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Development and evaluation of polyherbal formulation for memory enhancement: study on its antioxidant properties and synergistic effects

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Abstract

Centella asiatica, *Piper sarmentosum* and *Morinda citrifolia* is well-known for their memory enhancement contribution from traditional practices as well as recent researches. However, all three herbs are utilized individually and never mixed together. The nutrient data on the synergistic effect on all three herbs remain scarce. The aims of this research project were to apply Simplex-centroid mixture design in describing the study for the effect of polyherbal formulation on antioxidants properties and its synergistic effects. Based on results obtained, there was a significant difference in antioxidant properties of the polyherbal formulation. The results show the polyherbal formulation 3 and 13 (*M. citrifolia* only) has the highest antioxidant capacity when being tested with total phenolic content (TPC) (210.10 and 209.12 µg GAE/mL respectively) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (42.94 and 37.77%) assays. Contradict to this result, the highest in total flavonoid content (TFC) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) for describing antioxidant properties are formulations 1 and 11 (*C. asiatica* only). The TPC for formulation 1 and 11 are 479.8 µg RE/mL and 470 µg RE/mL respectively while ABTS radical inhibition for formulation 1 and 11 are 64.53% and 60% respectively. For ferric reducing ability of plasma (FRAP) assay, formulation 7 (2.11 µg FSE/mL) is the highest which have 1:1:1 ratio of each herb. In addition, TPC, TFC, and DPPH assays showed high antioxidant activities when only single herbs were added into the formulation. Meanwhile, ABTS and FRAP are prone to binary polyherbal formulation. Hence, this study showed an antagonism effect instead of synergistic effect since single formulation exhibited the highest for TPC, TFC and DPPH. All tests gave the lowest antioxidant properties when the mix was ternary polyherbal formulation.

1. Introduction

A memory improving activity is due to the presence of antioxidant properties in medicinal plants. A variety of botanical products have been reported to possess memory improving activity. The consequences of this study indicate that extracts of polyherbal formulation in powder form (oven-dried) of *C. asiatica*, *P. sarmentosum* and *M. citrifolia* mix with honey from honeybee breed (*Apis cerana*) owns a synergistic effect towards benefiting cognitive performance (Kapoor *et al*., 2013; Zainol Abidin *et al*., 2018).

Most people prefer herbal nootropics over synthetic nootropics because they have fewer side effects. For instance, synthetic drugs possess morphine-like and hepatotoxicity effect (Zimmerman, 1999). Herbal plant mainly acts by different ways, for instance, by increasing and replenishing neurotransmitter at high concentration in brain, anti-depression, adaptogenic and mood stabilization, by improved oxygen supply and brain energy, stimulation and brain cell protection. These nootropics or herbal drugs are used by many in cognitive enhancement regimens. Hence important to use optimization technique to evaluate the polyherbal formulation product acceptability (Kapoor *et al*., 2013).

The traditional medicinal plants and functional foods are defined to have ingredients that have additional benefits over and above the normal plant (Exarchou *et al*., 2006). The antioxidant activity of these plants is a part of interest both because of their beneficial

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physiological activity on human cells as well as potential they must replace the synthetic antioxidants used in medicine and food (Repetto and Llesuy, 2002). Several types of anti-inflammatory, digestive, anti-necrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant and/or antiradical scavenging mechanism as part of their activity. In the search for new sources of natural antioxidants, medicinal plants have been extensively studied for their antioxidant and radical scavenging activity (Desmarchelier and Ciccia, 2000).

This research will be for the finding of *A. cerana*, *C. asiatica*, *P. sarmentosum* and *M. citrifolia*'s antioxidant properties activity and memory improving effect as well as overall sensory acceptability (Lillrank, 2007; Martinez, 2010). This effect will surely facilitate the memory-boosting capacities and determine the acceptability in term of consuming them. In addition, the synergistic effect between all selected herbs that will be mixed together using honey will also be studied on. This research is the continuation of previous development and acceptance of polyherbal formulation for memory enhancement in term of sensory evaluation (Zainol Abidin *et al*., 2018).

