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LOCAL RESOURCE EXPLOITATION

Optimizing the extraction of hydrocolloids from the leaves of *Securidaca welwitschii* Oliv
Optimisation de l'extraction des hydrocolloïdes des feuilles de Securidaca welwitschii Oliv

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ABSTRACT:

This study aimed at determining the optimal extraction conditions for aqueous extraction of biopolymer from *Securidaca welwitschii* Oliv leaves and determining the physicochemical composition of the extract obtained. The chemical compositions of the leaves and extracts were evaluated by assessing the carbohydrates, polysaccharides, proteins, lipids, insoluble fiber and ash contents. A central composite design was used to study the effect of time, temperature, and solvent/solid ratio on the extraction yield. The extraction yields at optimum conditions were compared using microwave and ultrasound techniques. The results shown that carbohydrates (84.92 ± 4.0 %) are the main component of *S welwitschii* leaves. A second order polynomial model with interaction was used to predict hydrocolloids extraction with an R^2 (96.67 %) and adjusted R^2 (92.39 %), indicating that the model was valid to predict the biopolymer extraction yield. For linear effects, temperature and extraction time affect the extraction yield significantly ($p < 0.05$). The optimal extraction conditions for maximizing yield were 36 °C, 71 minutes and 4.22 g/200 mL, respectively for temperature, time and solid-liquid ratio. Under these conditions, theoretical and experimental extraction yields were 4.934 ± 0.01 % and 4.92 ± 0.01, respectively. The dry extract contains 1.95 % of protein and 83.88 % carbohydrates, indicating that the biopolymer extracted are mainly polysaccharides. The aqueous suspension of dried extract (0.42 g/150 mL distilled water) has a viscosity of 150.33 ± 0.57 mPa.s. The extraction of this hydrocolloids from *S. welwitschii* is an asset in the food industry, cosmetics and pharmaceutical fields.

Keywords: *Securidaca welwitschii* Oliv; Hydrocolloids; Extraction; Optimization; Polysaccharides.

RÉSUMÉ :

Cette étude visait à évaluer les conditions optimales d'extraction aqueuse des biopolymères des feuilles de *Securidaca welwitschii* Oliv et à faire une caractérisation des extraits obtenus. Les compositions chimiques des feuilles et des extraits ont été déterminés en évaluant les teneurs en glucides, polysaccharides, protéines, lipides, fibres insolubles et cendres. Un plan composite centré a été utilisé pour étudier l'effet du temps, de la température et du rapport solvant / solide sur le rendement d'extraction. Le rendement d'extraction dans des conditions optimales ont été comparés à l'aide de techniques micro-ondes et ultrasons. Les résultats ont montré que les glucides (84,92 ± 4,0 %) sont le principal composant des feuilles de *S welwitschii*. Un modèle polynomial de second ordre avec interaction a été utilisé pour prédire l'extraction des hydrocolloïdes avec un R^2 (96,67 %) et R^2 ajusté (92,39 %), indiquant que le modèle était valide pour prédire le rendement d'extraction du biopolymère. Pour les effets linéaires, la température et le temps d'extraction affectent le rendement d'extraction de manière significative ($p < 0,05$). Les conditions d'extraction optimales pour maximiser le rendement étaient de 36 °C, 71 minutes et 4,22 g/200 mL, respectivement pour la température, le temps et le rapport solide-liquide. Dans ces conditions, les rendements d'extraction théoriques et expérimentaux étaient respectivement de 4,934 ± 0,01 % et 4,92 ± 0,01 %. L'extrait sec contient 1,95 % de protéines et 83,88 % de glucides, indiquant que les biopolymères extraits sont principalement des polysaccharides. La suspension aqueuse d'extrait séché (0,42 g/150 mL d'eau distillée) a une viscosité de 150.33 ± 0,57 mPa.s. L'extraction de ces hydrocolloides des feuilles de *S. welwitschii* est un attout dans le domaines agroalimentaire, cosmétique et pharmaceutique.

