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Optimization of total polyphenol extraction from *Syzygium malaccense* seed

Optimización de la extracción de polifenoles totales a partir de la semilla de Syzygium malaccense

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Resumen

Los polifenoles, son metabolitos secundarios ampliamente distribuidos en el reino vegetal. Estos han sido empleados en la medicina natural en forma de extractos de plantas para aliviar diversas afecciones y enfermedades. Por esta razón, el objetivo del presente trabajo fue la optimización del proceso de extracción del contenido de polifenoles totales, a partir de la semilla de manzana malaya (*Syzygium malaccense*). Al material vegetal se le determinó el contenido de sustancias solubles, humedad, grasa y contenido de cenizas. Además, se corroboró la presencia de compuestos fenólicos mediante el tamizaje fitoquímico y posteriormente se optimizó el proceso de extracción en función del contenido de polifenoles totales. La mayor extracción de polifenoles totales se obtuvo con 30 % de etanol, tiempo de extracción de 2 h, temperatura de 65 ˚C y una relación droga disolvente de 1 g/5 ml. El extracto optimizado de la semilla de manzana malaya presentó 9071 mg/l de polifenoles totales y una capacidad antioxidante de 1023,3 µmol/g. Se recomienda la utilización del extracto en la formulación de suplementos dietéticos y productos fitoterapéuticos destinados a promover la salud cardiovascular, reducir el estrés oxidativo y fortalecer el sistema inmunológico.

Palabras clave: semilla; *Syzygium malaccense*; optimización; polifenoles; extracción

Abstract

Polyphenols are secondary metabolites widely distributed in the plant kingdom. They have been used in natural medicine in the form of plant extracts to alleviate various conditions and diseases. For this reason, the objective of the present work was the optimization of the extraction process of the total polyphenol content from the seed of Malayan apple (*Syzygium malaccense*). The content of soluble substances, moisture, fat and ash content were determined from the plant material. In addition, the presence of phenolic compounds was corroborated by phytochemical screening and then the extraction process was optimized according to the total polyphenol content. The highest extraction of total polyphenols was obtained with 30% ethanol, extraction time of 2 h, temperature of 65 ˚C and solvent drug ratio of 1 g/5 ml. The optimized Malaysian apple seed extract exhibited 9071 mg/l total polyphenols and an antioxidant capacity of 1023.3 µmol/g. The extract is recommended for use in the formulation of dietary supplements and phytotherapeutic products aimed at promoting cardiovascular health, reducing oxidative stress and strengthening the immune system.

Keywords: seed; *Syzygium malaccense*; optimization; polyphenols; extraction

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Introduction

Due to the high frequency of various degenerative diseases in recent decades[1], as well as the demand for natural products with potential for human health[2], have prompted the discovery of various phytochemical compounds that have extensive potential for exploitation in the food and pharmaceutical industry[3], among these compounds are polyphenols, which have the ability to modulate the activity of different enzymes and consequently interfere in signaling mechanisms in various cellular processes. Their antioxidant properties justify many of their beneficial effects[4], since they prevent diseases related to oxidative stress, increasing the effectiveness of the immune system considerably, avoiding diseases in internal organs or tissue lesions with the help of enzymes with antioxidant activity[5]. They also develop antimicrobial properties on bacteria such as *Escherichia coli* and *Salmonella typhimurium*, two common bacteria that frequently cause gastrointestinal infections in animals and humans[5].

Syzygium malaccense (*S. malaccense*), commonly known as Malay apple, is native to Malaysia[6], is among the group of medicinal plants, which are the richest biological resource of drugs in traditional systems of medicine[7], namely modern, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[8]. This plant has been used for the treatment of infections of the mouth (candidiasis), dysentery, diabetes, constipation, coughs, colds, headaches, and it is also used as an emmenagogue[6].

There are studies that reveal the presence of an enriched source of polyphenols in this genus (*Syzygium spp*.), as is the case of Camacho, Melgarejo, & De-la-Rosa[8] who studied the ripe fruit of *S. cumini*; Arencibia[9], in the fruit of *S. malaccense* and Camelo[10], in the fruit of *S. paniculatum* (G.). In addition, it has been demonstrated that there is a high concentration of polyphenols in seeds of *S. humilis*, studied by Flores *et al.*[11] Due to the little or almost no study on the content of total polyphenols (TPC) in the seed of *Syzyguim malaccense*, the process of extracting TPC from the seed of *S. malaccense* was optimized.