2. Materials and methods

The herbals were purchased from Ethno Resources Sdn. Bhd., Sungai Buloh, Selangor, Malaysia. The raw herbs were collected from local villagers and natives in Kedah, Malaysia and supplied to Ethno Resources Sdn. Bhd. The collected herbs were oven-dried at 40°C for 5 days. The dried herbs were then ground into powder form. Pure honey from *A. cerana* was purchased from Madu Kira Haq Global Marketing Sdn. Bhd., Malacca. Folin-Cioclateu reagent, sodium carbonate, rutin, aluminium trichloride, DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), acetate buffer, glacial acetic acid and TPTZ (2,4,6-tripyridyl-s-triazine) were purchased from Merck Germany. All the reagents were of analytical grade.

2.1 Polyherbal preparation

Three types of herbs from different families were selected for the polyherbal formulation. The part of powdered form herbs, *C. asiatica*, *P. sarmentosum*, *M. citrifolia* with respect to their high potential towards antioxidant properties which leads to memory enhancement was extracted and mixed with honey from honeybee (*A. cerana*). The formulations were as tabulated in Table 1. Polyherbal mix was extracted by decoction method where the temperature was set at 90°C for 10 mins and 10% concentration (10 g sample in 100 mL distilled water). The mixture was filtered via Whatman filter paper. About 5 g of honey was mixed with 1 mL filtrate of the polyherbal mix. These polyherbal mixtures were placed into small separate plastic packaging (polyethylene) in accordance with their desired ratio and the plastic was sealed with hot sealer.

2.2 Antioxidants properties

2.2.1 Total phenolic content

The content of total phenolic compounds in polyherbal was determined by Folin-Ciocalteu method (1927) with slight modification (Miliauskas *et al*., 2004). A calibration curve was constructed using gallic acid as standard. A total of 100 µL of sample was mixed with 2 mL sodium carbonate (2 g in 100 mL distilled water) and was left for 2 mins at room temperature. Then, it was mixed with Folin-Ciocalteu reagent and left for 30 mins. The experiment was done in triplicate. The absorbance reading was taken at 750 nm. The absorbance values were compared to gallic acid standard curve (Miliauskas *et al*., 2004).

2.2.2 Total flavonoid content

The content of flavonoids was determined following the method by Sheehata *et al.* (2014) with few modifications using rutin as standard. An aliquot (1 mL) of the sample was mixed with 1 mL of 2% aluminium trichloride. The samples were left to incubate for 15 mins. The experiment was done in triplicate and the absorption was read at 430nm. Total contents of flavonoids were calculated by comparing the absorbance value against rutin standard (Sheehata *et al*., 2014).

2.2.3 DPPH (2,2-diphenyl-2-picrylhydrazyl) assay

This radical scavenging activity extract was measured following the method by Miliauskas *et al.* (2004) with minor modifications. The DPPH solution (5.9 mg in 100 mL methanol) was prepared fresh. 3mL of DPPH solution was mixed with 77 µL of sample in cuvettes. The mixed samples were kept in the dark for 15 mins at room temperature and the absorbance was read at 515 nm. Gallic acid was used as standard curve. The experiment was done in triplicate. The radical scavenging activity was calculated using the following formula.

$$\%Inhibition = [(AB - AA)/AB] \times 100,$$

Where AB is the absorption of blank sample at t = 0 min; and AA is the absorption of tested extract solution at t = 15 mins.

2.2.4 ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) assay

For ABTS assay, the stock solution was prepared by dissolving the ABTS in water to 7 mmol concentration. ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS stock solution with 2.45 mmol potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hrs before usage. The solution was diluted by mixing 1 mL $ABTS^{+}$ solution with 60 mL distilled water to obtain an absorbance of 0.70 (±0.02) at 734 nm using the spectrophotometer. A total of 200 µL samples were allowed to react with 2000 µl of the $ABTS^{+}$ solution for 2 hrs in a dark condition. Absorbance reading was taken at 734 nm. The experiment was done in triplicate. The percentage inhibition of absorbance was calculated and plotted as a function of concentration of antioxidants. The ascorbic acid standard curve was used to calibrate the values (Roberta *et al*., 1999; Sheehata *et al*., 2014)

2.2.5 FRAP (Ferric Reducing Ability of Plasma) assay

Reagents included 300 mmol/L acetate buffer with pH 3.6, and 16 mL of glacial acetic acid; 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L HCl; 20mmol/L $FeCl_3 \cdot 6H_2O$. Working FRAP reagent was prepared as required by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution. A total of 3 mL freshly prepared FRAP reagent was warmed to 37°C and a reagent blank reading was taken at 593 nm. Sample of 100 µL was then added along with 300 µL distilled water. Absorbance reading was taken after 4 mins. The experiment was done in triplicate. The absorbance values were compared to ferrous sulphate standard curve (Benzie and Strain, 1996).

