Scientific Research Publishing

Food and Nutrition Sciences, 2025, 16(11), 1788-1807
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Phenolic Profile and Antioxidant Activities of Oil Cake Extracts of *Anisophyllea boehmii* and *Pycnanthus angolensis* from Burundi

Jonathan Niyukuri[1,2*], Séverin Sindayikengera[1,2], Albert Iribagiza[3,4], Emmanuel Banzubaze[2,5,6], Manirakiza Josiane[2,3], Ntunzwenimana Mélance[2,3]

[1]Food Science and Technology Research Center, CRSTA, University of Burundi, Bujumbura, Burundi
[2]East African Nutritional Sciences Institute (EANSI), University of Burundi, Bujumbura, Burundi
[3]Center for Animal, Crop and Environmental Sciences, CRAVE, University of Burundi, Bujumbura, Burundi
[4]Key Laboratory of Yak Breeding Engineering of Gansu Province, Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Lanzhou, China
[5]Department of Paraclinical Sciences, National Institute of Public Health, Bujumbura, Burundi
[6]Department of Paramedical Sciences, Pan-African University Les Mages, Bujumbura, Burundi
Email: *jonaniyu@gmail.com

How to cite this paper: Niyukuri, J., Sindayikengera, S., Iribagiza, A., Banzubaze, E., Josiane, M. and Mélance, N. (2025) Phenolic Profile and Antioxidant Activities of Oil Cake Extracts of *Anisophyllea boehmii* and *Pycnanthus angolensis* from Burundi. *Food and Nutrition Sciences*, **16**, 1788-1807.
https://doi.org/10.4236/fns.2025.1611105

Received: September 22, 2025
Accepted: November 25, 2025
Published: November 28, 2025

Abstract

This research aims to valorize two wild species: *Anisophyllea boehmii* and *Pycnanthus angolensis* from Burundi forests. The antioxidant activities were estimated using 2, 2-diphenyl-b-picrylhydrazyl free radical scavenger and the reducing power assay whereas total polyphenolic (TPC), flavonoids (TFC), and condensed tannins (CTC) contents were examined by colorimetric methods. High-Performance Liquid Chromatography-Mass Spectrometry analysis revealed 14 phenolic compounds in *A. boehmii* seeds, and 11 were identified. Fifteen phenolic compounds were found in *P. angolensis seeds*; among them, 12 compounds were identified. Proximate analysis revealed high contents of carbohydrates and proteins, respectively, 63.0% ± 4% and 24.1% ± 3% DM oilcake of *A. boehmii,* and 59.4% ± 2.4% and 25.6% ± 2.2% DM oilcake of *P. angolensis*. Both species revealed TPCs and effectiveness antiradical (EA) very interesting and they were respectively 874.97 ± 20.45 GAE/100g and 100 ± 5.6 ml/µg·mim for *A. boehmii* while 1089.89 ± 293.40 GAE/100g and 13.3 ± 0.5 ml/µg·mim were found in *P. angolensis extract.* This powerful antioxidant activity observed in both species may be due to the tannin compounds for *A. boehmii* and flavonoid compounds for *P. angolensis*. This study suggests that both species analyzed may be a potentially source of natural antioxidants.

Keywords

Anisophyllea boehmii, Antioxidant, Burundi, *Pcynanthus angolensis*, Polyphenols

1. Introduction

A. boehmii and *P. Angolensis* are respectively natives of Miombo woodland of sub-Saharan Africa [1] [2] and intertropical forests of Western and central Africa [3]-[5]. *A. boehmii*, family of Anisophylleaceae, and *P. Angolensis*, family of Myrtaceae, bear fleshy fruits with seeds enclosed in a fragile shell. From the shapefile of the natural regions of Burundi, the automatically calculated geometry with arcMap 10.4.1 revealed distributions around 12,475 km^2 for *A. boehmii* and 2513 km^2 for *P. Angolensis*. They are both wild plants, and only some trees left after clearing can be isolated in agricultural ecosystems. Both species are used as firewood and wood for construction of cowsheds. They are considered as plants with no great value and are part of the neglected species. Locally, the pericarp from *A. boehmii* fruit is edible.

Some previous investigations reported that they contain compounds of great interest. Extracts from all parts of *P. Angolensis* are characterized by different bioactivities. Many reports suggested that its leaves extracts have anthelmintic and antimicrobial activities [6] while the bark extracts show antimalarial [3] [7], antinociceptive, and antiulcer activities [8]. Tannin extracted from stem bark has been demonstrated to have a significant influence on the cellular physiology of human keratinocytes and dermal fibroblasts [4] while all flavonoids induce apoptosis in HuH-7 human hepatoma cells [9]. Furthermore, extract from stem bark and leaves is reported to have analgesic, hemostatic activities [10]; to treat hemorrhoids, jaunice, leprosy, and toothache [11] [12]; and to have anti-hemorrhagic and anti-rheumatic properties [13]. Regarding *A. boehmii,* almost no studies on its possible bioactivities were performed. Its bark infusion is reported to have an interesting antimalarial bioactivity [14]. Furthermore, [15] has reported to have property to protect against oil oxidation.

Seeds from these two species were already investigated on their seed oil content: 74% has been recorded in *P. Angolensis* [16] [17] and 29% in *A. boehmii* oils [16] [17]. While most of the studies were carried out on sensitive parts of the plant which may gradually leads to deforestation, the valorization of the seeds is an effective way of the preservation of the vegetal species and consequently constitutes one of the sustainable tools of the conservation and the protection of the environment. In addition to the primary metabolites found in seeds, they also contain valuable secondary metabolites, such as polyphenols [18].

While we observe that the diseases related to the oxidants increase in a worrying way [19]-[21], natural polyphenols from plants are reported to have powerful an-

tiradical and antioxidant activities that enhance human health [22]-[25] and food quality [26] [27]. However, to the best of our knowledge, polyphenol from the seeds of these species has not been investigated. The beneficial effects of polyphenols on human health are not limited only to their antioxidant capacity. It has been suggested that they are involved in treatment of autoimmune diseases [28]; in regulating of ncRNAs to exert antitumor effects [29]; in treatment/prevention of cataracts, age-related macular degeneration, diabetic retinopathy and glaucoma [30]; in controlling of infectious diseases caused by oral microorganisms [31]; and in reducing the risk of hypothalamic inflammation, mitochondrial dysfunction, and neurodegeneration [32].

The objectives of this study are: 1) the identification of the phenolic components from oilcake extracts using High-Performance Liquid Chromatography Mass Spectrometry (HPLC-MAS), 2) the evaluation of the antioxidant potential of seed extracts and 3) the estimation of proximate compounds of oilcake. This investigation will highlight nutritional and antioxidant potential of *P. Angolensis* and *A. boehmii* oilcakes, suggesting their functional food, cosmetic, pharmaceutical and nutraceutical domain uses.

2. Materials and Methods

2.1. Chemicals

Chemicals such as: 2,2-Diphenyl-1-picrylhdrazyl (DPPH), methanol, ethanol, sodium bicarbonate, vanillin, gallic acid, catechin, Folin-Ciocalteau reagent, potassium ferricyanide, and sodium hydroxide were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.). Aluminum chloride, iron (III) chloride, nitrite de sodium, phosphate buffer, and trichloro acetic were purchased from Merck (Germany).

2.2. Plant Materials

The seed samples of *A. boehmii* (vernacular name: Umushindwi) were harvested from three sites of three different eco-climatic zones of Burundi, namely: eastern depression (1200 - 1500 m of altitude), central trays (1400 - 2000 m of altitude) and foothills of Mumirwa (1000 - 1500 m of altitude). The seed of *P. angolensis* (vernacular name: umusurura) were harvested in the eco-climatic zone of the foothills of Mumirwa from different commune (Vyanda, Vugizo and Musigati). Harvest was carried out in July for *P. anglolensis* and October for *A. boehmii.* The identification of the plant species was performed at the herbarium of the University of Burundi (BJA) and the herbarium of the Burundian Office for the Protection of the Environment. The ripe fruits, identifiable by their respective color, were harvested manually. Nine trees were sampled on each specie and three samples were collected per site. The fruits were dried at room temperature in the Microbiology Laboratory of bioengineering's faculty at University of Burundi. After drying, the seeds were hulled manually.

2.3. Sample Preparation

The first step was the extraction of the oil from the seeds to have oilcake. Seeds were crushed using a Moulinex blinder (France) and then the extraction was performed with hexane as solvent in a Soxhlet apparatus under reflux for 8 h. Thus, we found 24% and 69% of oil respectively from *A. boehmii* and *P. angolensis.* Then, to 10 g of completely defatted cake, 50 ml of ethanol (80%) was added and homogenized for 30 minutes on a magnetic stirrer. Subsequently, the phase separation was done by centrifugation at the 4000 rpm for 20 minutes. The supernatant (ethanolic extract) was recovered in a flask and the pellet was again reextracted three times. The three ethanolic extracts thus obtained were mixed and evaporated to dryness under reduced pressure. The extract was recovered in a known amount of ethanol 80%.

