IJP (2024), Vol. 11, Issue 12 **(Research Article)**

Received on 19 November 2024; received in revised form, 28 December 2024; accepted, 29 December 2024; published 31 December 2024

TLC PROFILES, TOTAL PHENOLIC AND FLAVONOID CONTENTS, ANTIOXIDANT ACTIVITY OF *CYNOMETRA ANANTA*

Lanciné Traoré [1, * 2], N' Dri Séraphin Konan [1, 2], Kamagaté Mahamadou [1], Yao Arthur Kouassi [1], Benson Boua Boua [2] and Yves-Alain Békro [2]

UFR-Sciences et Technologies [1], Université de Man, BP 20 Man, Côte d'Ivoire.
Laboratoire de Chimie Bio Organique et de Substances Naturelles [2], UFR-SFA, Université Nangui Abrogoua, 02 BP801 Abidjan 02, Côte d'Ivoire.

Keywords:

Cynometra ananta, Phenolics, Flavonoids, DPPH, Sonication

Correspondence to Author:
Traoré Lanciné

UFR-Sciences et Technologies, Université de Man, BP 20 Man, Côte d'Ivoire.

E-mail: lancine.traore@univ-man.edu.ci

ABSTRACT: *Cynometra ananta* belongs to the Fabaceae family and is a source of biologically active secondary metabolites. In West Africa, *C. ananta* is renowned for its empirical application against a range of illnesses. The phytochemical composition, total phenolic and flavonoid levels, and *in-vitro* antioxidant activity of extracts made from *C. ananta* trunk bark were all examined in this work. TLC was used to detect phenolic compounds and total flavonoids ; spectrophotometric techniques were used to quantify the total phenolic and flavonoid contents; and the DPPH test was used to measure the antioxidant activity of extracts from maceration and sonication. Sonication produced the greatest levels of total flavonoid (14 ±0.7 µg EQ/mg of dry matter) and phenolic (482 ± 4.8 µg GAE/mg of dry matter) contents. Moreover, with a 92% trapping rate, sonication displayed the highest DPPH activity. *C. ananta* trunk bark is a significant source of phenolic compounds and flavonoids. Trunk bark has demonstrated therapeutic benefits in conventional settings, making it a promising candidate for alternative medicine.

INTRODUCTION: The ability of phenolic compounds and other antioxidants to protect food products against lipid degradation makes them very interesting [1]. In addition, phenolic compounds from foods items and other plant sources are of important for disease prevention, as many diseases, including diabetes and several types of cancer, are linked to oxidative stress [2]. Several animal studies revealed that polyphenols have an effect on energy metabolism that increases longevity and well-being while reducing the risk of aging-related chronic disorders [3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.11(12).687-91

Article can be accessed online on: www.ijpjournal.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.IJP.11(12).687-91

In the countries, they occursas part of the flora, *Cynometra* species are recognized for their use in traditional medicine. In order to cure a variety of illnesses, traditional practitioners typically produce medicine using different plant parts and diverse preparation techniques. It should be highlighted, although, that little was known about the specific application in treating various pathological signals or symptoms, as well as the chemical, pharmacological, and toxicological characteristics [5].

From an ethnobotanical perspective, indigenous inhabitants in the southern Côte d'Ivoire have empirically employed *Cynometra ananta* as a folk remedy to treat a variety of illnesses. Despite *C. ananta*'s demonstrated efficacy in conventional medicine, little is known about its phytochemical composition or pharmacological characteristics *in-vitro*. In light of these factors, we provide here a

study on the total phenolic and flavonoid contents and antioxidant activity of *C. ananta* utilizing extracts prepared by sonication and maceration, as measured by the DPPH test. To the best of our knowledge, this work is the first to highlight the antioxidant activity and phenolic and flavonoid contents of *C. ananta* trunk bark.

MATERIAL AND METHODS :

Plant Material: The stem bark of *C. ananta* was collected from the Moapé forest, in the Mé region, Côte d'Ivoire (Geographical coordinates: N006° 11'13.2" and W003°48'24.3"). This plant was identified at the National Floristic Center of Côte d'Ivoire (Herbarium number: UCJ009272).

