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ORIGINAL ARTICLE

Optimization of Ultrasound-Assisted Extraction of L-Ascorbic Acid from *Adansonia digitata* (Linn.) and Evaluation of its Antityrosinase Activity

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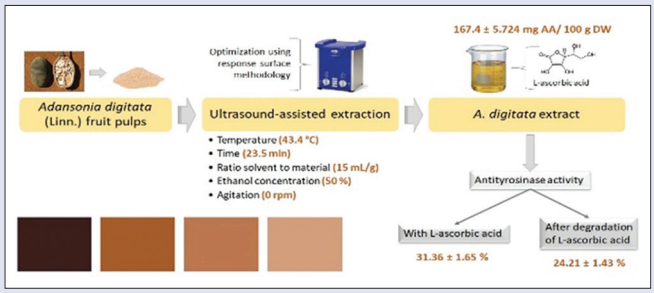
ABSTRACT

Background: *Adansonia digitata* (Linn.) is a widely distributed tree and its edible fruit pulp is well known to have L-ascorbic acid (AA) in high amounts. AA is essential for collagen biosynthesis and has been reported to have antioxidant and skin-lightening properties. **Objectives:** The aim of this study was to optimize the extraction of AA from the fruit pulp of *A. digitata* by ultrasound-assisted extraction and to evaluate the antityrosinase activity of the extract. **Materials and Methods:** A rotatable central composite design was used to investigate the effect of process variables by surface response methodology. The effect of key parameters of extraction temperature (30°C–40°C), extraction time (12–28 min), and ratio solvent to material (7–13 mL/g) was investigated on AA content. Then, mushroom tyrosinase was used to evaluate the antityrosinase activity of the extract. **Results:** The optimal conditions were obtained with a temperature of 43.4°C, in 23.5 min with a ratio solvent to material of 15.04 mL/g. Under these conditions, AA content from *A. digitata* (167.4 ± 5.724 mg AA per 100 g dry weight) was determined. Antityrosinase activity of *A. digitata* extract containing AA was 31.36% ± 1.65%. After degradation of AA, tyrosinase activity decreases to 24.21% ± 1.43%. **Conclusion:** AA contained in *A. digitata* extract show to increases significantly the inhibition of enzymatic tyrosinase activity. This extract may have a potential impact on skin depigmentation for the treatment of hyperpigmentation disorders.

Key words: *Adansonia digitata* (Linn.), antityrosinase, depigmentation, L-ascorbic acid, ultrasonic-assisted extraction

SUMMARY

- Ultrasound-assisted extraction parameters were optimized by surface response methodology to extract L-ascorbic acid from *Adansonia digitata* (Linn.) pulps and the antityrosinase activity of the optimized extract was evaluated.



Abbreviations used: *A. digitata*: *Adansonia digitata* (Linn.); RSM: Response surface methodology; UAE: Ultrasound-assisted extraction; AA: L-ascorbic acid; DW: Dry weight; SD: Standard deviation; X_1: Extraction temperature; X_2: Extraction time; X_3: Ratio solvent to material; R^2: Coefficient of determination; ANOVA: Analysis of variance; PBS: Phosphate buffer solution.

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INTRODUCTION

Adansonia digitata (L.) called the baobab tree is very characteristic of the Sahelian region. Baobab tree belongs to the Malvaceae family and is a multipurpose tree with various medicinal properties. Every part of the plant is reported to be useful.[1,2] The fruit pulp, which represents 14%–28% of the total fruit weight, contains organic acids such as citric, tartaric, malic, succinic, and L-ascorbic acid (AA). The pulp water content is lower than 15%.[3] The fruit pulp has a particularly high antioxidant property mainly due to its high natural AA content.[4] Recently, derivatives of baobab fruit (fruit pulp and seed oil) have been exported to Europe, Canada, and the United States. The popularity of *A. digitata* (Linn.) extract being grown indicates that the demand for this resource will grow in the coming years.[5]