2.4. Proximate and Elemental Analysis of Oilcake

Chemical composition of the samples namely ash, and crude protein of oilcake were determined according to the Association of Official Analytical Chemist [33] methods. Carbohydrate content in the samples was estimated using formula [100%-ash-protein].

2.5. Total Phenolic Content

Since Folin Ciocalteu can react with other compounds, applying this method does not give exact values of polyphenols, rather estimates. For this, some researchers tend to replace the designation of total polyphenol contents by the Folin-Ciocalteu reagent assay measures sample reducing capacity [34] while others continue to use it as estimators of total polyphenol contents [35]. Thus, the total phenol content of the samples was determined using the Folin-Ciocalteau reagent [36]. Briefly, an aliquot of 100 µl of ethanol extracts was added to 1.0 ml of distilled water and 0.5 ml of Folin-Ciocaleu reagent (1/10 v/v). After mixing, 1.5 ml of 2% sodium bicarbonate was added to the mixture. The absorbance was read using a spectrophotometer at 760 nm after 30 min of incubation in the dark. The total content of phenolic compounds was expressed as mg gallic acid equivalent (GAE) of extract/100 g dry matter of oilcake (DM).

2.6. Total Flavonoid Content (TFC)

Total flavonoids were assessed using a colorimetric test as described by [37]. 250 µl of the extract and 1 ml of distilled water were successively introduced into a tube. At the initial time (0 minutes), 75% µl of a Na NO_2 solution (5%) were added, after five minutes 75% µl of $AlCl_3$ (10%) were successively added to the mixture. The absorbance of the mixture obtained was directly measured with a UV-visible spectrophotometer at 510 nm against the blank.

2.7. Condensed Tannin Content

Condensed tannin concentrations were determined by a modified method of [38]

[38]. Ten microliters of samples were mixed with 200 µl of vanillin-HCl reagent (4% vanillin in methanol and 8% concentrated HCl in methanol). After 15 min, the absorbance of the mixture was determined at 500 nm against a blank solution. The condensed tannin content was expressed as catechin equivalents (CE) in milligrams per 100 gram (mg/g) of dray matter of oilcake (DM).

2.8. High-Performance Liquid Chromatography Mass Spectrometry (HPLC-MAS) Analysis

Qualitative LC-MS analysis was carried out using a Dionex UltiMate 3000 RSLC system coupled to a TSQ Endura triple quadrupole mass spectrometer, equipped with an H-ESI source working in negative mode. Mass spectra were acquired in profile mode with a setting of 30,000 resolutions at m/z 400. Operation parameters were as follows: source voltage, 4 kV; sheath gas, 60 (arbitrary units); auxiliary gas, 20 (arbitrary units); sweep gas, 2 (arbitrary units); and capillary temperature, 275˚C. Extract samples were analyzed in full scan mode at a resolving power of 30,000 at m/z 400 and data-dependent MS/MS events acquired at a resolving power of 15,000. The most intense ions detected during full scan MS triggered data-dependent scanning. Ions that were not intense enough for a data-dependent scan were analyzed in MSn mode with the Orbitrap resolution also set at 15,000 at m/z 400. An isolation width of 100 amu was used and precursors were fragmented by collision induced dissociation C-trap (CID) with a normalized collision energy of 40 V and an activation time of 10 ms. The mass range in FTMS mode was from m/z 100 to 1000. The data analysis was achieved using XCalibur software v4.0.27.42 (Thermo Fisher Scientific).

Phenolic acid separation was performed on a reverse phase C18 column (4.6 × 250 mm) and the compounds elution were monitored with diode array detector. The mobile phase used was 2.5% acetic acid (Solvent A) and acetonitrile (Solvent B. The following gradient was applied: initial 3% B; 9% B for 5 min; 5 - 15 min, 16% B; and 15 - 50 min, 50% B.

2.9. Antioxidant Activity

2.9.1. DPPH Radical Scavenging Activities Assay

The DPPH free radical scavenging test was measured as described by [39]. A volume of 0.1 ml of each of the solutions of the ethanolic extracts at different concentrations was mixed with 3.9 ml of methanolic solution (80%) of $DPPH^+$ (0.004%). The decrease in absorbance was determined at 515 nm at 0 min, and every 5 min until a steady state was reached. The inhibition of free radicals in percentage (I%) was calculated using the following formula: I% = [1 − (Abs test/Abs control)] × 100; where Abs test is the absorbance of the sample and Abs control is the absorbance negative control. The concentration of each extract and ascorbic acid necessary to decrease the initial DPPH concentration by 50% (Efficient Concentration = EC_{50}) was calculated graphically. The antiradical activity was expressed as EC_{50} (mg/ml). The kinetics of the reaction were evaluated according to [40] by calcu-

lating the time needed to reach a steady state with an antioxidant concentration corresponding to EC_{50} (TEC_{50}) and effectiveness antiradical (EA). The TEC_{50} was determined graphically. The AE, which involves the potency ($1/EC_{50}$) and the reaction time (TEC_{50}), was calculated using the following formula: $AE = 1/EC_{50} \times TEC_{50}$. The lower the EC_{50}, the lower the TEC_{50} and the higher the AE. All tests were performed in triplicate.

2.9.2. Reducing Power (FRAP) Assay

The reducing power was measured according to [41]. A volume of 0.5 ml of the sample was homogenized with 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). After incubation in a water bath (50˚C/20 min), 1.25 ml of trichloroacetic acid (10%) was added to the mixture. which was then centrifuged at 2000 rpm for 20 min. The upper layer of the solution (1.25 ml) was mixed with distilled water (1.25 ml). The absorbance was read at 700 nm after the addition of 0.25 ml of Iron (III) chloride (1%). The increase in absorbance indicates a high reducing power [42] and results were expressed as antioxidant gallic acid equivalents in mg per 100 g of product (mg GAE/100 g).

2.9.3. Statistical Analysis

Data analysis was performed using IBM SPSS statistic 20. Results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Correlation between various parameters was also investigated. Significance was determined at $p < 0.05$ and the results were expressed as mean values and standard error (SE) of the means. Comparisons were made between the results of the same species for three sites and another comparison between the results of these two species.

3. Results and Discussion

3.1. Proximate and Elemental Analysis

Results on proximate components of *A. boehmie* and *P. angolensis* oilcakes are illustrated in **Table 1**. The Overall trends of compound contents compared to dry matter in both oilcake species were found to be similar. The contents of carbohydrates, proteins, and ash were, respectively 70.08% ± 4%, 26.81% ± 3.2% and 3.92% ± 0.3% for *A. boehmii* and 66.52% ± 2.4%, 28.67% ± 2.2%, and 4.82% ± 0.6% for *P. angolens*. Furthermore, proximate compounds from *A. boehmii* oilcake, calculated including oil content (24%), were found to be similar to those reported on the same species by [43].

Thus, carbohydrate, crude protein, and ash contents were, 56.5%, 21.6%, and 2.5%, respectively. For *P. angolensis*, because of its high oil content (69%), the calculation includes the latter has remarkably lowered the proximate compound content at very low values: carbohydrate (39.36%), crude protein (16.96%), and ash (2.85%). Moreover, large discrepancies were observed for the levels of carbo-

hydrates and proteins reported in the previous study on *P. angolensis* [16]. Regarding origin influences on yields, significant differences ($p < 0.05$) were observed in the results of protein contents (foothills > depressions > trays) recorded for the *A. boehmii* oilcake extracts and ashcontents (Vyanda > Musigati > Vyanda) determined in *P. angolensis* oilcake extracts.

Table 1. Proximate compounds of *A. boehmii* and *P. angolensis* oilcakes from different sites: TR, trays; DP, depression; FH, foothills; Y, average; MS, Musigati; VG, Vugizo; VY, Vyanda.