2.2.6 Overall sensory evaluation

All 10 formulations were tested on 50-trained panelists for its overall sensory acceptability (Zainol Abidin *et al*., 2018).

2.3 Simplex-centroid mixture design

The mixture design of experiment was used to obtain the optimum composition between the polyherbal formulation for their antioxidant properties as well as overall sensory acceptance. Three components augmented simplex-centroid mixture design has been employed in which each component was studied in 6 types of responses which were TPC, TFC, DPPH, FRAP assay, ABTS assay and overall sensory acceptability. Design-Expert® Software Version 11.0 from State-ease Inc. was employed for experimental design, data analysis and model building (Scheffe, 1963).

2.4 Calculation and statistics

The statistical analysis was performed using Design-Expert® Software Version 11.0 from State-ease Inc. Values were performed as ANOVA, surface contours plot and triangular contour diagrams. The ANOVA was used to test for significant differences ($p<0.05$) between the responses.

3. Results and discussion

3.1 Experimental results

The design layout and experimental results of antioxidant properties in terms of TPC, TFC, DPPH, ABTS assay and FRAP assay, as well as overall sensory acceptability as per the experimental results, were presented in Table 1.

3.2 ANOVA analysis of antioxidant properties and overall acceptance

Table 2 summarizes the ANOVA of the tests performed. The value of "Prob > F" for responses TPC, TFC, DPPH, ABTS and FRAP are 0.0297, <0.0001,

Table 1. Design layout and experimental result for antioxidant properties and overall acceptance of sensory evaluation.

Std run no.	Factor 1	Factor 2	Factor 3	Response variables 1	2	3	4	5	6
	C. asiatica (%w/v)	*P. sarmentosum* (%w/v)	*M. citrifolia* (%w/v)	TPC (g/mL) in GAE	TFC (g/mL) in RE	DPPH (%)	ABTS (%)	FRAP (%)	Overall acceptance
1	1	0	0	134.33	479.8	34.92	64.533	1.19	7.12
2	0	1	0	125.7	406.6	27.08	53.6747	1.79	6.46
3	0	0	1	209.12	432.73	42.94	40.0664	1.26	5.78
4	0.5	0.5	0	161.6	457.53	29.67	43.1958	0.9	6
5	0.5	0	0.5	143.99	408.2	20.78	39.3551	1.69	6.54
6	0	0.5	0.5	130.57	459.8	26.14	59.3646	1.62	6.32
7	0.33	0.33	0.33	107.15	436.07	21.71	40.4457	2.11	6.34
8	0.66	0.17	0.17	167.15	453.8	18.88	41.2518	1.55	6.36
9	0.17	0.66	0.17	166.9	435.4	37.77	32.4798	1.5	6.48
10	0.17	0.17	0.66	110.85	441	23.38	55.192	2.1	6.48
11	1	0	0	133.33	470	30	60	1.2	6.98
12	0	1	0	124.3	405	25	55	1.75	6.33
13	0	0	1	210.1	433	42	45	1.3	5.5
14	0.5	0.5	0	131.2	450	30	43	0.85	6.2

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Table 2. ANOVA Table of antioxidant properties (TPC, TFC, DPPH, ABTS and FRAP) and overall sensory acceptance