Mots clés: *Securidaca welwitschii* Oliv; Hydrocolloïdes; Extraction; Optimisation; Polysaccharides

1. INTRODUCTION

Hydrocolloids have been used since ancient Egypt in human food (Ndjouenkeu et al., 1996; Hoefler, 2004). Hydrocolloids are nowadays used in different industrial sectors (agri-food, cosmetics, chemicals) (Liu et al., 2015; Sood et al., 2021) with an annual production of 2.3 million tons equivalent to more than 7.4 billion dollars (IMR International, 2018). Hydrocolloids beyond their main technological (gelling and thickening) and secondary (binding agent, encapsulation, emulsifiers) properties, have different health functions mainly as a regulator of satiety, colon functions, blood cholesterol, prevention of diarrhea and dehydration, antidiabetic and antioxidant activity) (Oulai Ali et al., 2019; Hamdani et al., 2018).

Hydrocolloids are obtained from various sources, including synthetic and natural sources. Synthetic polysaccharides have a high cost with potential toxicity, and their synthesis pollute environment (Sood et al., 2021). Natural polysaccharides from plants, microorganisms and algae are more exploited thanks to their availability, low cost, biocompatibility and non-toxicity (Sood et al., 2021). However, production technology of microbial polysaccharides is expensive, while some algae polysaccharides are potentially toxic such as low molecular weight caraghenanes (Engster and Abraham, 1976). Thus, plants (edible or not) are the most important natural source of polysaccharides (Laaman, 2011) and those intended for human consumption expose the world to food and health crisis, due to their use in production polysaccharides, hence, the need of valorization of new non-food sources like *Securidaca welwitschii*.

S. welwitschii is a creeping plant found in several African countries including Guinea, Cameroon, the Democratic Republic of Congo, Angola, and Uganda and is used in traditional medicine for eye treatment (Davreux and Delaude, 1971). In Cameroon, this plant is found in West Region where the gel like leave extract is used for stomach pain healing and to regulate heartbeat rate. Indeed, the aqueous live extract generally maintained overnight before consumption forms a gel like structure at room temperature. Despite its high potential as source of biopolymer there is lack of scientific data on the composition of the leaves of *S. welwitschii*.

Water is the main solvent used for extracting hydrocolloids from plant material. Moreover, several techniques for extracting hydrocolloids have been proposed (microwave extraction, ultrasound, extraction assisted with enzymes and mechanical extraction under agitation) (Kassakul et al., 2014; Rostami et al., 2016; Shang et al., 2019), mechanical agitation extraction being widely used because its higher extraction yields with most source of hydrocolloids (Samavati, 2013; Samavati et al., 2014). Therefore, the aim of this study is to extract and analyse the hydrocolloids from *S welwitschii* leaves.

2. MATERIALS AND METHOD

2.1. Raw materials and chemicals

The fresh leaves of *S welwitschii* (Figure 1) were collected in Bafang (West Region of Cameroon) in March 2020 and transported immediately to Enzymatic Engineering and Food Technology Laboratory (LAGETA) at ENSAI, University of Ngaoundéré, Cameroon for processing and analysis.

Figure 1: *S welwitschii* : (a) les feuilles ; (b) : la fleur (© Leutcha, 2020)

All the chemicals use were of analytical grade.

2.2. Production of leaves powder and physicochemical analysis

Once in the laboratory, the leaves were sorted and the fresh leaves without any damage dried in a ventilated oven (P.dominioni, italy, UFoscolo 17) ate 45 °C for 24h, crushed (Preethi zodiac 750 W, MG-218; India), sieved with a sive having 400µm pore size and the operation repeted on the residue until there was not more residue.

The pH (Panyoo et al., 2014), dry matter, and ash (AFNOR, 1984), protein (AOAC, 1990); soluble sugar (Fischer and Stein, 1961), total sugars (Dubois et al., 1956), total lipids content (Bourely, 1982) and crude fiber content (Wolff, 1968) of the leaves powder were determined.