Species	site	carbohyrdate	protein	ash
A. boehmii	TR	75.16 ± 9.0^{c}	20.92 ± 1.4^{a}	3.92 ± 0.3^{a}
	DP	68.72 ± 6.1^{a}	28.2 ± 0.5^{b}	3.08 ± 0^{a}
	FH	66.22 ± 3.8^{a}	31.45 ± 0.4^{c}	2.33 ± 0.1^{a}
	Y	**70.08 ± 4^{u}**	**26.81 ± 3.2^{u}**	**3.11 ± 0.5^{u}**
	MS	65.54 ± 1.4^{b}	28.48 ± 3.4^{b}	5.98 ± 0.0^{b}
P. angolensi	VG	63.16 ± 7.2^{b}	32.19 ± 2.8^{b}	4.65 ± 0.3^{ab}
	VY	70.96 ± 3.1^{b}	25.28 ± 2.7^{a}	3.76 ± 0.0^{a}
	Y	**66.52 ± 2.4^{u}**	**28.67 ± 2.2^{u}**	**4.82 ± 0.6^{v}**

3.2. Total Phenols, Flavonoids and Condensed Tannins Contents

The ethanolic extraction yields of *A. boehmii* and *P. angolensis* are mentioned in Figure 1(I) and are expressed as percentage (%) of dry matter from oilcake. The amount of TPC in samples was reported as mg of gallic acid equivalent (GAE) per 100 g. Figure 1(II) showed that *P. angolensis* exhibited the highest TPC content (1089.89 ± 293.40 GAE/100g) compared to *A. boehmii* (874.97 ± 20.45 GAE/100g). However, their differences were no significant at $p > 0.05$. TPC of *A. boehmii* oilcake extracts from different sites were also found not to be statistically significant ($p > 0.05$). These contents varied from 905.65 ± 37 mg GAE/100g obtained in the central trays region to about 850.44 ± 57.58 mg GAE/100g) in the seeds collected in the Mumirwa zone. Significant differences ($p < 0.05$) were observed for *P. angolensis* between TPC from Musigati (1530 ± 397.74 mg GAE/100g) > Vugizo (995.12 ± 59.35 mg GAE/100g) > Vyanda 744.56 ± 113.59 mg GAE/100g DM). Although *A. boehmii* seeds originated from climatically different regions (depressions, central trays, and Mumirwa), it seems not to have influenced the phenolic contents of this specie. Other studies reported a great environmental influence on TPC [44].

For *A. boehmii*, yields obtained from seeds originating from different regions were found not to be significantly different ($p > 0.05$). But, for *P. angolensis*, significant differences ($p < 0.05$) were observed in this order: Vyanda > Vugizo > Musigati. Overall extraction averages of *A. boehmii* (14.80% ± 0.18%) and *P. angolensis* (17.50% ± 2.35%) showed no significant differences ($p > 0.05$).

Trends in TPC and extraction yield of ethanolic soluble substances observed

between the two species were reversed in TFC and CTC. The results (Figure 1(III) and Figure 1(IV)) showed that TFC and CTC values were higher in *A. boehmii* oilcake extracts compared to these of *P. angolensis* oilcake extracts with significant difference at $p < 0.05$. Furthermore, significant differences were observed in *A. boehmii* oilcake extracts only. It was obvious that the environmental factor has influenced the accumulation of these molecules. The highest TFC was found to be in the seeds from Mumirwa (236.14 ± 43.22 mg CE/100g) followed by those from depressions (188.36 ± 10.43 mg CE/100g) and lowest were registered in those from central trays (132.88 ± 8.03 mg CE/100g) with significant difference at $p < 0.05$.

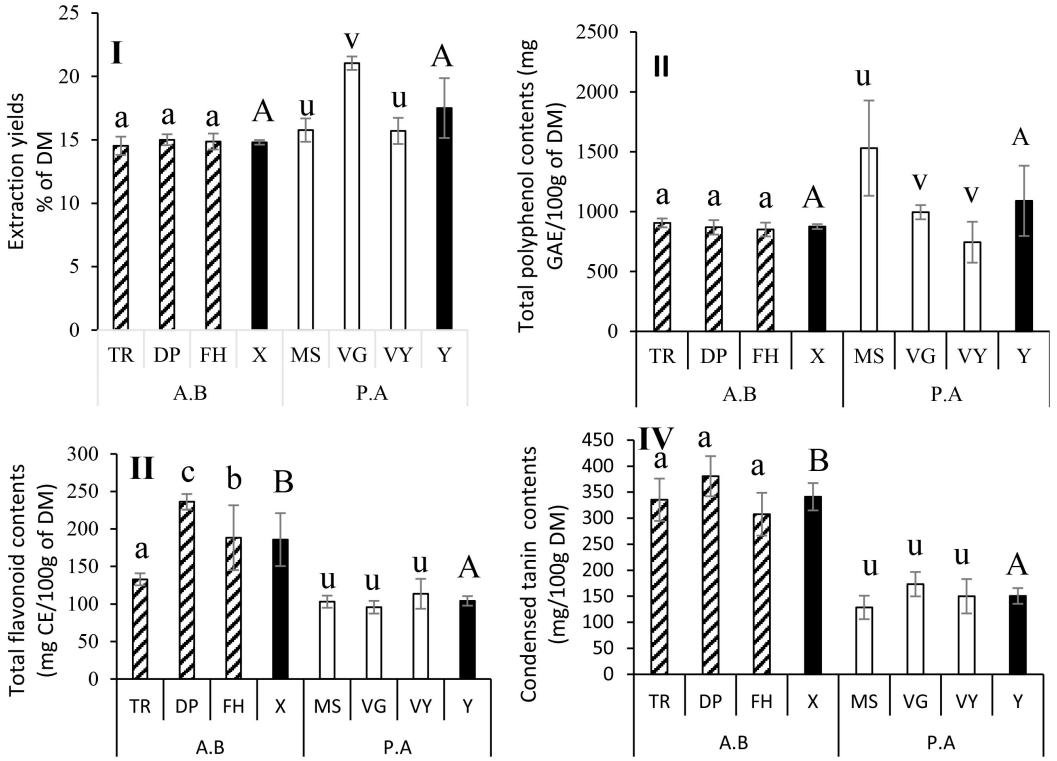


Figure 1. Ethanolic extraction yield (I), Total polyphenol (II), total flavonoids (III) and total condensed tannin content (IV) of *A. boehmii* (A.B) and *Pycnanthus angolensis* (P.A). In each of I, II, III and IV, results are expressed as the mean (standard deviation (n = 3). In each species, the bars sharing different letters were significantly different ($P < 0.05$) and their results were ranked in ascending order (a < b < c for A.B and u<v<w for P.A). The average of the three sites of each species (X for A.B and Y for P.A) was compared to that of the other where the non-sharing of the same letter (A and B such as A < B) gave them a significant difference ($P < 0.05$).

For *P. angolensis*, the highest were obtained in the seeds harvested from Vyanda commune site (113.59 ± 19.97 mg CE/100g) and the lowest these from Vugizo sites (95.69 ± 18.47 mg CE/100g). Results on CTC showed that the highest content (341.203 ± 40.15 mg CE/100g) determined in *A. boehmii* was twice as high than the highest obtained in the *P. angolensis extracts* (150.59 ± 26.34 g). The comparison of TFC and CTC with those of total polyphenols showed that it had no interdependence. Statistically, no correlation (Table 2) was recorded between the con-

tents of these two compounds (TFC and CTC) and those of TPC. Several studies, also reported that high content of polyphenols does not necessarily correlate with high content of flavonoids or condensed tannins [41] [45].

Table 2. Pearson's correlation between extract (Extr), polyphenols, flavonoids, FRAP and EA: EC50 (Extr), determined in crude extract; EC50 (TPC), calculated relative to the total polyphenol.

	TPC	TFC	CTC	FRAP	TEC_{50}	EC_{50} (Extr)	EC_{50} (TPC)	EA
Extr	−0.122	0.063	0.675**	0.347	−0.114	−0.452	−0.063	0.506*
TPC		−0.362	−0.362	−0.377	0.078	0.746**	0.593**	−0.358
TFC			0.642**	0.919**	−0.374	−0.657**	−0.203	0.845**
CTC				0.845**	−0.336	−0.812**	−0.280	0.856**
FRAP					−0.375	−0.827**	−0.285	0.934**
TEC_{50}						0.229	0.150	−0.258
EC_{50} (Extr)							0.571*	−0.794**
EC_{50} (TPC)								−0.282

* The correlation is significant at the 0.05 level (bilateral). ** The correlation is significant at the 0.01 level (bilateral).

3.3. Identification and Quantification of Phenolic Compounds by HPLC-MAS

3.3.1. Phenolic Compouds of *A. boehmii*

The identification of the phenolic compounds revealed that the extract of *A. boehmii* contained important amounts of tannins (**Table 3** and **Figure 2(a)**). Ellagitannins, the major tannins detected in *A. boehmii* extracts, are characterized by their hexahydroxydiphenoyl (HHDP) group which is released by acid hydrolysis and spontaneously lactonizes to ellagic acid [46] [47]. The sequential losses of galloyl (m/z = 152), gallate (m/z = 170) and HHDP (m/z = 301) residues of ellagitannin family [48] allowed to distinguish five compounds. The Hexahydroxydiphenoyl (HHDP)-hexoside (Peak 1), bis-HHDP-glucose isomer (Peak 2), galloyl-hexoside (Peak 3), HHDP-galloylglucose (Peak 5), galloyl-bis-HHDP-glucose (Peak 6), were identified according to other studies [49]-[52].