Methodology

The present work was developed in the laboratories of the Institute of Pharmacy and Food of the University of Havana. The fruits of *S. malaccense* were collected in May and June 2019 in Sagua la Grande, which were stored in boxes for subsequent transfer to the Institute of Pharmacy and Food of the University of Havana. Fruits were selected that presented, in general, the same characteristics of size, color and absence of spots, cracks and visible morphological alterations. For the development of the research it was necessary to separate the pulp from the seed; for this purpose a stainless steel scalpel No. 3 was used. Subsequently, the seeds were crushed with a mortar and dried in an oven (YDL-6000, AISET, China) at 40 ºC. The plant drug was then sieved to a particle size of less than 0.5 mm and deposited in amber-colored jars, placed in a desiccator with activated silica gel until further use.

The vegetable drug was determined the content of water-soluble substances, 60 and 90% (v/v) in hydroalcoholic mixture, in addition to the moisture content by gravimetric method, the ash content, and fat by Soxhlet method. These determinations were carried out according to Miranda & Cuellar[12].

The vegetable drug was subjected to a phytochemical screening for the identification of its composition. The system of crosses was used as a measurement criterion to specify the qualification of these secondary metabolites[12].

For the optimization of the extraction of total polyphenols from *S. malaccense* seed, the Design Expert 11.1.2.0 software (Stad-Ease Inc., Minneapolis, USA) was used. This was done in order to obtain the experimental design and subsequently the processing of the *S. malaccense* seed extracts, so that the selected extract would present the highest total polyphenol contents. The numerical optimization method was used through a response surface design I Optimum, generating a mathematical model that described the variations of the variables in each experimental run. The factors evaluated were ethanol percentage (A) in intervals of 30, 60 and 90% (v/v), extraction time (B) in intervals of 2, 6 and 12 h, temperature (C) in intervals of 25, 45 and 65 ºC and mass/solvent ratio (D) in intervals of 1 g/5 ml and 1 g/10 ml, while TPC was the response variable. The total number of combinations defined by the software was 24 runs, including 5 replicates and 5 points for model misfit.

Extracts were obtained by maceration with occasional shaking, according to the specifications of the design matrix (Table

3). At the end of the extraction time for each of the runs, the resulting mixture was filtered under vacuum and the solid residue was discarded. The filtrate was recovered to determine its TPC.

The quantification of polyphenols was performed according to the methodology proposed by Slinkard & Singlenton[13]. The assay consisted of adding 50 µl of *S. malaccense* extract and 2.5 ml of diluted Folin-Ciocalteu reagent solution (1:9). After 5 min, 2 ml of 7.5% (m/v) Na_2CO_3 was added and 2 h was waited for. After this time, the absorbance was measured at 765 nm in a UV-VIS spectrophotometer (Rayleigh UV-1601, Beijing). Gallic acid at concentrations between 100 and 500 mg/l was used as a standard for the calibration curve. A blank assay prepared with 50 µl of distilled water under the same conditions as the sample was also performed to calibrate the equipment and eliminate interference from absorbances produced by the solvents and reagents used in the technique. The concentration of polyphenols was expressed in mg of gallic acid equivalent/l of extract according to the calibration curve.

After the numerical optimization process, the TPC was determined from the optimized extract following the same methodology proposed by Slinkard & Singlenton[13] mentioned above.

For the antioxidant capacity, 10 mg of ABTS were weighed and dissolved to 1 ml to obtain a solution of ABTS (19.4 mM). Subsequently, an ammonium persulfate solution (438 mM) was prepared by weighing 100 mg of ammonium persulfate (PSA) and dissolving it with 1 ml of distilled water. Next, 180 µl of 19.4 mM ABTS was mixed with 3 µl of 438 mM PSA and made up to 500 µl with MilliQ water. The ABTS/PSA mixture was heated at 68 °C for 13 min to generate the stable ABTS radical. The ABTS radical preparation was then diluted until the absorbance at 734 nm was around 0.70 using distilled water as a blank[12].