2.3. Extraction of *Securidaca welwitschii Oliv* hydrocoloids

The composite central design with 3 factors was used to optimize the extraction of hydrocolloids from leaves powder (Table 1). Based on the results of the preliminary studies, temperature, time and solid/solvent ratio were chosen as factors. The agitation speed (200 trs/min), pH (7) and particle size (400 µm) were maintained constant.

Table 1: Experimental Factors and their real values

Factors	- alpha	Low level	Center	High level	+ alpha
Temperature (°C)	14.77	25	40	55	65.22
Time (Min)	39.77	50	65	80	90.22
Ratio Solid/Liquid (g/mL)	2.98	3.5	4.25	5	5.51

For the extraction, leaves powder (400 µm) were mixed 200 mL of distilled water under agitation (200 trs/min) during a time of extraction, extraction temperatures and solid/liquid ratio. to control the temperature, we used a digital thermometer (TP3001 DIGITAL THERMOMETER, -50 °C to +300 °C) and ice to regulate the temperature lower than or equal to 25 °C. After extraction, the mixture was cooled to room temperature (25 ± 2 °C) centrifuged (3600 trs/min; 20 min). The supernatant was precipitated by the addition of ethanol 95 % (2:1; v: v) for 1 hour at room temperature followed by centrifugation (3600trs/min; 20min) to collect the precipitate (bottom). The precipitate collected was at 30 °C for 8h based on preliminary tests. The simplified extraction diagram is presented in Figure 2.

