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Phytochemical Analysis and Antioxidant Activity of Aqueous and Hydroethanolic Extracts from Three Anticancerous Fabaceae of Northern Cameroon Pharmacopoeia

Halmata Mohamadou[1,2], Landry Lienou Lienou[2*], Richard Simo Tagne[3], Herve Pabamé Kada[4], Elisee Libert Enyengue Embolo[1], Grace Kembaou Nganwa[5], Jean Louis Oyono Essame[1], Piere Michel Dongmo Jazet[2]

[1]Anatomy and Cytology Pathology Laboratory, Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon
[2]Department of Biochemistry, Faculty of Science, University of Douala, Douala, Cameroon
[3]Department of Biomedical Sciences, University of Ngaoundere, Ngaoundere, Cameroon
[4]General Hospital of Garoua (HGG), Garoua, Cameroon
[5]Anatomo-Cytopathology Department of Biological Sciences, Faculty of Medicine and Pharmacy, University of Douala, Douala, Cameroon
Email: *lienoulandry@yahoo.fr

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Abstract

Background: Cancer continues to pose a significant threat to our society, representing one of the most pressing health concerns worldwide. This study aimed to evaluate the chemical composition and the antioxidant activity of aqueous and hydroethanolic extracts from *Acacia nilotica* (An), *Bauhinia reticulate* (Br), and *Tamarindus indica* (Ti) of Fabaceae family, traditionally used in Northern Cameroon for cancer treatment. **Methods:** The phytochemical screening of the three plants was conducted using conventional colorimetric methods, followed by the measurement of total phenol content, flavonoids, and tannins. The antiradical and antioxidant activities of both plant extracts were assessed through FRAP, ABTS, and DPPH methods. A principal components analysis was employed to correlate the quantities of the evaluated secondary metabolites with the activities. **Results:** Both types of extracts from the three plants contain alkaloids, saponins, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, coumarins, anthocyanins, and anthraquinones. The aqueous extracts of Br and An are significantly richer ($p < 0.05$) in phenolic compounds and flavonoids than their respective hydroethanolic extracts, while the opposite is observed with Ti. The FRAP antioxi-

dant potential was more pronounced in the aqueous extracts of Ti and Br than in their corresponding hydroethanolic extracts. However, An exhibited the highest potential with both types of extraction. The best DPPH scavenging activity was obtained with the aqueous extract of An, comparable to the reference. The same plant also demonstrated the highest activity in the ABTS test with both its extracts, more pronounced in the hydroethanolic extract, which was significantly better ($p < 0.05$) than the reference (gallic acid). The FRAP activity was highly correlated with the three classes of quantified secondary metabolites. **Conclusion:** The three Fabaceae plants from northern Cameroon, prepared in different solvents, can be utilized for their antiradical properties in cancer treatment.

Keywords

Cancer, Antioxidant, Fabaceae, Phenolic Compounds, Aqueous Extract, Hydroethanolic Extract

1. Introduction

According to the World Health Organization (WHO), cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumours and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis. Widespread metastases are the primary cause of death from cancer [1]. Cancer is one of the leading causes of death globally, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths [2].

The most commonly diagnosed cancers worldwide were female breast cancer (2.26 million cases), lung cancer (2.21 million cases), and prostate cancer (1.41 million cases). The most common causes of cancer death were lung (1.79 million deaths), liver (830,000 deaths), and stomach (769,000 deaths) cancers [3]. In 2023, it is expected that there will be 1,958,310 new cancer cases and 609,820 cancer deaths in the United States. This estimate is projected to increase in 2024, with 2,001,140 new cases of cancer and 611,720 cancer deaths expected in the United States [4]. Approximately 70% of cancer deaths occur in low- and middle-income countries [5].

In Africa, an estimated 801,392 new cases of cancer and 520,158 deaths from cancer occurred in sub-Saharan Africa in 2020. Breast (129,400 female cases) and cervical (110,300 cases) cancers accounted for three out of ten cancers diagnosed [6]. By 2040, the burden of all neoplasms is expected to increase to 2.1 million new cases and 1.4 million deaths in Africa [7].