For the determination of the antioxidant capacity, 980 µl of the ABTS radical solution was mixed with 20 µl of the optimized extract and incubated at 37 °C for 10 min. Finally, the absorbance at 734 nm was measured in a UV-VIS spectrophotometer (Rayleigh UV1601, Beijing). For the calibration curve, 13 mg of ascorbic acid was weighed and dissolved to 1 ml, thus obtaining a 73.8 mM ascorbic acid stock solution, then 5 µl of this solution was taken and made up to 500 µl to obtain a 738 µM ascorbic acid solution. From this solution, solutions were prepared at increasing and exactly known concentrations of the reference standard (100, 200, 300, 400, 500, 500, 600 and 700 µM). In addition, a blank assay was performed with distilled water to calibrate the equipment and eliminate interferences produced by the reagents used in the technique[12].

Density was determined according to Miranda *et al.*[12], using a capillary tube pycnometer. The refractive index and soluble solids were determined in triplicate in an Abbe refractometer with temperature correction[12]. The pH was determined using a pH meter model Basic 20+ (Crison, Spain). The readings were taken in triplicate[12]. In addition, the total solids content was determined by drying in a thermogravimetric balance MA-40 (Sartorius, Göttingen, Germany) at 105 ºC to constant mass.

Results and discussion

Table 1 shows the results obtained for the different physicochemical indicators evaluated in the vegetable drug. The value of water-soluble substances (16.51%) was lower than that reported by Flores *et al.*[11] in a study of the crude drug of *S. humilis* and the seeds of Mimusops splos, which were 11.46 % and 10.90%, respectively[14].

The highest percentage of soluble substances was obtained in the 60 % ethanol extract (16.86%), a value that is higher than that reported by Bulgarin *et al.*[15], in the study of 98% ethanol soluble substances from the bitter seed of *Nephelium lappaceum* L, and by Jimenez *et al.*[16] in the study of 70% ethanol extractable substances from the seeds of *P. quadrangularis*, which were 4.44% and 20.71%, respectively. These differences in the percentages of soluble substances in the different solvents can be attributed to the difference between the polarity of these, as well as the origin and chemical composition of the plant materials in question[17].

A moisture percentage similar to that obtained by Arias[18], which was 7.72% moisture in pomegranate seeds (*Punica granatum* L.), and lower than that reported by Panchana & Velásquez[14], which was 10.9% in *Mimusops sp*. seeds, was obtained. According to Miranda & Cuellar[12], moisture values should be between 8 and 10%, a characteristic that makes it stable to deterioration processes that require the presence of high water content to occur, such as hydrolysis[14], which is the range in which the one analyzed in this study is found.

García[19] obtained 3.89 ± 0.166 fat in the seeds of *Ebenopsis ebano*, which, in comparison with that studied in this work, is higher than the average that characterizes the Malayan apple. Douglas *et al.*[20] also analyzed the fat content (53.13%)

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Table 1. Physicochemical indicators of the plant drug.

Indicator (%)	Mean (standard deviation)
Aqueous soluble substances	16,51(0,22)
Ethanol soluble substances 60%	16,86(0,44)
Ethanol soluble substances 90%	7,73(0,22)
Moisture	8,19(2,80)
Fat	0,24(0,18)
Ash	3,08(0,03)

of corozo seeds, a value that is higher than that analyzed in this study, as well as Cervantes *et al.*[21] who analyzed the fat content (17.6%) of pumpkin seeds.

The ash content was found to be within the specification limits established by Pharmacopoeias for most medicinal plants, which should be less than 5%[22-24]. The values reported (1.65%) were lower than those analyzed in this study, coinciding with the study carried out on the ash content (2.10 ± 0.06%) of corozo seed by Douglas *et al.*[20] which was also lower. The values for ash content (5.29%) of squash seed reported by Cervantes & Torres[21] were higher. Total ash is an indicator of the quality of the material studied, since it is an essential element for determining the identity and purity of the drug by reporting the possible adulteration with inorganic matter or foreign bodies or the amount of these elements in its content, as stated by Evans[24] and Chanda[25].

Table 2. Phytochemical profile of S. malaccense seed.