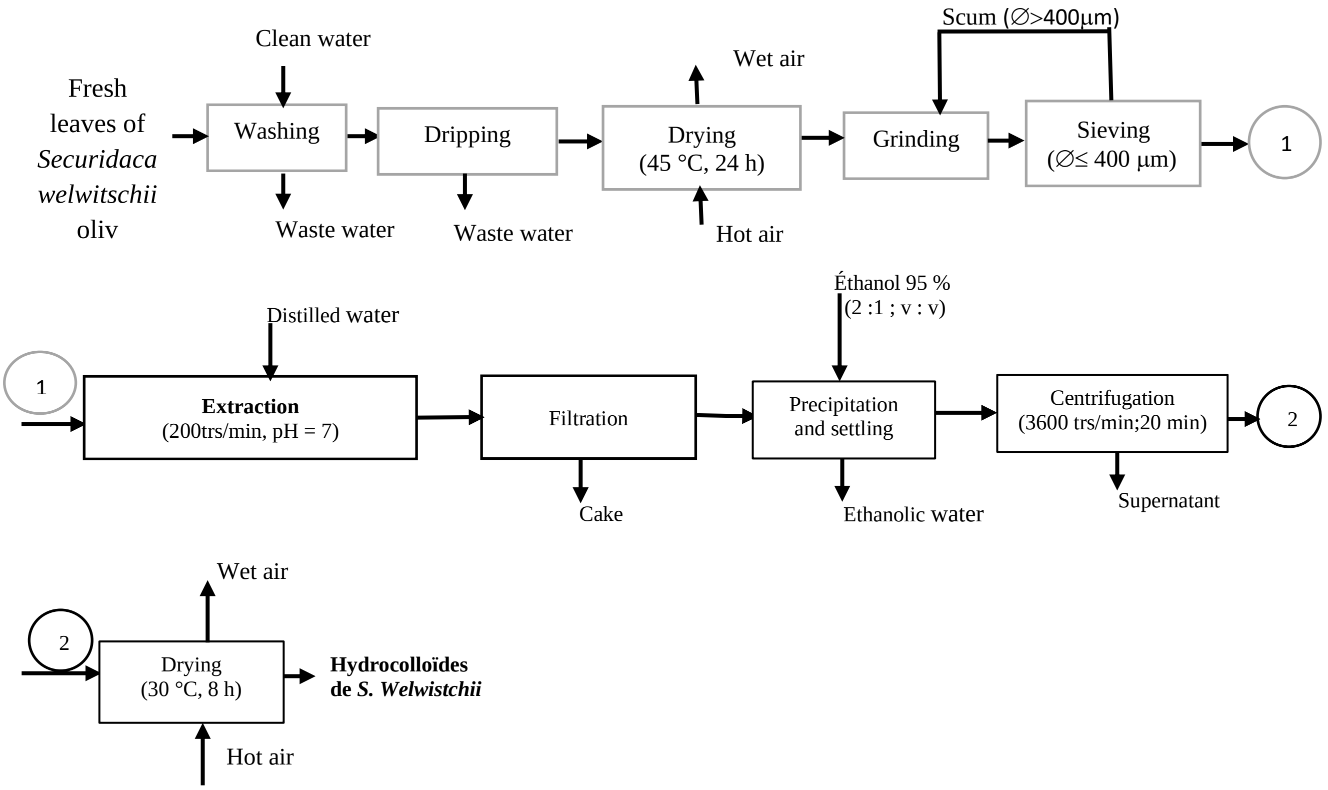


Figure 2. Schematic diagram of HSW extraction

2.4. Experimental design and statistical analysis

The Design Expert 11 software was used to generate the experimental matrix, for statistical analysis of ANOVA and optimization of the response (polysaccharides weight). The p-value of 5 % was used for significant effect. To predict optimal conditions, an empirical polynomial regression model of second order was used (equation 1).

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} b_{ij} X_i X_j \tag{1}$$

where Y is the colloid extraction yield, Xi and Xj represent the coded variables (extraction time, extraction temperatures and solid/liquid ratio); b0, bi (i 1.2, ...,k), bii (i 1.2,...,k) and bij (i 1.2, ..., k-1; j 2.3, ..., k) are respectively the regression coefficients for the central value, linear, quadratic and interaction and k the number of factors. The model evaluated the effects of each independent variable on response.

2.5. Chemical and rheological analysis of extracted hydrocolloids

HSW was obtained under optimal extraction conditions. Total protein (AOAC, 1990) and total sugar (Fischer and Stein, 1961) contents were determined. Viscosity was measured (temperature 25 °C; pH 7; 0,42 g/150 mL; 100 rpm), using a rotating viscometer (NDJ-5S viscometer, 10 mPa – 100000 mPa, 6 - 100 trs/min).

3. RESULTS AND DISCUSSION

3.1. Chemical composition and pH of the *S. welwitschii* leaves powder.

The chemical composition and the pH of *S. welwitschii* leaves powder is given in Table 2. The pH of the leaves is 6.41 ± 0.11. The pH of these leaves close to neutrality indicates that its aqueous extract could not be irritative (Somya et al., 2015) and could find application in food, cosmetic and pharmaceutical industries. The moisture content of the leaves of is 13.02 ± 0.10 g/100 g DW. This result is closed to those reported by Panyoo et al. (2014) (12.3 ± 0.11 g/100 g DW) in *Grewia mollis* powder, Singthong et al. (2009) and Oulai et al. (2019) with 10.42 % and 10.04 % respectively in the dry leaves of *Tiliacora triandra.*

Table 2. pH and proximate composition of *S. welwitschii* Leaves Powder.

Parameter	**Values**
pH	6.41 ± 0.11
Fiber content(g/100 g DW)	1.32 ± 0.008 %
Ash content(g/100 g DW)	2.41 ± 0.75 %
Fat content(g/100 g DW)	3.57 ± 0.22 %
Protein content(g/100 g DW)	7.48 ± 0.18 %
Total sugar content(g/100 g DW)	84.92 ± 4.00 %
Hydrocolloids content(g/100 g DW)	52.41 ± 2.99 %
Reducing sugar content(g/100 g DW)	32.51 ± 1.83 %
Water content (g/100 g DW)	13.02 ± 0.10 %

The fiber content is 1.32 ± 0.008 %, which represents the insoluble fiber content of the leaves, this reflects that the cell membrane of the *S. welwitschii* leaves is less rigid.

The lipid content (3.57 ± 0.22 % DW) is close to the general levels of 1 - 3 % in (Hsiang-Yun Lin and Lih-Shiuh Lai., 2009; Chadare et al., 2009). Singthong et al. (2009) obtained a lipid content of 5.1±0.6 % in the leaves of *Morus alba* L*;* 2.66 % in the leaves of *Tiliacora triandra* and 4.9 g/100 g in the baobab leaves.

