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Enhanced Extraction of Polyphenols, Flavonoids and Antioxidant Activity from *Cynometra ananta* Stem Bark using Ultrasonic Method: A Kinetic Modelling Approach

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Cynometra ananta stem bark is rich in bioactive phenolic compounds with antioxidant potential. This study evaluated the efficiency of ultrasound-assisted extraction (UAE) in comparison to conventional maceration at various solid-to-liquid ratios (1:60, 1:80, and 1:120 g/mL) and described the extraction kinetics using a second-order kinetic equation. The highest polyphenol yield was achieved with UAE at a 1/60 ratio (482 µg GAE/mg DM), surpassing maceration (420 µg GAE/mg DM). Flavonoid content also increased significantly with UAE, reaching 14.18 µg QE/mg DM *versus* 7.72 µg QE/mg DM with maceration. Antioxidant activity (FRAP) was enhanced by ultrasound, with a saturation concentration of 238 µg Trolox/mg DM. Kinetic modeling showed excellent correlation ($R^2 > 0.99$) across all conditions. The improved performance of UAE is attributed to enhanced mass transfer, solvent penetration and cavitation effects. These findings highlight ultrasound-assisted extraction as a powerful, cost-effective method for recovering polyphenols and flavonoids from *C. ananta*, with promising implications for natural antioxidant production.

Keywords: *Cynometra ananta*, Ultrasound-assisted extraction, Polyphenols, Flavonoids, Antioxidant activity, Second-order kinetics.

INTRODUCTION

Cynometra ananta Hutch. & Dalziel is one of the most important trees in West African countries such as Côte d'Ivoire, Liberia and Ghana. *Cynometra* species are generally recognized as being used in traditional medicines in the countries where they occur as part of the spontaneous flora. Traditional practitioners usually prepare medicines from different parts of the plant and in different ways to treat different ailments [1,2]. *C. ananta* is rich in a wide variety of phenolic compounds such as alkaloids (anantine, cynometrine and cynodine) and flavonoids (naringenin, apigenin, epigallocatechin, 3′,4′,7-trihydroxyflavone, *etc.*), along with monoterpenoids (limonene, α-pinene, myrcene and (*Z*)-β-ocimene, *etc.*), which may be associated with the significant biological activities and therapeutic properties [2].

Several techniques are employed to extract such compounds, including maceration (MAC), assisted solvent extraction (ASE), supercritical fluid extraction (SFE) and ultrasound-assisted extraction (UAE) [3]. However, the mechanisms and the optimal extraction time remain poorly defined or unclear. UAE is an effective and practical method for the large-scale industrial production of vegetable extracts. It offers a number of advantages, including a fast extraction rate, simplicity, increased yield, high efficiency, low cost and a short execution time [4]. UAE is a promising technique with the potential to reduce the energy and solvent consumption and to align with green chemistry technology and sustainable concepts [3,5].

UAE generate the acoustic waves in the kilohertz gamme for the purpose of producing cavitation bubbles, which travel through the solvent. Upon reaching the surface of the plant sample matrix, the bubbles burst, resulting in a shockwave that damages the plant cell wall. This enhances the mass transfer of phenolic compounds across cellular membranes into solution [6]. The fact that this technique can be performed at room temperature means that the oxidation and decomposition of target natural products can be prevented. UAE has been widely applied in the isolation of different natural products [7]. Hu

et al. [8], has successfully demonstrated the ability to extract polyphenols with antioxidant activity from *B. bicolor* in a time frame of 41 min through the use of UAE. The extraction rate is 2.1 times higher than that of maceration extraction in 24 h. Therefore, UAE requires less time to achieve better efficiency. In comparison to conventional extraction, the UAE technique was successfully used to extract phenolic antioxidants from olive pomace. The optimal conditions for phenolic extraction and antioxidant activity were achieved using 57.34% acetone and 2 min of extraction [9].