Conventional extraction such as maceration and Soxhlet have many drawbacks such as large amount of solvent utilization, long extraction time, and high extraction temperature for relatively low extraction yield.[6] To overcome these drawbacks, new techniques for the extraction of bioactive compounds have been developed, including ultrasound-assisted extraction (UAE), supercritical fluid extraction, microwave-assisted extraction, and accelerated solvent extraction.[7] However, among all these nonconventional techniques, UAE offers an inexpensive, environmentally friendly, less time-consuming, and efficient alternative to conventional extraction techniques.[8] A simple cleaning bath can be used to perform ultrasonic-assisted extraction on a laboratory scale.[9] Ultrasonic waves may cause some undesirable chemical effects such as changes in chemical composition, possible degradation of targeted compounds, and production of free radicals within the gas

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bubbles.[10] Therefore, extraction conditions should be optimized to avoid or reduce these undesirable effects. Degradation of AA depends on many factors (oxygen, heat, light, storage temperature, and storage time) and proceeds both aerobic and anaerobic pathways.[11] Said *et al*.[12] found that the highest yield of AA from banana peels (0.04939 ± 0.00080 mg) is obtained with an extraction temperature of 30°C, an extraction time of 15 min and a ratio solvent to material of 5 mL/g using UAE. Neto *et al*.[13] showed that the solubility of Vitamin C in water, ethanol, and water + ethanol was high compared to that with propan-1-ol and water + propan-1-ol.

Fives parameters affecting the extraction efficiency of AA were identified such as ethanol concentration, agitation speed, ratio solvent to material, extraction time, and temperature. One-factor-at-a-time approach was used to optimize ethanol concentration and agitation speed. To optimize the other parameters, response surface methodology (RSM) was used to evaluate multiple parameters and their interactions with a reduced number of experimental trials.[14] RSM allows the collection of mathematical and statistical technique for the development, improvement and optimization of processes.[15,16] RSM is a faster and economical method used for gathering research results instead of classic one-variable-at-a-time or full factors in experimentation.[17]

AA is the main biologically active form of Vitamin C and is reversibly oxidized to form L-dehydroascorbic acid, which also shows biological activity. However, further oxidation generates diketogulonic acid, which has no biological activity.[18] Discoveries show that AA plays important role in the health and beauty of the skin. In addition, AA has been tested extensively and is reported to inhibit melanogenesis.[19] AA allows the interruption of a key step of melanogenesis. It interacts with copper ions at the tyrosinase-active site and inhibits the action of the tyrosinase enzyme, which triggers the production of melanin.[20] Inhibition of enzymatic tyrosinase activity is one of the most commonly used ways for finding new depigmenting agents.[21] Natural extracts are increasingly used to treat hyperpigmentation disorders because of their efficiency and lack of harmful side effects.[22] However, antityrosinase activity of *A. digitata* fruit pulp have never been evaluated.

The aim of this study was to optimize the ultrasonic-assisted extraction of AA from *A. digitata* fruit pulp and evaluate its antityrosinase activity. Parameters such as extraction temperature, extraction time, and ratio solvent to material were optimized by employing a rotatable central composite design to maximize the amount of AA in the extract. Antityrosinase activity was done before and after degradation of AA in order to highlight the inhibition contribution of AA.

MATERIALS AND METHODS

Plant materials

A. digitata (Linn.) pulps were harvested in Dakar (Senegal). The pulps were turned into powder manually until fine and homogenous particles were obtained. All reagents and chemicals were purchased from Sigma-Aldrich (Germany).

Preliminary study

A preliminary study was carried out in order to determine a suitable agitation speed and ethanol concentration for *A. digitata*. For agitation speed, extraction yield was considered, while for ethanol concentration, maximum recovery of AA was considered. In the preliminary experiments, each parameter was optimized by fixing all the other parameters. It was observed for *A. digitata* that the optimum agitation speed and ethanol concentration were 0 rpm and 50%, respectively.

