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Research Article

Season and Ecotype Variation on Phenolic Contents, Antioxidant and Antidiabetic Activities of Diospyros Mespiliformis

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ABSTRACT

Phenolic compounds in plants act as free radical scavengers and contribute to diabetes prevention. However, the production of plants is influenced by various factors. This study investigates the impact of season and ecology on the phenolic compound content, as well as the antioxidant and anti-diabetic activities of Diospyros mespiliformis (D. mespiuliformis) extracts.Samples (leaves, stem bark, and roots) were collected from three locations during two seasons. Phenolic compound contents were quantified using the FolinCiocalte ureagent and flavonoid content with aluminum trichloride methods. Antioxidant activity was assessed using chromogen $DPPH^{\bullet}$, $ABTS^{+\bullet}$, and Fe^{3+}. The anti-diabetic potential of the plant was evaluated through α-amylase inhibition and glucose adsorption tests. In leaves and roots, extracts from dry-season samples showed the highest total flavonoid content. In contrast, the wet season was favorable for the production of these compounds in the bark. The $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ radical scavenging of the extracts varied according to the season and the place where the samples were harvested ($p < 0.05$). Fe^{3+}chelation activity was higher in dry season samples collected at Dirabakoko. The ethylacetate fraction exhibited the strongest inhibition of α-amylase (IC_{50}=0.185 ± 0.069 mg/mL), while the diethylether fractions demonstrated greater glucose adsorption capacity (366.66 ± 11.57 mM/g). The biomolecules in the ethylacetate and diethylether fractions of that plant could potentially be utilized to prevent oxidative stress and diabetes.

Keywords: flavonoid, glucose adsorption, alpha-amylase

INTRODUCTION

One of the main complicating factors in diabetes is the aggression of free radicals against the body's cells (Chakrabortyet al., 2013 ;Punnagaiand Glory Josephine, 2018)causing an imbalance known as oxidative stress (Sieset al. 2017 ;Balde, 2018 ; Checkourietal. 2020). The body's natural defenses, obtained from the diet, play a crucial role in counteracting this situation. Plants, being potential sources of antioxidants, can contribute to preventing diabetes by controlling oxidative stress with bioactive compounds. Studies have confirmed that plant extracts, rich in polyphenols, act as effective scavengers of free radicals, offering the potential to prevent diabetes through their antioxidant power (Marwahet al. 2007). Polyphenols, known for their antioxidant and hypoglycemic properties, are key compounds in this context (Punnagaiand Glory Josephine, 2018 ;Joshi et al. 2021).Notably, plants like D. mespiliformis contain polyphenols and possess antioxidants(Nacoulma, 1996 ;Belemtougriet al. 2006 ;Ahmed and Mahmud, 2017 ;Adamuet al. 2020). The polyphenol content of a plant is influenced by various factors(Radušienėet al. 2012 ;Pacifico et al. 2015), leading to variability in both quality and quantity of chemical compounds (Pacifico et al. 2015;Ahmed and Mahmud, 2017). This variability may impact the biological properties of plants. However, existingstudies on D.mespiliformis have primarily focused on samples from a single site or period. Therefore, this study aims to assess how seasonal and ecological variations influence the

polyphenol content and biological activities of D. mespiliformis.

MATERIALS AND METHODS

Chemicals, reagent and instruments

Several solvents were used in this study. These were methanol (CHEM-LAB, Belgium),ethanol (CARLO-ERBA, France), ethylacetate (CARLO-ERBA, France), petroleumether (CARLO-ERBA, France) and diethylether (CARLO-ERBA, France).
The reagents used in this study consisted of 3,5-dinitrosalicylic acid (Sigma-Aldrich-Chemie, Steinheim, China), FolinCiocalteu Reagent (Merck KGaA, HC9059050I, Germany), 2,2-diphenyl-picrylhydrazyl radical (Thermo Fisher, P19F002, Germany), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonates (Sigma-Aldrich-Chemie, Steinheim, China), aluminium trichloride,trichloroacetic acid (CARLO-ERBA, France), ascorbic acid (Sigma-Aldrich-Chemie, Steinheim, China), gallic acid (Sigma-Aldrich-Chemie, Steinheim, China), quercetin (Sigma-Aldrich-Chemie, Steinheim, China), starch (CARLO-ERBA, France), glucose, potassium hexacyanoferrate (CARLO-ERBA, France), ferrictrichloride (CARLO-ERBA, France) and calcium chloride.
The megamylase (3000 U. CEIP) was obtained from FRILAB, Geneve. The GOD KIT was purchased from Lab-Kit, Spain. A centrifuge (Hettich MIKRO 220R) was acquired from Hettich Mikro, Germany. Elisamicroplate readers were procured from Biobase, BK-EL10C, MBY10C22040496, China. A spectrophotometer (Mindray BA, 88A), was obtained from Shenzhen Mindray bio-medical electronics, Hamburg-Germany. A hot plate (701546-Economy hot plate, 1500 W; 230 V) was purchased from Gerash. An electrothermalin cubator (Model DNP) was procured from Zhenjiang Huaying instrument and electrical equipment, in China.