Metabolite	Assay	Ethereal Extract	Ethanolic Extract	Aqueous Extract
Fatty compounds	Sudan	+		
Alkaloids	Dragendorff	+	-	-
Lactone grouping	Baljet	+-	+	
Triterpenes / steroids	Lieberman. B	-	-	
Catechins	Catechins		+	
Resins	Resins		-	
Reducing sugars	Fehling		-	+
Saponins	Espuma		+	+
Phenolic compounds	Ferric (III) chloride		+	+
Free amino acids / amines	Nihydrin		-	
Quinones / benzoquinones	Bortranger		+	
Flavonoids	Shinoda		+-	+-
Cardiotonic glycosides	Kedde		-	
Anthocyanins	Anthocyanidins		-	
Mucilages	Mucilages			-
Bitter principles	Bitter principles			+

+: Presence, +-: Slight presence, -: Absence, Blank spaces: test not performed.

Table 2 shows the absence of triterpenes in the ethereal extract, coinciding with Camacho *et al.*[8] in the chromatographic analysis of the ethereal extract of the ripe fruit of *S. cumini*, reporting the following compounds: β-guaiene, triterpene,

trans-caryophyllene, β-pinene and α-pinene. Arencibia[9] reported the presence of fatty compounds in the fruit of *S. malaccense*, coinciding with that analyzed in this work, except for the presence of alkaloids and lactonic groups.

The ethanolic extract reported the absence of reducing sugars and anthocyanins. The presence of polyphenols was demonstrated, coinciding with Camelo[10] in the study of the ethanolic extract in the fruit of *Syzygium paniculatum* species and that reported by Arencibia[9] in the fruit of *S. malaccense*.

The results reported in the aqueous extract coincide with the phytochemical screening carried out on the whole seed of *S. humilis* by Flores *et al.* [11] except for flavonoids and saponins; these differences between the results are due to the fact that the plant belongs to different species, as well as the influence of the stress conditions to which the plant has been subjected, among others[17].

Table 3 shows the experimental design and the result for each variable, as a function of TPC. The highest TPC extraction was obtained in run number 23; these differences can be attributed to the decrease in ethanol content and the increase in temperature[9]. The study replicates 1 and 22; 4 and 12; 6 and 11; 18 and 19 differed significantly in total polyphenol content, while replicates 1 and 4; 7 and 13 did not differ significantly in TPC.

Table 3. Total polyphenol content of S. malaccense extracts

Run	Ethanol (%)	Extraction time (h)	Temperature (ºC)	Drug/solvent ratio (m/V)	Total polyphenols (mg/L)
1	60	6	45	2	3027 (106) g
2	90	12	65	2	1492 (42) c
3	60	2	25	1	3487 (32) i
4	30	6	45	1	5717 (119) n
5	60	2	65	2	3249 (93) h
6	60	6	25	1	3724 (114) j
7	60	12	45	1	6530 (62) ñ
8	90	6	65	1	1252 (72) ab
9	60	6	45	2	3120 (64) gh
10	30	12	25	1	4729 (106) l
11	60	6	25	1	3975 (86) k
12	30	6	45	1	5500 (56) m
13	60	12	45	1	6420 (162) ñ
14	60	6	45	2	3005 (162) g
15	30	12	65	2	3716 (21) j
16	90	2	25	2	1359 (81) bc
17	30	2	25	2	1943 (37) d
18	60	12	25	2	2513 (60) f
19	60	12	25	2	2240 (17) e
20	90	2	45	2	1181 (45) a
21	60	12	65	1	7450 (51) p
22	60	6	45	2	3182 (114) h
23	30	2	65	1	6730 (142) o
24	90	12	25	1	1445 (56) c

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Mean standard deviation n = 3. Different letters indicate significant differences for $p \leq 0.05$.

The responses obtained for each of the runs were related to the four independent variables through a quadratic equation for the TPC: The best fit of the variables to the models was:

TPC=+ 4083,35 - 1498,96 A + 439,79 B + 774,03 C - 1001,87 D+146,56 AB - 572,08 AC + 814,83 AD + 375,47 BC - 439,29 BD - 305,85 CD - 1535,42 A^2 + 394,51 B^2 - 224,13 C^2 (Ec.1)

Where:

TPC: Total polyphenol content, A: % Ethanol, B: Extraction time, C: Temperature, D: Mass/solvent ratio.