The protein content of *S. welwitschii* leaves is 7.48 ± 0.18 %. Similar results were reported by Panyoo et al. (2014) (7. 8±0.55 g/100 g DW) in *grewia mollis* and by Singthong et al. (2009) (10.04 %) in *Tiliacora triandra* leaves powders.

S. welwitschii leaves contain mainly sugar with 84.9 ± 24.0 g/100 g DW of total carbohydrate. This value is higher than that obtained by Singthong et al. (2009) (59.5 %) in *Tiliacora triandra* leaves. The difference between total sugar content and reducing sugar (32.51 ± 1.83) determines the approximate content of hydrocolloids (52.41 ± 2.99 %), higher than several literature works and could be due to difference in species.

3.2. Optimizing Hydrocolloids of *Securidaca welwitschii* Oliv (HSW) extraction settings

3.2.1. The experimental design

The experimental design used consisted of 17 experimental runs with extraction yield as main response (Table 3). The ANOVA of the influence of extraction conditions of HSW is presented on Table 4.

Table 3. Experimental design with Response

Trial number	Temperature (C)	Time (min)	Ratio S/L (g/mL)	Yields (%)
1.	40	65	4.25	4.94
2.	65.22	65	4.25	1.19
3.	40	65	4.25	4.47
4.	55	50	3.5	1
5.	14.77	65	4.25	3.65
6.	40	65	2.98	1.12
7.	55	80	3.5	2.54
8.	25	50	3.5	1.39
9.	55	50	5	1.6
10.	40	65	4.25	4.92
11.	55	80	5	1.78
12.	25	80	3.5	2.91
13.	40	39.77	4.25	2.47
14.	25	50	5	2.12
15.	40	90.22	4.25	3.97
16.	40	65	5.51	1.14
17.	25	80	5	2.46

A second order polynomial equation was used to describe the effect of the different factors on the hydrocolloid yield (Y) (equation 2):

$$Y = 4.79 - 0.4470 A - 0.4480 B - 0.0100 C - 0.8606 A^2 - 0.5788 B^2 - 1.32 C^2 - 0.176 AB - 0.0546 AC - 0.3181 BC \quad (2)$$

Y: hydrocolloid yield; A: Extraction temperature; B: Extraction time; C: Solid/liquid ratio.

Table 4. ANOVA of the Influence of Extraction Conditions on HSW extraction

Source	Sum of squares	Ddl	Medium square	F-value	P-value
Model	28.71	9	3.19	22.59	**0.0002**
A-temperature	2.73	1	2.73	19.34	**0.0032**
B-time	2.74	1	2.74	19.41	**0.0031**
C-ratio S/L	0.0014	1	0.0014	0.0097	0.9244
AB	0.0025	1	0.0025	0.0176	0.8982
AC	0.0239	1	0.0239	0.1691	0.6932
BC	0.8096	1	0.8096	5.73	**0.0478**
A2	8.35	1	8.35	59.14	**0.0001**
B2	3.78	1	3.78	26.75	**0.0013**
C2	19.57	1	19.57	138.58	**0.0001**
Residual	0.9883	7	0.1412		
Lack of Fit	0.8467	5	0.1693	2.39	0.3206
Pure Error	0.1416	245	0.0708		
Cor Total	29.70	287			

The model's low P-value (P =0,0001), low value of the coefficient of variation (14.62 %), high R^2 (0.97), high adjusted R^2 (0.92) and the low F-value (2.39) of Lack of Fit indicate that the model equation is adequate to predict extraction yield under these experimental conditions. A summary of the variance analysis of the experimental results is presented in Table 4. From table 4, the coefficients of factors temperature (A), time (B), the interaction between time and ratio S/L (BC) and the quadratic effect of the different factors (A^2, B^2, C^2) with the P-value below 0.05 Significantly affect the extraction yield (Y).

