Bioactive compounds and antioxidant activity of *Leucaena leucocephala* leavescollected in Côte d'Ivoire

ABSTRACT

Aims: In a context of increasing valorization of tropical plant resources as potential sources of bioactive compounds, *Leucaena leucocephala*, a pantropical legume widely distributed in Côte d'Ivoire, represents a model of interest still insufficiently explored for its phytochemical and antioxidant properties. The present study aimed to determine the contents of condensed tannins, total tannins and total polyphenols, as well as the antioxidant activity of *Leucaena leucocephala* leaf extracts, in order to valorizing its nutraceutical potential.

Study Design:*Leucaena leucocephala* leaves were collected at the vegetative stage in the locality of Yamoussoukro, dried under shelter at room temperature and then crushed. The powder obtained was used for the various analysesat the Chemistry laboratory of the Institut National Polytechnique Félix HOUPHOUËT-B0IGNY in Yamoussoukro.

Place and Duration of Studies: After the harvest, the analyses were carried out from March to June 2023.

Methodology:Maceration and decoction were used to extract the phenolic compounds. Maceration extraction was done using a mixture of distilled water/ethanol (30/70, v/v) and distilled water/methanol (30/70, v/v) as solvent while decoction was done using distilled water only.

Results: The condensed tannin contents obtained in *Leucaena leucocephala* leaves were 137.52 ± 92.74, 140.22 ± 92.74, and 32.63 ± 7.13 mg Cat E/g extract, for methanolic, ethanolic and aqueous solutions, respectively. The total polyphenols contents were 5.74 ± 0.22, 4.01 ± 0.22, and 1.32 ± 0.11 mg GAE/g extract, respectively. The total tannin contents were 209.05 ± 7.49, 207.92 ± 7.49, and 55.85 ± 7.49 mg TAE/g extract, respectively. The antioxidant activities were30.99±1.95, 19.75±0.740, and 12.21±2.63 mg TE/g extract, respectively.

Conclusion: It was concluded that *Leucaena leucocephala* leaves collected in Côte d'Ivoire contain bioactive compounds and has antioxidant activity with variations depending on the extraction solvent.

Keywords:antioxidant activity, condensed tannins, *Leucaena leucocephala,* total polyphenols,total tannins.

1. INTRODUCTION

Tropical legumes represent an unexplored source of bioactive compounds with major interest for pharmaceutical and nutraceutical fields(Ahmad *et al.*, 2023). Plant secondary metabolites, including phenolic compounds such as tannins, harbor significant antioxidant and therapeutic properties, the exploration of which remains a crucial scientific challenge (Kim and In, 2017,Karima, 2021). Condensed tannins and total polyphenols are recognized for their multiple biological properties, including antioxidant, anti-inflammatory and antiproliferative effects (Falleh *et al.* , 2021,Kouamé *et al.* , 2021). Several recent studies have highlighted the therapeutic potential of phenolic compounds from tropical plants, highlighting the importance of precisely characterizing their biochemical profiles (Sayago-Ayerdi *et al.*, 2021).

The bioactive compounds contained in *Leucaena leucocephala* leaves, including its polyphenols and tannins, exhibit significant antioxidant and anti-inflammatory properties, with studies demonstrating their ability to neutralize free radicals and reduce oxidative stress(Ahmad *et al.*, 2023). In addition, extracts from this legume have revealed promising antimicrobial activities against certain bacterial strains and potential immune system modulation activity, opening interesting perspectives in phytotherapy and traditional medicine(Sayago-Ayerdi *et al.*, 2021).

Despite the wide distribution of *Leucaena leucocephala* in Côte d'Ivoire, few studies have been devoted to the characterization of its secondary metabolites (Fantodji *et al.* , 2009). This study aimed to characterize the phytochemical profile of *Leucaena leucocephalaleaves*in Côte d'Ivoire, by comparing different methods of extracting bioactive compounds.

2. MATERIALS AND METHODS

2.1. Plant material

Leucaena leucocephala leaves were collected at the vegetative stage in the District of Yamoussoukro, located in central Côte d'Ivoire, between 6°49'00'' north latitude and 5°16'60'' west longitude. After the harvest in March 2023, the leaves were dried under shelter at room temperature and then crushed.The floury ground material obtained was used for the various analyses.