To date, no study has been conducted on the application of kinetic modelling to investigate the optimal extraction time, as well as the kinetics of maceration and ultrasound assisted extraction of polyphenols, flavonoids and antioxidant activity. The objective of this study was to enhance the extraction process of polyphenols and flavonoids from *C. ananta*, a medicinal plant indigenous to West Africa that has not been the subject of extensive prior research. The initial step involved monitoring the extraction kinetics of polyphenols and flavonoids, with the objective of identifying the optimal extraction time and solid-liquid ratio. Subsequently, the second-order rate constant (k) and concentration saturation (C_S) were deduced using a model plot. FRAP assays were conducted to investigate the antioxidant activity and the presence of free radicals. The second-order rate model was used to describe the extraction kinetics of polyphenols, flavonoids and the antioxidant activity of the extracts derived from *C. ananta* stem bark.

EXPERIMENTAL

The chemicals used in this study, 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ), 1,1-diphenyl-2-picrylhydrazyl (free radical), Folin-Ciocalteu′s reagent, sodium acetate, Trolox, gallic acid, quercetin and aluminum chloride were purchased from Sigma-Aldrich (Saint Louis, USA). Sodium carbonate was obtained from Fisher Chemicals (France).

Collection and identification of plant material: The plant material (stem bark of *C. ananta*) was collected from the Moape locality (coordinates: N 006º11′13.2″ and W 003º48′24.3″) in the Mé region of Côte d'Ivoire and subsequently verified at the National Center of Floristics, where it was assigned the following voucher no. UCJ009272. The stem bark samples were prepared, air-dried at room temperature and pulverized. The resulting powder was stored in an amber glass bottle until required for use. The moisture and total ash contents of the powder were found to be 13.38 ± 0.34% (w/w) and 6.95 ± 0.59% (w/w), respectively.

Maceration (MAC) and ultrasound-assisted extraction (UAE) methods: Aliquots of *C. ananta* dried stem bark powder were extracted with an acetone/water mixture (60/40, v/v) at 40 ºC with different solid to solvent ratios (*i.e.*, 1/60, 1/80, 1/120 g/mL). The UAE was conducted using an ultrasonic bath (240 W PS-40 AC110/220 V) with a digital timer and temperature control. Then, the extractions were conducted at 2.5 and 5 min and with intervals of 5 min up to 30 min. Finally, the extracts were subjected to centrifugation at 3000 rpm for a period of 10 min, the obtained supernatant was kept for further analysis.

Total phenolic contents (TPC): In brief, 100 µL of crude extract was combined with 500 µL of 10% (w/v) Folin-Ciocalteu reagent. After 10 min, 500 µL of Na_2CO_3 solution was added to the mixture and incubated at room temperature for 40 min. Then, the absorbance was quantified utilizing a single-beam UV-Vis spectrophotometer (ONDA spectrophotometer, UV-30SCAN, China) at 765 nm, with a blank. The resulting data were expressed as micrograms of gallic acid equivalent (GAE) per milligram of dry matter (µg GAE/mg DM) [10].

Total flavonoid contents (TFC): TFC in the supernatant extract was determined in accordance with Galgano method [10]. An aliquot of 2 mL of supernatant extract and a quercetin standard solution (*i.e.*, 3.125; 6.25; 12.5; 25 and 50 µg/mL) were mixed with 1 mL of 10% w/v $AlCl_3$ solution in methanol. The mixture was then incubated for 30 min at room temperature, after which the absorbance was measured at 434 nm against the blank, using a UV spectrophotometer (ONDA spectrophotometer, UV-30SCAN, China). The quercetin standard was used and the resulting data were expressed as micrograms of quercetin equivalent per milligram of dry matter (µg QE/mg DM).

Ferric reducing antioxidant power (FRAP): FRAP assay was assessed using acetate buffer (300 mM, pH = 3.6), 10 mM TPTZ in 40 mM HCl and a solution of 20 mM $FeCl_3$ in a ratio 10:1:1 (v/v/v). Then, 200 µL of the supernatant was mixed with 3 mL of freshly prepared FRAP reagent. Then, the mixture was incubated for 10 min at room temperature. Absorbance was measured at λ_{max} 593 nm against a blank. A standard curve was plotted using a series of Trolox concentrations. The results were expressed as micrograms of Trolox equivalent per milligram of dry mass (µg Trolox/mg DM). The analyses were conducted in triplicate [11].