Response Variables	Sum of square	Degree of freedom	Mean Square	F value	Probability	
TPC-Special quartic						
Regression	12354.36	8	1544.29	6.22	0.0297	significant
Residual	1241	5	248.2			
Lack of fit	776.96	1	776.96	6.7	0.0608	not significant
Pure error	464.04	4	116.01			
Cor total	13595.36	13				
R^2						0.9087
Adj R^2						0.7627
Adeq Precisior						8.831
TFC-Quadratic						
Regression	6.743.96	5	1348.79	50.09	<0.0001	significant
Residual	215.42	8	26.93			
Lack of fit	137.73	4	34.43	1.77	0.2964	not significant
Pure error	77.69	4	19.42			
Cor total	6959.38	13				
R^2						0.969
Adj R^2						0.9497
Adeq Precisior						20.869
DPPH-Special quartic						
Regression	739.66	8	92.46	23.9	0.0014	significant
Residual	19.34	5	3.87			
Lack of fit	4.58	1	4.58	1.24	1.24	not significant
Pure error	14.76	4	3.69			
Cor total	759.01	13				
R^2						0.9745
Adj R^2						0.9337
Adeq Precisior						15.581
ABTS-Special quartic						
Regression	1188.78	8	148.6	25.75	0.0012	significant
Residual	28.85	5	5.77			
Lack of fit	5.51	1	5.51	0.94	0.3862	not significant
Pure error	23.34	4	5.84			
Cor total	1217.64	13				
R^2						0.9763
Adj R^2						0.9384
Adeq Precisior						14.906
FRAP-Special quartic						
Regression	1.99	8	0.25	245.66	<0.0001	significant
Residual	5.059	5	1.012			
Lack of fit	2.279	1	2.278	3.28	0.1444	not significant
Pure error	2.78	4	6.95			
Cor total	1.99	13				
R^2						0.9975
Adj R^2						0.9934
Adeq Precisior						49.043
Overall acceptability-Quadratic [2]						
Regression	2.04	5	0.41	14.26	0.0008	significant
Residual	0.23	8	0.029			
Lack of fit	0.15	4	0.038	1.95	0.2667	not significant
Pure error	0.077	4	0.019			
Cor total	2.27	13				
R^2						0.8991
Adj R^2						0.8361
Adeq precisior						12.074

0.0014, 0.0012 and <0.0001 respectively which are less than 0.05.

The lack of fit for the dependent variables was insignificant. This is desirable, as we want a model that fits. The R^2 value calculated for TPC, TFC, DPPH, ABTS and FRAP are 0.9087, 0.9690, 0.9745, 0.9763 and 0.9975 respectively, reasonably close to 1, which is acceptable.

R^2 value is the first criteria to see the appropriateness of the model from the determination coefficient. It can also reveal the total variation of the observed values of activity about its mean. It also implies that about more than 91%, 97%, 98% and 99.7% of the variability in the data are explained by the special quartic model (TPC, DPPH, ABTS and FRAP) while 97% by quadratic model (TFC).

The predicted R^2 is in reasonable agreement with the adjusted R^2. Adequate precision compares the range of the predicted values at the design points to the average prediction error. In this case, the value is well above 4 which indicate adequate model discrimination. ANOVA analysis of overall sensory acceptance was explained in the previous report (Zainol Abidin *et al*., 2018).

The following equation is the final empirical models in term of coded factors of TPC, TFC, DPPH, ABTS, FRAP and overall acceptability. Where, CA is *C. asiatica*; PS is *P. sarmentosum*; MC is *M. citrifolia*.

a) TPC = 134.81CA + 125.98PS + 210.59MC + 71.88 CA*PS – 99.11CA*MC – 135.13PS*MC + 21277.77CA2*PS*MC + 2901.77CA*PS2*MC – 6181.70CA*PS*MC2
b) TFC = 475.84CA + 404.95PS + 433.72MC + 51.47CA*PS – 176.71CA*MC + 157.12PS*MC
c) DPPH = 32.54CA + 26.12PS + 42.55MC + 2.64CA*PS – 65.83CA*MC – 31.55PS*MC – 780.80CA2*PS*MC + 1297.48CA*PS2*MC – 527.58CA*PS*MC2
d) ABTS = 62.18CA + 54.25PS + 42.45MC – 61.15CA*PS – 53.17CA*MC + 42.72PS*MC - 248.13CA2*PS*MC – 1630.40CA*PS2*MC + 1388.18CA*PS*MC2
e) FRAP = 1.19CA + 1.77PS + 1.28MC – 2.44CA*PS + 1.78CA*MC + 0.35PS*MC + 16.27CA2*PS*MC – 6.38CA*PS2*MC + 46.63CA*PS*MC2
f) Overall acceptability = 7.01CA + 6.41PS + 5.67MC – 2.45CA*PS+0.89CA*MC + 1.67PS*MC [2]

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Examination of the fit summary output revealed that the special quartic model was statistically significant for the TPC, DPPH, ABTS, FRAP while the quadratic model was statistically significant for TFC and overall acceptance for sensory test. Therefore, this model was used to represent each of the response for further analysis. This can be observed from Table 2 where ANOVA Tables for both antioxidant properties and sensory evaluation were presented.