Ultrasound-assisted extraction

UAE was applied to the extraction of AA from *A. digitata* pulps. UAE was performed in an ultrasonic bath Elmasonic S type S 15/H type S 15 (produce by Elma Hans Schmidbauer GmbH and Co. KG, Germany, bath frequency 37 KHz, power 280 W inside the bath). The setup allowed the control of temperature. The fruit pulp powder was placed directly into the ultrasonic bath with agitation according to the results obtained from the preliminary study. Then, reaction mass was filtered, and the filtrate was collected in volumetric flask to be used for the determination of AA content.

Determination of L-ascorbic acid content

Determination of AA was carried out by the AOAC's official titrimetric method (AOAC, 1990)[23] and expressed as mg AA/100 g dry weight (DW). This titration method only determines AA and not L-dehydroascorbic acid. 2 mL of the extract and 5 mL of the meta-phosphoric acid-acetic acid solution were titrated with indophenol solution (50 mg DCIP and 42 mg $NaHCO_3$ in 200 mL water) until a light, but distinct rose pink color appears and persists for >5 s. The indophenol solution was standardized twice daily with AA solution. The data are presented as the average of triplicate analyses.

Experimental design

A three-factor (X_1, X_2, and X_3) and three levels (−1, 0, and +1) central composite design was employed for this study using a rotatable second-order design with four replicates in the center of the experimental domain. The independent variables were (X_1) extraction temperature (°C), (X_2) extraction time (min), and (X_3) ratio solvent to material (mL/g). The response variable was AA content (mg AA/100 g DW). The conditions of the independent variables studied were X_1 in the range (30°C–40°C), X_2 in the range (12–28 min), and X_3 in the range (7–13 mL/g) according to the study done by Said *et al*.[12] The different levels of the three parameters are resumed in Table 1.

The experiment was performed in triplicate in order to prove the reproducibility of the UAE. Multiple regression equations were generated in order to develop an empirical model, which correlated the response to the independent variables.

Analysis was carried out in triplicates and the experimental results were expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) was done for the response variables to test the model significance with 95% confidence level. All statistical analyses were conducted using the software Statgraphics Plus software (version 5.1, Statpoint Technologies Inc., Warrenton, VA, USA).

Antitysosinase assay

Antityrosinase activity was determined as described previously by El Khoury *et al*. 2018.[24] Solutions of mushroom tyrosinase (150 U/mL), L-tyrosine (0.5 mg/mL), and *A. digitata* extract were prepared in phosphate buffer solution pH 6.6. Kojic acid was used as reference standard inhibitor for comparison. The reaction was carried out using 96-well microplate and Thermo scientific microplate reader (multiskan GO). Briefly, 50 µL of tyrosinase solution was mixed with 50 µL of L-tyrosine solution and 50 µL of extract (with and without AA) or kojic acid solution. AA was allowed to be degraded naturally after 2 weeks of exposure to ambient

Table 1: Range of coded and actual values for the central composite design

Independent variables	Symbol	Uncoded and coded levels				
		−1.68	−1	0	1	+1.68
Extraction temperature (°C)	X_1	26.6	30	35	40	43.4
Extraction time (min)	X_2	6.56	12	20	28	33.44
Ratio solvent to material (mL/g)	X_3	4.96	7	10	13	15.04

temperature, air, and light. The final concentrations of *A. digitata* aqueous extracts with and without AA were 1, 2, 3, 4, and 5 mg/mL. Then, the mixture was incubated at 37°C for 60 min. The amount of dopachrome produced during this reaction was determined by reading the optical density at 475 nm. The antityrosinase activity was expressed as percentage of inhibition as determined by optical density in comparison to a witness, which has no extract. All results are expressed as average values ± SD of three independent analyses.

RESULTS AND DISCUSSION

Fitting the models

The experimental design and the results for extraction of AA from *A. digitata* are shown in Table 2. The average values of the response and variance expressed by SD are presented. AA content in *A. digitata* pulps extracts varied from 114.39–167.00 mg AA/100 g DW.