Second-order rate model: In order to gain further insight into the kinetic mechanism of polyphenol total, flavonoid total and antioxidant compound release from *C. ananta* stem bark, their follow-ups were investigated in accordance with the second-order rate model, with reference to previous studies [12-16]. Indeed, the second-order velocity model provides the best fit of the release profile and an adequate explanation of the solid-liquid extraction process.

The equation for the second-order kinetic model is given by eqn. 1:

$$\frac{dC_t}{dt} = k(C_S - C_t)^2 \quad (1)$$

The second-order kinetic model, which posits that the rate of release of a component is dependent upon its concentration, is presented in eqn. 2:

$$C_t = \frac{C_S^2 kt}{1 + C_S kt} \quad (2)$$

where C_t is the concentration of the compounds at time t, C_S is the concentration of the compounds at saturation, k is the second order extraction rate constant.

Statistical analysis and model evaluation: The data were subjected to statistical analysis using RStudio software version 2024.12.1+563. However, differences in relative abundance were calculated using Least-Significant Difference (LSD) test. A *p*-value of 0.05 was considered to be the threshold for stati-

stical significance. Furthermore, kinetic data were analyzed using regression analysis.

The consistency between the values of model parameters and graph plots was examined using the coefficient of correlation (R^2) and root mean squared error (RMSE) criteria, defined as follows (eqns. 3 and 4), respectively:

$$R^2 = 1 - \frac{\sum_{j=1}^{N}(q_{exp} - q_{cal})^2}{\sum_{j=1}^{N}(q_{exp} - \bar{q}_{cal})^2} \quad (3)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{t=1}^{N}(\bar{q}_{exp} - \bar{q}_{cal})^2} \quad (4)$$

The correlation between the values of the model parameters and the graph plots was enhanced when R^2 was higher and RMSE was minimal.

RESULTS AND DISCUSSION

The total polyphenol extraction quantities from *C. ananta* stem bark with or without ultrasound (MAC) at varying solid-liquid ratios are illustrated in Fig. 1. Overall, the saturation concentrations (C_S) were reached within 10 min with or without ultrasound, irrespective of the solid-liquid ratio. However, the optimum quantities were observed at a solid-liquid ratio of 1/60 g/mL, regardless of the extraction system (Fig. 1a). Indeed, the saturation concentration of total polyphenols increased from 186 to 420 µg GAE/mg DM for the system without ultrasound (Fig. 1a-c), while in the system with ultrasound, they increased from 210 to 482 µg GAE/mg DM, thus demonstrating the efficacy of the ultrasonic system. The increase in the extraction rate with ultrasound can be attributed to the power and frequency of the ultrasound, as well as the extraction temperature. A number of studies have demonstrated that increasing the temperature and power of ultrasound improved the extraction rate of polyphenols. This is due to an increase in material porosity, higher solvation and mass transfer [17-19]. In a study by Dzah *et al.* [3], it was demonstrated that a reduction in surface tension and viscosity in extracts also results in an improvement in the extraction yield. Moreover, Dai & Mumper [20] also observed that the viscosity and surface tension of solvent-sample mixtures decrease at higher temperatures, thereby increasing matrix penetration and polyphenol

