第 49 卷 第 8 期
2022 年 8 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 8
August 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.8.1

Optimization of a Functional Beverage Formula with Antioxidant Properties

Sawita Srisawat[1], Chutikan Sakphisutthikul[2]*

[1] Doctor of Public Health Program, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand

[2] Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand

Abstract: *Morus alba* L., *Cnidoscolus chayamansa* Mc.Vaugh, *Moringa oleifera*, and *Stevia rebaudiana* Bert. are widely used as ingredients in a traditional Thai beverage. This beverage is known for its beneficial properties for human health, such as its high antioxidant capacity, which give it the potential to be developed as a functional beverage. The objective of this study is to determine the optimum antioxidant properties of polyherbal formulations of leaf extracts using an in vitro method. There are 22 formulations consisting of various proportions of the extracts designated using Design Expert 6.0.4. The antioxidant study of the individual extracts and their different combinations was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, a Ferric Reduction Antioxidant Potential Assay (FRAP), and a Total Phenolic Content (TPC) assay. The results showed that *Morus alba* L. has higher antioxidant properties in most of the assays when tested individually, as compared to *Cnidoscolus chayamansa* Mc.Vaugh, *Moringa oleifera*, and *Stevia rebaudiana* Bert. A synergistic effect was exhibited in most of the polyherbal combinations. From the optimization process, it was revealed that the quadratic model was statistically significant for the FRAP and the TPC, while the special cubic model was statistically significant for the DPPH. The optimum mixture for antioxidant properties was found to contain an extract of 24.87 percent of *Cnidoscolus chayamansa* Mc.Vaugh, 0.64 percent of *Moringa oleifera*, and 73.46 percent of *Morus alba* L., with a desirability of 0.988. In conclusion, this present study justifies the promising antioxidant properties of the polyherbal mixtures. Thus, it is sensible to use a studied herbal formulation for the development of any antioxidant supplements or food products.

Keywords: optimization, functional beverage, antioxidant properties.

具有抗氧化特性的功能性饮料配方的优化

摘要：莫鲁斯*阿尔巴 L.，[医]鼻窦查亚曼萨司仪。沃,辣木[医]油,和甜叶菊雷巴迪亚纳伯特. 被广泛用作传统泰国饮料的配料。这种饮料以其对人类健康有益的特性而闻名，例如其高抗氧化能力，这使其具有开发为功能性饮料的潜力。本研究的目的是使用体外方法确定叶提取物的多叶制剂的最佳抗氧化性能。有 22 种配方,由使用设计专家 6.0.4 指定的提取物的各种比例组成.使用 2,2-二苯基-1-吡啶基(DPPH)自由基清除活性、铁还原抗氧化电位测定(FRAP)和总酚含量(TPC)测定对单个提取物及其不同组合进行了抗氧化研究。结果表明，与[医]鼻窦查亚曼萨司仪相比，莫鲁斯*阿尔巴 L.在大多数检测中具有更高的抗氧化性能。沃,辣木[医]油,和甜叶菊雷巴迪亚纳伯特. 在大多数多叶组合中表现出协同效应。从优化过程中发现，二次模型对 FRAP 和 TPC 具有统计学意义，而特殊三次模型对 DPPH 具有统计学意义。发现抗氧化性能的最佳混合物含有 24.87%的[医]鼻窦查亚曼萨司仪提取物。0.64%的辣木[医]油和 73.46%的莫鲁斯*阿尔巴 L.，可取性为 0.988。总之，这项研究证明了多叶虫,多叶虫混合物的有希望的抗氧化性能。因此，它是明智的使用研究草药配方的任何抗氧化剂补充剂或食品的发展。

关键词：优化，功能性饮料，抗氧化性能。

Received: May 4, 2022 / Revised: June 7, 2022 / Accepted: July 12, 2022 / Published: August 30, 2022

About the authors: Sawita Srisawat, Doctor of Public Health Program, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand; Chutikan Sakphisutthikul, Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand

Corresponding author Chutikan Sakphisutthikul, chusak@kku.ac.th

1. Introduction

A high margin of safety, cost-effectiveness, eco-friendliness, and ready availability have led to the increasing development of herbal supplements involving traditional medicinal plants [1]. An antioxidant is a compound that plays an important role as a protective factor that may reduce the risk of oxidative stress-related diseases and may be able to provide a health enhancing effect on the human organism [2]. The main characteristic of an antioxidant is its ability to trap free radicals, which are oxygen-centered molecules that contain a single electron at the outermost orbit. Highly reactive free radicals and oxygen species are present in biological systems, as they come from a wide variety of sources [3]. Thus, the study of the biological activity and chemical composition of medicinal plant extracts as a potential source of natural antioxidants is becoming a trend in the development of products. *Cnidoscolus chayamansa* Mc.Vaugh (from the Euphorbiaceae family) is a well-known herb in Thailand that has been reported to have various properties including antioxidant effects [4]. Meanwhile, *Moringa oleifera* is from the family Moringaceae, and the extract from this plant has been widely used in the preparation of various Thai folk remedies [5]. Another herb, *Morus alba* L., is locally known as "bai-mon" and belongs to the family Moraceae. The leaves exhibit dynamic pharmacological properties such as strong antioxidant potency and total phenolic content [6]. *Stevia rebaudiana* Bert., which belongs to the family Asteraceae, has been used in various formulations mainly to improve the palatability of the formulation, in which it acts as a natural sweetener [7]. Nevertheless, the extract has the ability to quench the DPPH radical, which indicates that the extract is also a good antioxidant with radical scavenging activity [8]. Basically, plant extracts are natural components that might employ synergistic, antagonistic, additive, and indifferent effects depending on the interaction of the phytochemicals [9]. Those effects should be taken into account during the development of supplements or food products from natural sources, or when they are used as a replacement for synthetic antioxidants, which can contribute to harmful effects on human health [10]. A previous study reported that commercial statistical software was used to discover the experimental conditions that produce the best possible analytical performance [11]. Therefore, in this present study, commercial statistical software package Design Expert 6.0.4 was used to determine the optimum antioxidant properties of polyherbal formulations of the aqueous extract of the leaves of *Morus alba* L., *Cnidoscolus chayamansa* Mc.Vaugh, *Moringa oleifera*, and *Stevia rebaudiana* Bert. using the in vitro method.

2. Materials and Methods

2.1. Chemicals and Instruments

DPPH, methanol, Folin-Cioclateu reagent, sodium carbonate, acetate buffer, glacial acetic acid, and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Merck Germany. The reagents were analytical grade and were procured from local sources. The instrument used was a UV–visible (UV-Vis) spectrophotometer (T60u, PG Instrument, United States of America) located in the Food Analysis Laboratory.

2.2. Collection and Preparation of Plant Materials

The leaves of *Morus alba* L., *Cnidoscolus chayamansa* Mc.Vaugh, *Moringa oleifera*, and *Stevia rebaudiana* Bert. were used in the polyherbal formulations. The dried forms of the samples were purchased from a local company. The preparation of the leaves' extracts was accomplished with slight modifications [12]. The dried leaves of selected extracts were grinded into powdered form using a standard laboratory blender. Next, 100 g of the powder was brought to a boil in 1,000 mL of distilled water until the volume of the mixture was reduced to about a third of the original volume. It was then immersed in the hot water at a range of 80°C to 90°C for 15 minutes. The extract was filtered separately using a sterile Whatman grade 1 filter paper to separate the supernatant mixture from the extraction solution, and the residues were stored under a cool condition for later use. For the development of the polyherbal formulations, the plant extracts were mixed in 22 separate portions that were designated using Design Expert 6.0.4. The simplex-centroid mixture design was chosen for the experiments because all the components have the same range of 0 to 100. There were no constraints on the design space [13].