When analyzing equation 1, it is evident that the coefficients of linear factors A and D were negative, while the coefficients of linear factors B and C were positive. In addition, it can be observed that the interactions between AC, BD and CD, as well as factor A2, presented negative coefficients, therefore, an increase in temperature during the extraction process will decrease the TPC since these factors turned out to be significant ($p \leq 0.05$) (Table 4). In the case of AD and BC, they were linear and their coefficients were positive, therefore, an increase in these variables will increase the TPC in the extraction process.

Table 4. Analysis of variance of the models coded for the extraction process

Source	F-value	p-value
Model	189,19	< 0,0001
A-Ethanol	646,00	< 0,0001
B- Extraction time	63,18	< 0,0001
C- Temperature	235,18	< 0,0001
D- Drug/solvent ratio	546,88	< 0,0001
AB	4,37	0,0630
AC	68,44	< 0,0001
AD	147,90	< 0,0001
BC	45,84	< 0,0001
BD	54,40	< 0,0001
CD	38,83	< 0,0001
A2	342,24	< 0,0001
B2	12,14	0,0059
C2	3,98	0,0741
Lack of fit	4,26	0,0523
R2		0,9960
R2 Adjusted		0,9907

Table 4 shows that the independent variables (A, B, C, D), which significantly affected the TPC during the extraction process. For the interactions between AC, AD, BC, BD and CD, they turned out to be significant because their values are below $p \leq 0.05$, while the interaction AB, turned out not to be significant, so it could not be affirmed that there will be an increase or decrease of the TPC when interacting the independent variables A and B during the extraction process.

An $R^2 = 0.9960$ and an adjusted $R^2 = 0.9907$ were obtained, values close to 1 that indicate linearity and precision. The lack of adjustment was not significant, because the F value is 4.26, which implies that there is a 5.23% probability that the process was affected by some interference outside the extraction process[26].

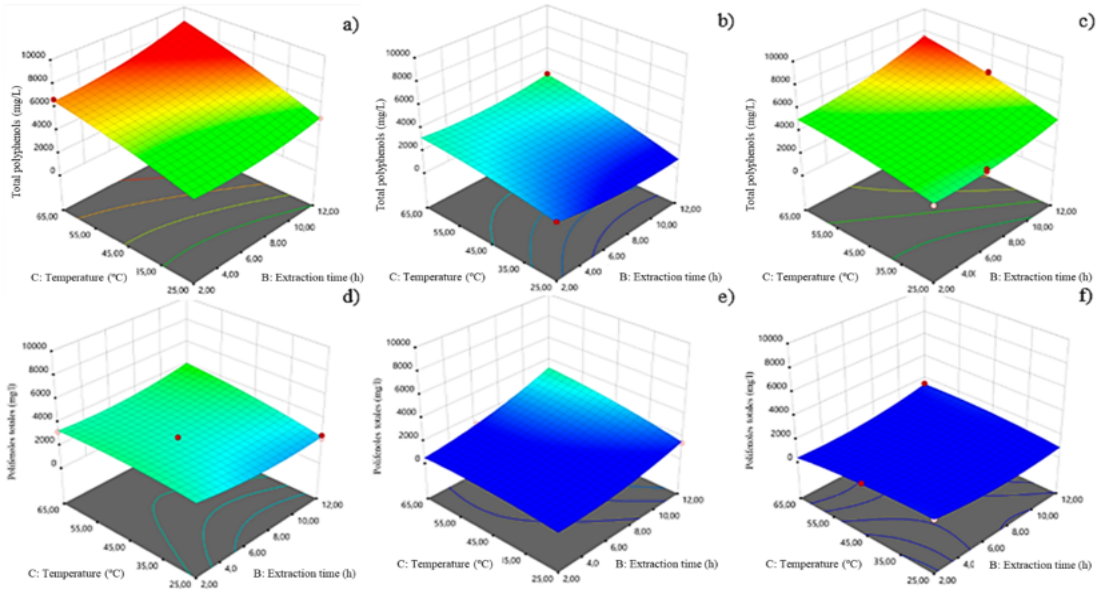

Figure 1. Influence of ethanol percentage, extraction time, mass/solvent ratio and temperature on the TPC. (a) 1 g/5 ml as mass/solvent ratio and 30% ethanol; (b) 1 g/10 ml as mass/solvent ratio and 30% ethanol; (c) 1 g/5 ml as mass/solvent ratio and 60% ethanol; (d) 1 g/10 ml as mass/solvent ratio and 60% ethanol; (e) 1 g/5 ml as mass/solvent ratio and 90% ethanol; (f) 1 g/10 ml as mass/solvent ratio and 90% ethanol.