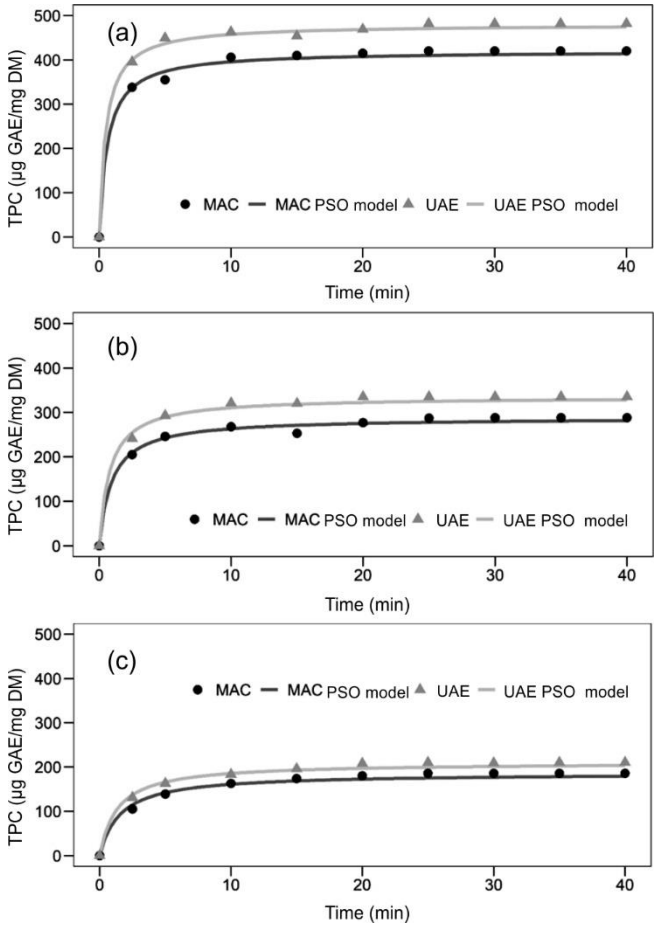

Fig. 1. Comparison between extraction techniques for total polyphenols with (triangle) and without ultrasound (circle) at different solid-liquid ratios: (a) 1/60 (b) 1/80 and (c) 1/120 g/mL (W/V). Experimental conditions: UAE power = 240W, pH = 5.8-6. MAC (Maceration); UAE (Ultrasound-assisted extraction); MAC PSO model (Maceration pseudo second order model); UAE PSO model (ultrasound-assisted extraction pseudo second order model)

extraction rates. As reported by Jovanovic *et al.* [21], the maximum polyphenol yield was achieved at 60 min in maceration (*i.e.*, 18.1 µg GAE/mL) and at 15 min in UAE (*i.e.*, 23.1 µg GAE/mL), respectively.

Kinetic and statistical parameters of polyphenols: Table-1 exhibits the model and statistical parameters associ-

TABLE-1
KINETIC AND STATISTICAL PARAMETERS FOR THE TOTAL POLYPHENOL EXTRACTION METHODS AT DIFFERENT SOLID-LIQUID RATIOS USING DIVERSE METHODS

Extraction method Solid-liquid ratio (g/mL)	Model parameters		Statistical parameters		
	Extraction constant k (mg DM/µg GAE min)	TPC at C_S (µg GAE/mg DM)	R^2	SEE	RMSE
1/60					
Without ultrasounds	3.859×10^{-3}	420	0.997	3.852×10^{-4}	3.398×10^{-4}
With ultrasounds	4.207×10^{-3}	482	0.999	5.325×10^{-4}	4.696×10^{-4}
1/80					
Without ultrasounds	3.695×10^{-3}	288	0.984	1.887×10^{-3}	1.664×10^{-3}
With ultrasounds	3.784×10^{-3}	335	0.997	6.300×10^{-4}	5.5556×10^{-4}
1/120					
Without ultrasounds	3.457×10^{-3}	186	0.996	1.386×10^{-3}	1.226×10^{-3}
With ultrasounds	3.595×10^{-3}	210	0.999	1.434×10^{-3}	1.265×10^{-3}

ated with the polyphenol extraction. Indeed, the rate and the constant second-order extraction decrease while the solid-liquid ratio increase from 1/60 to 1/120 g/mL (w/v), with a better correlation indicated by the R^2 and RMSE values achieved. In contrast to maceration extraction, the yield of ultrasound-assisted extraction increased to 13%, 19% and 11% for solid-liquid ratios of 1/60, 1/80 and 1/120 g/mL (w/v), respectively. Moreover, the highest yield, *i.e.*, 19%, was attained at a solid-liquid ratio of 1/80 g/mL. The determining the optimum amount of solvent to use is economically important, as it is also crucial to improve the effectiveness and efficiency of the extraction process. The optimal solid-to-liquid ratio can be attributed to the reduced density of the extraction medium, which enhances ultrasonic wave propagation, reduces acoustic attenuation, and improves energy transmission throughout the medium. Moreover, the reduction in mixture density led to an increase in the incidence of cavitation, a distinctive phenomenon linked to the efficacy of ultrasound treatment [3,19,22]. However, the yield of the ultrasound-assisted extraction process was found to decrease to 11% when the volume of the mixture solvent was increased to 120 mL. This is due to the fact that a considerable number of phenolic compounds are susceptible to the hydrolysis at higher concentrations.