In Cameroon, the statistics on cancer remain alarming. According to the

WHO, in 2020, 13,199 people died from cancer in Cameroon, and 20,745 new cases were diagnosed, including 12,235 primarily gynecological (breast and cervical cancer) cases [8].

There are several types of cancer treatment worldwide, including surgery, radiotherapy, chemotherapy, targeted therapies, hormone therapy, and immunotherapy [9]. However, these modern treatments face several challenges, such as the lack of selectivity of chemotherapy products, which can cause undesirable effects such as vomiting, death of bone marrow cells, alteration of cell division at the level of the intestinal epithelium, decline in white blood cells, red blood cells and platelets, and congestive heart failure, among others [10].

In Cameroon, modern cancer treatments are costly compared to the population's budget and remain unavailable in several localities. This difficulty in easily accessing modern treatment and the safety of medicinal plants are the main motivations for the use of phytotherapy, specifically the use of plants known for their medicinal value.

Several ethnobotanical surveys have revealed families of plants used to treat cancer. In particular, ethnobotanical surveys carried out in Western Palestine identified several families of plants used in the treatment of cancer, and in Nigeria, the Fabaceae family was the most cited [11]. Similarly, a recent ethnobotanical study conducted by our research team through Northern Cameroon found the Fabaceae family as the most cited family of medicinal plants in the treatment of cancers.

The Fabaceae family, one of the well-known plant families of ethno-pharmacological importance, includes approximately 770 genera and 19,500 species recorded in almost every biome in the world except Antarctica and the High Arctic. This family is a great source of phytochemicals, namely flavonoids, lectins, saponins, alkaloids, carotenoids, and phenolic acids, which have anticancer properties, and the use of these phytochemicals is increasing over time [12]. Pharmacological studies have shown that some species exhibit potent anticancer, antioxidant, antimicrobial, anti-inflammatory, analgesic, antiulcer, antidiabetic, antirheumatic, cytotoxic, and antiparasitic activities, among others [2] [12].

A previous study conducted in Northern Cameroon presented *Acacia nilotica*, *Bauhinia reticulata*, and *Tamarindus indica* as the Fabaceae species most commonly used locally for the treatment of cancers, which motivated the present study on these three plants. Phytochemical studies on these three plants demonstrated that the pod of *A. nilotica* is a potential source of antioxidant polyphenols, tannins, saponins, flavonoids, and carbohydrates [13] [14]. The hydroethanolic extract of the bark of *Bauhinia reticulata* showed very high contents of phenols and flavonoids [15]. *Tamarindus indica* is a medicinal plant reported as a source of antioxidants, rich in phenolic compounds, tannins, and flavonoids [16].

These previous studies have shown that all these secondary metabolites present in the three species are linked to antioxidant activities, which could

present a strong anticancer potential given the strong involvement of oxidation in the occurrence and maintenance of the cancerous condition. The present study, therefore, aimed to evaluate the chemical composition in some phyto-antioxidants and the antioxidant activity of *Acacia nilotica*, *Bauhinia reticulata*, and *Tamarindus indica*, traditionally used in the treatment of cancer in Cameroon.

2. Methods

2.1. Collection of Plant Material and Identification

The plants utilized in this study (An, Br and Ti), were collected in February 2023 from the northern part of Cameroon. Specifically, *Bauhinia reticulata* was gathered from the Adamaoua region, while the other two species were collected from the North region. The identification of these plants was subsequently confirmed at the National Herbarium of Cameroon, where they were assigned the respective codes 36688 SRFCam, 14247 SRFCam, and 49571 SRFCam (as shown in Table 1).

2.2. Extraction of Plants

The pods of *Acacia nilotica*, along with the bark of *Bauhinia reticulata* and *Tamarindus indica*, were collected based on the information provided by traditional medicine practitioners. These plant parts were cleaned, dried at room temperature, and shielded from sunlight. They were then ground into fine powders. Subsequently, one thousand grams of each powder were macerated at room temperature (22˚C ± 2˚C) in 3.2 liters of ethanol and water mixture (70/30 ratio). The addition of 30% distilled water to ethanol significantly enhances its extraction power for polyphenols [17]. This mixture was stirred continuously for three days using an agitator. Every 24 hours, the resulting mixture was filtered, and a second maceration was initiated. The filtrate was then transferred to an evaporation flask and concentrated using a rotative evaporator. The resulting crude hydroethanolic extracts were dried, and the resulting powders were stored at 4˚C until further use.