Ellagic and gallic acid are phenolic acids which are indispensable compounds in the tannin structures [48] [53]. Therefore, the identification of tannins must be accompanied by that of gallic acids and ellagic acids. Thus, peak 10 was suggested as ellagic acid due to its mass spectrometry at m/z (MS m/z = 301) and its fragments with mass spectrometry at m/z (MS/MS m/z = 257, 229) as were reported by [54]. The Compound of peak 4 was proposed as gallic acid (MS m/z = 169, MS/MS m/z = 125) after being compared with a commercial standard and furthermore, a similar fragmentation pattern (Peak 4) was previously described [53], [55].

Table 3. Phenolic compound tentative identification of *A. boehmii*.

Peak number	Rt (min)	λmax (nm)	Molecular ion [M-H] (m/z)	MS/MS fragments (m/z)	Identification
1	3.73	261	481	191, 301, 275	Hexahydroxydiphenoyl (HHDP)-hexoside
2	4.28	263	783	301, 481	bis-HHDP-glucose isomer
3	5.07	262	331	169	galloyl-hexoside
4	5.84	271	169	125	Gallic acid
5	9.63	226, 268	633	301, 463, 257, 275	HHDP-galloylglucose
6	12.69	271	935	301, 275, 633	galloyl-bis-HHDP-glucose
7	20.31	273	575		not identified
8	21.93	262	981		not identified
9	23.09	260, 359	477	315	Isorhamnetin hexoside
10	24.09	252, 365	301	257, 229	Ellagic acid
11	24.88	264, 351	991	845, 653, 639, 301	Quercétine diméthyl éther 3-hydroxyferuoyl-glucosylglucoside-7-O-rhamnoside
12	26.37	253, 358	447	314, 315	Dérivé monoglycosylé de l'isorhamnétine
13	26.71	357	447	301, 179, 151	Quercetin 3-O-rhamnoside
14	28.63	357	575	315	not identified

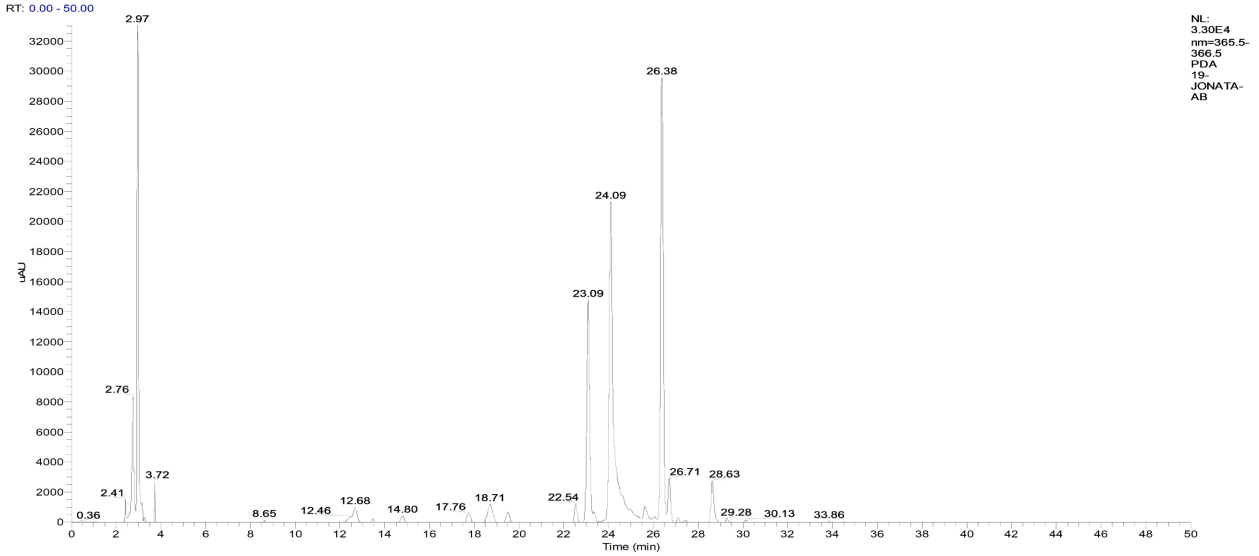
Chromatogram analysis showed also that *A. boehmii* contained also flavonoids, but less important than tannins. whereas peak 9 with MS at m/z 477 and fragments at m/z 315 and 151, was suggested as Isorhamnetin hexoside [54]. Peak 13 was proposed to be Quercetin 3-O-rhamnoside (MS m/z = 447, MS/MS m/z = 301, 179, 151). Previously, such features (MS and MS/MS) have been described [56] on raisins muscadine phenolic compound. Peak 11 was identified as Quercétine diméthyl éther 3-hydroxyferuoyl-glucosylglucoside-7-O-rhamnosid (MS m/z = 991, MS/MS m/z = 845, 653, 639, 301) whereas peak 12 was proposed to be monoglycosylated derivative of isorhamnetin (MS m/z = 447, MS/MS m/z = 314, 315).

3.3.2. Phenolic Compound of *P. angolensis*

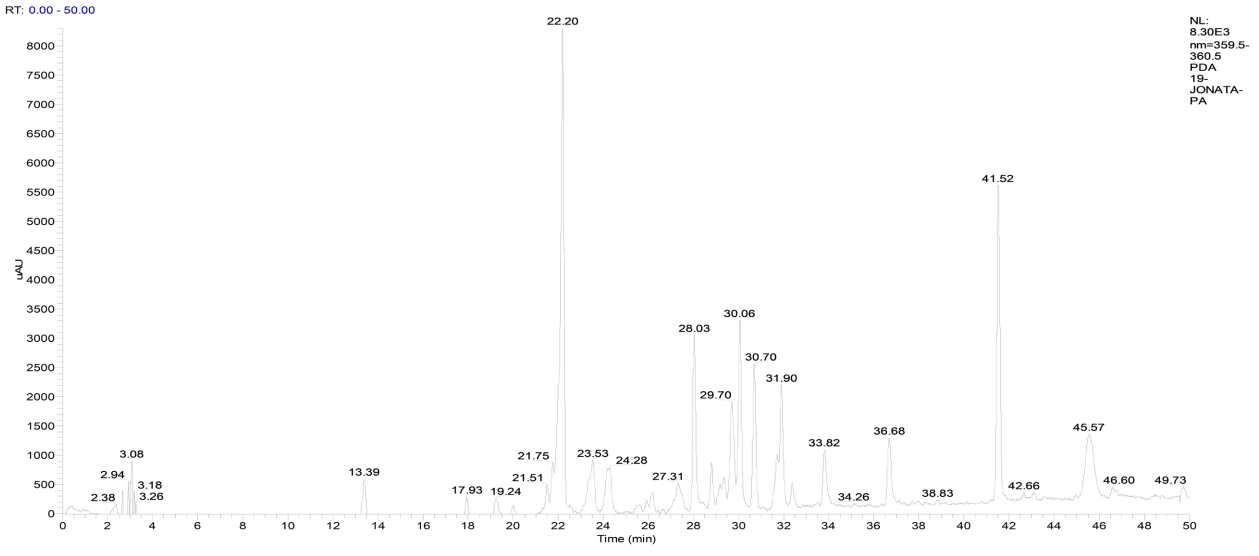
The phenolic composition of *P. angolensis* extract was different from those of *A. boehmie*. For *P. angolensis*, flavonoids were the most prevalent than condensed tannins (**Table 4** and **Figure 2(b)**). However, the colorimetric analyses showed that the two families of polyphenols have almost the similar content (**Figure 2**).