Plant material and extraction

Sample collection and preparation

The work focused on leaves, trunkbark and roots of D. mespiliformis. The samples were collected in september and november at Poundou (12°11′28″ N), Dindéresso(11°16′34.507″N) and Diarabakoko (10°46′73.933″ N). Septemberis a wet season and novemberis a dry season in Burkina Faso (Thiombiano and Kampmann, 2010 ; **c**limate knowledge portal.worldbank.org). This specie was identified at the Plant Biology and Ecology Laboratoryof the Nazi BONI University, where specimens of D. mespiliformis (UNB959) are on deposit. Following harvest, the specimens were washed with distilled water and dried underlaboratory conditions for three weeks. Once dried, they were pulverized and ground into a powder.

Crude extraction

The process of Ranillaet al. (2010)was used for extracting potentially bioactive compounds, with a slight modification. To this end, the sample powder (5g) wash omogenized in distilled water (100ml) and boiled for 30 minutes on a hot plate. Filtration was performed using Wathman paper n°1, and the solution was subsequently centrifuged at 6530 r/min at 4 °C for 30 min. The resulting supernatant under went concentration and drying in an incubator at 45 °C.

Organic fractions

In an Erlenmeyer flask, 20 g of sample powder was soaked in 200 ml of a 70 % methanol solution for 48 h. The obtained filtrate was treated with 30 ml of petroleumether to eliminate chlorophyll and lipids. After settling, the aqueous phase was collected. To this phase, 30 ml of diethylether was added, and after further decantation, the ether phase was recovered, constituting the diethylether fraction. The aqueous phase from the ether fraction wasthen mixed with 30 ml of ethylacetate and stirred for 10 min. This fraction underwent acid hydrolysiswith 1 ml of 7 % sulfuric acid for 2 h. After decantation, the ethylacetate phase was recovered, constituting the fraction rich in monoglycosylatedflavonoids.

Determination of total phenolic contents

Thereaction mixture, consisting of extract (50µl ; 1mg/ml), ethanol (50µl ; 95%), distilled water (25µl) and Folinciocalteureagent (25µl; 1N), was incubated at room temperature for 5 min.Next, Na_2CO_3 (50 µL ; 5%) wasadded to the previous solution and the whole set was incubated in the dark for 60 min. After incubation, absorbances were measured at 725 nm using an ELISA plate reader spectrophotometer. A blank was prepared similarly, substituting $Na_2CO_3$with distilled water. The results, expressed as milligram equivalents of gallic acid per gram of dry extract (mg EAG / g), were obtained from three readings. A calibration curve($y = 10.84x - 0.039$; $R^2 = 0.996$) based on gallic acid (0.00625-0.2 mg/ml) was used(Ranillaet al. 2010).

Total flavonoidsassay

Aluminumtri chloride was used to quantify flavonoids in the various extracts. In 96-well plates, the reaction mixture of extract (100µl;

1mg/ml) and $AlCl_3$ (2%) was incubated for 10 min. After incubation, absorbances wereread at 430 nm using an ELISA plate reader spectrophotometer. Flavonoid content was obtained from the calibration curve($y = -0.1095x + 1.2653;\ R^2 = 0.9625$) established with quercetin (0-50 mg/l). Assays were performed in triplicate and results were expressed in milligrams of quercetin equivalent per 1 g of dry extract (QE / g)(Zengin andAktumsek, 2014).