In a separate process, another thousand grams of each powder (from the three plants) were mixed at room temperature (22˚C ± 2˚C) in 5 liters of boiling water at 100˚C for 24 hours. The extract was subsequently filtered and freeze-dried.

For all quantitative assays, working solutions were prepared by dissolving each dry extract powder in distilled water to achieve a concentration of 1 mg/mL.

Table 1. Identification of the three studied medicinal plants.

Scientific name	Local name	Voucher Number
Acacia nilotica	Gabde	36688 SRFCam
Bauhinia reticulata Dc	Barkedji	14247 SRFCam
Tamarindus indica	Gulum djabé	49571 SRFCam

2.3. Analytical Phytochemistry of the Studied Plants

A qualitative phytochemical analysis was conducted to identify the presence of alkaloids, triterpenes, saponins, flavonoids, tannins, and anthraquinones. This analysis was performed in accordance with the method described by Egbuna *et al.* [18].

2.4. Quantitative Analysis of the Plants Contents in Phenolic Compounds

The total phenolic compounds, flavonoids, and tannins in the extracts were quantitatively determined using various methods:

- **Total Phenolic Compounds:** The total phenolic compounds in each extract were determined using the Folin-Ciocalteu reagent, with catechin used as the standard. This method was proposed by Vinson *et al.* [19].
- **Total Flavonoid Content:** The total flavonoid content was measured using the aluminum chloride colorimetric assay, following the method proposed by Zhinshen *et al.* [20].
- **Tannins:** The tannin content was determined using the spectrophotometric method of acidified vanillin, as described by Bainbridge *et al.* [21].

2.5. Evaluation of Antioxidant Potential of the Extracts of the Three Studied Plants

Three methods were utilized to determine the antioxidant potential: FRAP (Ferric Reducing Antioxidant Power), DPPH (Diphenyl-1,2-picryl hydrazyl), and ABTS (2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonic acid) radical scavenging effect).

- FRAP: The antioxidant power of the extracts was determined using the FRAP method as described by Benzie and Strain [22]. The FRAP reagent was composed of ten parts of acetate buffer (300 mM, pH 3.6), one part of TPTZ 10 mM in 400 mM of HCl Sigma, and one part of ferric chloride (10 mM). Following the preparation, 2 ml of the freshly prepared FRAP solution was added to 75 µl of the extract, and the mixture was incubated for 15 minutes. Catechin of varying concentrations (50 - 300 µM) was used as the standard, and the preparation was carried out as previously described for the extract. The optical density of the extracts/standard was read at 593 nm.
- DPPH: The radical scavenging assay was carried out using the DPPH free radical test, employing the method of Blois [23].
- ABTS: The 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonic acid) scavenging effect of the extracts was analyzed in accordance with the method of Arnon *et al.* [24].

In all measurements of antioxidant potential, gallic acid was used as the standard.

2.6. Statistical Analysis

Measurements were conducted three times, and the results were expressed as the

mean ± standard deviation (sd). The data were analyzed using SPSS software (version 22). Value comparisons were made using the Duncan test, and differences between values were considered significant at a probability threshold of $p < 0.05$. Correlations were established between the antioxidant capacities, as determined by various methods, and the quantitative phytochemical composition of the extracts. This was achieved using Pearson's multivariate principal component analysis.

3. Results

3.1. Analytical Phytochemistry of the Studied Plants

The results revealed that all the aqueous and hydroethanolic extracts studied contain alkaloids, saponins, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, coumarins, anthocyanins, and anthraquinones. However, there are exceptions. The aqueous extracts of An and Br do not contain terpenoids. Additionally, the aqueous extract of Br and the hydroethanolic extract of An lack saponins (as shown in Table 2).

3.2. Phenolic Compounds Contents of the Different Extracts of the Studied Plants

The antioxidant activity of the extracts was determined through the dosages of total polyphenolic compounds, flavonoids and tannins.

Table 2. Phytochemical screening of the aqueous and hydroethanolic extracts of the studied plants.