2.3. DPPH Radical Scavenging Activity Assay

The radical scavenging activity of the polyherbal formulations against the stable DPPH chemical compound was determined spectrophotometrically. When DPPH reacted with an antioxidant compound, which can donate hydrogen, it was reduced. The changes in color (from deep violet to light yellow) were measured at 517 nm on a UV–Vis spectrophotometer. The radical scavenging activities of each extract and the polyherbal combination were measured according to the previous method with slight modifications [14, 15]. The DPPH solution (5.9 mg in 100 mL methanol) was prepared the day before the UV measurement. Then, 3 mL of the DPPH solution was

mixed with 77 µL of sample-in cuvettes. The mixed samples were kept in darkness for 15 min at room temperature, and then the decrease in absorption was measured. The absorption of the blank sample containing the same amount of methanol and DPPH was prepared and measured. The experiment was conducted in triplicate. The radical scavenging activity was calculated by the following formula:

% Inhibition = [(AB – AA)/AB] x 100 (1)

where AB-absorption of the blank sample was t = 0 min, and the AA-absorption of tested extract solution was t = 15 min.

2.4. FRAP Assay

The FRAP assay was carried out as described by Benzie and Strain, with minor modifications [16]. Reagents included 300 mmol/L acetate buffer, pH 3.6, and 16 mL glacial acetic acid per liter of buffer solution; 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L HCl; and 20 mmol/L $FeCl_3$•$6H_2O$. The working FRAP reagent was prepared as required by mixing 25 mL acetate buffet, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3$•$6H_2O$ solution. The 3 mL freshly prepared FRAP reagent was warmed to 37°C, and a reagent blank reading was taken at 593 nm. Then, 100 µL of the sample was added along with 300 µL H_2O. The absorbance reading was taken after 4 min. The experiment was conducted in triplicate. The change in absorbance between the final reading and the blank reading was calculated for each formulation and compared to the standard curve. A standard of known Fe (II) concentrations was carried out using several concentrations from 100 to 1000 µM. A standard curve was plotted by plotting the FRAP value of each standard against its concentration. The FRAP values for the samples were determined using this standard curve. The results obtained were calculated using the equation below:

FRAP value of sample (µM) = (a/b) × FRAP value of standard (1000 µM) (2)

where a – change in absorbance of sample from 0 to 4 min; b – change in absorbance from 0 to 4 min.

2.5. TPC Assay

The content of the phenolic compounds in polyherbal formulations was determined by Folin-Ciocalteu colorimetric method with a slight modification [15]. For the preparation of the calibration curve, 1 mL aliquots of 10, 20, 40, and 80 µg/mL gallic acid solutions were mixed with 5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 4 mL (75 g/L) sodium carbonate. The absorption was read after 30 minutes at 20°C at 750 nm, and the calibration curve was drawn. 100 µL of the sample was mixed with 2 mL sodium carbonate (2 g in 100 mL distilled water) and will be left for 2 minutes at room temperature. Then, it was mixed with the same Folin-Ciocalteu reagent and was left for 30 minutes. The experiment was done in triplicate. The absorbance reading was taken at 750 nm. The absorbance values were compared to the standard curve.

2.6. Statistical Analysis

The Design Expert® 6.0.4 software was used in determining the optimum polyherbal formulation. Values expressed are means of the three replicate determinations. Variance analysis (one-way ANOVA) was applied to mean data obtained from each antioxidant assay using SPSS 16.00 for Windows. Differences were considered significant when $p < 0.05$ ($\alpha = 0.05$).

3. Results and Discussion

3.1. Model Fitting

Table 1 presents the results of mixture design studies of antioxidant properties. The independent variables and the runs were generated and arranged randomly by the Design Expert software. The independent and dependent variables were fitted to linear, quadratic, special cubic, and cubic models, and residual plots were generated to check the goodness of model fit. Table 2 presents the important values examined to determine the adequate model for each dependent variable. Since the standard deviation is important for checking data distribution, the predicted sum of squares for measuring the model's predictive ability and the predicted R-squared were calculated and compared. The best model has a low standard deviation, predicted sum of squares, and high predicted R-squared [17]. Therefore, the fit summary output examination revealed that the quadratic model was statistically significant for the FRAP and TPC (Table 2). Meanwhile, the special cubic model was statistically significant for DPPH. Thus, this model was used to represent each response for further analysis.