ANOVA was used to evaluate the quality of the fitted model [Table 3]. To evaluate how well a model fits the experiment data, the parameter such as coefficient of determination (R^2), lack-of-fit, *P* value, and *F* value should be used.

The R^2 is calculated by dividing the sum of squares due to regression by the total sum of squares, and the result is interpreted as the proportion of the variability in the data explained by the ANOVA.[25] For a good fit of the model, R^2 should be at least 80%.[26] $P > 0.05$ for lack of fit test indicated the suitability of models for accurate prediction of the variation in the results.[27] The R^2 was 90.089% with no significant lack of fit at $P > 0.05$, indicating that the predicted model could explain 90.09% of the results and only 9.91% of the total variance was not explained by the model. The value of R^2 adjusted showed also the significance of the model. Adjusted R^2 is obtained after the elimination of unnecessary model terms of R^2. In case of many nonsignificant terms in the model, adjusted R^2 value could be very small compared to R^2 value.[28] In this study, R^2 adjusted (87.49%) was very close to the R^2 value. The significance of the regression coefficients, which were determined at $P < 0.05$, was determined by *F*-test and *P* value resulting from the ANOVA [Table 3]. A coefficient is more significant when the magnitude of *F* value is larger and the *P* value is smaller.[25] Moreover, the reproducibility of UAE was proved from the *P* value of "blocks" with a *P* value for blocks >0.05 which means that there was a small block effect.[29]

Table 2: The central composite design matrix and the experimental data for the response for *Adansonia digitata*

Run	Factors			L-AA content					
	X_1*	X_2†	X_3‡	mg AA/100 g DW§	SD	mg AA/100 g DW	SD	mg AA/100 g DW	SD
1	30	12	7	127.08	3.32	130.92	0.00	129.00	3.32
2	40	12	7	132.38	3.23	128.65	0.00	134.25	0
3	30	28	7	114.39	3.08	114.39	3.08	116.17	0
4	40	28	7	118.85	0.00	117.27	2.74	118.85	0
5	30	12	13	145.93	5.74	149.25	0.00	159.20	0
6	40	12	13	165.83	5.51	165.82	5.52	166.21	0
7	30	28	13	164.29	5.81	160.93	0.00	167.64	5.81
8	40	28	13	167.00	6.49	155.87	0.00	167.00	0
9	26.59	20	10	130.26	2.72	128.69	2.72	128.69	2.72
10	43.41	20	10	154.60	0.00	161.04	0.00	161.04	0
11	35	6.55	10	131.04	6.91	131.04	6.91	131.04	6.91
12	35	33.45	10	128.54	5.14	130.26	2.97	130.26	5.94
13	35	20	4.95	96.29	2.98	99.73	2.98	96.29	2.98
14	35	20	15.05	153.67	12.24	153.67	12.24	157.67	12.24
15	35	20	10	143.18	2.99	141.45	2.99	141.45	2.99
16	35	20	10	136.37	2.85	136.37	2.85	136.37	2.85
17	35	20	10	127.75	3.03	129.50	3.03	131.25	0
18	35	20	10	133.60	2.64	133.60	0.00	133.60	2.64

*Extraction temperature; †Extraction time; ‡Ratio solvent to material; §mg L-AA per 100 g of DW. SD: Standard deviation; DW: Dry weight; AA: L-ascorbic acid

Table 3: Analysis of variance for the second-order polynomial model for *Adansonia digitata* pulps

Source	*Adansonia digitata* L-AA content				
	Sum of squares	Degree of freedom	Mean square	*F*-ratio	*P*
X_1: Temperature	1061.44	1	1061.44	37.27	0.0002
X_2: Time	84.0959	1	84.0959	2.95	0.1198
X_3: Ratio solvent to material	13482.1	1	13482.1	473.41	<0.0000
X_1^2	950.902	1	950.902	33.39	0.0003
X_1X_2	83.3655	1	83.3655	2.93	0.1213
X_1X_3	20.5165	1	20.5165	0.72	0.418
X_2^2	1.04208	1	1.04208	0.04	0.8525
X_2X_3	530.63	1	530.63	18.63	0.0019
X_3^2	64.0532	1	64.0532	2.25	0.1679
Blocks	48.8031	2	24.4015	0.86	0.4564
Lack-of-fit	1557.8	33	47.2062	1.66	0.215
Pure error	256.307	9	28.4785		
Total (correlation)	18304	53			
R^2	90.089 percent				
R^2 adjusted	87.4932 percent				