In the present study, 7 flavonoids were observed at absorbance around 366 nm. Compound of peak 2 was presented as pelargonidin-3, 5-diglucoside (MS m/z = 595, MS/MS m/z = 433, 271) by referring on [49] while that of peak 3 was proposed to be proanthocyanidin B1(MS m/z = 595, MS/MS m/z = 433, 271) as describe by [54] Compounds shown by peak 4 and 8 were suggested as kaempferol 3-glucuronide (MS m/z = 463, MS/MS m/z = 287) and cyanidin hexoside (MS m/z = 449, MS/MS m/z = 327, 287) respectively. The examination of the chromatograms and data of [56] led to the identification of peak 9 as myricetin rhamnoside compound (MS m/z = 464, MS/MS m/z = 317). Peaks 7, due to its fragments ob-

served, was proposed to be Apigenin 6-C-arabinosyl-8-C-glucoside (MS m/z = 563, MS/MS m/z = 503, 473, 383, 353). According to [57] in their study performed on determining of *Duchesnea indica* phenolic compound showed that neutral losses of 60, 90 and 180 corresponding to apigenin (270) + hexose (162) + pentose (132). Peak 13 was suggested as Procyanidin dimer (MSm/z = 577, MS/MS m/z = 451, 425, 407, 289) [47]. Regarding tannins, two compounds HHDP-hexoside (Peak 10) and HHDP-glucose (Peak 14) were tentatively identified basing on the procedure used for those of *A. boehmie* and the literature data [49] [58]. Others compounds detected were peak 5 and 6 identified as ellagic acid (MS m/z = 301, MS/MS m/z = 301) and 1 as citric acid derivative (MS m/z = 391, MS/MS m/z = 217, 191, 373) [49] [59].



(a)



(b)

Figure 2. HPLC-MS profiles of phenolic compounds at 366 nm for *A. boehmii* (a) and *P. angolensis* (b) oilcake.

Table 4. Phenolic compound tentative identification of *P. angolensis*.

Peak number	Rt (min)	λmax (nm)	Molecular ion [M-H] (m/z)	MS/MS fragments (m/z)	Identification
1	13.19	366	391	217, 191, 373	Citric acid derivative
2	22.14	366	595	433, 271	Pelargonidin-3,5-diglucoside
3	23.47	366	579	453, 427, 409, 301	Proanthocyanidin B1
4	24.27	366	463	287	Kaempferol 3-glucuronide
5	27.27	366	301	301, 257, 229, 185	Ellagic acid
6	28.02	366	301	301	Ellagic acid
7	28.78	366	563	503, 473, 383, 353	Apigenin 6-C-arabinosyl-8-C-glucoside
8	30.03	366	449	327, 287	Cyanidin hexoside
9	30.7	366	464	317	Myricetin rhamnoside
10	31.72	366	481	301, 275	HHDP-hexoside
11	31.89	366	268		Not identified
12	36.68	366	268		Not identified
13	41.53	366	577	451, 425, 407, 289	Procyanidin dimer
14	43.11	366	481	301	HHDP-glucose
15	45.58	366, 280	807		Not identified

3.4. Antioxidant Activity

The scavenging effect of *A. boehmii*, *P. angolensis* oilcake extracts against the DPPH radical was evaluated using a spectrophotometric method. Thus, the evaluation of the antioxidant activity was performed in two approaches: the determination of the amount of antioxidant necessary to reduce 50% of DPPH• after 60 minutes (Table 5) and the time needed to reach a steady state for a given concentration of extract (Figure 3).

Table 5. Potential for antioxidant activity of seed oilcake extracts of *Anisophyllea boehmii* and *Pycnanthus angolensis*: FRAP, TEC50, EC50 expressed as mg GAE/ml was calculated relative to the total polyphenol, EC50 expressed as mg/ml was determined in crude extract, EA calculated relative to the concentration of crude extract.

Extract/ standard	site	FRAP	DPPH			
		mg GAE/100g MS	EC_{50} (mg GAE/ml)	EC_{50} (mg/ml)	TEC_{50} (min)	EA (ml/µg·mim)
A. boehmii	Trays	3212.1 ± 59.75^{b}	0.01 ± 0.00	0.10 ± 0.01^{a}	10.2 ± 0.3	100 ± 5.6^{b}
	Depression	2537.7 ± 139.0^{c}	0.01 ± 0.00	0.07 ± 0.00^{ab}	11.7 ± 0.4	145.8 ± 21.5^{ab}
	Foothills	3731.0 ± 117.9^{a}	0.01 ± 0.00	0.05 ± 0.00^{b}	13.7 ± 0.2	194.4 ± 21.5^{a}
	Average	3160.3 ± 415.0^{u}	0.01 ± 14.9^{u}	0.08 ± 0.00^{u}	11.8 ± 1.2^{u}	146.7 ± 31.7^{u}
P. angolensis	Musigati	946.9 ± 18.6^{b}	0.02 ± 0.00	1.50 ± 0.46^{a}	20 ± 1.3	13.3 ± 0.6^{b}
	Vugizo	1155.3 ± 3.8^{a}	0.01 ± 0.00	1.06 ± 0.90^{b}	15 ± 0.9	14.1 ± 0.3^{ab}
	Vyanda	877.0 ± 5^{b}	0.01 ± 0.00	1.33 ± 0.65^{b}	16.7 ± 0.1	12.5 ± 0.6^{a}
	Average	993.0 ± 108.1^{v}	0.01 ± 0.00^{u}	1.29 ± 0.15^{v}	17.03 ± 1.9^{v}	13.3 ± 0.5^{w}
Acide ascorbique		-	0.09 ± 0.00^{v}	0.09 ± 0.00^{u}	5.00 ± 0.02^{w}	52.63 ± 3.7^{v}

Results are mean of three replicates (from 3 sites for samples) with standard errors (Mean ± S.E, n = 3, -, not determined). In each column different letters mean significant difference ($p < 0.05$): the letters decreasing in value, a > b > c, were used to compare the regions for one specie while u > v > w were used to compare the global values of the two species between them one hand, and between that of the ascorbic acid on the other hand.

The overall results revealed that the activity in *A. boehmii* extract (0.08 ± 0.00 mg/ml) was higher compared to *P. angolensis* EC_{50} (1.29 ± 0.15 mg/ml) and almost similar to that of the ascorbic acid (0.09 ± 0.00 mg/ml). However, no significant difference ($p < 0.05$) was observed between EC_{50} from *A. boehmii* extract and that from ascorbic acid. Regarding EC_{50} calculated based on total polyphenols, results on *A. boehmii* and *P. angolensis* were the same at 0.01 mg GAE/ml and they were very significantly ($p < 0.05$) higher than that of ascorbic acid. This showed that both species contain significant amounts of bioactive molecules. According to our TEC_{50} results, *A. boehmii* extract (11.8 ± 1.2 minutes) reached a steady state at a time that was not significantly different ($p > 0.05$) from that of *P. angolensis* (17.03 ± 1.9 minutes) while ascorbic acid (5 ± 0.6 minutes) was very significantly effective than both species. Results obtained on EA, by combining EC_{50} and TEC_{50} parameters, were in the following order: *A. boehmii* > ascorbic acid > *P. angolensis*. The statistical test revealed significant difference ($p < 0.05$) between them. Furthermore, a good correlation was found between EC_{50} and EA ($p < 0\ 0.01$, $r^2 = 0.6306$). However, TEC_{50} was not found to be correlated with EA ($R^2 = 0.066$).

Significant differences were also observed between the results of extracts from seeds originating from different regions. Thus, EA of *A. boehemii* harvested from foothills showed the highest activity, followed by those from depression then those from trays with significant differences at $p < 0.05$. The same trend of difference significations observed above was also observed between EC_{50} determined in extracts: foothills > depression > trays. There was no significant difference at $p < 0.05$ between TEC_{50} and EC_{50} calculated in reference to the polyphenol concentrations.

Concerning *P. angolensis*, EA determined in oilcake from Vugizo was found to be significantly higher than those from other sites; order being Vugizo > Musigati > Vyanda ($p < 0.05$). EC_{50} analyzed in oilcake extracts showed highest activity in oilcake from foothills in comparison of the other sites and followed the order as: foothills > trays > depression and significantly different at $p < 0.05$. Value EC_{50} calculated in reference to polyphenol contents and TEC_{50} were almost the same in their respective sites. No significant differences ($p > 0.05$) between these sites for each of these parameters. After the involvement of all parameters in DPPH assay, *A. boehmii* extract was found to be the most powerful antioxidant than that *P. angolensis* with EA of 146.75 ± 34.67 ml/µg·min while *P. angolensis* was 13.3 ± 0.5 ml/µg·mim. This is probably due to the high tannin content of *A. boehmii*. Particularly ellagitannins who is reputed to have important antioxidant, antiviral, antimicrobial, immunomodulatory, antitumor, and hepatoprotective activities [60] [61].