Table 5 presents the low value of the coefficient of variation (CV %, 14.62) clearly suggested a very high degree of accuracy and a great reliability of the experimental values. The "Adeq. Precision" was used to measure the signal-to-noise ratio. It is normally desirable when a ratio is greater than 4. The "Adeq. Precision'' of 13,5603 indicated that this model could be used to navigate the design space.

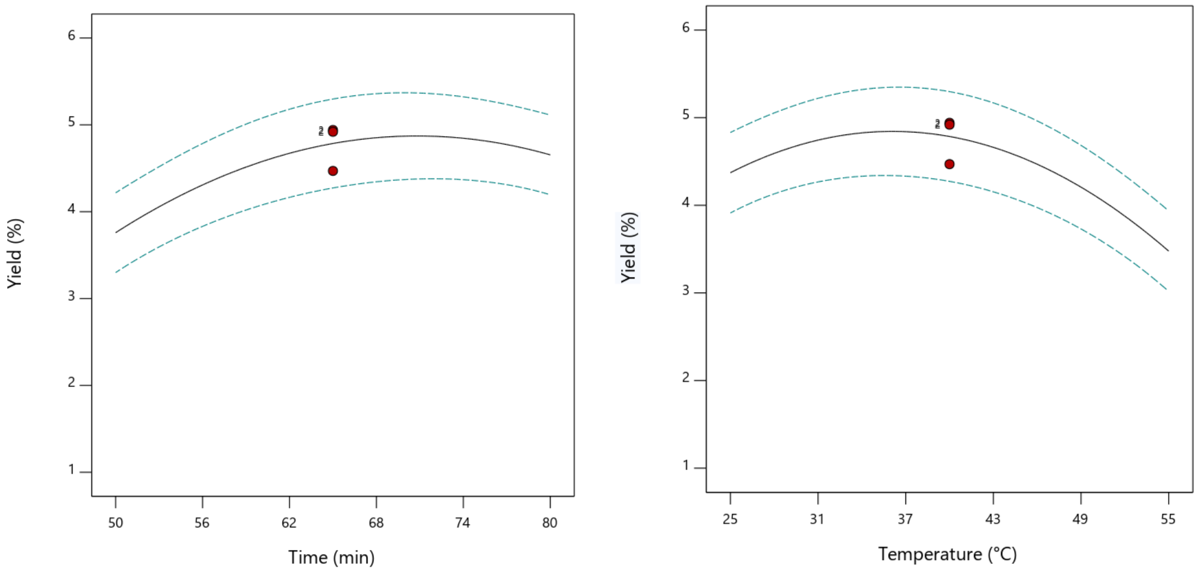
Table 6. Variance Analysis for the quadratic polynomial model of HSW extraction.

Paramètres	S.d.	Mean	C.V.%	Press	R^2	R^2 Adj	R^2_{Pred}	Adeq.précision
Valeurs	0.37	2.57	14.62	0.67	0.9667	0.9269	0.7724	13.5603

The effects of temperature and time on extraction yield are presented in Figure 3(b). The results revealed that the increase in temperature from 25 °C to 37 °C improves the extraction yield of hydrocolloid from 3.9

% to 4.92 % probably by softening the plant tissue, improving the diffusion rate of the hydrocolloid out of the leaves accelerated by the effect of agitation (Maran et al., 2013). However, a further increase in temperature (38 - 55 °C) leads to decreased extraction efficiency by affecting the permeability of the solvent in the environment due to the appearance of insoluble compounds and decreases extraction efficiency (Gan and Latiff, 2011). This temperature there may be degradation of these polysaccharides thus reducing extraction yield.

The effect of time on hydrocolloid extraction yield is presented in Figure 3(a). The results revealed that the extraction yield increased linearly with extraction time up to 70 min then decrease. This time had allowed the solvent to diffuse in the plant cell and by putrescence, increase the diameter of the membrane cell pore favorizing diffusion of the hydrocolloid in water, thus increasing the extraction yield (Prakash and Manikandan, 2012). The decrease in extraction yield above 70 min could be due to the cell lysis and releasing of other component of cell (polyphenols, phytate, pectin…) which can reduce the solubility of the hydrocolloid extracted in aqueous environment.