Table 1 Design layout and experimental results for antioxidant properties

Run No.	Factor (%)				Antioxidant assay		
	C. chayamansa	*M. oleifera.*	*M. alba.*	*S. rebaudiana*	DPPH	FRAP	TPC
1	0	0	0	100	48.32^{g}	60.81^{d}	39.26^{d}
2	100	0	0	0	64.16^{def}	152.64^{a}	132.61^{ab}
3	33.33	33.33	33.33	0	86.78^{ab}	142.74^{ab}	131.35^{ab}
4	0	33.33	33.33	33.33	86.72^{ab}	142.82^{ab}	132.45^{ab}
5	50	50	0	0	88.65^{a}	120.58^{abc}	117.98^{c}
6	50	0	0	50	57.86^{efg}	142.68^{ab}	131.79^{ab}
7	62.5	12.5	12.5	12.5	69.84^{cde}	129.96^{ab}	126.48^{ab}

Continuation of Table 1

8	12.5	12.5	62.5	12.5	77.15^{bcd}	142.45^{ab}	133.13^{ab}
9	12.5	12.5	12.5	62.5	67.10^{cde}	141.87^{ab}	133.56^{ab}
10	0	0	0	100	53.38^{fg}	67.368^{cd}	23.81^{d}
11	0	100	0	0	87.34^{a}	131.46^{bcd}	107.41^{bc}
12	0	0	100	0	73.99^{bcd}	142.27^{ab}	132.20^{ab}
13	100	0	0	0	65.38^{def}	141.63^{ab}	124.86^{ab}
14	0	0	100	0	80.71^{bcd}	140.64^{ab}	131.33^{ab}
15	0	50	0	50	91.23^{a}	135.85^{ab}	134.41^{ab}
16	50	0	50	0	90.13^{a}	141.38^{ab}	132.05^{ab}
17	0	0	50	50	67.31^{cdef}	140.69^{ab}	132.71^{ab}
18	33.33	33.33	0	33.33	66.39^{def}	142.86^{ab}	134.88^{ab}
19	33.33	0	33.33	33.33	70.64^{cde}	137.26^{ab}	135.17^{ab}
20	12.5	62.5	12.5	12.5	69.24^{cde}	143.35^{ab}	132.18^{ab}
21	0	50	50	0	77.86^{bcd}	143.92^{ab}	135.22^{ab}
22	25	25	25	25	65.47^{def}	142.37^{ab}	136.68^{a}

$^{a–g}$ Mean within each column with different letters differ significantly ($p < 0.05$)

3.2. Modeling of Antioxidant Properties

The following equation is the final empirical model for coded factors of DPPH, FRAP, and TPC, where A for *C. chayamansa*, B for *M. oleifera*, C for *M. alba*, and D for *S. rebaudiana*.

a) DPPH = 51.28*A + 70.59*B + 78.95*C + 64.81*D + 111.39*A*B + 5.32*A*C + 5.61*A*D + 10.35*B*C + 93.23*B*D-14.47*C*D-250.93*A*B*C-613.54*A*B*D-189.84*A*C*D + 26.46*B*C*D (3)

b) FRAP = 65.34*A + 131.67*B + 141.20*C + 146.74*D + 100.51*A*B + 139.73*A*C + 138.05*A*D + 33.18*B*C-6.02*B*D-30.84*C*D (4)

c) TPC = +34.12*A + 108.69*B + 131.60*C + 128.53*D + 182.02*A*B + 184.21*A*C + 194.58*A*D + 41.80*B*C + 49.93*B*D-10.98*C*D (5)

3.3. Evaluation of Interaction Effect

The equation is useful for determining the type of interaction of the factors by comparing the factor coefficients, such as the synergistic and antagonistic effects. By default, the high levels of the factors are coded as +1, and the low levels of the factors are coded as -1. For DPPH, Equation 3 showed a synergistic effect of the combination of AC, AD, BC, and BD. The highest synergistic effect was shown in the combination of AB. Meanwhile, for FRAP (Equation 4), only two of the polyphyto formulations showed antagonistic interactions: BD and CD. The highest synergistic effect is clearly shown in the AC combinations. On the other hand, the equation of TPC (Equation 5) showed that only one formulation had an antagonistic effect: a combination of CD.

