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Development and Acceptance of Polyherbal Formulation for Memory Enhancement

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Abstract: Simplex-centroid mixture design has been applied to investigate the acceptance on the polyherbal formulation for memory enhancement. Polyherbal formulation consists of three different herbs (*Centella asiatica*, *Piper sarmentosum* and *Morinda citrifolia*) as independent variables and mixed with honey bee (Apis cerana) as the medium was developed. Consumer acceptability was measured in terms of nine dependent variables by 50 semi-trained panelists using a 9-point hedonic scale. Results showed that overall acceptability was affected mostly by changes in the interaction of *Centella asiatica* and *Piper sarmentosum*, *Centella asiatica* and *Morinda citrifolia*, *Piper sarmentosum* and *Morinda citrifolia* ($p<0.05$). Optimization suggested a polyherbal formulation containing *Centella asiatica* only as the best proportion of these mixtures.

Key words: Simplex-Centroid Mixture Design (SCMD), polyherbal formulation, *Centella asiatica*, *Piper sarmentosum*, *Morinda citrifolia*, Honey *(Apis cerana)*, product development, sensory acceptability

INTRODUCTION

Herbal treatments are the most popular form of traditional medicin (WHO., 2016). Scientific literature is continuously reporting plant drugs having neuromodulatory activity. Traditional herbs may be useful for neural regeneration and synaptic plasticity (Shah and Goyal, 2001). The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings (WHO., 2004). By identifying what can induce the memory abilities, enhancement towards higher Intelligence Quotient (IQ) level can be met and realised.

The selections of plants are based on their antioxidant activities and antiacetylcholinesterase effects by research done previously (Dalimartha, 2000). *In vitro* studies, *Centella asiatica* is recognized as a reputed plant species for its traditional use in ayurvedic and Chinese medicines (Howes and Houghton, 2003) and also against Alzheimer disease (Orhan *et al*., 2007). Besides that, Praveen and Yellamma (2014) has concluded in their study that drug molecules presents in *Morinda citrifolia* will interact with the active sites of proteins and inhibit the activity of Acetylcholinesterase enzyme, hence, have the neuroprotective effect against Alzheimer's disease to enhance memory ability. Throughout the tropics, a range of piper have been used for many purposes (Barrett, 1994; Sengupta and Sengupta, 1987). Ridtitid *et al*. (1998) found that the extract of *Piper sarmentosum* elicited a marked dose-related neuromuscular blocking activity at neuromuscular junction of skeletal muscle indicating a strong AChE inhibitory activity (Ridtitid *et al*., 1998). The plants are also selected based on their utilities in folk medicines, a traditional herbs used to treat diseases during the old age.

According to previous studies done by Bhutada *et al*. (2011), chronic treatment with antioxidant present in berberine significantly decreased the cholinesterase enzyme activity in the cortex of lab rat. Raghavendra and Kulkarni (2001) studied 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.

Considering the fact that the 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. 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 Shrinivas, 2001).

Statistical mixture designs are special class of response surface designs where the proportions of the

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components or factors are considered important. It involves the use of different combinations between the components for changing mixture composition and exploring how such changes will affect a specific response. The interactions between the components of a mixture for maximizing the response are studied using mixture design approach (Rao and Baral, 2011).

The study described here was carried out to apply simplex centroid mixture design in describing the sensory acceptance of polyherbal formulation consisting *Centella asiatica*, *Piper sarmentosum* and *Morinda citrifolia* with respect to their high potential towards cholinergic activity for memory enhancement by 9-point hedonic test (Shah and Goyal, 2011).

MATERIALS AND METHODS

The herbals were purchased from Ethno resources Sdn Bhd., Sungai Buloh, Selangor. The raw herbs were collected from local villagers and natives in Kedah area and supplied to Ethno Resources Sdn Bhd. The collected herbs were oven dried at 40°C for 5 days. The dried herbs were then grinded into powder form. Pure honey from *Apis cerana* was purchased from Madu Kira Haq Global Marketing Sdn Bhd., Malacca.