2.2. Analytical methods

2.2.1. Extraction of secondary compounds from the floury ground material of *Leucaena leaves leucocephala*

Two methods, maceration and decoction were used to extract the phenolic compounds. Maceration extraction was done using distilled water/ethanol (30/70, v/v) and distilled water/methanol (30/70, v/v) as solvent while decoction required the use of distilled water only.

2.2.2. Extraction by maceration method

The maceration technique was performed according to the protocol described by Yao *et al.* , (2023). One gram of the sample was macerated at room temperature for 45 min with 60 mL of hydroalcoholic solution. The Erlenmeyer flasks containing the solutions were placed on a magnetic stirrer. Magnetic bars were immersed in the solution while maintaining the speed of the apparatus at 1,100 rotations per minute. The solution obtained was filtered with cotton and stored in the refrigerator at 4 ° C until use.

2.2.3. Extraction by decoction method

The decoction required a device described by Tiho *et al.* , (2017)using a closed circuit system composed of a heating system topped by a cooling system. One gram of the sample was added to flasks containing 120 mL of water. The mixture was placed on an electronic oven stirrer (Agimatic -N, Germany) at a temperature of 100 ° C. After observing boiling, the time was taken into account and the mixture was allowed to boil for up to 45 min. After this time, the apparatus was turned off and the flask containing the solution remained under the apparatus for 15 minutes. The solution obtained was filtered and the filtrate was refrigerated at 4 ° C until analysis.

2.2.4. Determination ofcondensed tannins

Condensed tannins were determined by the acid vanillin method described by Price *et al.*, (1978). It is based on the ability of vanillin to react with condensed tannin units in the presence of acid to produce a colored complex measured at 500 nm. Indeed, the reactivity of vanillin with tannins involves only the first unit of the polymer. Three mL of 4% methanolic vanillin are added to a quantity of 50 µL of hydroalcoholic extract. Then, the mixture obtained was stirred and completed with a volume of 1.5 mL of concentrated hydrochloric acid. Finally, the solution was left to react for 15 minutes in the dark before reading. The tests were repeated three times. The absorbance was measured at 500 nm against a blank consisting of a 4% vanillin solution in methanol by spectrophotometer. Catechin was used as a standard and the results were expressed as micrograms of catechin equivalent per milliliter (µg Cat E / mL). These results were then converted to milligrams of catechin equivalent per gram of dry matter (mg CatE /g) (equations 1 and 2).

$$Ce(mg\ Cat\ Eg^{-1}) = \frac{C_L}{0.546} \qquad \text{(Equation 1)}$$

Where:*Ce,* is thecontent of condensed tannins in the sample; CL, is the concentration read by spectrophotometer

$$Ce(mg\ Cat\ Eg^{-1}) = \frac{10^3}{109.2} C_L \qquad \text{(Equation 2)}$$

Where: *Ce* is the content of condensed tannins in the sample; CL is the concentration read by spectrophotometer

2.2.5. Determination of total polyphenols

The determination of total polyphenols was carried out according to the method described by Kouamé *et al.* , (2021) **.** A quantity of 30µL of extract was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted 1/10 th. The whole was left in the dark at room temperature for about 2 minutes. Then, 2 mL of 75% sodium carbonate (NaCO3) solution was added to the mixture. The solution obtained was incubated at 50°C for 15 minutes and cooled to room temperature. The absorbance was read with a UV-visible spectrophotometer with a wavelength of 760 nm against a blank consisting of 5 mL of Folin-Ciocalteu reagent diluted 1/10 thand 4 mL of 75% sodium carbonate solution. The analyses were repeated three times and gallic acid at different concentrations was used as a reference standard to establish the

calibration curve. The total polyphenol contents were expressed in milligrams of gallic acid equivalent per liter of extract (mg GAE /L of extract). These contents were converted into milligrams of gallic acid equivalent per gram of dry matter (mg GAE /g of extract) (equations 3 and 4).