Kinetic model and quantity of total flavonoids extracted: The flavonoids content extracted from *C. ananta* stem bark with or without ultrasound at different solid-liquid ratios are shown in Fig. 2. Indeed, the saturation concentration (C_S) was also observed at the 20 min mark, regardless of the solid-liquid ratio, with or without the application of ultrasound. Yet, the optimal amount was attained at a solid-liquid ratio of 1/60 g/mL, irrespective of the extraction system (Fig. 2a). In fact, the saturation concentration of flavonoids increased from 3.34 to 7.72 µg GAE/mg DM for the system without ultrasounds, whereas that with ultrasounds exhibited a marked increase, passing from 4.29 to 14.18 µg GAE/mg DM (Fig. 2a-c), which substantiates the efficacy of the ultrasonic system. The elevated flavonoid content resulting from ultrasound extraction is assigned to the acceleration of oscillatory motion between solid and liquid particles. This results in the rapid dispersion of solutes within the solvent. The application of ultrasound allows to increase in the mechanical oscillations of the liquid, which in turn enhances the penetration of the solvent into the cellular material, thereby improving mass transfer [23,24]. For instance, Kostic *et al.* [25], achieved higher levels of total flavonoid extraction (*i.e.* 70.08 µg QE/mg DM) through ultrasound than maceration (*i.e.* 56.09 µg QE/mg DM) of mulberry fruit. A comparable outcome was also observed by Jovanovic *et al.* [21], who reported a flavonoid yield of 14.3 mg QE/L using maceration and 16.7 mg QE/L using UAE from *Thymus serpyllum* L. herb extraction.

Kinetic and statistical parameters of flavonoid contents: The model and statistical parameters pertinent to flavonoid extraction are presented in Table-2. It can be observed that as the solid-liquid ratio increased from 1/60 to 1/120 g/mL (w/v), the rate and the constant second-order extraction both decreases. Furthermore, the correlation indicated by the R^2 and RMSE values achieved is more significant. In contrast to maceration extraction, the yield of ultrasound-assisted extraction increased to 45.55%, 32.71% and 22.14% for solid-liquid ratios of 1/60, 1/80 and 1/120 g/mL (w/v), respectively. Moreover, the highest yield, *i.e.* 45.55%, was achieved at a solid-liquid ratio of 1/60 g/mL. The yield of flavonoid extraction decreased progressively with increasing solvent concentration. It can be suggested that an appropriate solvent concentration is beneficial in increasing the efficiency of flavonoid extraction from *C. ananta* stem bark. The most likely reason is that the lower the solvent concentration (below 60 mL), the higher the solubility of flavonoids in the solvent solution. However, a higher solvent concentration may directly affect the solubility of acetone soluble flavonoids [26,27]. It was clearly found that there was a satisfactory agreement between the experimental and predicted values of the concentration of flavonoids according to the second order constant rate and the experimental data, as well as the correlation coefficient R^2 (Table-2). According to the above results for polyphenols, ultrasound improves the solubility of flavonoids in the solvent [28,29], hence the increase in the extraction rate at 45.55%.

