Optimization of ultrasounds assisted extraction of polyphenols and antioxidant activity from *Tectona grandis* L. leaves, using central composite design

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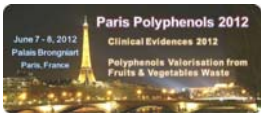


❑ INTRODUCTION

***Tectona grandis* L.** (Verbenaceae), commonly called Teak, is grown in Côte d'Ivoire for its wood of high commercial value. Leaves are also used by rural communities to prepare water-extracts used as traditional medicinal beverages. Local recipes require boiling Teak leaves for a long time (3h) which lead to a low polyphenol content in the extract (average of 1250 µmol.g^{-1} GAE), with low level of antioxidant activity (AOA).

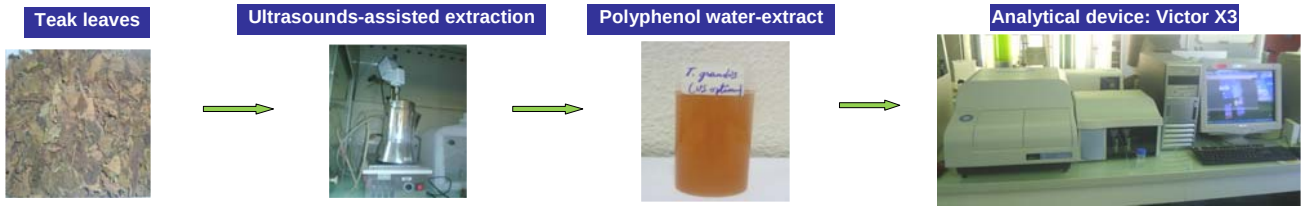
To optimize polyphenol content and AOA, an ultrasounds-assisted extraction technology was used. The extraction parameters were: leaves to water ratio (X_1), extraction time (X_2), and water acidification (X_3) using citric acid. The extraction conditions were optimized using a central composite (CC) statistical model. The responses were polyphenol content (Y_1) and AOA (Y_2) of the extract obtained.

❑ Materials and Method

- Entire young Teak leaves were harvested in the lake area of Côte d'Ivoire (Yamoussoukro area). They were dried under a shade of the laboratory (30±2 °C)., then cut in strips of 10cm of wide.
- Central composite design used for extraction optimization of active polyphenols is shown in table 1. The responses were : polyphenol content and AOA.
- Total Polyphenol contents were determined at 760 nm using to the Folin-Ciocalteu analytical method.
- AOA was measured according to the Oxygen Radical Absorbance Capacity assay (ORAC). The fluorescence was measured at 485 nm for emission and at 535 nm for excitation..

Table 1: Independent variables (X_i) with real and coded values used for statistical optimization

Independent variables	Symbols	Coded levels				
		-1.68	-1	0	1	1.68
Vegetal to water ratio (g/L)	**X1**	5	8	12.5	18	20
Extraction time (min)	**X2**	15	21	30	39	45
Citric acid (10^{-3} N)	**X3**	0	3.5	5	6.5	10



Extraction and analysis of Teak leaves

❑ RESULTS

❖ Statistical model

Polyphenol content (**Y1**) and **AOA** (**Y2**) in the extracts obtained, ranged from 420 to 1290µmol.g^{-1} GAE and 160 to 430 µmol.g^{-1} TE, respectively. Response and test variables were related by a 2nd order polynomial equation found in both cases:

Y1= 1147 + 113 **X1** + 171 **X2** + 143 **X3** – 72 **X12** – 89 **X22** – 30 **X32** – 80 **X2X3**
with R^2=0.99, lack of fit (p-value)=0.59(ns)

Y2 = 354 + 21 **X1** + 45 **X2** + 44 **X3** – 12 **X12** – 33 **X22** – 18 **X1X2** – 24 **X2X3**
with R^2=0.93, lack of fit (p-value)=0.12 (ns)

❖ Relation between polyphenol content and AOA

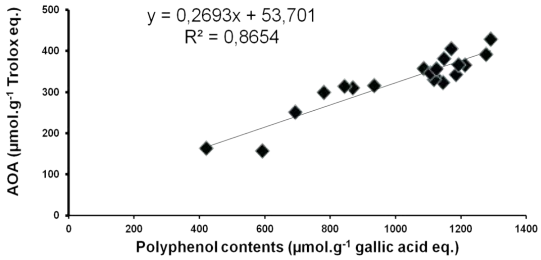


Fig. 1 : Correlation between AOA and Polyphenol content in the extracts

A good correlation between polyphenol content and AOA was confirmed.

❖ Effects of variables on antioxidant activity

Using the quadratic model, response surfaces were plotted keeping constant at the zero level one variable, and allowing the two other two to vary over the chosen experimental ranges (fig. 2)

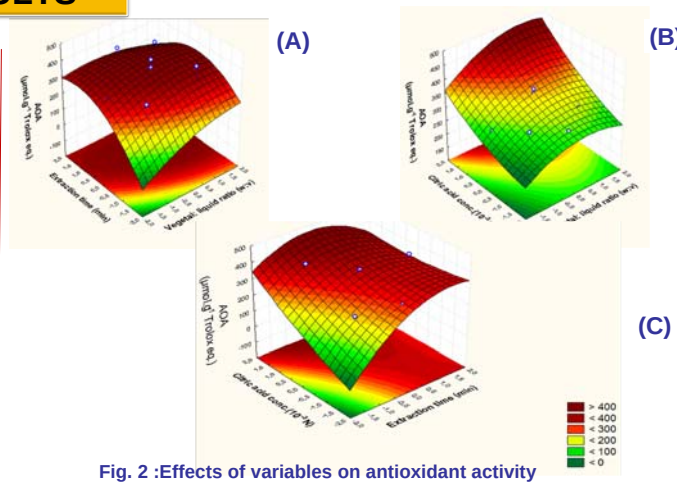


Fig. 2 :Effects of variables on antioxidant activity

A : Interaction between vegetal / liquid ratio (**X1**) and extraction time (**X2**)
B : Interaction between vegetal / liquid ratio (**X1**) and citric acid concentration (**X3**)
C : Interaction between extraction time (**X2**) and citric acid concentration (**X3**).

❖ Verification of the predictive model

Under the optimized extraction conditions determined (vegetal / water ratio set at 16.25 g.L^{-1}, citric acid conc. at 10^{-2} N, and extraction time at 37.5 min), the predicted and experimental values for polyphenol content and for AOA are shown in table 3.

Table 3: Validation of the optimized model

Model responses	Predicted value	Experimental value
Polyphenol content (µmol.g^{-1}, GAE)	1300	1310
AOA (µmol.g^{-1}, TE)	429	420

Experimental and predicted results were very close, meaning that the regression model was correct.

❑ CONCLUSION

The ultrasounds-assisted extraction technology was successfully optimized using a statistical design to obtain a *Tectona grandis* leaf water-extract, with optimized polyphenol content and AntiOxidant Activity. The process applied under optimized extraction conditions, performed better than the local extraction recipes. The extracts was obtained in a shorter extraction time, and were found slightly richer in polyphenol content with an interesting AAO, than the traditional Teak leaf extracts prepared and used as local medicinal beverages. This allows to further explore comparatively these novel processed water-extracts as organic and healthy beverages to fight against the cardiovascular diseases.

❑ REFERENCES

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