moisture	5.92%	-
Crude fiber	9.33%	-
Ash	8.34%	-
Vitamin C	0.91 mg%	-
Ca	0.69%	26.2
K	1.32%	69.5
Mg	0.4%	29.0
Mn	72.3 mg%	1.30
P	0.25%	6.77
Asiaticoside	0.684%	46.7

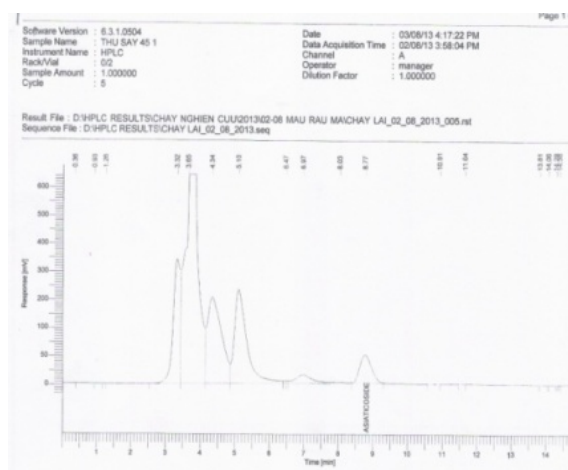
Chromatogram of asiaticoside in the dried *Centella asiatica* leaf and its extract by HPLC as follow:



a)



b)



c)

Fig 5: Chromatogram of asiaticoside

a) Chromatogram of asiaticoside standard

b) Chromatogram of asiaticoside in the dried *Centella asiatica* leaf

c) Chromatogram of asiaticoside in the extract from the dried *Centella asiatica* leaf

3.5 Comparison of mineral content from the extract of dried *Centella asiatica* leaf and extract of green tea

Comparison of mineral content from the extract of dried *Centella asiatica* leaf and extract of green tea is conducted in hot water 90 ºC, 30 minutes, 100 mL distilled water with 1 gram of its material having moisture content 5÷6 %. Extract of the dried *Centella asiatica* leaf has green color, the same color of extract from green tea, with specific flavor and aroma. Mineral contents in both these extracts are illustrated in table 8. Mn and P are similar in both tow extracts; Ca and Mg in extract of dried *Centella asiatica* leaf are higher than in extract of green tea; meanwhile K is lower. Dried *Centella asiatica* leaf can be used as a new kind of tea containing saponin asiaticoside benefit to human health, especially for heart.

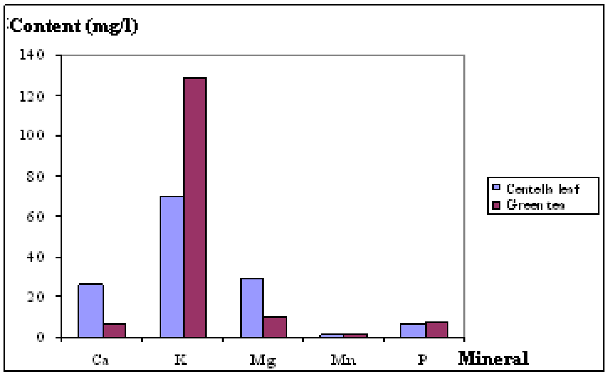


Fig 6: Several minerals in extract of dried *Centella asiatica* leaf and green tea

4. Conclusion

In the dried *Centella asiatica* leaf production, the blanching and drying has been obviously affected to product quality. It should be blanched in water at 85 °C in 30s and then dried to 6% moisture content at drying temperature 76.5 °C in 3.47 h. Soluble content in its finished product is 24.27%. Mineral content of K, Mg, Ca in Centella leaf is also similarly high as in green tea. Moreover, asiaticoside in dried leaf is 0.684% with extraction yield 68.27%.

5. References

1. Pittella F, Dutra RC, Junior DD, Lopes MTP, Barbosa NR. Antioxidant and Cytotoxic Activities of *Centella asiatica* (L) Urb. International Journal of Molecular Sciences 2009; 10:3713-3721.
2. Hashim P. Minireview *Centella asiatica*in food and beverage applications and its potential antioxidant and neuroprotective effect. International Food Research Journal 2011; 18:1215-1222.
3. Mahanom H, Azizah AH, Dzulkifly MH. Effect of different drying methods on concentrations of several phytochemicals in herbal preparation of 8 medicinal plants leaves. Malaysia Journal of Nutrition 1999; 5: 47-54.
4. Mohd Zainol MK, Abdul-Hamid A, Bakar FA. Dek SP. Effect of different drying methods on the degradation of selected flavonoids in *Centella asiatica*. International Food Research Journal 2009; 16:531-537.
5. Zainol NA, Voo SC, Sarmidi MR, Aziz RA. Profiling of *Centella asiatica.* L (Urban) extract. The Malaysian Journal of Analytical Sciences 2008; 12:322-327.
6. Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa. Pharmaceutical biology 2005; 43:249-252.
7. Hashim P, Sidek H, Helme MM, Helan, Sabery A, Palanisamy UD *et al*. Triterpene Composition and Bioactivities of *Centella asiatica*. Molecules 2011; 16:1310-1322.
8. Singh S, Gautam A, Sharma A, Batra A. *Centella asiatica* (L.): a plant with immense medicinal potential but threatened. International Journal of Pharmaceutical Sciences Review and Research 2010; 4:9-17.
9. Trirattanapikul W, Phoungchandang S. Microwave blanching and drying characteristics of *Centella asiatica* (L.) urban leaves using tray and heat pump-assisted dehumidified drying. Journal of Food Science and Technology 2012; 9:1-12.