In order to ensure a good model, test for significance of the regression model, test for significance on individual model coefficients and test for lack of fit needed to be performed. An ANOVA Table for the response special quartic model for TPC, DPPH, ABTS and FRAP, quadratic model for TFC and overall acceptance on sensory evaluation were observed based on the value of F-ratio. The larger the magnitude of the F -value and the smaller the P-value, the more significant is the corresponding coefficient. The lack of fit can also be said to be insignificant.

The antioxidant properties vary considerably among herbs. The antioxidant properties of the polyherbal formulation were investigated using TPC, TFC, DPPH, ABTS and FRAP and the acceptance was determined by structured hedonic test. The reduction of ABTS and DPPH by antioxidants in the polyherbal are expressed as the percentage of radical scavenging activity and TPC, TFC and FRAP antioxidant capacity were ranked (from highest to lowest) as in Table 3.

The overall results indicate a strong association between antioxidative activities and phenolic compound, suggesting that phenolic compounds are probably responsible for the antioxidative activities of the herbs, *C. asiatica*, *P. sarmentosum* and *M. citrifolia*. A similar finding was reported that phenolic compounds are the major contributors to the antioxidative activity of apple, pineapple and vegetable juices (Gardner *et al*., 2000). It was also reported that phenolic compounds were responsible for the antioxidative activity in some selected fruits, vegetables and grains tested. Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants (Rice-Evans *et al*., 1995; Velioglu *et al*., 1998).

Similarly, it was also reported that naturally occurring phenolics exhibit antioxidative activity. Thus, the antioxidant properties of polyherbal extracts may possibly be attributed to the phenolic compounds present. Although they are found to be the major

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Table 3. The ranking for the highest to lowest value; including polyherbal formulation

Rank/Values	TPC (g/mL) in (GAE)	TFC (g/mL) in (RE)	DPPH (% scavenging effect)	ABTS (% scavenging effect)	FRAP (µmol/g)
1	3- 209.12	1- 479.80	3- 42.94	1- 64.53	7- 2.11
2	8- 167.15	6- 459.80	9- 37.77	6-59.36	10- 2.10
3	9- 166.90	4- 457.53	1- 34.92	10- 55.19	2- 1.79
4	4- 161.60	8- 453.80	4- 29.67	2- 53.67	5- 1.70
5	5-143.99	10- 441.00	2- 27.08	4- 43.20	6- 1.62
6	1- 134.33	7- 436.07	6- 26.14	8- 41.25	8- 1.55
7	6- 130.57	9- 435.40	10- 23.38	7- 40.45	9- 1.50
8	2- 125.70	3- 432.73	7- 21.71	3- 40.07	3- 1.26
9	10- 110.85	5- 408.20	5- 20.78	5- 39.36	1- 1.20
10	7- 107.15	2- 406.60	8- 18.88	9- 32.48	4- 0.90