Polyherbal preparation: Three types of herb from different families were selected for the polyherbal formulation. The part of powdered-form herbs, *Centella asiatica*, *Piper sarmentosum* and *Morinda citrifolia* with respect to their high potential towards will be extracted and mixed in honey from honey bee (*Apis cerana*). The antioxidants from the polyherbal were extracted by pasteurisation method at 90°C for 10 min and 10% concentration (10 g sample in 100 mL distilled water). The mixture was filtered via. whatman filter paper. About 5g of the honey was mixed with 1mL of the filtered extract. These polyherbal mixtures were into small plastic packaging and the plastic was sealed with hot sealer.

Simplex-centroid mixture design: The mixture design of experiment was used to obtain the optimum composition between the polyherbal formulation (*Centella asiatica*, *Piper sarmentosum* and *Morinda citrifolia*) for their acceptance. A three component augmented simplex-centroid design has been employed in which each components is studied in 6 types of responses which were colour, flavour, odour, liquid thickness, sweetness and overall acceptability. The Design-Expert® 10.0 Software from State-Ease Inc. was employed for experimental design, data analysis and model building (Scheffe, 1963).

Calculation and statistics: The statistical analysis were performed using the Design-Expert® 10.0 Software from State-Ease Inc. values are expressed as ANOVA, surface contours plot and triangular contours diagrams. The ANOVA was used to test for significant differences ($p<0.05$) between the responses.

RESULTS AND DISCUSSION

The overall acceptance of the polyherbal formulation was studied for the development and evaluation of polyherbal formulation. In this study, six attributes are evaluated to determine the overall acceptance towards polyherbal formulation on sensory evaluation. When a product is experienced, the sensory characteristics perceived by a consumers and expectation are combined into an overall product quality evaluation. Hedonic measurements (expected linking evaluation) are usually performed in order to study how intrinsic and extrinsic characteristics interact in the global perception. Hence, the acceptance of target consumers on the product is developed (Lange *et al*., 2002).

Consumer acceptability was measured in terms of nine response variables by 50 semi-rained panelists using a 9-point hedonic scale. Results showed that overall acceptability was affected mostly by changes in the interaction of *Centella asiatica* and *Piper sarmentosum*, *Centella asiatica* and *Morinda citrifolia* and *Piper sarmentosum* and *Morinda citrifolia* ($p<0.05$). Optimization suggested a polyherbal formulation containing *Centella asiatica* only as the best proportion of these components.

The literature on sensory evaluation for both *Centella asiatica* and *Piper sarmentosum* are very limited. Limited previous research on the sensory acceptance itself proved that the overall acceptance of *Centella asiatica* and *Piper sarmentosum* are non-significant issues. As the result obtained, panelists prefer the formulation with *Centella asiatica* only because as compare to the other two herbs, *Centella asiatica* emits the most faint smell and aftertaste. The faint smell and taste was masked by the taste of honey therefore, increasing the acceptance value. Meanwhile, *Piper sarmentosum* has a strong bitter aftertaste and smell. Honey were unable to mask the smell and taste of *Piper sarmentosum*.

For *Morinda citrifolia* because the part used in this formulation was the fruit, it has a foul taste and a soapy smell when the fruit matured. There was a research done by previous researcher where they tried to mask the taste of *Morinda citrifolia* with lemon. (Saniah and Hasimah, 2008). This showed that the acceptance towards the taste of *Morinda citrifolia* was considerably low and it needed

Table 1: Design layout and experimental result

Factors (w/v%)			Response variables					
Centella asiatica (1)	*Piper sarmentosum* (2)	*Morinda citrifolia* (3)	Colour (1)	Flavour (2)	Odour (3)	Liquid thickness (4)	Sweetness (5)	Overall acceptance (6)
1.00	0.00	0.00	7.24	6.90	6.80	6.62	7.20	7.12
0.00	1.00	0.00	7.20	6.10	6.74	6.72	5.90	6.46
0.00	0.00	1.00	6.98	5.48	6.44	6.60	6.80	5.78
0.50	0.50	0.00	6.89	5.68	6.48	6.52	6.80	6.00
0.50	0.00	0.50	7.10	7.40	6.58	6.58	6.10	6.54
0.00	0.50	0.50	7.10	5.94	6.58	6.54	6.50	6.32
0.33	0.33	0.33	7.28	6.24	6.96	6.38	6.00	6.34
0.66	0.17	0.17	7.18	6.74	6.94	6.62	6.60	6.36
0.17	0.66	0.17	7.26	5.88	6.82	6.58	5.50	6.48
0.17	0.17	0.66	7.14	6.02	6.68	6.62	6.60	6.48
1.00	0.00	0.00	7.19	7.20	6.78	6.55	7.30	6.98
0.00	1.00	0.00	7.10	6.21	6.66	6.80	5.70	6.33
0.00	0.00	1.00	7.00	5.60	6.50	6.58	6.50	5.50
0.50	0.50	0.00	6.98	5.70	6.54	6.60	5.98	6.20

to be covered. Hence, from this sensory evaluation, the most preferred formulation is when honey is mixed with *Centella asiatica* only.