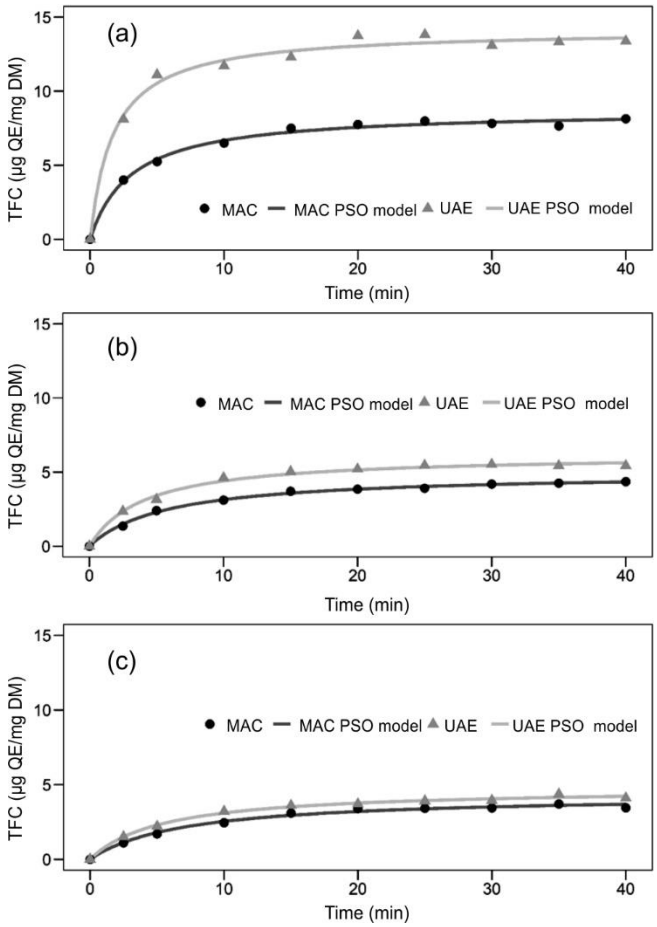

Fig. 2. Comparison between extraction techniques for total flavonoids with (triangle) and without ultrasound (circle) at different solid-liquid ratios: (a) 1/60 (b) 1/80 and (c) 1/120 g/mL). Experimental conditions: UAE power = 240 W, pH = 5.8-6. MAC (Maceration); UAE (Ultrasound-assisted extraction); MAC PSO model (maceration pseudo second order model); UAE PSO model (ultrasound-assisted extraction pseudo second order model)

Kinetic model for antioxidant activity (FRAP): Fig. 3 shows the evolution of the antioxidant activity of *C. ananta* stem upon maceration and ultrasound-assisted extraction following TPC extraction. Indeed, higher activity was observed

TABLE-2
KINETIC AND STATISTICAL PARAMETERS FOR FLAVONOID EXTRACTION METHODS AT DIFFERENT SOLID-LIQUID RATIOS USING DIVERSE METHODS

Extraction method Solid-liquid ratio (g/mL)	Model parameters		Statistical parameters		
	k (mg DM/µg QE min)	C_S (µg QE/mg DM)	R^2	SEE	RMSE
1/60					
Without ultrasounds	3.732×10^{-2}	7.72	0.996	9.422×10^{-2}	8.310×10^{-2}
With ultrasounds	4.084×10^{-2}	14.18	0.997	5.326×10^{-2}	4.697×10^{-2}
1/80					
Without ultrasounds	3.432×10^{-2}	3.97	0.998	1.217×10^{-1}	1.074×10^{-1}
With ultrasounds	3.931×10^{-2}	5.90	0.997	1.268×10^{-1}	1.118×10^{-1}
1/120					
Without ultrasounds	3.237×10^{-2}	3.34	0.989	3.404×10^{-1}	3.002×10^{-1}
With ultrasounds	3.823×10^{-2}	4.29	0.994	2.167×10^{-1}	1.911×10^{-1}

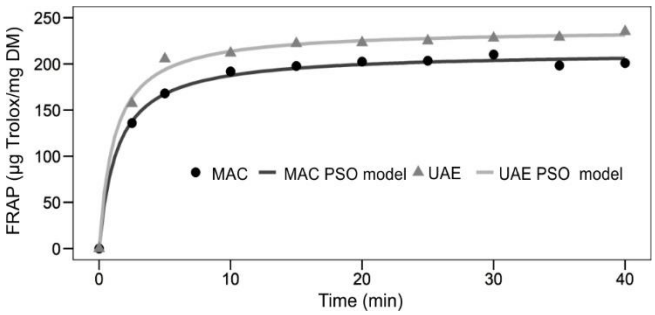

Fig. 3. Evolution of antioxidant activity from *Cynometra ananta stem* upon maceration and ultrasound-assisted extraction following TPC extraction. Experimental conditions: Solid-liquid (w/v): 1/60 g/mL; UAE power = 240 W; pH = 5.8- 6. MAC (Maceration); UAE (ultrasound-assisted extraction); MAC PSO model (maceration pseudo second order model); UAE PSO model (ultrasound-assisted extraction pseudo second order model)