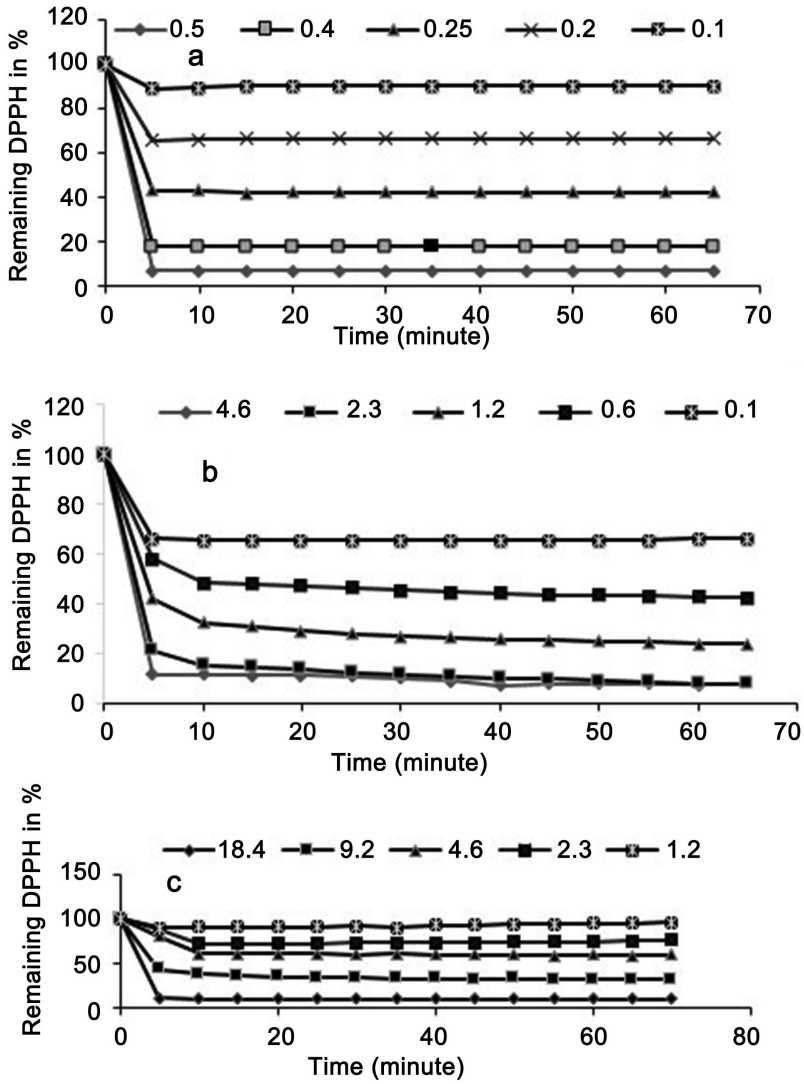


Figure 3. Example for kinetics of Ascorbic Acid (a), *A. boehmii* (b) from foothill of Mirwa and *P. angolensis* (c) from Vyanda comune (A point of the curve is the mean corresponding to the three measurements of each concentration).

Antioxidant activities of a crude extract may be due to different compounds acting through different mechanism to prevent the oxidation of sensitive organic substances. Ferric reducing antioxidant power (FRAP) assay was as complement to the DPPH assay. FRAP assay is also reported to have his high sensitivity and reliability [62].

Through the results of **Table 3**, the same trend observed in DPPH assay has been reproduced in FRAP assay where *A. boehmii* oilcake extract exhibited highest reducing power (3160.3 ± 415.0 mg GAE/100g) compared to *P. angolensis* oilcake extract (993.0 ± 108.1 mg GAE/100g). Furthermore, a highly significant correlation was observed between FRAP and TFC ($p < 0.01$, $r^2 = 0.8446$), and between FRAP and CTC ($p < 0.01$, $r^2 = 0.7132$). Furthermore, in both specie, significant difference between zones was recorded. For *A. boehmii,* results recorded on oil-

cake from foothills were found to be the highest Foothills > Trays > Depression with significant difference at $p < 0.05$. FRAP result of *P. angolensis* extracts from sites were also significantly different ($p < 0.05$) in the following descending order: Vugizo > Musigati > Vyanda. There is no research which is already performed on the evaluation of the antioxidant activity of these wild species using the FRAP method.

The results obtained using these two methods revealed that both species content valuable potent antioxidants. Probably that was due to the high content of the polyphenols. It has been reported that antioxidant activity is correlated strongly with polyphenol content [63] [64], especially with flavonoid phenolic acids, and tannins contents [65] [66]. In this study, flavonoids represent 21.33 and 10% of the total polyphenols, respectively, in *A. boehmii* oilcake extract and in *P. angolensis* oilcake extract while condensed tannins had occupied, in the same order, 39.00% and 15.31%. However, there are lack of studies performed on ethnobotanical and biochemical activity of *A. boehmii*.

4. Conclusion

This study highlights the potential, of two wild species, to have a considerable amount of phenolic compounds and an impressive bioactivity. *A. boehmii* and *P. angolensis* oilcake had been characterized by high contents of total polyphenols respectively 874.97 mg GAE/100g *and* 1089.89 mg GAE/100g. *A. boehmii* contained more tannin compounds of ellagitannin family (5 of 12 identified) while in *P. angolensis* predominated flavonoids (7 of 13 identified). Both species may be exploited as feeding in livestock or human nutrition. In light of these results, *A. boehmii* and *P. angolensis* can provide raw materials as food additives, health supplements and nutraceuticals. The highest proximate component contents were carbohydrate and crude protein, respectively, 63.0% ± 4% and 24.1% ± 3.2% for *A. boehmii,* and 59.49% ± 2.4% and 25.61% ± 2.2 for *P. angolensis*. Further research is required to study toxicity and extract active ingredients.

Acknowledgements

The authors thank the Moroccan-Burundian cooperation for financing this work through a scholarship. University of Burundi (BJA) and Burundian Office for the Protection of the Environment Burundian Office for Environmental Protection (OBPE) are acknowledged for facilitating access to its herbariums.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Kalaba, F.K., Quinn, C.H. and Dougill, A.J. (2013) The Role of Forest Provisioning Ecosystem Services in Coping with Household Stresses and Shocks in Miombo Woodlands, Zambia. *Ecosystem Services*, **5**, 143-148.

https://doi.org/10.1016/j.ecoser.2013.07.008

[2] Jew, E.K.K., Dougill, A.J., Sallu, S.M., O'Connell, J. and Benton, T.G. (2016) Miombo Woodland under Threat: Consequences for Tree Diversity and Carbon Storage. *Forest Ecology and Management*, **361**, 144-153. https://doi.org/10.1016/j.foreco.2015.11.011

[3] do Céu de Madureira, M., Paula Martins, A., Gomes, M., Paiva, J., Proença da Cunha, A. and do Rosário, V. (2002) Antimalarial Activity of Medicinal Plants Used in Traditional Medicine in S. Tomé and Príncipe Islands. *Journal of Ethnopharmacology*, **81**, 23-29. https://doi.org/10.1016/s0378-8741(02)00005-3

[4] Agyare, C., Asase, A., Lechtenberg, M., Niehues, M., Deters, A. and Hensel, A. (2009) An Ethnopharmacological Survey and *in Vitro* Confirmation of Ethnopharmacological Use of Medicinal Plants Used for Wound Healing in Bosomtwi-Atwima-Kwanwoma Area, Ghana. *Journal of Ethnopharmacology*, **125**, 393-403. https://doi.org/10.1016/j.jep.2009.07.024

[5] Hubau, W., Van den Bulcke, J., Kitin, P., Mees, F., Baert, G., Verschuren, D., *et al.* (2013) Ancient Charcoal as a Natural Archive for Paleofire Regime and Vegetation Change in the Mayumbe, Democratic Republic of the Congo. *Quaternary Research*, **80**, 326-340. https://doi.org/10.1016/j.yqres.2013.04.006

[6] Onocha, P.A. and Otunla, E.O. (2010) Biological Activities of Extracts of Pycnanthus angolensis (WELW). *Archives of Applied Sciences Research*, **2**, 186-190.