Experimental results: The design layout and experimental results of sensory evaluation in terms of colour, flavor, odour, liquid thickness, sweetness and overall acceptance as per the experimental result are presented in Table 1.

ANOVA analysis of overall acceptance: An ANOVA table as shown in Table 2 has summarized the tests performed. The value of "Prob.>F" for responses colour, flavour, odour, sweetness and overall acceptance are <0.05 which indicates that the term in the model has a significant effect on the responses. Meanwhile for liquid thickness, the p-value is 0.0921 which is >0.05. This indicates that the term in the model has no significant effect on the responses. Honey was used as the medium in this polyherbal formulation. The concentration and volume of honey used was kept constant even though the formulation was of different concentration. Hence, the panelists were unable to differentiate the difference. The lack of fit for the dependent variables was insignificant. This is desirable as we want a model that fits. Table 2 also shows the R^2 value calculated for overall acceptability is 0.8991, reasonably close to 1 which is acceptable. 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. The following equation is the final empirical models in term of coded factors of overall acceptance:

$$\text{Overall acceptability} = 7.01CA+6.41PS+5.67MC - 2.45CA * PS +0.89CA * MC + 1.67PS * MC$$

Generally, the coefficient with negative sign in the fitted model indicates the ability of its associated factor to decrease the response, while the positive sign display the ability of a factor to decrease the response variable (CA, *Centella asiatica*; PS, *Piper sarmentosum*; MC, *Morinda citrifolia*).

Surface contours plot and triangular-dimensional contours diagrams: The three dimensional surface plots demonstrate the effect of different formulation on overall acceptance and they are depicted in Fig. 1a. Surface plots were then generated at each of the herbs (*Centella asiatica*, *Piper sarmentosum* and *Morinda citrifolia*) increased along the line from the centroid (1:1:1) toward its vertex (1:0:0). 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 three responses are depicted in Fig. 1b to present more detailed interactions related to the regression models on the desirability of polyherbal formulation optimized by SCMD. The diagrams illustrate 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.

Optimum overall acceptance: The optimum overall acceptance of polyherbal mix was determined through the highest mean obtained from overall acceptance response in sensory evaluation. Based on Table 2, 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

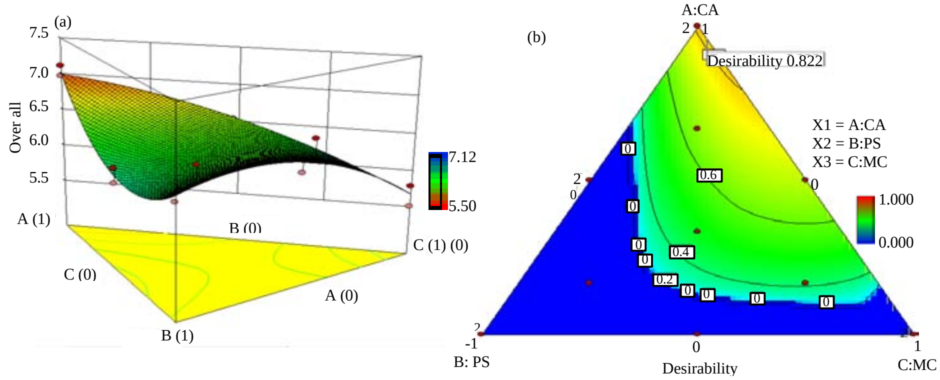

Fig. 1: a) Surface plot of overall acceptability; b) Triangular-dimentional contours diagram for the desirability of polyherbal mix

Table 2: ANOVA table for acceptance (overall acceptance, colour, flavour, odour, liquid thikness and sweetness)