AA: L-ascorbic acid

For the ultrasonic-assisted extraction of AA from *A. digitata*, X_1, X_3, X_1^2 and X_2, X_3, were the most significant parameters because of $P < 0.05$. However, X_2, X_1X_2, X_1X_3, and X_2^2 and X_3^2 had less effect, with a $P > 0.05$.

Multiple regression equations were obtained in terms of coded factors to describe the effect of the three independent parameters. The equation generated allows the prediction of AA extraction efficiency by an empirical model equation (1). According to equation (1), the linear term of temperature (X_1) for AA showed the highest value and negative signal; consequently, it was considered to be of greater importance on the response. The negative values in the models indicate that an increase of factor values tends to decrease the response values.

$$\text{L-ascorbic acid content} = 302.452 - 12.683\ X_1 - 0.611\ X_2 + 2.858\ X_3 + 0.200\ X_1^2 - 0.047\ X_1X_2 + 0.062\ X_1X_3 + 0.00258\ X_2^2 + 0.196\ X_2X_3 - 0.144\ X_3^2 \quad (1)$$

Response surface analysis of L-ascorbic acid from *Adansonia digitata*

The relationship between extraction parameters and AA content was investigated by surface response plots. These graphs are drawn by maintaining one factor constant and varying the other two factors. Figures 1-3 show the effect of temperature, time, ratio solvent to material, and their mutual interaction on the AA content.

The highest AA content was observed at lower times and higher temperature [Figure 1]. High temperatures may increase the diffusion and solubility rate of many compounds. Furthermore, at higher temperature, the extraction yield was increased due to the high number of cavitational nucleus formed during higher extraction temperatures as a result of high cavitation threshold.[30] However, AA degrades when subjected to higher temperatures during a long extraction time. Long extraction period followed by temperature >30°C will increase the possibility of oxidation of AA and degradation of AA due to heat.[12] The increase of time at the lowest temperatures, increase AA content at a fixed ratio solvent to material [Figure 1]. At lower temperatures, a higher extraction time allows mass transfer to be completed and equilibrium of diffusion to be reached. The effect of ratio solvent to material and time represented in Figure 2 demonstrates that AA content always increased, with increasing ratio solvent to material regardless of the extraction time. AA is a polar molecule because of the numerous hydroxyl groups present in its structure. This observation is consistent with the mass transfer principle. The driving force during the mass transfer is the concentration gradient between the material and the solvent, which is greater when a higher ratio solvent to material is employed.[31] Moreover, compared to ethanol and many others solvents, water is a smaller and more polar molecule capable of achieving equilibrium more easily with AA by establish hydrogen bonds, favoring the increase of its solubility.[13] AA content seems to decrease when the extraction time increase due to the relatively high temperature which is fixed at 35°C. This result was in agreement with the previous conclusions about extraction time and temperature in Figure 1. Nevertheless, for high ratio solvent to material, AA content was not affected by the temperature even for a long period of extraction. Figure 3 shows the effect of temperature and ratio solvent to material on the ultrasonic-assisted extraction of AA from *A. digitata*. At fixed extraction time, AA content increase with the increasing of ratio solvent to material. AA content was not affected by the increasing of temperature in a short period of time (20 min). These final observations were consistent with all previous observations. The relationship between extraction parameters of ultrasonic-assisted extraction and AA from *A. digitata* was well described by the surface response plots.