Antioxidant assays

DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) free radical scavenging assay

The method used by Kwonet al. (2006)was inspired to assess the DPPH• radical scavenging capacity of extracts. However,small modifications were made. A mixture of DPPH solution (750µl; 60 µM) and extract (0.020-5 mg/ml) wasincubated in the dark for 15 min. Absorbances were read at 517nm after incubation using an ELISA plate reader spectrophotometer. A control using the same procedure but excluding the extract was used. Assays were performed five times usingascorbic acid and quercetin as standard. The inhibition percentage (I %), which corresponds to the percentage of DPPH discoloration in ethanol solution, was calculated from the following formula.

$$I\,\% = \frac{(Absblank - Abstest)}{Absblank} x100$$

Where

Abs control: absorbency at 517nm

ABS test: extractabsorbency at 517nm

The IC_{50}, indicating the extract values causing the loss of 50% of DPPH• free radicals, was determined using GraphPad_Prisme_8.0.2 software.

ABTS$^{+\bullet}$ radical discolorationtest

A reaction medium comprisingextract (20 µl) and $ABTS^{+\bullet}$ solution(1980 µl) was prepared and incubated in the dark for 15 min. After incubation, absorbances were read at 734 nm using an ELISA plate reader spectrophotometer (Biobase, BK-EL10C, MBY10C22040496, China). Ascorbic acid (0-100 µg / ml) wasused to produce the standard curve of equation($y = -0.834x + 0{,}654;\ R^2 = 0{,}990$). The tests were repeated five times.Free radical scavenging capacitywasexpressed in micromolascorbic acid equivalent per 1 g dry extract (µmolEAA / g)(Bharadwaj, 2019 ;Gonçaloet al. 2020).

Ferricreducing antioxidant power (FRAP) assay

The modified method of ability of Hinneburget al. (2006)was used with a few modifications. A mixture consisting of extract (0.25 ml), 0.625 mL phosphate buffer (0.2 M, pH 6.6) and 0.625 ml potassium hexacyanoferrate (1%) was prepared and incubated for 30 min at 50°C. A volume of 0.625 mltrichloroacetic acid (10%) wasa dded and the whole was centrifuged at 3000 rpm for 10 min. After adding 0.625ml of distilled water to the supernatant (0.625ml), 0.125ml of irontrichloride (0.1%) was added and absorbances wereread at 700 nm. Ascorbic acid (0.00625-0.2 µg/ml) was used to produce the calibration curve($y = 6.149x + 0.245;\ R^2 = 0.998$.Determination of Fe^{3+}reducing activity was carried out five times, and the average was expressed as µmol ascorbic acid equivalent per gram of dry extract (µmoL EAA) / g ES).

Antidiabeticassays

Glucose adsorption test

Glucose adsorption capacity was assessed based on the method used by Rehmanet al. (2018). A few modifications were made. Each extract (50 µg/ml) was prepared in glucose (5, 10, 15, 20 and 30 mM) respectively. After 6 h incubation at 37°C, the solutions were centrifuged at 4800 rpm for 20 min. The GOD KIT (CHEMELEX LABKIT) was then used to determine glucose concentration using a spectrophotometer (Mindray BA, 88A). The average of threereadingswasused to determine the amount of glucose adsorbed, using the formula below$\text{Glucose bond} = \frac{(G1-G6)xV}{m}$

Where:

- G1 represents glucose concentration at the initial time.
- G6 is glucose concentration after 6 h of incubation.
- V is the volume of the solution.
- m is the mass of the extract.

α-amylase inhibition assay

The method of Dastjerdiet al. (2015)was employed with adaptations to evaluate alpha-amylase inhibitory activity. Thus, a reaction mixture consisting of 7 mlstarch (1%), 2 ml sodium phosphate buffer (20 mM, pH 6.9), 1 mlextract at different concentrations (1; 0.75; 0.5; 0.375 and 0.25 mg/ml), and 100 µL α-amylase (3 U/ml) wasincubated at 37°C for 60 min. After incubation, 1 ml DNSA (96 mM) was added to 1.5 ml of this mixture and heated in a water bath at 100°C for 5 min. Aftercooling, the resulting absorbance wasmeasured at 540 nm.

Each assay was performed in triplicate. Results were expressed as percentage inhibition (%I) according to the formula below:

$$\%\,I = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{x}100$$

The control wasp repared in the same way except that extract was replaced by PBS. For the extract blank, buffer (100 µl) was used instead of enzyme. Starch and PBS made up the control blank.

Statistical analysis

Results were presented as mean ± SD from five trials.Phenolic compound levels were compared by harvest period and harvest site. Analysis of variance ANOVA followingTurkey's multiple comparison test was used. Means are considered statistically significant at the $p < 0.05$ threshold. All graphs were produced using GraphPad_Prisme_9.2.0.332x64 software.