3.4. Validation of the Model

The experimental data were fitted into equations (3)-(5), and the optimum proportions were found to be 24.87% of *Cnidoscolus chayamansa* Mc. Vaugh, 0.64% of *Moringa oleifera*, and 73.46% of *Morus alba* L. At these optimum formulations, the experimental value obtained for DPPH was 93.95%, which is close to the predicted value of 91.23%, while the experimental value for FRAP was 149.12% and reasonably close to the predicted, which is 148.52%. The predicted value for TPC is 141.85%, and the value obtained for the validation of TPC was 145.42%. In all the responses obtained, less than 10% of errors indicate that the formulations were validated. These results were comparable with the evaluation of antioxidant properties for each assay. *Morus alba* L. is in higher percentage in the polyherbal formulations as it contributes strong potential of antioxidant properties as stated in the previous study. Therefore, these proportions of the polyherbal formulations were suitable conditions for antioxidant assay.

Table 2 Model summary statistics for DPPH, FRAP, and TPC

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	Predicted Sum of squares
DPPH					
Linear	9.77	0.410	0.311	0.077	2,687.18
Quadratic	7.42	0.773	0.602	-0.099	3,200.81
Special cubic	4.84	0.936	0.831	-2.199	9,313.34
Cubic	4.48	0.966	0.854	-9.032	29,202.84
FRAP					
Linear	14.70	0.656	0.599	0.426	6,489.10
Quadratic	5.09	0.973	0.952	0.894	1,203.08
Special cubic	4.82	0.984	0.957	0.554	5,043.23
Cubic	4.20	0.993	0.967	0.765	2,660.42
TPC					
Linear	21.37	0.567	0.490	0.219	14,683.48
Quadratic	6.96	0.969	0.946	0.873	2,383.16
Special cubic	7.73	0.975	0.933	0.209	14,876.04
Cubic	5.88	0.991	0.961	0.618	7,172.79

4. Conclusion

The proportion of each substrate is important to ensure the efficiency of the antioxidant properties of polyherbal formulations. Different amounts of *Morus alba* L., *Cnidoscolus chayamansa* Mc. Vaugh, *Moringa oleifera*, and *Stevia rebaudiana* Bert. gave different interactions on antioxidant properties since their contents are different. The mixture design was used to identify the best formulations through an optimization process that provides the best condition for the antioxidant properties.

References

[1] WONGYAI S. Standardization of Medicinal Plants for the Sustainable Exploitation of Biodiversity. *The Journal of Chulabhorn Royal Academy*, 2020, 2(2): 1-8. https://he02.tci-thaijo.org/index.php/jcra/article/view/241564

[2] PIZZINO G., IRRERA N., CUCINOTTA M., PALLIO G., MANNINO F., ARCORACI V., SQUADRITO F., ALTAVILLA D., and BITTO A. Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, 2017, 2017: 8416763. https://doi.org/10.1155/2017/8416763

[3] APAK R., CALOKERINOS A., GORINSTEIN S., SEGUNDO M., HIBBERT D., GÜLÇIN İ., DEMIRCI ÇEKIÇ S., GÜÇLÜ K., ÖZYÜREK M., ÇELIK S., MAGALHÃES L., and ARANCIBIA-AVILA P. Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (IUPAC Technical Report). *Pure and Applied Chemistry*, 2022, 94(1): 87-144. https://doi.org/10.1515/pac-2020-0902