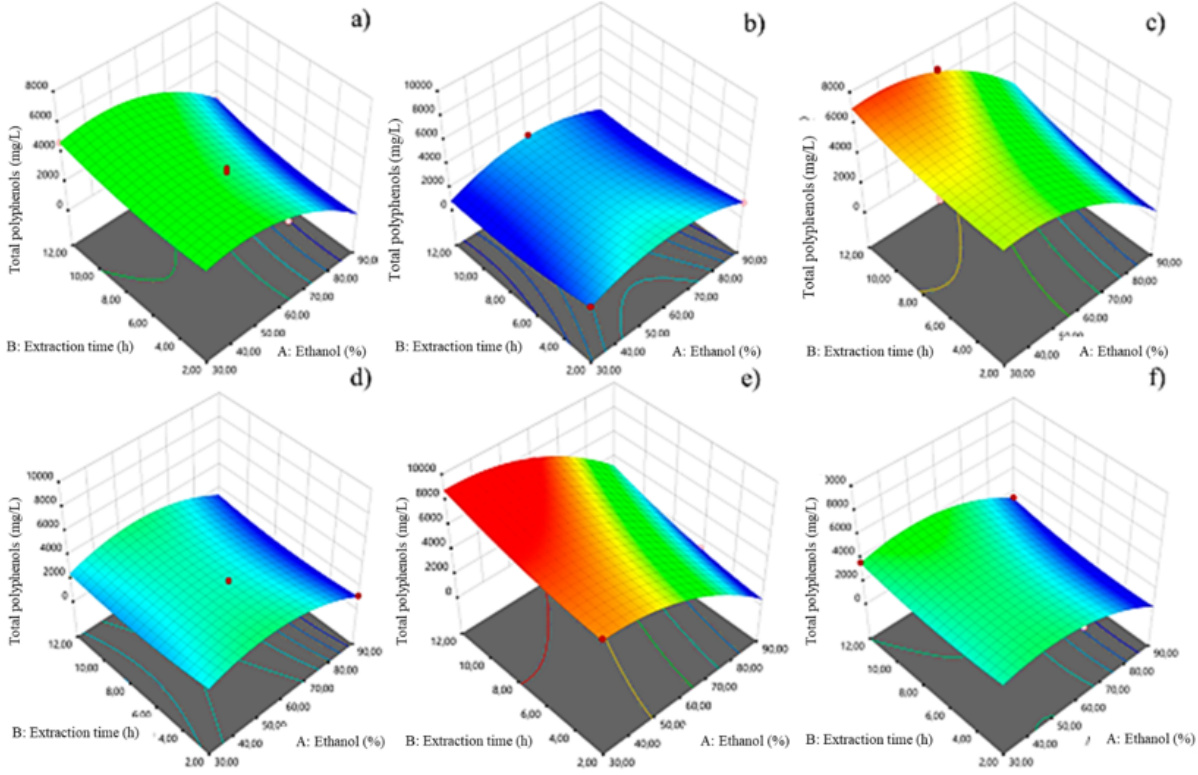

Figure 2. Influence of ethanol percentage, extraction time, mass/solvent ratio and temperature on TPC. (a) 1 g/5 ml as mass/solvent ratio and 25 ºC; (b) 1 g/10 ml as mass/solvent ratio and 25 ºC; (c) 1 g/5 ml as mass/solvent ratio and 45 ºC; (d) 1 g/10 ml as mass/solvent ratio and 45 ºC; (e) 1 g/5 ml as mass/solvent ratio and 65 ºC; (f) 1 g/10 ml as mass/solvent ratio and 65 ºC.

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Figure 1 (a) shows that the highest TPC was obtained with 30% ethanol, temperature between 55 ˚C and 65 ˚C, extraction time from 8 to 12 h and solvent drug ratio 1 g/5 ml. Arencibia *et al.*[9] determined the TPC to a liquid pink dye, from Malayan apple fruit with the same drug/solvent ratio 1 g/5 ml, the highest extraction of total polyphenols was obtained with 75% ethanol, extraction time of 6 h and 60 ˚C temperature, values similar to those obtained in this work, except for the percentage of ethanol and extraction time. The latter may be due to the fact that this variable, after a certain time, may not be significant for the extraction of polyphenols, according to Fick's first law of diffusion, which states that there will be a final equilibrium between the solute in the solid matrix and the extraction solvent[28].