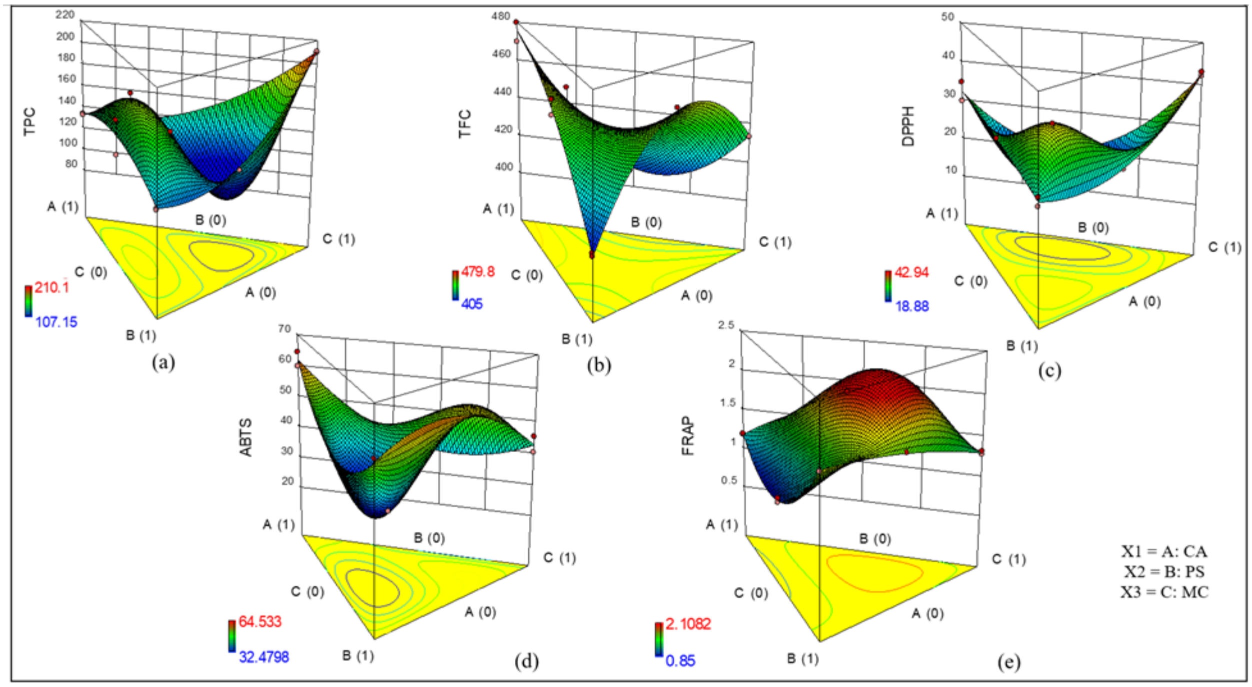

Figure 1. Surface plot of (a) TPC (b) TFC (c) DPPH (d) ABTS (e) FRAP

contributors to the antioxidative activity in *M. citrifolia*, *P. sarmentosum* and *C. asiatica*, the identity of these phenolic compounds remains unknown (Rice-Evans *et al*., 1995; Shahidi and Naczk, 1995; Velioglu *et al*., 1998; Granato *et al*., 2018).

3.3 Surface contours plot and triangular-dimensional contours diagram

The three-dimensional surface plots demonstrate the effect of different formulation on TPC, TFC, DPPH, ABTS and FRAP were depicted in Figure 1 (a), (b), (c), (d) and (e) respectively. Surface plots were then generated at each of the herbs (*C. asiatica*, *P. sarmentosum* and *M. citrifolia*) increased along the line from the centroid (1:1:1) toward its vertex (1:0:0) with honey as constant. The sensory acceptance's result was reported previously by Zainol Abidin *et al*. (2018). The contour plot is a two-dimensional (2D) representation of the response plotted against combinations of numeric factors and/or mixture components. It can show the relationship between the responses, mixture components and/or numeric factors (Rao and Baral, 2011). The mixture contour plots of the responses are depicted in Figure 2 to present more detailed interactions related to the regression models on the acceptance of polyherbal formulation. The diagrams illustrated three variations of interactions on the responses, in which the dark red areas represent the highest acceptance value. Meanwhile, green and blue indicate medium and the lowest acceptance value towards the response against the factors.

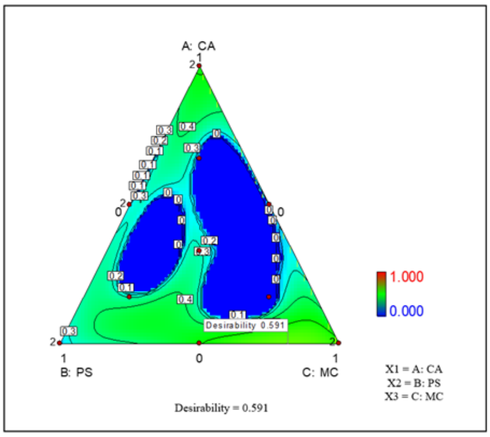

Figure 2. Desirability and optimisation of antioxidant properties

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3.4 Optimised polyherbal mix by simplex-centroid mixture design: antioxidant properties and overall sensory acceptance

Optimization of the levels to get best combination was resolved through graphical optimisation procedure of design expert software by giving criteria to maximise the TPC, TFC, DPPH, ABTS and FRAP as shown in Figure 2. Higher desirability value or the value closer to 1 indicates how best the criteria given for optimization are meeting.