	Aqueous extract			Hydroethanolic extract		
Tested metabolites	**An**	**Br**	**Ti**	**An**	**Br**	**Ti**
Alkaloids	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Terpenoids	−	−	+	+	+	+
Sterols	−	−	−	−	−	−
Tannins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Anthraquinones	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Anthocyanins	+	+	+	+	+	+
Saponins	+	−	+	−	+	+

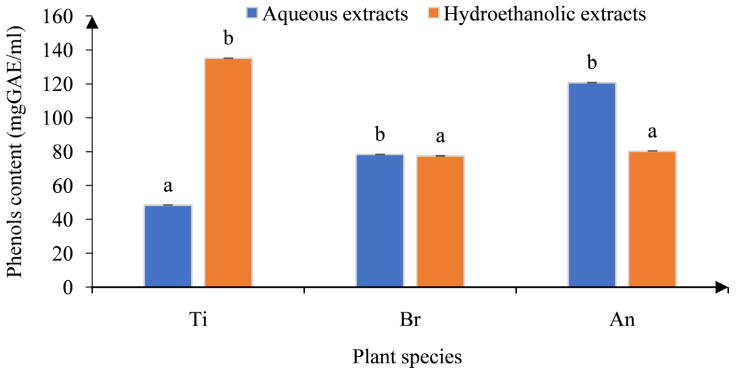
An = *Acacia nilotica*; Br = *Bauhinia reticulata*; Ti = *Tamarindus indica*; + = Présence; − = absence.

3.2.1. Amount of Total Phenolic Compounds in the Plants Extracts

The results indicate that the polyphenol content varies depending on the extract being tested. Upon analyzing the data presented in Figure 1, it is evident that the aqueous extracts of Br and An contain significantly higher quantities of polyphenols compared to their respective hydroethanolic extracts. Conversely, for the Ti extracts, the hydroethanolic mixture results in a more potent extraction of polyphenols.

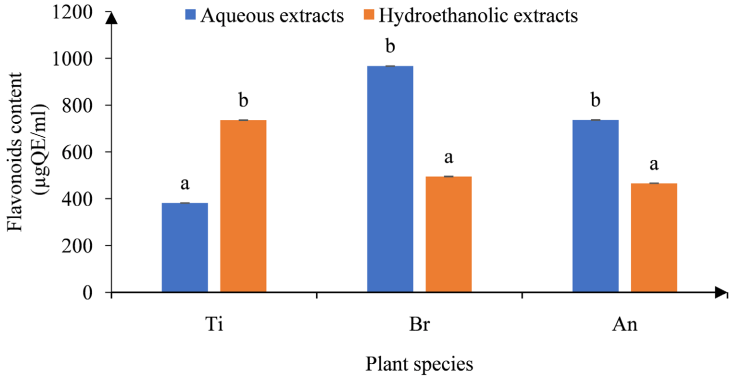
3.2.2. Amount of Flavonoids in the Extracts of Studied Plants

The outcomes of this assay align with those of the polyphenols, exhibiting the same significant variations. Notably, the highest value was recorded with the aqueous extract of Br (*Bauhinia reticulata*), which reached a peak at 967.05 ± 0.00 µg/ml of the dissolved extract, as depicted in Figure 2.



"An" refers to *Acacia nilotica*, "Br" to *Bauhinia reticulata,* and "Ti" to *Tamarindus indica*. Each histogram represents the mean value ± standard deviation (sd) derived from three measurements. The significance of differences ($p < 0.05$) was determined using the Duncan Test, and is indicated by histograms labeled with different letters.

Figure 1. Polyphenols contents of the studied plants extracted with water or hydroethanolic solvents.



"An" refers to *Acacia nilotica*, "Br" to *Bauhinia reticulata,* and "Ti" to *Tamarindus indica*. Each histogram represents the mean value ± standard deviation (sd) derived from three measurements. The significance of differences ($p < 0.05$) was determined using the Duncan Test, and is indicated by histograms labeled with different letters.

Figure 2. Flavonoids contents of the studied plants extracted with water or hydroethanolic solvents.