[4] KURI-GARCÃ-A, A., CHÃ¡VEZ-SERVÃ-N, J. L., and GUZMÃ¡N-MALDONADO, S. H. Phenolic profile and antioxidant capacity of *Cnidoscolus chayamansa* and *Cnidoscolus aconitifolius*: A review. *Journal of Medicinal Plants Research*, 2017, 11(45): 713-727. https://doi.org/10.5897/JMPR2017.6512

[5] MEIRELES D., GOMES J., LOPES L., HINZMANN, M., and MACHADO, J. A review of properties, nutritional and pharmaceutical applications of *Moringa oleifera:* integrative approach on conventional and traditional Asian medicine. *Advances in Traditional Medicine*, 2020, 20: 495–515. https://doi.org/10.1007/s13596-020-00468-0

[6] PRZEOR M., FLACZYK E., KMIECIK D., BUCHOWSKI M. S., STANIEK H., TOMCZAK-GRACZYK A., KOBUS-CISOWSKA J., GRAMZA-MICHAŁOWSKA A., and FOKSOWICZ-FLACZYK J. Functional Properties and Antioxidant Activity of *Morus alba* L. Leaves var. Zolwinska Wielkolistna (WML-P)—The Effect of Controlled Conditioning Process. *Antioxidants*, 2020, 9(8): 668. https://doi.org/10.3390/antiox9080668

[7] AHMAD J., KHAN I., BLUNDELL R., AZZOPARDI J., and MAHOMOODALLY M. F. *Stevia rebaudiana* Bertoni.: an updated review of its health benefits, industrial applications and safety. *Trends in Food Science & Technology*, 2020, 100: 177-189. https://doi.org/10.1016/j.tifs.2020.04.030

[8] BALIYAN S., MUKHERJEE R., PRIYADARSHINI A., VIBHUTI A., GUPTA A., PANDEY R.P., and CHANG C.-M. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*, 2022, 27: 1326. https://doi.org/ 10.3390/molecules27041326

[9] CAESAR L. K. and CECH N. B. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. *Natural Product Reports*, 2019, 36(6): 869-888. https://doi.org/10.1039/C9NP00011A

[10] LOURENÇO S. C., MOLDÃO-MARTINS M., and ALVES V. D. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules*, 2019, 24(22): 4132. https://doi.org/10.3390/molecules24224132

[11] BELOUAFA S., HABTI F., BENHAR S., BELAFKIH B., TAYANE S., HAMDOUCH S., BENNAMARA A., and ABOURRICHE A. Statistical tools and approaches to validate analytical methods: methodology and practical examples. *International Journal of Metrology and Quality Engineering*, 2017, 8: 9. https://doi.org/10.1051/ijmqe/2016030

[12] SALEHI B., ATA A., ANIL KUMAR N. V., SHAROPOV F., RAMÍREZ-ALARCÓN K., RUIZ-ORTEGA A., ABDULMAJID AYATOLLAHI S., TSOUH FOKOU P. V., KOBARFARD F., AMIRUDDIN ZAKARIA Z., IRITI M., TAHERI Y., MARTORELL M., SUREDA A., SETZER W. N., DURAZZO A., LUCARINI M., SANTINI A., CAPASSO R., OSTRANDER E. A., ATTA-UR-RAHMAN, CHOUDHARY M. I., CHO W. C., and SHARIFI-RAD J. Antidiabetic Potential of Medicinal Plants and Their Active Components. *Biomolecules*, 2019, 9(10): 551. https://doi.org/10.3390/biom9100551

[13] ALMANZAR M. M. S. *An Optimizing Method for Screening in A Mixture Design Experiment.* Master's thesis, Rochester Institute of Technology, 2017. https://scholarworks.rit.edu/cgi/viewcontent.cgi?article=10837&context=theses

[14] PERUMAL N., NALLAPPAN M., SHOHAIMI S., KASSIM N. K., TEE T. T., and CHEAH Y. H. Synergistic antidiabetic activity of *Taraxacum officinale* (L.) Weber ex F.H.Wigg and *Momordica charantia* L. polyherbal combination. *Biomedicine & Pharmacotherapy*, 2022, 145: 112401. https://doi.org/10.1016/j.biopha.2021.112401