$Ce(mg\ GAEg^{-1}) = \frac{10^{-4}}{9.06} C_L$ (Equation 3)

Where: *Ce,* is the Total polyphenol content in the sample; CL, is the concentration read by spectrophotometer

$Ce(mg\ GAEg^{-1}) = \frac{10^{-3}}{181.2} C_L$ (Equation 4)

Where: *Ce,* is the Total polyphenol content in the sample; CL, is the concentration read by spectrophotometer

2.2.6. Determination of total tannins

The total tannins of the samples were determined according to the method described by Hossain *et al.* , (2020). Seven-point five mL of distilled water were added in 100 µL of extract. Then, 0.5 mL of pure Folin-Ciocalteu and 1 mL of 35% sodium carbonate were added in the obtained solution. Then, 0.9 mL of distilled water was made up to the volume of the solution after adding the reagents. The mixture was stirred well and kept for 30 minutes at room temperature. Finally, the absorbance was read at 700 nm in the UV-visible spectrophotometer against a blank consisting of distilled water. A set of tannic acid concentrations was used to plot the calibration curve. The total tannin contents were expressed in microgram equivalent of tannic acid per liter of extract (µg TAE/L). These contents were converted (equation 8 and 9) into milligrams of tannic acid equivalent per gram of dry matter (mg TAE /g of extract).

$Ce(\text{mg TAE } g^{-1}) = \frac{1}{0.6} Cl$ (Equation 8)

Where: Ce, is the total tannin content in the sample; CL, is the concentration read on the spectrophotometer

$Ce(\text{mg TAE } g^{-1}) = \frac{1}{120} C_L$ (Equation 9)

Where: Ce, is the total tannin content in the sample; CL, is the concentration read on the spectrophotometer

2.2.7. Determination of antioxidant activity

The antioxidant activity of the samples was determined according to the method described by Dienget al. (2017) . The ABTS*+ cation radical was produced by reacting 8 millimole (mM) ABTS (87.7mg in 20mL water) and 3mM potassium persulfate (0.01262g in distilled water) in a ratio of 1:1 (v/v). This mixture was incubated for 12 to 16h in the dark and at room temperature. Before using the ABTS*+ solution, it was diluted with methanol to an absorbance of 0.700 ± 0.02 at 734nm.

Then, 3.9 mL of this diluted ABTS*+ was mixed with 100 µL of the sample and the mixture was incubated in the dark for 6 minutes at room temperature. Finally, the absorbances were measured at 734 nm with a UV-visible spectrophotometer and should be between 20 and 80% of the absorbance of the blank. The concentrations were expressed in µmol Trolox equivalent per liter of extract (µmol Trolox E /L of extract). The inhibition rate I (%) of ABTS*+ was obtained with equation 5.

$I(\%) = \frac{Abscontrol - AbsExtract}{\text{Abscontrol}} * 100$ (Equation 5)

Where:Abscontrol is the diluted ABTS absorbance, AbsExtract is the ABTS+ diluted sample absorbance.

The concentrations expressed in µmol Trolox equivalent per liter of extract (µmol Trolox E/L of extract) (equations 6 and 7) were converted to milligram Trolox equivalent per gram of extract (mg Trolox E/g of extract).

$Ce(\text{µmol Trolox E } g^{-1}) = \frac{1}{0.24} Cl$ (Equation 6)

Where:Ce, is the antioxidant activity content in the sample; CL, is the concentration read on the spectrophotometer

$Ce(\text{µmol Trolox E } g^{-1}) = \frac{10^6}{48} C_L$ (Equation 7)

Where: Ce, is theantioxidant activity content of the sample; CL, is the concentration read on the spectrophotometer

3. RESULTS AND DISCUSSION

3.1. Total polyphenol content of *Leucaena leucocephala*leaves

Polyphenol contents obtained were better with methanolic and ethanolic solutions, with values of 4.01 and 5.74 mg GAE/g extract, respectively, compared to the aqueous solution (Table 1). The total polyphenol content of the methanolic extract of *Leucaena leucocephala* leavesobtained in this study is higherthan that obtained with a hydroalcoholic solution containing 70% acetone (Zarin et al. , 2016**)**. This difference may be due to the difference in extraction conditions, plant age and solvent used (Hossain *et al.* , 2020).