3.2.3. Amount of Tannins in the Extracts of Studied Plants

Figure 3 showcases the tannin contents of the aqueous and hydroethanolic extracts of the three plants. It is evident that the hydroethanolic extracts of Ti and An are significantly richer in tannins compared to their respective aqueous extracts. Conversely, for the extracts of Br, the aqueous extract exhibited the highest concentration of tannins. The distribution peaked at a value of 772.13 ± 0.63 µg/ml of extract.

3.3. Antioxidant Activities of Aqueous and Hydroethanolic Extracts of the Three Plants of Interest

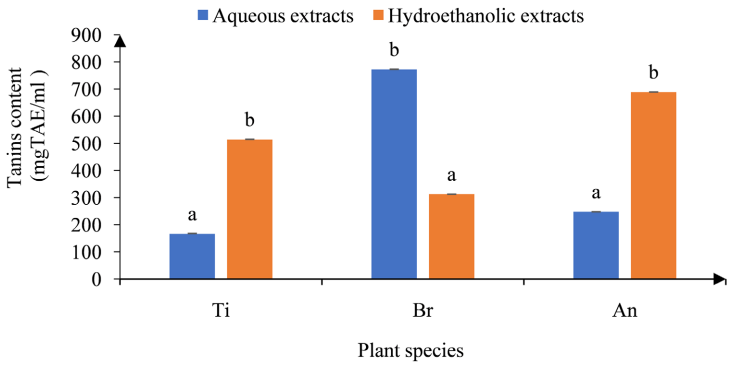
3.3.1. Determination of Antioxidant Concentration by the FRAP Method

As depicted in Figure 4, the most active aqueous (202.44 ± 0.17 µg/ml) and hydroethanolic (205.23 ± 0.06 µg/ml) extracts are those derived from An (*Acacia nilotica*). However, Br (*Bauhinia reticulata*) exhibited a significantly higher antioxidant content in its aqueous extract compared to its hydroethanolic extract ($p < 0.05$). This indicates a notable difference in the antioxidant potential between the two types of extracts for Br.

3.3.2. Evaluation of the Anti-Radical Activity of Plant Extracts by the DPPH and ABTS Methods

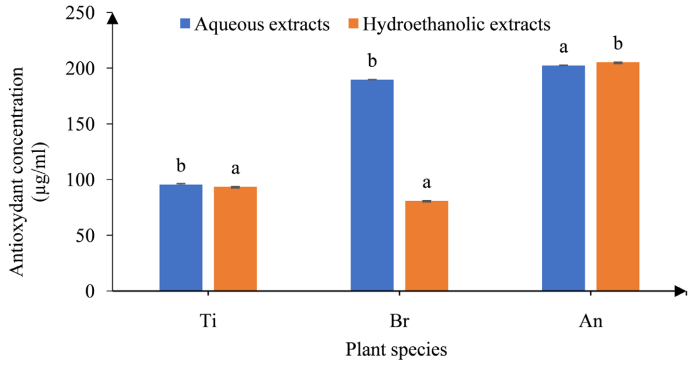
The antiradical activity of the extracts was assessed using the DPPH and ABTS radical scavenging methods. Upon analyzing the data presented in Table 3, it was observed that the most active aqueous extract against DPPH was that of An, with a value of 5.54 ± 1.17 µg/ml. This value is comparable to that of the reference anti-free radical.

Moderate activity was recorded with Br for the same type of extraction, and with Ti for the hydroethanolic extraction. On the other hand, the ABTS activity was notably high with the hydroethanolic extract of An. Its IC50 value was significantly lower ($p < 0.05$) than that of the reference anti-radical agent.



"An" refers to *Acacia nilotica*, "Br" to *Bauhinia reticulata,* and "Ti" to *Tamarindus indica*. Each histogram represents the mean value ± standard deviation (sd) derived from three measurements. The significance of differences ($p < 0.05$) was determined using the Duncan Test, and is indicated by histograms labeled with different letters.

Figure 3. Tannins contents of the studied plants extracted with water or hydroethanolic solvents.



"An" refers to *Acacia nilotica*, "Br" to *Bauhinia reticulata,* and "Ti" to *Tamarindus indica*. Each histogram represents the mean value ± standard deviation (sd) derived from three measurements. The significance of differences ($p < 0.05$) was determined using the Duncan Test, and is indicated by histograms labeled with different letters.