(a)

(b)

Figure 3. Influence of time (a) and temperature (b) on extraction yield

Figure 4 presents the global effect of time and temperature (individual factor and their interaction) on the extraction yield. The results revealed that there is interaction between factors B and C because the effect of B on the response depends on the level of C or vice versa. As a result, when time is set at these different levels and the ratio is varied, the increase in the S/L ratio produces a difference in concentration gradient between leaf particles and the outer solvent, which may eventually increase solvent spreadivity in plant matter and polysaccharides solubilization, accelerate the rate of mass transfer and increase extraction yield (Pompeu et al., 2009). However, starting from a ratio threshold, the yield decreases, this results in saturation of the solute solvent, negatively alters the rate of mass transfer, hinders the diffusion of polysaccharide in the solvent and reduces polysaccharide yield (Amid and Mirhosseini., 2012)**.** to have a better compromise between time and ratio, it is necessary either to increase the time (68min) and reduce the ratio g/200 mL (approximately 4.1g) or reduce the extraction time (60min) and increase the ratio g/200 mL (4.5 minutes).

These phenomena had been observed by Maran et al., (2016) during the extraction of polysaccharides from *gossypium seeds arboreum* L.

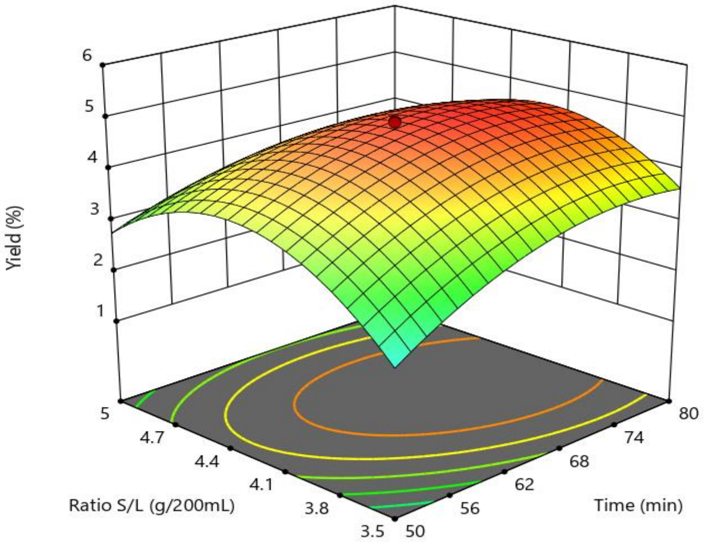


Figure 4. Surface curve response for the influence of the interaction ratio (S/L) and time

3.2.2. Confirmation test

Optimal extraction conditions (Temperature 36.07 °C; time 71.02 min; Ratio S/L 4.22 g/g) for hydrocolloid extraction yield were estimated using the regression model equation. Under these conditions, the theoretical maximum extraction yield is 4.93 % and the real extraction yield value of 4.92 %, confirming the accuracy of the model to predict extraction yield.