Table 1 Total polyphenol content of *Leucaenaleucocephala leaves*depending on the solvent

Solvents	Total polyphenol contents (mg GAE/g)
Ethanol	4.01 ±0.16
Methanol	5.74 ±0.90
Water	1.32 ±0.11

3.2. Condensed tannin content of *Leucaena leucocephalaleaves*

The condensed tannin content in Leucaena leucocephala leaves was determined for methanolic, ethanolic, and aqueous extracts. The results were 140.22 mg CE/g, 137.52 mg CE/g, and 32.63 mg CE/g, respectively (Table 2). Notably, the aqueous extract yielded significantly lower results compared to the methanolic and ethanolic extracts. Methanol proved to be the most effective solvent, followed closely by ethanol. This variation can be attributed to the chemical nature of the condensed tannins and the solvent used (Deba *et al.*, 2008). However, these findings contradict those of Mahmoudi *et al.* (2013), who reported that aqueous decoction is more efficient for extracting condensed tannins. This discrepancy may be due to differences in the nature of condensed tannins among plant species(Mboko *et al.*, 2017).

Table 2 Condensed tannin content of *Leucaena leucocephala leaves*according to the solvent

Solvents	Condensed tannin content (mg Cat E/g)
Ethanol	140.22 ±23.33
Methanol	137.52±44.33
Water	32.63±7.13

3.3. Total tannin content of *Leucaena leucocephala*leaves

The total tannin contents of Leucaena leucocephala leaves are presented in Table 3. The values were 209.06 mg TAE/g, 207.93 mg TAE/g, and 55.85 mg TAE/g for the methanolic, ethanolic, and aqueous extracts, respectively. Notably, the methanolic and ethanolic extracts yielded similar and high total tannin contents, while the aqueous extract had the lowest content. This study supports the effectiveness of methanol and ethanol as solvents for extracting total tannins from plant leaves, consistent with findings by (Hamzah *et al.*, 2024).

Table 3 Total tannin content of *Leucaena leucocephala* leavesdepending on the solvent

Solvents	Total tannin content (mg TAE/g)
Ethanol	207.928 ±7.492
Methanol	209.056±7.492
Water	55.85±0.36

3.4. Antioxidant activities

In Leucaena leucocephala leaves, the methanolic extract exhibited the highest antioxidant activity, followed by the ethanolic extract, while the aqueous extract showed the lowest value (Table 4). Notably, the antioxidant activity of the ethanolic extract in this study was lower than that reported by Kim and In (2017) using the same solvent. This discrepancy may be attributed to various factors, including agro-climatic conditions, cultivar type, geographical origin, plant part, genetic factors, maturity level, storage conditions, and analytical methods(Naco *et al.*, 2024;.Al-Tememi and Al-Janabi, 2023).

Table 4. Antioxidant activity of the extract as a function of the solvent

Solvents	Antioxidant activities (mg T E/g)
Ethanol	19.75±0.740
Methanol	30.99±1.95
Water	12.21±2.63

4. CONCLUSION

This comparative study of *Leucaena leucocephala* leaf extracts revealed significant variations depending on the extraction solvent used. Notably, methanol emerged as the most effective solvent for extracting total polyphenols, with a yield of 5.740 mg GAE/g, and for producing remarkable antioxidant activity, at 30.99 mg TE/g. These findings suggest that *Leucaena leucocephala*collected in Côte d'Ivoire is a promising source of bioactive compounds, offering interesting perspectives for potential applications in pharmacology and nutrition. The diversity in phytochemical profiles underscores the critical importance of solvent selection when optimizing extraction for target compounds.

REFERENCES

Abu Zarin M., Wan H. Y., Isha A., & Armania N. (2016). Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from Leucaena leucocephala hybrid-Rendang. *Food Science and Human Wellness*, **5** (2), 65□75.

Ahmad Wani S., Elshikh M. S., Al-Wahaibi M. S., & Naik H. R. (2023). Functional Foods: Technological Challenges and Advancement in Health Promotion. 1re éd. CRC Press, Boca Raton.

Al-Tememi H. F., & Al-Janabi N. M. (2023). Phenols and Flavonoids as an Indicator for Selecting the most Suitable Solvent for Resveratrol Flavonoid Extraction. In: IOP Conference Series: Earth and Environmental Science. Vol. 1262. p. 062040. IOP Publishing. Consulté à l'adresse https://iopscience.iop.org/article/10.1088/1755-1315/1262/6/062040/meta

Deba F., Xuan T. D., Yasuda M., & Tawata S. (2008). Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from Bidens pilosa Linn. var. Radiata. *Food control*, **19** (4), 346□352.