[15] CHANTHASRI W., PUANGKEAW N., KUNWORARATH N., JAISAMUT P., LIMSUWAN S., MANEENOON K., CHOOCHANA P., and CHUSRI S. Antioxidant capacities and total phenolic contents of 20 polyherbal remedies used as tonics by folk healers in Phatthalung and Songkhla provinces, Thailand. *BMC Complementary and Alternative Medicine*, 2018, 18(73): 73. https://doi.org/10.1186/s12906-018-2131-y

[16] BENZIE I. and DEVAKI M. The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. In: APAK R., CAPANOGLU E., and SHAHIDI F. (eds.) Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications. John Wiley & Sons, 2017. https://doi.org/10.1002/9781119135388.ch5

[17] CHICCO D., WARRENS M. J., and JURMAN G. The coefficient of determination R-squared is more informative than SMAPE, MAE, MAPE, MSE and RMSE in regression analysis evaluation. *PeerJ Computer Science*, 2021, 7: e623. https://doi.org/10.7717/peerj-cs.623

参考文:

[1] WONGYAI S. **可持**续开发生物多样性的药用植物标准化。**朱拉布霍恩皇家学院学**报，2020, 2(2): 1-8. https://he02.tci-thaijo.org/index.php/jcra/article/view/241564

[2] PIZZINO G.，IRRERA N.，CUCINOTTA M.，PALLIO G.，MANNINO F.，ARCORACI V.，SQUADRITO F.，ALTAVILLA D. 和 BITTO A. 氧化应激：对人类健康的危害和好处。氧化医学与细胞长寿，2017，2017：8416763。https://doi.org/10.1155/2017/8416763

[3] APAK R.，CALOKERINOS A.，GORINSTEIN S.，SEGUNDO M.，HIBBERT D.，GÜLÇIN I.，DEMIRCI ÇEKIÇ S.，GÜÇLÜ K.，ÖZYÜREK M.，ÇELIK S.，MAGALHÃES L. 和 ARANCIBIA-AVILA P. 评估抗氧化剂对活性氧和氮物种的清除活性的方法（IUPAC 技术报告）。纯化学和应用化学，2022, 94(1): 87-144. https://doi.org/10.1515/pac-2020-0902

[4] KURI-GARCÃA，A.，CHÃ¡VEZ-SERVÃN，J.L. 和 GUZMÃ¡N-MALDONADO，S.H. [医]鼻窦查亚曼萨和[医]鼻窦[医]阿科尼提弗利乌斯的酚谱和抗氧化能力：综述。药用植物研究杂志，2017, 11(45): 713-727. https://doi.org/10.5897/JMPR2017.6512

[5] MEIRELES D.，GOMES J.，LOPES L.，HINZMANN，M. 和 MACHADO，J. 辣木[医]油的特性，营养和药物应用综述：传统和传统亚洲医学的综合方法。传统医学进展，2020，20：495-515。https://doi.org/10.1007/s13596-020-00468-0

[6] PRZEOR M.，FLACZYK E.，KMIECIK D.，BUCHOWSKI M.S.，STANIEK H.，TOMCZAK-GRACZYK A.，KOBUS-CISOWSKA J.，GRAMZA-MICHAŁOWSKA A. 和 FOKSOWICZ-FLACZYK J. 莫鲁斯*阿尔巴 L. 叶瓦尔的功能特性和抗氧化活性。大叶玳瑁—WML-P）-受控调理过程的效果。抗氧化剂，2020, 9(8): 668. https://doi.org/10.3390/antiox9080668

[7] AHMAD J.，KHAN I.，BLUNDELL R.，AZZOPARDI J.和 MAHOMOODALLY M.F. 甜叶菊雷巴迪亚纳贝尔托尼。：对其健康益处，工业应用和安全性的最新回顾。食品科技趋势，2020，100：177-189。https://doi.org/10.1016/j.tifs.2020.04.030