RESULTS

Totalphenolic and flavonoid contents

The results indicate varying total phenolic content among different organs of D. mespilifor misextracts (Figure 1). Notably, leaves (Figure 1, Leaves) exhibited the highest levels, in september (223.54 ± 32.25 mgGAE/g dry extract) and november (215.85 ± 36.79 mgGAE/g dry extract) from Dindéresso. However, there were no significant differences in content with in each organ based on the month of harvest ($p > 0.05$). Furthermore, no significant differences between the september ($p > 0.05$) and november ($p > 0.05$), harvests across the three localities.

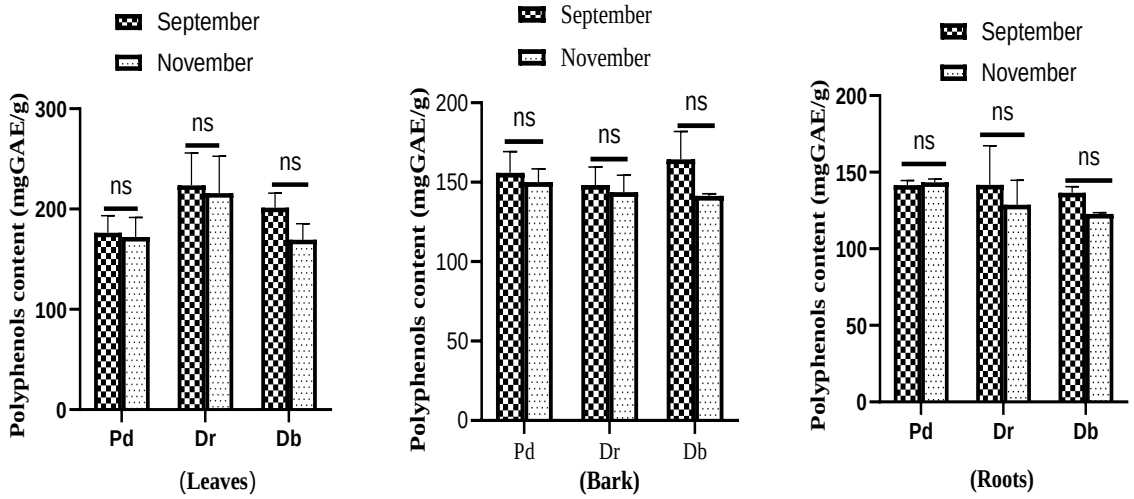


Figure 1: Total phenolic content of aqueousdecoction extracts of leaves, bark, and roots of D.mespiliformis sampled during two seasons and in threedifferent ecotypes. Results are means ± SD (n=3) and expressed in milligram equivalents of gallic acid per gram (mgGAE/g) of dry extract. Phenolic contents were compared with each other according to harvesting period and site, using ANOVA variance with Turkey's multiple comparison test. Means are considered statistically significant at the $p < 0.05$ threshold. The symbols ns indicatethere are no differences in content. Pd denotes the Poundou site; Dr denotes the Dindéresso site; Dbdenotes the Diarabakoko site.

Concerning flavonoids in the plant (Figure 2), the highest content wasfound in the leaves (Figure 2, Leaves). The extract from Poundou collected in November (57.83 ± 3.77 mgQE/g dry extract) exhibited the highest content. Variability in content based on the harvesting period was observed ($p < 0.05$). The dry period favored biosynthesis of these compounds in leaves and roots (Figure 2, Roots), while in the barks (Figure 2, Barks), the wet period produced the richest flavonoid samples. Flavonoid content in the leaves and bark at the Diarabakoko site remained consistent across harvesting periods. However, for the same sampling period in September ($p > 0.05$) and november ($p > 0.05$) respectively, flavonoid content in leavesdid not change at each site. In bark and roots, a significant difference in content between the three sites was observed for the septemberharvest ($p < 0.05$). This variability persisted for the november harvesting period at each site ($p < 0.05$).