Dieng S. I. M., Fall A. D., Diatta-Badji K., Sarr A., Sene M., Sene M., Mbaye A., Diatta W., & Bassene E. (2017). Evaluation of the antioxidant activity of hydro-ethanolic extracts of the leaves and barks of Piliostigma thonningii Schumach. International Journal of Biological and Chemical Sciences, 11 (2), 768-776.

Falleh H., Hafsi C., Mohsni I., & Ksouri R. (2021). Evaluation of different extraction processes of phenolic compounds from a medicinal plant: Verbena officinalis. Biologie Aujourd'hui, 215 (3-4), 133-142.

Fantodji A., Traore B., & Kouame L. (2009). Influence of Brewers' Grains and Leucaena leucocephala on the Growth of Thryonomys Swinderianus in Captivity. African Agronomy, 15 (1), 39□50.

Hamzah B., Kartayasa I. W., Said I., Jura M. R., Ningsih P., Hardani M. F., & Hardani R. (2024). Determination of thick tannine extract levels of Guava leaves (Psidium guajava L.) by using acetone solution, ethanol, and methanol. *AIP Conference Proceedings*, **3058** (1), 050009.

Hossain M., Disha N., Shourove J., & dey P. (2020). Determination of Antioxidant Activity and Total Tannin from Drumstick (Moringa oleifera Lam.) Leaves Using Different Solvent Extraction Methods. *Turkish Journal of Agriculture - Food Science and Technology*, **8** , 2749□2755.

Karima Y. (2021). Floristic and Ethnobotanical study of medicinal plants in the Tell-Sétifien (Tamntout forest, Beni Aziz), Essay on the biological and pharmacopoeial characterization of the species Erica arborea L. (Working Paper). Accessed at http://dspace.univ-setif.dz:8888/jspui/handle/123456789/3720

Kim D. C., & In M.-J. (2017). Antioxidative ability of ethanol extract from the leaves of Leucaena leucocephala (Lam.) de Wit. Journal of Applied Biological Chemistry, 60 (2), 185-190.

Kouame T. K., Siaka S., Kassi A. B. B., & Soro Y. (2021). Determination of total polyphenols, total flavonoids and tannins contents in young unopened leaves of Piliostigma thonningii (Caesalpiniaceae). International Journal of Biological and Chemical Sciences, 15 (1), 97□105.

Mahmoudi S., Khali M., & Mahmoudi N. (2013). Study of the extraction of phenolic compounds from different parts of the artichoke flower (Cynara scolymus L.). Nature & Technology, (9), 35.

Mboko A. V., Matumuini F. N. E., Tendonkeng M., Miégoué E., Lemoufouet J., Akagah A. A., Boukila B., & Pamo E. T. (2017). Chemical composition of forage shrubs (Albizia lebbeck, Leucaena leucocephala, Morinda lucida, Senna siamea) in the dry season in Gabon. Livest Res Rural Develop, 29 (1), 23□29.

Naco M. E. B., Coulibaly D. B., Tirera H., Mariko M., Koumare B. Y., Ndiaye B., Sarr S. O., Sanogo R., & Diop A. (2024). Study of phytochemical composition and antioxidant activities of Moringa oleifera leaves. Lam (Moringaceae) in the Kita (Mali) and Thiès (Senegal) regions. International Journal of Biological and Chemical Sciences, 18 (4), 1554□1568.

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry, 26 (5), 1214□1218.

Sayago-Ayerdi S., García-Martínez D. L., Ramírez-Castillo A. C., Ramírez-Concepción H. R., & Viuda-Martos M. (2021). Tropical Fruits and Their Co-Products as Bioactive Compounds and Their Health Effects: A Review. Foods, 10 (8), 1952.

Tiho T., Yao N. J. C., Brou Y. C., & Adima A. A. (2017). Drying temperature effect on total phenols and flavonoids content, and antioxidant activity of Borassus aethiopum Mart ripe fruits' pulp. *Journal of Food Research*, **6** (2), 50□64.

Yao K. A., Tiho T., Silué N., Assidjo N. E., & Koné K. Y. (2023). Total Polyphenols, Total Flavonoids, Condensed Tannins, and Antioxidant Activity of Borassus aethiopum (Arecaceae) ripe fruits' Peels, and Peel-Pulps, Dried at Different Temperatures. *Asian Journal of Chemical Sciences*, **13** (2), 20□33.