Response variables	Sum of square	df	Mean square	F-values	Probability	Results
Overall acceptability-quadratic						
Regression	2.040	5	0.410	14.26	0.0008	Significant
Residual	0.230	8	0.029			
Lack of fit	0.150	4	0.038	1.95	0.2667	Not significant
Pure error	0.077	4	0.019			
Cor total	2.270	13				
R^2						0.8991
Adj R^2						0.8361
Adeq precisior						12.074
Colour-special quartic						
Regression	0.180	8	0.023	7.97	0.0175	Significant
Residual	0.014	5	2.863			
Lack of fit	6.645	1	6.645	0.19	0.6818	Not significant
Pure error	0.014	4	3.413			
Cor total	0.200	13				
R^2						0.9273
Adj R^2						0.8110
Adeq precisior						8.7310
Flavour-quadratic						
Regression	4.700	5	0.940	58.91	<0.0001	Significant
Residual	0.130	8	0.016			
Lack of fit	0.069	4	0.017	1.18	0.4369	Not significant
Pure error	0.058	4	0.015			
Cor total	4.830	13				
R^2						0.9736
Adj R^2						0.9570
Adeq precisior						21.676
Odour-special quadratic						
Regression	0.350	8	0.044	23.44	0.0015	Significant
Residual	9.444	5	1.889			
Lack of fit	2.444	1	2.444	1.40	0.3028	Not significant
Pure error	7.000	4	1.750			
Cor total	0.360	13				
R^2						0.9740
Adj R^2						0.3125
Adeq precisior						14.914
Liquid thickness-quadratic						
Regression	0.075	5	0.015	2.83	0.0921	Not significant
Residual	0.042	8	5.284			
Lack of fit	0.033	4	8.305	3.67	0.1179	Not significant
Pure error	9.050	4	2.263			
Cor total	0.120	13				
R^2						0.6391
Adj R^2						0.4135
Adeq precisior						5.016
Sweetness-linear						
Regression	2.170	2	1.090	7.13	0.0103	Significant
Residual	1.670	11	0.150			
Lack of fit	1.270	7	0.180	1.78	0.3007	Not significant
Pure error	0.410	4	0.100			
Cor total	3.850	13				
R^2						0.9740
Adj R^2						0.9325
Adeq precisior						14.914

Table 3: Average amount single, binary and ternary polyherbal formulation

Polyherbal formulation	Colour	Flavour	Odour	Liquid thickness	Sweetness	Overall acceptance
Single	7.118	6.248	6.653	6.645	6.5667	6.3617
Binary	8.725	7.600	8.165	8.190	8.0450	7.7650
Ternary	7.215	6.220	6.850	6.550	6.1750	6.4150

components is the one containing *Centella asiatica* only. This formulation with only *Centella asiatica* only also obtained the highest mean score in flavour (7.20), sweetness (7.3) and overall acceptance. While it is amongst the highest for colour (7.24) after formulation 7 and 9 with mean value of 7.28 and 7.26 respectively as well as for odour (6.80) after formulation 7 (6.96), 8 (6.94) and 9 (6.82). It showed that formulation 11 was the most preferred by consumer.

Optimisation by simplex-centroid mixture design: The most preferred polyherbal formulation as the best proportion of these components is the one containing *Centella asiatica* only. This can be supported by Fig. 1 where optimisation of the polyherbal formulation was done and the result shown was 96.04% *Centella asiatica*; 0% *Piper sarmentosum*; 3.96% *Morinda citrifolia* of the herbal concentration where the desirability value indicates high criteria (0.822).

Optimization of the levels to get best combination was resolved through graphical optimisation procedure of design expert software by giving criteria to maximise the colour, flavour, odour, liquid thickness, sweetness, hence, the overall acceptance. 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 0.822 which indicates high met of optimisation criteria. It showed that this formulation was the most preferred by consumer and was supported optimised variable and desirability as shown in Table 3.

According to Table 3, the 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* are able to mask the unacceptability of the other two herbs while by itself being the most preferred.

CONCLUSION

Centella asiatica can be used in the formulation of the polyherbal product's development since it helps to obtain better sensory attributes for the product. In regards to the optimization results it is to be concluded that the polyherbal formulation containing only *Centella asiatica* was most accepted. In addition, according to previous studies towards its memory enhancement capabilities, able to contribute the commercial value towards the polyherbal product.

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