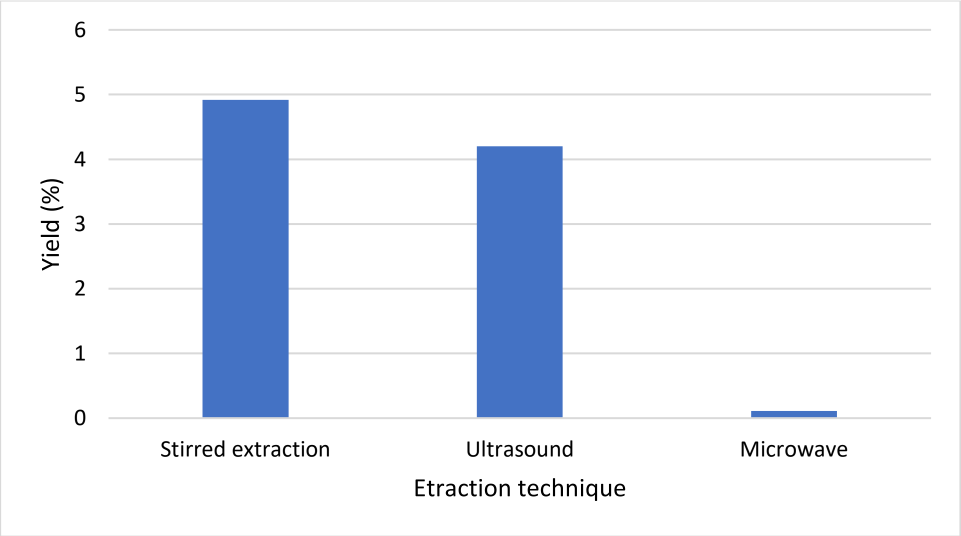


Figure 5. Comparison of Extraction Yields

Figure 5 shows the different extraction yields obtained by mechanical extraction under agitation, ultrasound (60.90 W) and microwave (400W) under the above conditions. The results obtained show that the yields of the three extraction methods are significantly different (P<0.05). Among three methods, stirred extraction performed better (4.92 ± 0.01 %), followed by ultrasound extraction (4.2 ± 0.019 %) and microwave extraction (0.11 ± 0.1 %). However, opposite results have been obtained by several authors. Unconventional extraction can significantly improve the extraction efficiency of hydrocolloids, including the cavitation effect (ultrasound extraction) and dielectric heating (microwave extraction) can accelerate cell wall rupture and promote the dissolution and diffusion of polysaccharides from cells (Wu et al., 2019). However, the process by which bubbles form, develop, and undergo an implosive collapse called "cavitation", and dielectric heating greatly increases temperature (about 5000K), and pressures (about 2000atm) are locally affected which can cause hydrolysis of polysaccharides over time (Mandal et al., 2015), which justifies low ultrasound and microwave yield.

3.3. Chemical and rheological properties of the dry extract

The extract obtained was dried and analyzed, the dry extract contains 1.95 % of protein and 83.88 % of carbohydrate. the high carbohydrate content of the extract indicates that the hydrocolloids extracted are mainly polysaccharides. The protein content is like those reported by Nep et al. (2016) (2.2 %) in the polysaccharides of sesame leaves *(Sesamum radiatum*), and Yamazaki et al. (2008) (5.5 %) in *Corchorus olitorius* hydrocolloids.

The viscosity of the *securidaca welwitschii* biopolymer in aqueous medium (0.28 % w/w) was 150.33 ± 0.57 mPa.s. This viscosity obtained in an aqueous medium at room temperature suggests that this bioplymer could have a high molecular weight and a linear structure with free hydroxyl groups which easily form bonds with the water molecules making it possible to increase the viscosity in an aqueous medium (Hoefler, 2004). This viscosity is close to that obtained by Panyoo et al. (2014) 0.39 Pa·s (2.5 % w/w) in the powder of Grwia mollis.The carbohydrate content of *S. welwitschii* aqueous leaves extract is close to reported by Yamazaki et al. (2008) (62.2 %) in *Corchorus olitorius* hydrocolloids, Nep et al. 2016 (67 %) in the of sesame leaves polysaccharides.

4. CONCLUSION

The objectives of this work were to determine the chemical composition of the leaves of *S welwitschia* and the effects of time, temperature and solid/liquid ratio on the hydrocolloid extraction yield from the leaves of *S welwitschii* by means of the response surface methodology. It was also a question of carrying out the extraction with microwaves and ultrasound to make the choice of the best method of extraction. It emerges from the characterization of the leaves of *S welwitschii* that carbohydrates occupy an important place in the composition of the *S welwitschii* leaves (84.92 ± 4.0 %). The optimum conditions (maximization) of the extraction yield were extraction temperature (36 °C); extraction time (71 min) and ratio (4.22 g/200 mL) which provide an extraction yield of 4.92 ± 0.01 %, composed of 1.95 % protein, 83.88 % total sugar.

5. CONFLICTS OF INTEREST DECLARATION

The authors declare that there are no conflicts of interest.

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