[7] Abrantes, M., Mil-Homens, T., Duarte, N., Lopes, D., Cravo, P., Madureira, M., *et al.* (2008) Antiplasmodial Activity of Lignans and Extracts from *Pycnanthus angolensis*. *Planta Medica*, **74**, 1408-1412. https://doi.org/10.1055/s-2008-1081317

[8] Sofidiya, M.O. and Awolesi, A.O. (2015) Antinociceptive and Antiulcer Activities of Pycnanthus Angolensis. *Revista Brasileira de Farmacognosia*, **25**, 252-257. https://doi.org/10.1016/j.bjp.2015.05.004

[9] Mansoor, T.A., Ramalho, R.M., Luo, X., Ramalhete, C., Rodrigues, C.M.P. and Ferreira, M.U. (2011) Isoflavones as Apoptosis Inducers in Human Hepatoma Huh-7 Cells. *Phytotherapy Research*, **25**, 1819-1824. https://doi.org/10.1002/ptr.3498

[10] Diehl, M.S., Atindehou, K.K., Téré, H. and Betschart, B. (2004) Prospect for Anthelminthic Plants in the Ivory Coast Using Ethnobotanical Criteria. *Journal of Ethnopharmacology*, **95**, 277-284. https://doi.org/10.1016/j.jep.2004.07.012

[11] Temitope Israel Borokini, (2012) Phytochemical and Ethnobotanical Study of Some Selected Medicinal Plants from Nigeria. *Journal of Medicinal Plants Research*, **6**, 1106-1118. https://doi.org/10.5897/jmpr09.430

[12] Ashidi, J.S., Houghton, P.J., Hylands, P.J. and Efferth, T. (2010) Ethnobotanical Survey and Cytotoxicity Testing of Plants of South-Western Nigeria Used to Treat Cancer, with Isolation of Cytotoxic Constituents from Cajanus Cajan Millsp. Leaves. *Journal of Ethnopharmacology*, **128**, 501-512. https://doi.org/10.1016/j.jep.2010.01.009

[13] Akendengué, B. (1992) Medicinal Plants Used by the Fang Traditional Healers in Equatorial Guinea. *Journal of Ethnopharmacology*, **37**, 165-173. https://doi.org/10.1016/0378-8741(92)90075-3

[14] Chinsembu, K.C. (2015) Plants as Antimalarial Agents in Sub-Saharan Africa. *Acta Tropica*, **152**, 32-48. https://doi.org/10.1016/j.actatropica.2015.08.009

[15] Niyukuri, J., Sindayikengera, S., Nteziryayo, V., Karikurubu, J.F., Muvunyi, R. and Hafashimana, A. (2022) Potential of Indigenous Plants Seed Extracts of *Anisophyllea boehmii* and *Aframomum sanguineum* from Burundi to Protect Against Oil Oxidation. *Brazilian Journal of Biology*, **82**, 1-6.

[16] Nagre, R.D., Ellis, W.O. and Oduro, I. (2012) Yield and Quality of Pycnanthus Kombo Kernel Butter as Affected by Temperature-Moisture Interactions. *African Journal of Food Science*, **6**, 224-231.

[17] Nkengurutse, J., Mansouri, F., Bekkouch, O., Ben Moumen, A., Masharabu, T., Gahungu, G., *et al.* (2019) Chemical Composition and Oral Toxicity Assessment of Anisophyllea Boehmii Kernel Oil: Potential Source of New Edible Oil with High Tocopherol Content. *Food Chemistry*, **278**, 795-804. https://doi.org/10.1016/j.foodchem.2018.11.112

[18] Jin, L., Zhang, Y., Yan, L., Guo, Y. and Niu, L. (2012) Phenolic Compounds and Antioxidant Activity of Bulb Extracts of Six Lilium Species Native to China. *Molecules*, **17**, 9361-9378. https://doi.org/10.3390/molecules17089361

[19] Perez, M., Robbins, M.E., Revhaug, C. and Saugstad, O.D. (2019) Oxygen Radical Disease in the Newborn, Revisited: Oxidative Stress and Disease in the Newborn Period. *Free Radical Biology and Medicine*, **142**, 61-72. https://doi.org/10.1016/j.freeradbiomed.2019.03.035

[20] Ndrepepa, G. (2019) Myeloperoxidase—A Bridge Linking Inflammation and Oxidative Stress with Cardiovascular Disease. *Clinica Chimica Acta*, **493**, 36-51. https://doi.org/10.1016/j.cca.2019.02.022

[21] Zhong, S., Li, L., Shen, X., Li, Q., Xu, W., Wang, X., *et al.* (2019) An Update on Lipid Oxidation and Inflammation in Cardiovascular Diseases. *Free Radical Biology and Medicine*, **144**, 266-278. https://doi.org/10.1016/j.freeradbiomed.2019.03.036

[22] Pandya, S., Iyer, P., Gaitonde, V., Parekh, T. and Desai, A. (1999) Chemotaxis of Rhizobium SP.S2 Towards Cajanus Cajan Root Exudate and Its Major Components. *Current Microbiology*, **38**, 205-209. https://doi.org/10.1007/pl00006788

[23] Morita, M., Naito, Y., Yoshikawa, T. and Niki, E. (2017) Antioxidant Capacity of Blueberry Extracts: Peroxyl Radical Scavenging and Inhibition of Plasma Lipid Oxidation Induced by Multiple Oxidants. *Journal of Berry Research*, **7**, 1-9. https://doi.org/10.3233/jbr-170152

[24] Santhakumar, A.B., Bulmer, A.C. and Singh, I. (2014) A Review of the Mechanisms and Effectiveness of Dietary Polyphenols in Reducing Oxidative Stress and Thrombotic Risk. *Journal of Human Nutrition and Dietetics*, **27**, 1-21. https://doi.org/10.1111/jhn.12177

[25] Oak, M., Auger, C., Belcastro, E., Park, S., Lee, H. and Schini-Kerth, V.B. (2018) Potential Mechanisms Underlying Cardiovascular Protection by Polyphenols: Role of the Endothelium. *Free Radical Biology and Medicine*, **122**, 161-170. https://doi.org/10.1016/j.freeradbiomed.2018.03.018

[26] Shahidi, F. and Alexander, D.M. (1998) Green Tea Catechins as Inhibitors of Oxidation of Meat Lipids. *Journal of Food Lipids*, **5**, 125-133. https://doi.org/10.1111/j.1745-4522.1998.tb00112.x

[27] Zheng, L., *et al.* (2012) Antioxidant Activity of Lotus (Nelumbo Nucifera Gaertn.) Receptacles of Eleven Cultivars Grown in China. *Journal of Medicinal Plants Research*, **6**, 1902-1911.

[28] Khan, H., Sureda, A., Belwal, T., Çetinkaya, S., Süntar, İ., Tejada, S., *et al.* (2019) Polyphenols in the Treatment of Autoimmune Diseases. *Autoimmunity Reviews*, **18**, 647-657. https://doi.org/10.1016/j.autrev.2019.05.001

[29] Yi, J., Li, S., Wang, C., Cao, N., Qu, H., Cheng, C., *et al.* (2019) Potential Applications of Polyphenols on Main Ncrnas Regulations as Novel Therapeutic Strategy for Cancer. *Biomedicine & Pharmacotherapy*, **113**, Article 108703.

https://doi.org/10.1016/j.biopha.2019.108703

[30] Xu, Z., Sun, T., Li, W. and Sun, X. (2017) Inhibiting Effects of Dietary Polyphenols on Chronic Eye Diseases. *Journal of Functional Foods*, **39**, 186-197. https://doi.org/10.1016/j.jff.2017.10.031

[31] Li, Y., Jiang, X., Hao, J., Zhang, Y. and Huang, R. (2019) Tea Polyphenols: Application in the Control of Oral Microorganism Infectious Diseases. *Archives of Oral Biology*, **102**, 74-82. https://doi.org/10.1016/j.archoralbio.2019.03.027

[32] Samodien, E., Johnson, R., Pheiffer, C., Mabasa, L., Erasmus, M., Louw, J., *et al.* (2019) Diet-induced Hypothalamic Dysfunction and Metabolic Disease, and the Therapeutic Potential of Polyphenols. *Molecular Metabolism*, **27**, 1-10. https://doi.org/10.1016/j.molmet.2019.06.022

[33] AOAC (1990) Official Methods of Analyses of Association of Analytical Chemist. 15th Edition, AOAC.

[34] Górnaś, P., Dwiecki, K., Siger, A., Tomaszewska-Gras, J., Michalak, M. and Polewski, K. (2016) Contribution of Phenolic Acids Isolated from Green and Roasted Boiled-Type Coffee Brews to Total Coffee Antioxidant Capacity. *European Food Research and Technology*, **242**, 641-653. https://doi.org/10.1007/s00217-015-2572-1

[35] Kouka, P., Chatzieffraimidi, G., Raftis, G., Stagos, D., Angelis, A., Stathopoulos, P., *et al.* (2018) Antioxidant Effects of an Olive Oil Total Polyphenolic Fraction from a Greek Olea Europaea Variety in Different Cell Cultures. *Phytomedicine*, **47**, 135-142. https://doi.org/10.1016/j.phymed.2018.04.054

[36] Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, **16**, 144-158. https://doi.org/10.5344/ajev.1965.16.3.144

[37] Amezouar, F., Badri, W., Hsaine, M., Bourhim, N. and Fougrach, H. (2013) Évaluation des activités antioxydante et anti-inflammatoire de Erica arborea L. du Maroc. *Pathologie Biologie*, **61**, 254-258. https://doi.org/10.1016/j.patbio.2013.03.005

[38] Zou, Y., Chang, S.K.C., Gu, Y. and Qian, S.Y. (2011) Antioxidant Activity and Phenolic Compositions of Lentil (Lens Culinaris Var. Morton) Extract and Its Fractions. *Journal of Agricultural and Food Chemistry*, **59**, 2268-2276. https://doi.org/10.1021/jf104640k