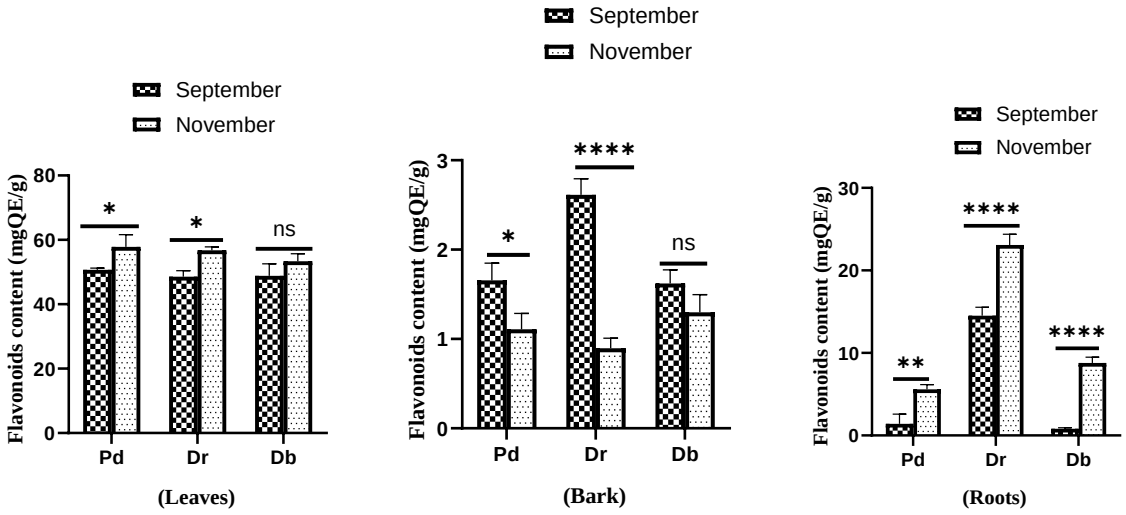
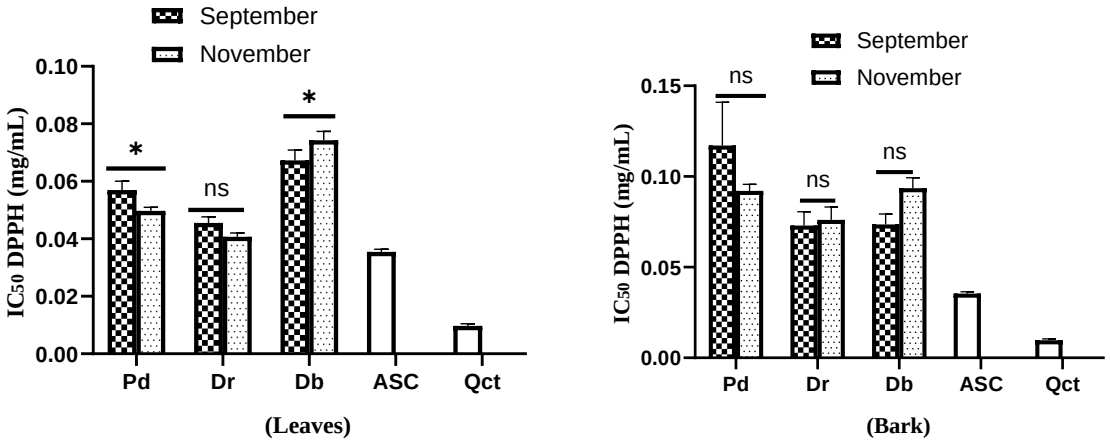


Figure 2: Total flavonoid content of aqueous decoction extracts of leaves, bark, and roots of D.mespiliformis sampled during two seasons and in three different ecotypes. Results are means ± SD (n=3) and expressed in milligramquercetin equivalent per gram dry extract (mgQE/g). Total flavonoid levels were compared with each other according to harvesting period and site, using ANOVA variance withTurkey's multiple comparison test. Means are considered statistically significant at the p < 0.05 threshold. The symbols ns indicate there are no differences in content. Significant differences are noted * if p < 0.05; ** if p < 0.01; * if p < 0.001; **** if p < 0.0001. Pd denotes the Poundou site; Dr denotes the Dindéresso site; Dbdenotes the Diarabakoko site.**

Effect of seasonal and ecotype variation on antioxidant activities

DPPH• scavenging activity

DPPH• free radical inhibition wasobserved (Figure 3). The most effective extracts against DPPH•radicals have been obtained with leaf samples (Figure 3, Leaves). The extract from Dindéresso sampled in November exhibited the highest DPPH• reduction (IC_{50}=37.5 ± 0.00 µg/ml) statistically comparable to ascorbic acid (35.45 ± 0.00 µg/ml) used as the standard. A significant difference was observed between the IC_{50} values of leaf extracts from samples harvested in September and November of Poundou and Diarabakoko respectively ($p < 0.05$). This trend was extended to Poundou root extracts (Figure 3, Roots) ($p < 0.05$). However, in barkextracts (Figure 3, Bark), no significant difference was noted in the trapping activity between the September and November extracts.The analysis between sites revealed a significant variation in the activity of extracts collected in the same season ($p < 0.05$).