Figure 2 e), shows that at a temperature of 65 ˚C, 30% to 60% ethanol, and 8 to 12 h extraction time with a drug/solvent ratio 1 g/5 ml, the highest TPC was obtained. According to Arencibia *et al.*[9] the highest TPC was obtained at a temperature of 60 ˚C, with ethanol percentage between 60% and 66%, with extraction time from 6 h to 24 h and a drug/solvent ratio 1 g/5 ml. As could be observed, these data coincide with the ranges of the study variables of this work, except for the factors temperature and ethanol, which were found to be significant for $p \leq 0.05$.

In Figure 3 e), the highest TPC was obtained, with an extraction time of 12 h, with temperature of 45 to 65 ˚C, and 30 to 60% ethanol, coinciding with a study by Arencibia *et al.*[9] to a Malaysian apple liquid pink dye.

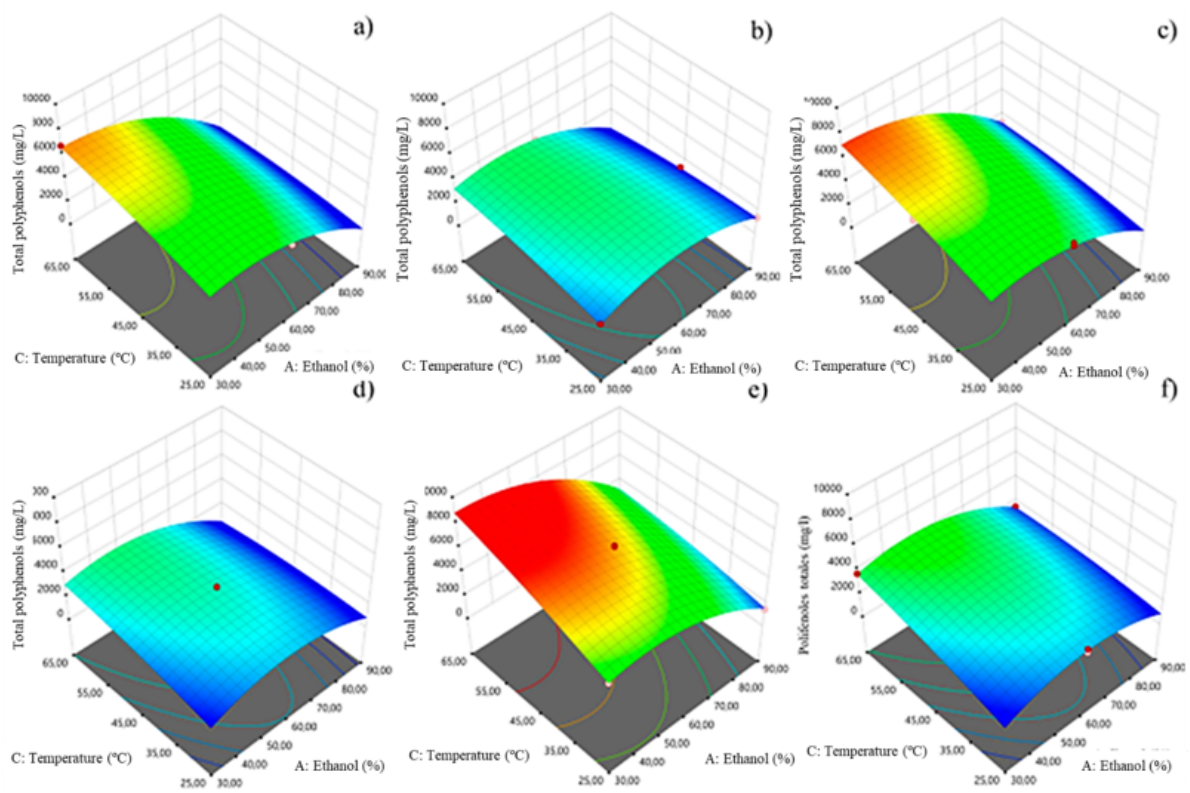

Figure 3. Influence of ethanol percentage, extraction time, mass/solvent ratio and temperature on TPC. (a) 1 g/5 ml as mass/solvent ratio and 2 h; (b) 1 g/10 ml as mass/solvent ratio and 2 h; (c) 1 g/5 ml as mass/solvent ratio and 6 h; (d) 1 g/10 ml as mass/solvent ratio and 6 h; (d) 1 g/5 ml as mass/solvent ratio and 12 h; (f) 1 g/10 ml as mass/solvent ratio and 12 h.