[39] Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Science and Technology*, **28**, 25-30. https://doi.org/10.1016/s0023-6438(95)80008-5

[40] Sánchez-Moreno, C., Larrauri, J.A. and Saura-Calixto, F. (1998) A Procedure to Measure the Antiradical Efficiency of Polyphenols. *Journal of the Science of Food and Agriculture*, **76**, 270-276. https://doi.org/10.1002/(sici)1097-0010(199802)76:2<270::aid-jsfa945>3.3.co;2-0

[41] Mouhoubi-Tafinine, Z., Ouchemoukh, S. and Tamendjari, A. (2016) Antioxydant Activity of Some Algerian Honey and Propolis. *Industrial Crops and Products*, **88**, 85-90. https://doi.org/10.1016/j.indcrop.2016.02.033

[42] Balasundram, N., Sundram, K. and Samman, S. (2006) Phenolic Compounds in Plants and Agri-Industrial By-Products: Antioxidant Activity, Occurrence, and Potential Uses. *Food Chemistry*, **99**, 191-203. https://doi.org/10.1016/j.foodchem.2005.07.042

[43] Nkengurutse, J., Houmy, N., Mansouri, F., Ben Moumen, A., Serghini Caid, H. and Khalid, A. (2016) Preliminary Chemical Characterization of Amashindwi (Anisophyllea Boehmii Engl.) Kernels and Kernel Oil. *Journal of Materials and Environ-*

mental Science, **7**, 1996-2005.

[44] Anttonen, M.J. and Karjalainen, R.O. (2005) Environmental and Genetic Variation of Phenolic Compounds in Red Raspberry. *Journal of Food Composition and Analysis*, **18**, 759-769. https://doi.org/10.1016/j.jfca.2004.11.003

[45] Marinova, D., Ribarova, F. and Atanassova, M. (2005) Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. *Journal of University of Chemical Technology and Metallurgy*, **40**, 255-260.

[46] Karlińska, E., Pecio, Ł., Macierzyński, J., Stochmal, A. and Kosmala, M. (2019) Structural Elucidation of the Ellagitannin with a Molecular Weight of 2038 Isolated from Strawberry Fruit (Fragaria Ananassa Duch.) and Named Fragariin A. *Food Chemistry*, **296**, 109-115. https://doi.org/10.1016/j.foodchem.2019.05.191

[47] Regueiro, J., Sánchez-González, C., Vallverdú-Queralt, A., Simal-Gándara, J., Lamuela-Raventós, R. and Izquierdo-Pulido, M. (2014) Comprehensive Identification of Walnut Polyphenols by Liquid Chromatography Coupled to Linear Ion Trap–orbitrap Mass Spectrometry. *Food Chemistry*, **152**, 340-348. https://doi.org/10.1016/j.foodchem.2013.11.158

[48] Hukkanen, A.T., Kokko, H.I. Buchala, A.J., *et al.* (2007) Benzothiadiazole Induces the Accumulation of Phenolics and Improves Resistance to Powdery Mildew in Strawberries. *Journal of Agricultural and Food Chemistry*, **55**, 1862-1870.

[49] Mena, P., Calani, L., Dall'Asta, C., Galaverna, G., García-Viguera, C., Bruni, R., *et al.* (2012) Rapid and Comprehensive Evaluation of (Poly)phenolic Compounds in Pomegranate (*Punica granatum* L.) Juice by UHPLC-MS. *Molecules*, **17**, 14821-14840. https://doi.org/10.3390/molecules171214821

[50] Simirgiotis, M.J. and Schmeda-Hirschmann, G. (2010) Determination of Phenolic Composition and Antioxidant Activity in Fruits, Rhizomes and Leaves of the White Strawberry (Fragaria Chiloensis Spp. Chiloensis Form Chiloensis) Using HPLC-DAD–ESI-MS and Free Radical Quenching Techniques. *Journal of Food Composition and Analysis*, **23**, 545-553. https://doi.org/10.1016/j.jfca.2009.08.020

[51] John, L.M. and Gene, J.G. (1991) Ellagic Acid, an Anticarcinogen in Fruits, Especially in Strawberries: A Review. *HortScience*, **26**, 10-14.

[52] Aaby, K., Ekeberg, D. and Skrede, G. (2007) Characterization of Phenolic Compounds in Strawberry (*Fragaria* × *ananassa*) Fruits by Different HPLC Detectors and Contribution of Individual Compounds to Total Antioxidant Capacity. *Journal of Agricultural and Food Chemistry*, **55**, 4395-4406. https://doi.org/10.1021/jf0702592

[53] Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, D.M. and Kader, A.A. (2000) Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. *Journal of Agricultural and Food Chemistry*, **48**, 4581-4589. https://doi.org/10.1021/jf000404a

[54] Abu-Reidah, I.M., Ali-Shtayeh, M.S., Jamous, R.M., Arráez-Román, D. and Segura-Carretero, A. (2015) HPLC–DAD–ESI-MS/MS Screening of Bioactive Components from Rhus Coriaria L. (Sumac) Fruits. *Food Chemistry*, **166**, 179-191. https://doi.org/10.1016/j.foodchem.2014.06.011

[55] Romani, A., Campo, M. and Pinelli, P. (2012) HPLC/DAD/ESI-MS Analyses and Anti-Radical Activity of Hydrolyzable Tannins from Different Vegetal Species. *Food Chemistry*, **130**, 214-221. https://doi.org/10.1016/j.foodchem.2011.07.009

[56] Lee, J., Johnson, J.V. and Talcott, S.T. (2005) Identification of Ellagic Acid Conjugates and Other Polyphenolics in Muscadine Grapes by HPLC-ESI-MS. *Journal of Agricultural and Food Chemistry*, **53**, 6003-6010. https://doi.org/10.1021/jf050468r

[57] Zhu, M., Dong, X. and Guo, M. (2015) Phenolic Profiling of Duchesnea Indica Combining Macroporous Resin Chromatography (MRC) with HPLC-ESI-MS/MS and ESI-IT-MS. *Molecules*, **20**, 22463-22475. https://doi.org/10.3390/molecules201219859

[58] Mullen, W., Yokota, T., Lean, M.E.J. and Crozier, A. (2003) Analysis of Ellagitannins and Conjugates of Ellagic Acid and Quercetin in Raspberry Fruits by LC-MSN. *Phytochemistry*, **64**, 617-624. https://doi.org/10.1016/s0031-9422(03)00281-4

[59] Cristofori, V., Caruso, D., Latini, G., Dell'Agli, M., Cammilli, C., Rugini, E., *et al.* (2011) Fruit Quality of Italian Pomegranate (*Punica granatum* L.) Autochthonous Varieties. *European Food Research and Technology*, **232**, 397-403. https://doi.org/10.1007/s00217-010-1390-8

[60] Feldman, K.S. (2005) Recent Progress in Ellagitannin Chemistry. *Phytochemistry*, **66**, 1984-2000. https://doi.org/10.1016/j.phytochem.2004.11.015

[61] Miyamoto, K., Nomura, M., Sasakura, M., Matsui, E., Koshiura, R., Murayama, T., *et al.* (1993) Antitumor Activity of Oenothein B, a Unique Macrocyclic Ellagitannin. *Japanese Journal of Cancer Research*, **84**, 99-103. https://doi.org/10.1111/j.1349-7006.1993.tb02790.x

[62] Shahidi, F. and Zhong, Y. (2015) Measurement of Antioxidant Activity. *Journal of Functional Foods*, **18**, 757-781. https://doi.org/10.1016/j.jff.2015.01.047

[63] Zheng, W. and Wang, S.Y. (2001) Antioxidant Activity and Phenolic Compounds in Selected Herbs. *Journal of Agricultural and Food Chemistry*, **49**, 5165-5170.

[64] Chu, Y., Sun, J., Wu, X. and Liu, R.H. (2002) Antioxidant and Antiproliferative Activities of Common Vegetables. *Journal of Agricultural and Food Chemistry*, **50**, 6910-6916. https://doi.org/10.1021/jf020665f

[65] Tcheghebe, O.T., Seukep, A.J. and Tatong, F.N. (2016) A Review on Traditional Uses, Phytochemical Composition and Pharmacological Profile of Canarium Schweinfurthii Eng. *Natural Sciences*, **14**, 17-22.

[66] Son, S. and Lewis, B.A. (2002) Free Radical Scavenging and Antioxidative Activity of Caffeic Acid Amide and Ester Analogues: Structure-Activity Relationship. *Journal of Agricultural and Food Chemistry*, **50**, 468-472. https://doi.org/10.1021/jf010830b