The desirability value of this design is 0.591 which indicates medium met of optimisation criteria. Responses with no goal are often shown with gray, but in this study, none was found. The optimised formulation is when 0% *C. asiatica*; 18.5% *P. sarmentosum*; 81.5% *M. citrifolia* was applied as the formulation for the polyherbal. There is a total of five optimised solutions with desirability value ranging from 0.344 to 0.591 as shown in Table 4.

Table 4. The Desirability with optimised proportions factors

Rank	Desirability	Factors (%)		
		C. asiatica	*P. sarmentosum*	*M. citrifolia*
1	0.591	0	18.5	81.5
2	0.526	0	0	1
3	0.516	100	0	0
4	0.463	6.85	63.8	29.35
5	0.344	20.04	79.96	0

The optimum overall acceptance of polyherbal mix was determined through the highest mean obtained from the overall acceptance response in sensory evaluation. It can be observed that the highest mean score 7.00 followed by 6.98 and 6.74 were obtained from formulation 11, 1 and 8 respectively. The most preferred polyherbal formulation as the best proportion of these components is the one containing *C. asiatica* only. This can be supported by previous research done where optimisation of the polyherbal formulation results shown 96.04% *C. asiatica*; 0% *P. sarmentosum*; 3.96% *M. citrifolia* of the herbal concentration where the desirability value indicates high criteria (0.822). The formulation with only *C. asiatica* only also obtained the highest mean score (Zainol Abidin *et al*., 2018).

3.5 Effect of single, binary and ternary polyherbal formulation on antioxidant properties and overall sensory acceptance

Table 5 indicates the average of TPC, TFC, DPPH, ABTS and FRAP in different polyherbal formulation which the formulations were for single; honey mixed with single herbs, binary; honey mixed with two kind of herbs while ternary; honey mixed with three types of herbs. TPC, TFC, and DPPH assays showed high antioxidant activities when only single herbs were added into the formulation. Meanwhile, ABTS and FRAP are prone to binary polyherbal formulation. It is reported that herbal combination often produces a promising effect in treatment of diseases over a single drug (Ramaiah *et al*., 2013).

Naturally occurring herbs and herbal ingredients organized into certain formula have been shown to have potential interaction effects. These include mutual enhancement, mutual assistance, mutual restraint and even mutual antagonism (Ramaiah *et al*., 2013). Hence, in this study, the results showed an antagonism effect instead of synergistic effect since single formulation exhibited the highest for TPC, TFC and DPPH. All tests gave the lowest antioxidant properties when the mix was ternary polyherbal formulation. Since there is limited research on the synergistic effect on exact herbs *C. asiatica*, *P. sarmentosum* and *M. citrifolia*, solid evidence to support this statement is limited. For sensory evaluation, panelists prefer binary formulation compared to single and ternary. Previously stated that the most preferred polyherbal formulation is the one containing *C. asiatica* only. *M. citrifolia* single polyherbal formulation is the least preferred among single formulation and even among the binary formulation when it is mixed with *P. sarmentosum*. But when *M. citrifolia* was mixed with *C. asiatica*, the overall acceptability increased. Hence, it can be concluded that *C. asiatica* was able to mask the unacceptability of the other two herbs while by itself is the most preferred (Zainol Abidin *et al*., 2018).

3.6 Relationship between antioxidant properties and memory enhancement

Acetylcholinesterase, the major cholinesterases in human blood, muscle and brain cells, which primarily hydrolases acetylcholine (Matinez, 2010). This neurotransmitter has been shown to be important for memory, attention and learning. Acetylcholine has functions both in the peripheral nervous system (PNS) and in the central nervous system (CNS) as a neuromodulator. In the peripheral nervous system, acetylcholine activates muscles and is a major neurotransmitter in the autonomic nervous system

Table 5. Average of TPC, TFC, DPPH, ABTS, FRAP in different polyherbal formulation

Polyherbal formulation	TPC (g/mL) in (GAE)	TFC (g/mL) in (RE)	DPPH (% scavenging effect)	ABTS (% scavenging effect)	FRAP (µmol/g)	Overall sensory acceptance
Single	156.1467	33.6567	53.04568	437.855	1.415428	6.3617
Binary	141.84	26.6475	46.22888	443.8825	1.264328	7.765
Ternary	138.0125	25.435	42.34233	441.5675	1.814915	6.415