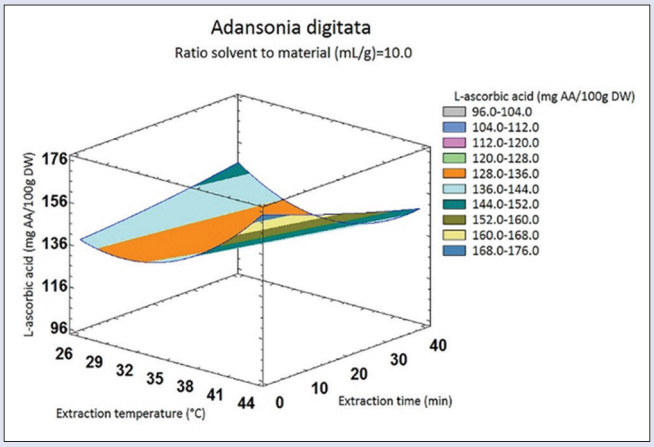

Figure 1: Response surface plots for the effect of temperature/time on the ascorbic acid (mg L-ascorbic acid/100 g dry weight) for *Adansonia digitata* (Linn.)

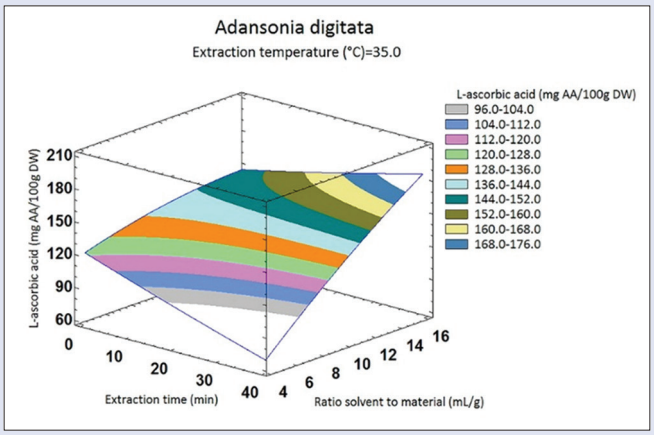

Figure 2: Response surface plots for the effect of time/ratio on the ascorbic acid (mg L-ascorbic acid/100 g dry weight) for *Adansonia digitata* (Linn.)

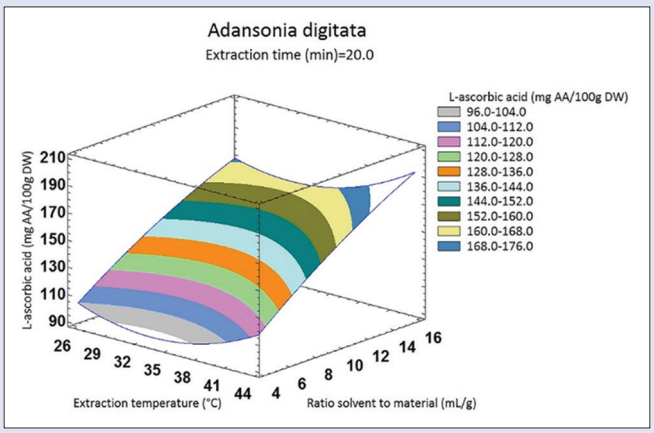

Figure 3: Response surface plots for the effect of temperature/ratio on the ascorbic acid (mg L-ascorbic acid/100 g dry weight) for *Adansonia digitata* (Linn.)

Determination of optimum conditions

In this study, the optimal conditions for the ultrasonic-assisted extraction of AA from *A. digitata* using surface response methodology

were determined. The experiments were conducted in triplicates at the optimum conditions and the average values are shown in Table 4.

At first sight, the experimental results for *A. digitata* seem to disagree with the optimum value provided by the empirical model. To understand this inconsistency, the total amount of AA present in the *A. digitata* pulp was determined. Three successive solid-liquid extraction in a beaker with stirring was carried out for 10 g of pulp with 100 mL of 50% ethanol (168.625 ± 0 mg AA/100 g DW). Then, extraction with 50 mL of solvent was carried out and dosed separately (2.8104 ± 0.973 mg AA/100 g DW). A final extraction with 50 mL of solvent was identical to the blank and did not contain AA in detectable quantity. This indicates that the *A. digitata* pulp used in this study contains a total amount of AA of 171.436 mg AA/100 g DW. Therefore, the optimum conditions obtained are accepted independently of the maximum amount of AA indicated by the empirical model.