with ultrasound-assisted extraction, *i.e.* nearly 238 µg Trolox/mg DM, which decreased to 180 µg Trolox/mg DM, thereby underscoring that ultrasound improved the extraction efficiency and the biological activity of extracts. However, it has been used to extract flavonoids, which showed higher antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* compared to conventional extraction methods [30]. Abdelkebir *et al.* [31] are also demonstrated the positive effect of ultrasound on the extraction of phenolic compounds, with a higher level of bioactivity on the bacteria tested and strong antiviral activity. The findings are related to the release of more metal ions due to the use of ultrasound.

Kinetic and statistical parameters of antioxidant activity: The kinetic and statistical parameters associated with antioxidant activity during total polyphenol extraction using different methods are presented in Table-3. In fact, the better correlation (*i.e.*, R^2 = 0.999) was obtained with ultrasound assisted extraction method compared to maceration method (*i.e.*, R^2 = 0.997). Furthermore, the kinetic model parameters such as Cs (*i.e.* 238 µg Trolox/mg DM) and k (*i.e.*, $3.680.10^{-3}$ mg DM/µg Trolox min) are also better than those of maceration (*i.e.*, 213 µg Trolox/mg DM) and k (*i.e.*, $3.451.10^{-3}$ mg DM/µg Trolox min) (Table-3), demonstrating the impact of ultrasound on the biological activities. This result could be explained by polyphenols acting as antioxidants, either by metal chelation, hydrogen atom transfer, single electron transfer, sequential proton loss and electron transfer [32]. However, the highest extraction yield of flavonoids was obtained at 1/60 g/mL due to the type and level of flavonoids extracted by ultrasound and/or the presence of different antioxidants promoted by this extraction method [33]. In addition, under the same conditions as those used for total polyphenol extraction, the saturation concentrations decreased two-fold, both with and without ultrasound, thereby highlighting the contribution of free radical scavenging activity during the process.

Conclusion

This study demonstrated that ultrasound enhanced the extraction of total polyphenols and flavonoids, as well as the antioxidant activity, from *Cynometra ananta* stem bark at various solid-to-liquid ratios (1:60, 1:80, and 1:120 g/mL). In fact, a superior extraction yield was attained with the 1/60 g/mL ratio, regardless of the compound at 10 and 20 min for polyphenols and flavonoids, respectively. According to the RStudio software, the second order rate constant (k) and concentration saturation (Cs) investigated during the kinetics, showed that these increased while the solid-liquid ratio decreased. The fit between the kinetic model and the experimental data was very good, regardless of the type of extraction technique and compound extracted, with R^2 correlation coefficients greater than 0.99. Otherwise, the amounts of total polyphenols extracted were greater than that of flavonoids, regardless of the solid-liquid ratio. Under the same conditions (*i.e.* ratio

TABLE-3
KINETIC AND STATISTICAL PARAMETERS FOR ANTIOXIDANT ACTIVITY IN THE TOTAL POLYPHENOL EXTRACTION USING DIFFERENT METHODS

Extraction method Solid-liquid ratio (g/mL)	Kinetic model parameters		Statistical parameters		
	k (mg DM/µg Trolox min)	C_S (µg Trolox/mg DM)	R^2	SEE	RMSE
1/60					
Without ultrasound	3.451×10^{-3}	213	0.997	3.091×10^{-3}	2.726×10^{-3}
With ultrasound	3.680×10^{-3}	238	0.999	1.380×10^{-3}	1.217×10^{-3}

solid-liquid: 1/60 g/mL) as the total polyphenol extraction, the saturation concentrations with or without ultrasound decreased 2-fold in FRAP analysis, thereby underscoring the free radical scavenging effect of the process. This ultrasound assisted extraction may promote the development of a cost-effective technology of compound extraction.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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