Preparation of Extracts : The bark of the trunk of *C. ananta* was air-dried and crushed into powder using a mortar. A total of 20 mg of powder was weighed and extracted by maceration (12 mL) with acetone/water (60/40, v/v) by two methods: without ultrasound (MAC) and with ultrasound (ULT). The obtained extracts were then centrifuged and analyzed.

Thin-layer Chromatography: 20 x 20 cm TLC sheets coated with 0.25 mm layers of silica gel 60 F254 (Merck) were used for TLC. The extract was applied, and the sheets were then developed in paper-lined, all-glass chambers that had been let at least 15 min to equilibrate.

Total Phenolic and Flavonoid Contents: For the purpose of determining the total phenolic content, the Folin-Ciocalteu method was utilized, slightly modified from a previously published article [6] and employing gallic acid as the standard (0-500 µg/mL). In brief, 500 µL of 10% aqueous Folin-Ciocalteu solution was combined with 0.5 mL of the extracts, agitated, and kept to stand for 10 minutes.

Next, 500 µL of a satured Na_2CO_3 solution was added. A UV-Vis spectrophotometer (ONDA spectrophotometer, UV-30SCAN, China) was used to measure the absorbance at a wavelength of 765 nm after the combination was subsequently incubated at room temperature for 40 min. Ultrapure water was used as the blank. The aluminum chloride method was used for total flavonoid content [6] evaluation using quercetin as standard (0-50 µg/mL) in methanol.

A total of 2 mL of each extract was mixed with 1 mL of 5% $AlCl_3$ (in methanol) followed by incubation for one hour and the absorbance was measured at 434 nm using UV-Vis spectrophotometer with methanol as the blank.

DPPH Radical Scavenging Assay: The DPPH antioxidant activity of extracts was determined following the reported papers [7]. A 0.5 mL of 0.1 mM DPPH was added to 500 µL of extracts (maximum dissolved concentration) and the mixture was diluted with 2 mL of ethanol.

After 10 min of incubation at room temperature, the absorbance of the mixture was assessed at a wavelength of 520 nm (As). The absorbance of the blank was also measured at the same wavelength (Ab). The radical inhibitory activity was calculated using the equation :

$$(Ab-As) / Ab \times 100\%$$

RESULTS AND DISCUSSION:

TLC Profiles: For polyphenols and flavonoids, the mobile phase chloroform/toluene/ethylacetate (5/5/3 ; v/v/v) demonstratedad equate resolution and separation of the sample's constituents.

The sample's TLC profile in the visible with Folin's reagent (polyphenols) is displayed in **Fig. 1A**, whereas the sample at 366 nm with $AlCl_3$ (flavonoids) is displayed in **Fig. 1B**.

The literature data were used as the basis for assigning spots. Four polyphenols or groups of polyphenols appeared as blue spots in the visible after revelation by Folin's reagent [8]. Blue or brown staining spots are characteristic of flavonoids under UV light after spraying with $AlCl_3$ [9, 10].

Flavonoids are a class of plant compounds that fall under the bioactive category and have drawn a lot of attention from researchers due to their potential advantages to human health in scenarios including altered physiology and disease [11].

Numerous diseases, including asthma, cancer, dementia, diabetes, glaucoma, hepatitis, malaria, and several others, can be treated and/or managed with the help of flavonoids [12].

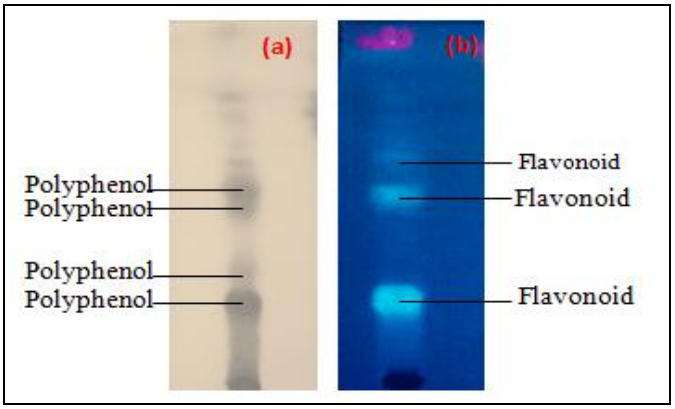

FIG. 1: TLC PROFILES OF *C. ANANTA* (A) POLYPHENOLS REVEALED WITH FOLIN'S REAGENT AT VISIBLE LIGHT; (B) FLAVONOIDS REVEALED WITH $AlCl_3$AT 366 NM

Total Phenolics and Total Flavonoids Contents : This study examined the total amounts of flavonoids and phenolic components in extracts made from macerating and sonicating *Cynometra ananta* trunk bark. The total phenolic content values were expressed using the equation of a standard curve y = 0.0044x, R^2 = 0.9982, as shown in **Fig. 2A** in µg of gallic acid equivalents per mg of dry matter.