Figure 4. Antioxidant content of the plant extracts according to FRAP test.

Table 3. Inhibition concentration 50 (IC_{50}) values of the different extracts of studied plants.

	Aqueous extracts			Hydroethanolic extracts			Reference
Species activity	**Ti**	**Br**	**An**	**Ti**	**Br**	**An**	**Gallic acid**
DPPH (µg/ml)	95.75 ± 3.65^{e}	34.46 ± 0.93^{b}	5.54 ± 1.17^{a}	35.79 ± 0.80^{b}	81.27 ± 5.62^{d}	39.69 ± 0.22^{c}	6.7 ± 0.11^{a}
ABTS (µg/ml)	82.56 ± 1.32^{g}	49.16 ± 0.43^{d}	25.54 ± 0.41^{c}	53.80 ± 1.17^{e}	62.37 ± 2.14^{f}	10.73 ± 1.63^{a}	17.44 ± 0^{b}

An = *Acacia nilotica*, Br = *Bauhinia reticulata Dc*, Ti = *Tamarindus indica.* The different superscripts in lines present significant differences between the values (Duncan Test); each value presents the mean ± standard deviation of three values.

For this same activity, An also demonstrated the best value for aqueous extraction, although it was significantly higher than that of the reference. The aqueous extraction of Ti was found to be the least effective for both activities. A similar observation was made with the hydroethanolic extraction of Br.

4. Discussion

This study focused on highlighting the antioxidant activities of three medicinal plants (*Acacia nilotica*, *Bauhinia reticulata* and *Tamarindus indica*), traditionally used in anti-cancer treatment in the northern part of Cameroon. The qualitative phytochemical data revealed that the aqueous and hydroethanolic extracts of these three plants contain various secondary metabolites such as alkaloids, saponins, flavonoids, phenolic compounds, tannins, glycosides, coumarins, anthocyanins, and anthraquinones.

Among these secondary metabolites, phenolic compounds, particularly flavonoids and tannins, are frequently associated with anticancer activity [25] [26]. Similarly, tannins, a heterogeneous class of polyphenolic natural products, show promising chemo-preventive and therapeutic potential against cancer [27]. These findings could explain the use of these three plants in cancer treatment by traditional practitioners in Cameroon.

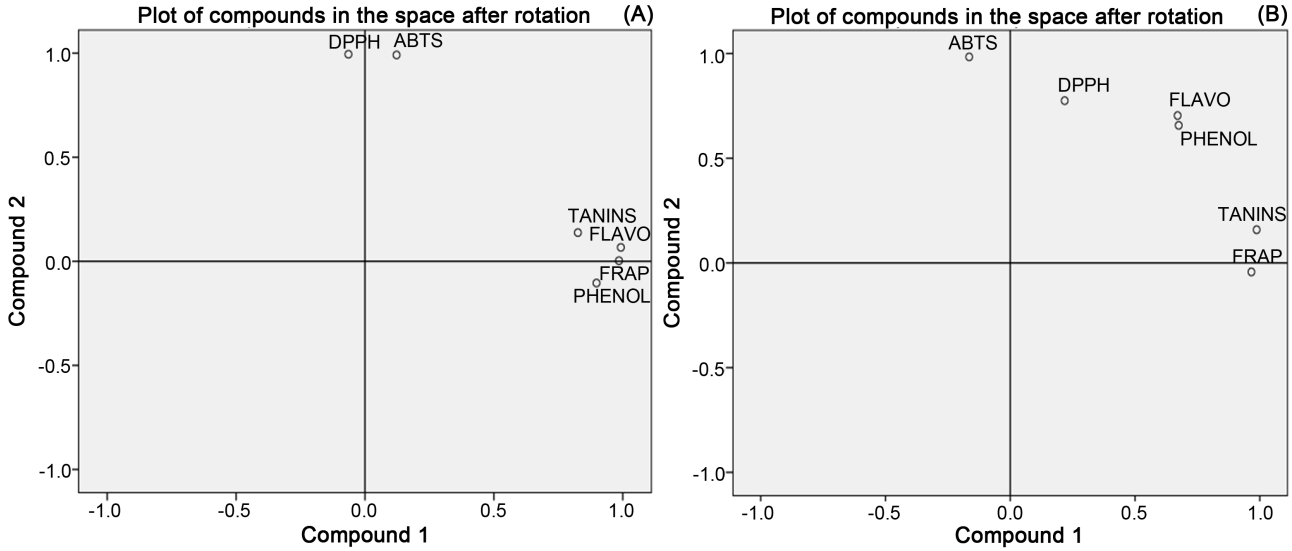
The quantitative analysis of the extracts from these three plants in phenolic compounds, flavonoids, and tannins showed varied content depending on the solvent and extraction method. This variation could be attributed to the part of the plant used, as the content of secondary metabolites in various plant organs varies depending on plant metabolism and various external factors related to its botany [28].