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(Ullrank, 2007). According to previous studies, chronic treatment with antioxidant present in berberine significantly decreased the cholinesterase enzyme activity in the cortex of lab rat (Bhutadaa *et al*., 2011). In addition, studies on oxidative damage and claimed that oxidation was considered a likely cause of age-associated brain dysfunction because the brain is believed to be particularly vulnerable to oxidative stress due to a relatively high rate of oxygen free radical generation without commensurate levels of antioxidative defenses (Raghavendra and Kulkarnia, 2001).

Since amnesia associated with increased brain oxidative stress during brain aging or other brain stress, it can be reversed by antioxidants. Cognitive deficits such as learning impairment and delayed amnesia are the debilitating consequences of aging (Cao *et al*., 1997). The age-associated impairment of cognitive and motor functions has been hypothesized as due to oxidative molecular damage. Antioxidant protected the brain cells against increased oxidative stress-induced during aging or by other chronic treatment (Raghavendra and Kulkarnia, 2001). It can also remove free radical production and/or intercept dissemination of autoxidation (Taslimi and Gulcin, 2018). Therefore, high antioxidant properties of a polyherbal formulation can help in memory enhancement.

3.7 Relationship between antioxidant properties and overall sensory acceptance

Optimization for sensory evaluation suggested a polyherbal formulation containing *C. asiatica* only as of the best proportion of these components. Meanwhile, the optimised polyherbal formulation for the highest antioxidant properties is when 0% *C. asiatica*; 18.5% *P. sarmentosum*; 81.5% *M. citrifolia* was applied. The result for antioxidant properties and sensory evaluation contradict one another. While the consumers will prefer *C. asiatica* only, we can provide better formulation to increase the antioxidant properties. Hence, we had concluded for the product development evolution, in vision of health-conscious society with little care about the taste, the formulation with 0% *C. asiatica*; 18.5% *P. sarmentosum*; 81.5% *M. citrifolia* is the ideal as the highest antioxidant properties. Likewise, for the society who prefer taste over health, the formulation with only *C. asiatica* is ideal. But, in order to optimise the antioxidant properties to tally with the overall acceptance of the product, further research needs to be done to come out with an ultimate formulation to mask the unacceptable sensory attributes.

4. Conclusion

Simplex-centroid mixture design was determined for antioxidant properties (TPC, TFC, DPPH, ABTS and FRAP) and sensory evaluation for the development and evaluation of polyherbal formulation. The results obtained that the formulation 3 and 13 which are the repetition of formulation with *M. citrifolia* only has the highest antioxidant capacity when being tested with TPC (210.10 and 209.12 µg GAE/mL respectively) and DPPH (42.94 and 37.77% inhibition) assays. Contradict to this result, the highest TFC and ABTS antioxidant properties are formulations 1 and 11 which are the repetitive formulation of *C. asiatica* only. The TPC for formulation 1 and 11 are 479.8 µg RE/mL and 470 µg RE/mL respectively) while ABTS radical inhibition for formulation 1 and 11 are 64.53% and 60% respectively. For FRAP assay, the highest antioxidant activity is formulation 7 (2.11 µg FSE/mL) which have 1:1:1 ratio of each herbal concentration. In addition, TPC, TFC, and DPPH assays showed high antioxidant activities when only single herbs were added into the formulation. Meanwhile, ABTS and FRAP are prone to binary polyherbal formulation. Hence, in this study the results showed an antagonistic effect instead of synergistic effect since single formulation exhibited the highest for TPC, TFC and DPPH. All tests gave the lowest antioxidant properties when the mix were ternary polyherbal formulation. Hence, through all the results obtained, SCMD had optimised the polyherbal formulation to come up with the highest antioxidant properties formulation. The most preferred is the one containing 0% *C. asiatica*; 18.5% *P. sarmentosum*; 81.5% *M. citrifolia* in the polyherbal formulation. In the aspect of sensory evaluation, the most preferred polyherbal formulation as the best proportion of these components is the one containing *C. asiatica* only.

Conflict of Interest

Authors declare no conflict of interest.

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