Based on the information provided in the description and discussion in Figures 1 a), 2 e) and 3 e) and comparison with the study by Arencibia *et al.*[9] some partial conclusions can be derived for the optimization process in the extraction of total

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polyphenols from Malaysian apple seed. The results suggest that the optimum temperature range for achieving high CPT is between 55˚ C and 65˚ C, although some specific variations may be acceptable without significantly compromising the extraction efficiency on CPT. It is noted that the percentages of ethanol used for extraction can vary between 30% and 60%, this range can be effective, providing some flexibility in the choice of solvent. The study suggests that after a certain point, the extraction time may not significantly influence the extracted CPT due to diffusion principles so that an extraction time in the range of 8 to 12 hours is adequate to obtain a high CPT. The similarities between the results of this study and the study of Arencibia *et al.*[9] provide some cross-validation and reinforce the reliability of the experimental results.

Table 5. Physicochemical indicators evaluated to the optimized extract of *S. malaccense*

Indicator	Mean (standard deviation)
Total polyphenols (mg/l)a	9071(93)
Antioxidant capacity (µmol/g)b	1023,3(7,4)
Density (g/ml)	1,163(0,04)
pH	5,4(0,02)
Total solids (%)	1,73(0,37)
Soluble solids (˚Brix)	6,23(0,01)
Refractive index	1,1258(0,0001)

a: expressed as mg of gallic acid equivalent per liter of extract, b: µmol of ascorbic acid per gram of extract.

The optimized extract of *S. malaccense* presented 9071 mg/l of total polyphenols, a value higher than that reported by Arencibia *et al.*[9] (1667 mg/l) in the fruit of *S. malaccense* and also higher than that reported by Muller[30] in black (295 mg/l) and white (185.91 mg/l) chia seeds. These differences can be attributed to the fact that the comparison was made between different species and anatomical parts of the plants, in addition to the interferences of organic acids and sugars that lead to an overestimation of the content of phenolic compounds[17].

Camacho *et al.*[30] determined the antioxidant capacity of a 99% ethanol extract of S. cumini seed (88909 mg ascorbic acid/100 g of fruit); these results were higher than those reported in this study (1023.3 µmol/g = 1822.4 mg ascorbic acid/100 g of fruit).

The refractive index can be used as a quality parameter, as it gives an indirect criterion of the concentration of the extract in relation to its total solids content, and therefore its density[9,12]. In this sense, the density of the concentrated extract corresponded to its high total solids content. The extract presented a pH with a basic tendency, since a value of 5.4 ± 0.02 was obtained.

The total solids content was evaluated because it is of great relevance and an important requirement in the analysis of the quality control of extracts obtained from medicinal plants[12].

Conclusions

The highest percentage of soluble substances (16.86%) was obtained in the 60% ethanol extract. *S. malaccense* seed is stable against deterioration processes such as hydrolysis, given its low moisture content. The highest extraction of compounds was favored by high polarity solvents, being higher for the hydroalcoholic mixture. Phytochemical screening yielded the presence of fatty compounds, alkaloids, lactonic clustering, catechins, reducing sugars, saponins, phenolic compounds, quinones and bitter principles. The significant influence of temperature, extraction time, ethanol percentage

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and drug/solvent ratio on the extraction of total polyphenols was demonstrated. Evaluation of these parameters indicates that, to optimize the extraction of total polyphenols from Malaysian apple seed, temperatures between 55 ˚C to 65 ˚C, ethanol percentages between 30% and 60%, and extraction times of 8 to 12 hours should be considered. The content of total polyphenols present in the optimized *S. malaccense* seed extract was 9071 mg/l. These conclusions can serve as a starting point for further optimization, considering other aspects such as costs, efficiency and specific applications of the extracted polyphenols.

Conflictos de interés

The authors declare no conflicts of interest.

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