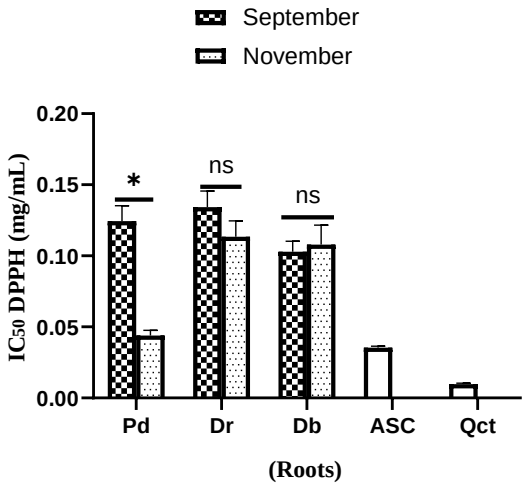


Figure 3: DPPH•scavenging activity of aqueous decoction extracts of leaves, bark, and roots of D.mespiliformis. These results, given at 50 % radical inhibitory concentration (IC_{50}) and expressed as in milligrams per milliliter (mg/ml) are means ± SD (n=5). They are compared by season and harvesting site and by standard (Ascorbic acid and quercetin). Means are considered statistically significant at the p < 0.05 threshold. The symbols ns indicate there are no differences in content, and * if p < 0.05 represented the level differences obtained. Pd: Poundou; Dr: Dindéresso; Db: Diarabakoko; ASC: Ascorbique Acid; Qct: Quercetin.

$ABTS^{+\bullet}$ inhibition

In $ABTS^{+\bullet}$ inhibition (Figure 4), leaf extracts (Figure 4, Leaves) demonstrated the highest activity. The Dindéresso site presented the most active extract (815.01 ± 10.28 µmolAAE/g dry extract) harvested in november, although not statistically different from September extracts of Poundou and Diarabakoko ($p < 0.05$). In roots (Figure 4, Roots), the highest activities were observed with extracts sampled in the dry period, showing a significant difference between the september and november extracts. However, this variability, based on the sampling period, is site and organ-dependent. For the same harvesting site, variability was observed in leaves ($p < 0.05$). Starting from the same harvesting period in all sites in september, there were no significant differences in eachorgan ($p > 0.05$). However, for the November period, the season variation influenced $ABTS^{+\bullet}$ inhibition activity in the plant ($p < 0.05$).

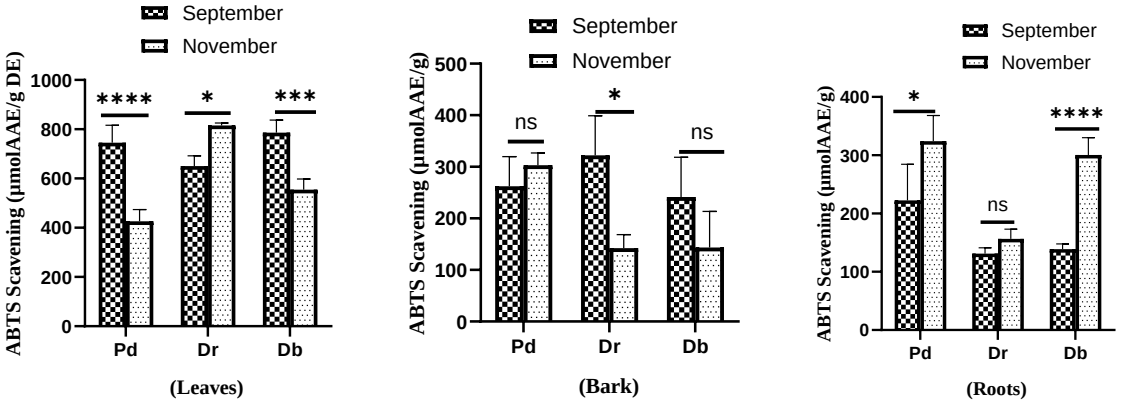


Figure 4: $ABTS^{+\bullet}$scavenging activity of aqueous decoctione xtracts (leaves, bark, and roots) of D.mespiliformis. Results are means ± SD (n=5) and expressed as micromoles of ascorbic acid equivalent per gram of dry extract. The symbols ns indicate there are no differences in content betweenthe two seasons and ecotypes. The statically different averages are given by * if p < 0.05; ** if p < 0.01; * if p < 0.001; **** if p < 0.0001represented the leveldifferencesobtained. Pd: Poundou; Dr: Dindéresso; Db: Diarabakoko; µmolAAE: micromoles ascorbic acid equivalent**