The highest concentration of phenolic compounds (482 ± 4.8 µg GAE/mg of dry matter) was demonstrated by sonication. Conversely, the maceration had the lowest teneur in phenolic compounds (420 ± 4.2 µg GAE/mg of dry matter) (**Fig. 3**).

According to this finding, the phenolic components of *C. ananta* were less prevalent in the maceration extract and more concentrated in the sonication extracts. This result has been supported by earlier research, which found that sonication extracts have higher polyphenol concentrations [13–15].

Flavonoid contents were expressed as quercetin equivalent using the equation y = 0.00447x : R^2 = 0.9972, as shown in **Fig. 2B.** The sonication extract had the highest flavonoid content (14 ± 0.7 µg EQ/mg of dry matter) while the maceration extract was the lowest (8 ± 0.4 mg EQ/g of dry matter) (**Fig. 3)**. It was observed that flavonoid compounds accumulated more easily in maceration extracts and less in sonication extracts [16-18].

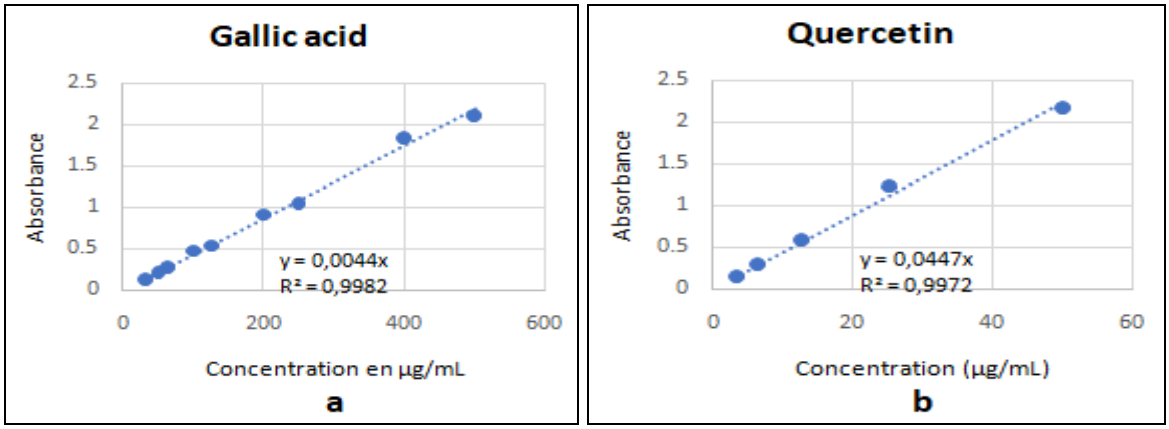

FIG. 2: STANDARD CURVES FOR GALLIC ACID (A) AND QUERCETIN (B)