[8] BALIYAN S.，MUKHERJEE R.，PRIYADARSHINI A.，VIBHUTI A.，GUPTA A.，PANDEY R.P. 和 CHANG C.-M. 通过 DPPH 自由基清除活性和无花果宗教的定量植物化学分析测定抗氧化剂。分子，2022, 27: 1326. https://doi.org/10.3390/分子 27041326

[9] CAESAR L.K. 和 CECH N.B. 天然产物提取物中的协同作用和拮抗作用：当 1+1 不等于 2 时。天然产品报告，2019, 36(6): 869-888. https://doi.org/10.1039/C9NP00011A

[10] LOURENÇO S.C.，MOLDÃO-MARTINS M. 和 ALVES V.D. 天然植物来源的抗氧化剂：从来源到食品工业应用。分子，2019, 24(22): 4132. https://doi.org/10.3390/molecules24224132

[11] BELOUAFA S.，HABTI F.，BENHAR S.，BELAFKIH B.，TAYANE S.，HAMDOUCH S.，BENNAMARA A. 和 ABOURRICHE A. 验证分析方法的统计工具和方法：方法学和实际例子。国际计量与质量工程杂志，2017，8：9。https://doi.org/10.1051/ijmqe/2016030

[12] SALEHI B.，ATA A.，ANIL KUMAR N. V.，SHAROPOV F.，RAMÍREZ-ALARCÓN K.，RUIZ-ORTEGA A.，ABDULMAJID AYATOLLAHI S.，TSOUH FOKOU P.V.，KOBARFARD F.，AMIRUDDIN ZAKARIA Z.，IRITI M.，TAHERI Y.，MARTORELL M.，SUREDA A.，SETZER W.N.，DURAZZO A.，LUCARINI M.，SANTINI A.，CAPASSO R.，OSTRANDER E. A.，ATTA-UR-RAHMAN，CHOUDHARY M. I.，CHO W. C. 和 SHARIFI-RAD J. 药用植物及其活性成分的抗糖尿病潜力。生物分子，2019, 9(10): 551. https://doi.org/10.3390/biom9100551

[13] ALMANZAR M.M.S. 在混合物设计实验中筛选的优化方法。罗切斯特理工学院硕士论文，2017 年。https://scholarworks.rit.edu/cgi/viewcontent.cgi?article=10837&context=theses

[14] PERUMAL N.，NALLAPPAN M.，SHOHAIMI S.，KASSIM N.K.，TEE T.T. 和 CHEAH Y.H. 蒲公英 n.办公室,办公室（L.）韦伯前 F.H.威格和莫莫迪卡*查兰蒂亚 L. 多叶虫,多叶虫组合的协同抗糖尿病活性。生物医学／药物治疗，2022，145：112401。https://doi.org/10.1016/j.biopha.2021.112401

[15] CHANTHASRI W.，PUANGKEAW N.，KUNWORARATH N.，JAISAMUT P.，LIMSUWAN S.，MANEENOON K.，CHOOCHANA P. 和 CHUSRI S. 泰国[医]帕塔隆和宋卡省民间治疗师用作补品的 20 种多叶疗法的抗氧化能力和总酚含量。BMC 补充和替代医学，2018, 18(73): 73. https://doi.org/10.1186/s12906-018-2131-y

[16] BENZIE I. 和 DEVAKI M. 非酶抗氧化能力的铁还原/抗氧化能力（FRAP）测定：概念，程序，限制和应用。在：APAK R.，CAPANOGLU E. 和 SHAHIDI F.（编辑。）抗氧化活性和容量的测量：最新趋势和应用。约翰*威利的儿子，2017。https://doi.org/10.1002/9781119135388.ch5

[17] CHICCO D.，WARRENS M.J. **和** JURMAN G. **在回归分析评估中，测定系数** R **平方比斯梅普，梅，玛佩，** MSE **和** RMSE **更具信息性。佩尔杰计算机科学**，2021，7：e623。https://doi.org/10.7717/peerj-cs.623