FRAP activity

Regarding fe^{3+}chelation activity, all extracts exhibited substantial antioxidant activity (Figure 5). The Niangoloko site presented the mostreduction in eachorgan, particularly in extracts obtained during the dry season. Significant differences in leaf extracts (Figure 5, Leaves) reduction were noted based on season variation ($p < 0.05$). This variability extended to bark (Figure 5, Bark) and root (Figure 5, Roots) extracts, except for Poundou extracts ($p > 0.05$). Moreover, variability based on ecological differences waso bserved in Fe^{3+}reduction activity ($p < 0.05$). However, the bark extracts from November in all three sites showed non-significantly different activities ($p > 0.05$).

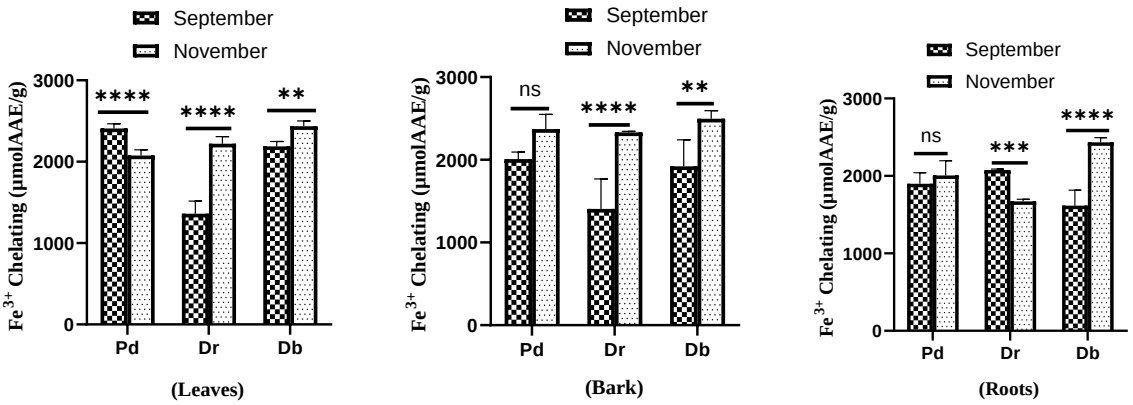
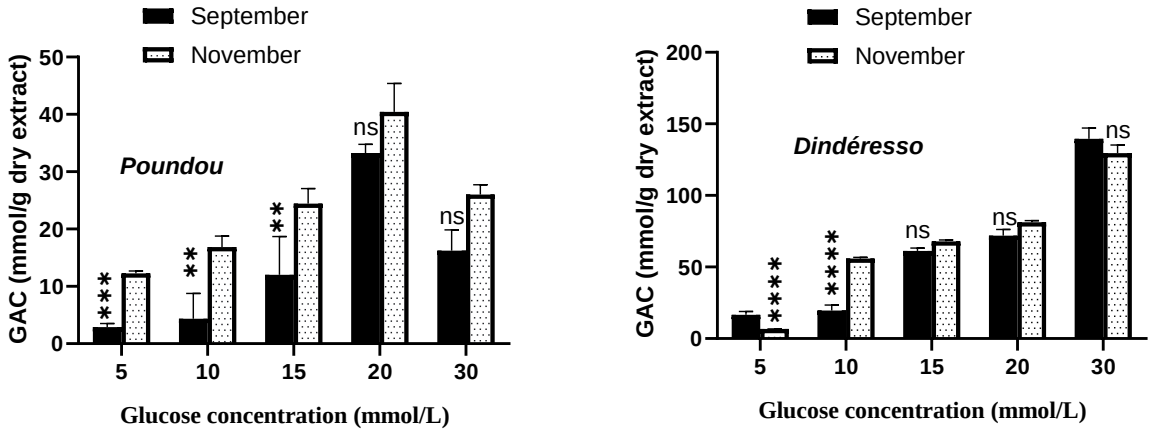