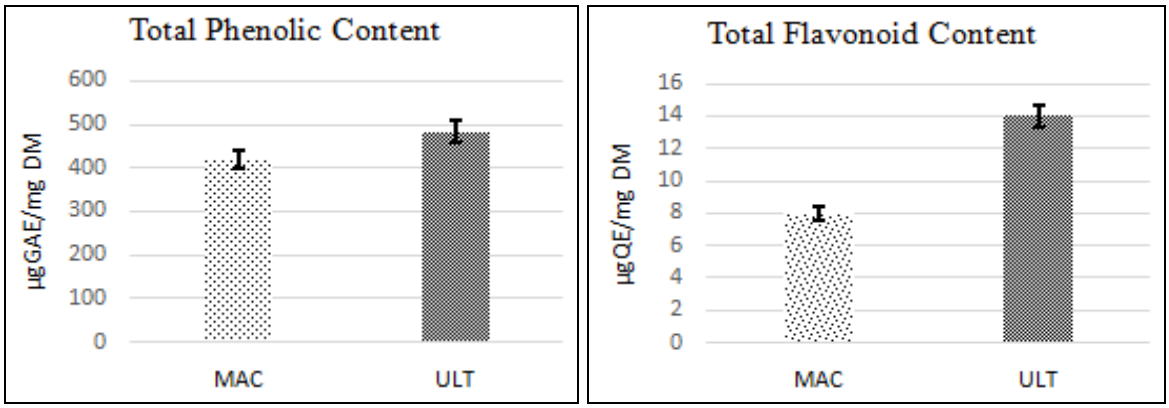

FIG. 3: TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS IN THE EXTRACT FROM MACERATION (MAC) AND SONICATION (ULT)

Radical-scavenging Activity (DPPH Assays): The *in-vitro* DPPH method was used to conduct the antioxidant experiment. The authors have utilized this technique to assess the ability of several natural product extracts to scavenge free radicals.

Based on the DPPH method, **Fig. 4** displays the free radical scavenging activity at a concentration of 20 mg/12 mLaftermaceration and sonication.

Maceration showed a lower inhibition rate (89%) compared to sonication (92%), suggesting a good potential for DPPH radical scavenging.

Previously, other authors had shown the improvement by ultrasound of extraction yields and antioxidant activity of the polysaccharide from the peels of *Garcinia mangostana* L. and phenolic and flavonoid compounds from the fruit of the *Rosa rugosa* Thunb [19, 20].

These findings demonstrated the DPPH scavenging ability of *Cynometra ananta* trunk extracts, which are attributed to the general content of flavonoids and polyphenols [21].

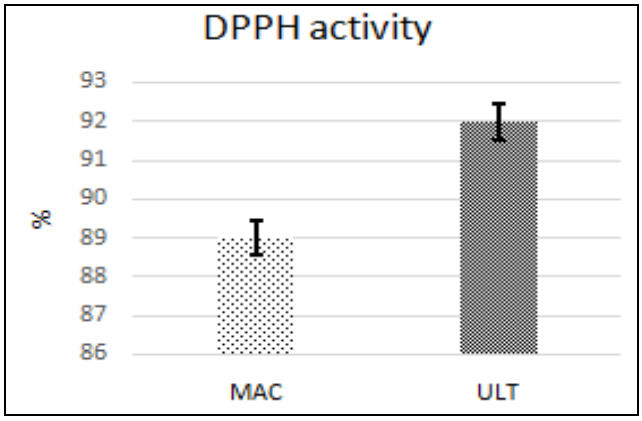

FIG. 4: ACTIVITY OF THE DPPH RADICAL IN THE EXTRACT FROM MACERATION (MAC) AND SONICATION (ULT)

CONCLUSION : The total phenolic and flavonoid content, total phenolic and flavonoid detection, and *in-vitro* antioxidant activity of *C. ananta* stem bark employing an acetone/water (60/40, v/v) method are reported for the first time in this work. The purpose of comparing sonication to maceration was to evaluate how sonication affected the extraction of these phytocompounds. Polyphenols and flavonoids were identified using qualitative analysis (TLC), demonstrating that *C. ananta* is a promising source of possible bioactive metabolites for use in medicine and pharmaceuticals. The phenolic and flavonoid content of the extract made by sonicating the stem bark of *C. ananta* was found to be greater. Furthermore, this extract showed increased antioxidant activity with DPPH *in-vitro*. For understanding the phenolic chemical and flavonoid extraction kinetics from kinetic models, more research is required.

ACKNOWLEDGEMENT: The authors are indebted to the Strategic Support Program for Scientific Research (PASRES in French) in Côte d'Ivoire, for its financial support

CONFLICT OF INTEREST: The authors declareno conflict of interest.

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How to cite this article:
Lanciné T, Séraphin KND, Kamagaté M, Arthur KY, Benson BB and Yves-Alain B: TLC profiles, total phenolic and flavonoid contents, antioxidant activity of *Cynometra ananta*. Int J Pharmacognosy 2024; 11(12): 687-91. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.11(12).687-91.