Antityrosinase activity of *Adansonia digitata* extract

A. digitata aqueous extract with and without AA were tested for antityrosinase activity at different concentrations [Figure 4]. The aqueous extracts showed a significant and dose-dependent potential to inhibit enzymatic activity of tyrosinase. *A. digitata* extract containing AA show a good inhibition of tyrosinase activity with approximately 31.36% ± 1.65% inhibition at 5 g/L. At the same concentration, the inhibition percentage of aqueous extract without AA was 24.21 ± 1.43 g/L. Aqueous extracts containing AA showed significantly higher inhibition of tyrosinase activity compared to the aqueous extract without AA. The contribution of AA in increasing the inhibition of tyrosinase activity was significant. The total inhibitory activity exhibited by *A. digitata* extract is, therefore, mainly related to high content of AA. In analogy, *Phyllanthus emblica* extract is widely used in skin-lightening products and has a high contain in AA, which is the main biologically active compound.[32]

CONCLUSION

In this article, ultrasonic-assisted extraction was studied for the extraction of AA from *A. digitata* and its aqueous extract was evaluated for its antityrosinase activity. On the one hand, surface response methodology was employed to investigate the effects of extraction parameters (temperature, time, and ratio solvent to material) on the AA content. To achieve the maximum extraction of AA from *A. digitata* by UAE, an extraction temperature of 43.4°C, an extraction time of 23.5 min, and a ratio solvent to material of 15.04 mL/g should be employed as optimal operating conditions. With a quantity of 167.376 ± 5.724 mg AA/100 g DW, the pulp of *A. digitata* can be considered as a good source of AA. On the other hand, inhibition of enzymatic activity of tyrosinase was evaluated using mushroom tyrosinase. AA contains in the aqueous extract showed to have an important contribution in inhibiting by 31.36% ± 1.65% the activity of tyrosinase enzyme. This result indicated that *A. digitata* aqueous extract could be potentially used as depigmenting agents in cosmetic and dermatologic products designed to treat hyperpigmentation disorders. Further studies are needed in order to stabilize the AA in the extract and evaluate its cytotoxic effect at effective concentrations.

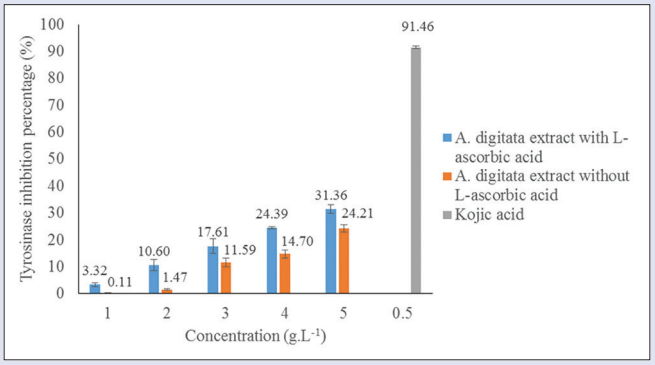

Figure 4: Antityrosinase activity of *Adansonia digitata* fruit pulp extract with and without L-ascorbic acid in comparison to kojic acid

Table 4: Predicted and experimental values of the responses at optimum conditions for *Adansonia digitata* pulps

Optimal levels of process parameters	L-AA content (mg AA/100 g DW)	
	Predicted value	Experimental values
X_1 (°C)=43.4	188.5	168.7±0.000
X_2 (min)=23.5		161.1±6.490
X_3 (mL/g)=15.04		172.4±6.490

DW: Dry weight; AA: L-ascorbic acid

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Conflicts of interest

There are no conflicts of interest.

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