Flavonoids, as a major class of phenolic compounds, have been extensively studied for their therapeutic potential. Mutha *et al.* [29] provide a comprehensive overview of the exploration of natural phenolic compounds, with a focus on flavonoids and their therapeutic potential. They highlight the protective effects of long-term intake of dietary foods rich in phenolic compounds against the development and management of various diseases, including cancer. This aligns with the findings mentioned, suggesting that flavonoids, being the main phenolic compounds in plants, logically correlate with total phenols and contribute significantly to antioxidant activities.

Furthermore, the structural diversity of flavonoids and tannins allows for varied mechanisms of antioxidant action [30] [31]. Kováč *et al.* [32] discuss the therapeutic potential of flavonoids and tannins in managing infectious diseases, indicating their broad antimicrobial activity. Although this review focuses on oral diseases, the antioxidant properties of these compounds can be extrapolated to their anti-cancer potential, as antioxidants play a crucial role in combating cellular oxidation, a major cause of cancer.

In addition to the in vitro and in vivo antioxidant activities of phenolic compounds, their anticancer potential is also well-documented [33]. Anantharaju *et al.* [34] provide an overview of the role of dietary phenolic compounds in the treatment of cancers, demonstrating how plant-derived phenolic compounds modulate genes regulating key processes such as oncogenic transformation, tumor growth and development, angiogenesis and metastasis. This supports the notion that secondary compounds with antioxidant and anti-radical activities, such as flavonoids and tannins, could be key in combating cancer through their protective effects against cellular oxidation, one of the major causes of cancer [35] [36]

To further substantiate the specific action of secondary metabolites, it was indeed necessary to correlate the content in phenolic compounds with anti-radical and antioxidant activities. The principal component analysis (**Figure 5**) is a valuable tool for establishing a composition/activity coupling of the extracts. The correlation between water extraction and FRAP type activity with quantified phenolic compounds suggests that these agents are primarily antioxidant compounds that act by reducing reactive oxygen species, thus protecting cells against oxidation and potential cancerization. These findings thus emphasize the importance of verifying the specific anticancer potential of these extracts through further research on their effects on cancerous cell lines and the evaluation of their selective cytotoxicity. Further studies are needed for understanding the complex interactions between plant extracts' phenolic content and their biological activities, particularly their role in cancer prevention and treatment.



FLAVO = Flavonoids; PHENOL = Phenolic compounds; TANINS = Tannins; FRAP, DPPH, ABTS represent the values of antioxidant and antiradical properties through the three methods.

Figure 5. Principal components analysis of the aqueous. (A) and hydroethanolic; (B) extracts of the three plants.

5. Conclusion

Indeed, the aqueous and hydroethanolic extracts of *Acacia nilotica* (An), *Bauhinia reticulata* (Br), and *Tamarindus indica* (Ti) exhibit a high content of phenolic compounds, tannins, and flavonoids. Their pronounced antioxidant activity could potentially make them compelling candidates for anticancer treatment. These findings highlight the potential of these plants to contribute to the development of new therapeutic strategies for cancer. However, further research, including in vivo studies and clinical trials, would be necessary to confirm their efficacy and safety in cancer treatment.

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Authors' Contributions

Conceptualization (JDPM, LLL); Data curation (LLL, TSR, NKG); Investigation (HM, KPH); Methodology (LLL, HM); Project administration (JDPM); Resources (HM); Software (HM, LLL); Supervision (JDPM, TSR, EOJL); Validation (JDPM); Writing—original draft (HM, LLL); Writing—review & editing (All the authors).

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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