Figure 5: Fe^{3+}reductionactivity of aqueous decoction extracts (leaves, bark, and roots) of D.mespiliformis sampled during two seasons and in three different ecotypes. Results are means ± SD (n=5) and expressed as micromoles of ascorbic acid equivalent per gram of dry extract. The symbols ns indicate there are no differences in content, and * if $p < 0.05$; ** if $p < 0.01$; * if $p < 0.001$; **** if $p < 0.0001$represented the leveldifferencesobtained. Pd: Poundou; Dr: Dindéresso; Db: Diarabakoko; µmolAAE: micromoles ascorbic acid equivalent.**

Antidiabeticactivity

The study indicated that adsorption varied with glucose concentration, and at the lowest glucose concentration used, the extracts effectively trapped glucose (Figure 6, Poundou, Dindéresso, Diarabakoko). Dindéresso (Figure 6, Dindéresso) and Poundou (Figure 6, Poundou) sites exhibited maximum adsorption at 20 mM and 30 mM respectively. Site-specific variability in activity was observed depending on the sampling period ($p < 0.05$). Notably, the activity of november extracts was higher than that of september across sites ($p < 0.05$), with Dindéresso extracts showing the highest adsorption capacities in both periods. Consequently, the extract of november from Dindéresso wasused to obtain flavonoid fractions (Figure 6, Fractions). However, diethylether and acetate fractions demonstrated higher adsorptions than the crude extract ($p < 0.05$), with the diethylether fraction exhibiting higher activity at all concentrations ($p < 0.05$).



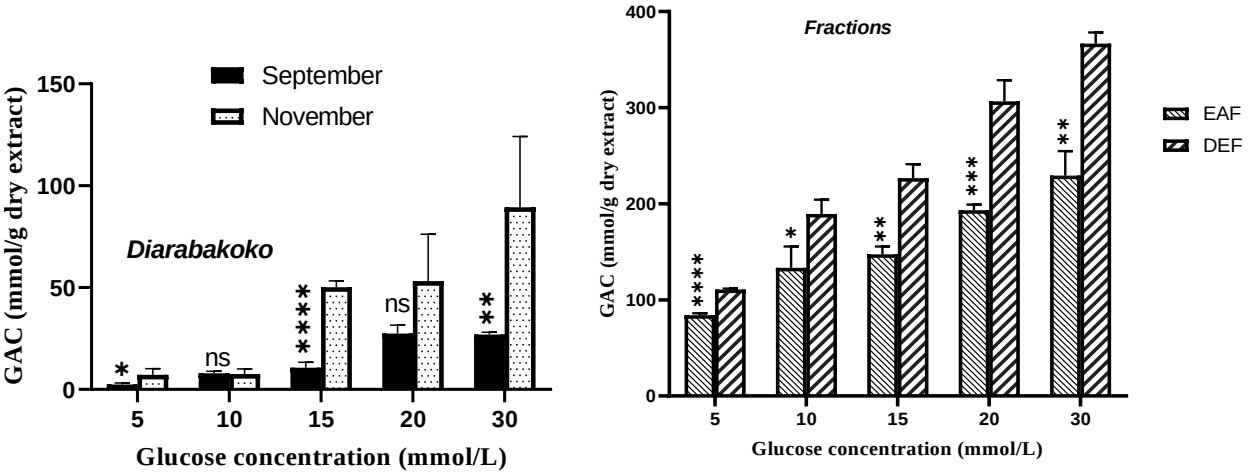


Figure 6: Glucose adsorption activity of aqueousdecoction of Poundou, Dindéresso, and Diarabakoko site samples extracts. The variability of activity according to site and harvesting season was estimated by using ANOVA variance withTurkey's multiple comparison test. Results are means ± SD (n=5) and expressed as millimole of glucose per gram of dry extract (mmol/g). GAC: glucose adsorption capacity; CE: crudeextract; DEF: diethylether fraction; EAF: ethylacetate fraction. Different symbols ns P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001indicate significant differences in the extract compared with the high concentration.

The enzyme inhibition activity test results (Figure 7), indicated dose-dependent inhibition of the alpha-amylase enzyme by both fractions and crude extract. The ethylacetate fraction (EAF) showed the lowest IC_{50}values, approximately0.185±0.069 mg/ml.

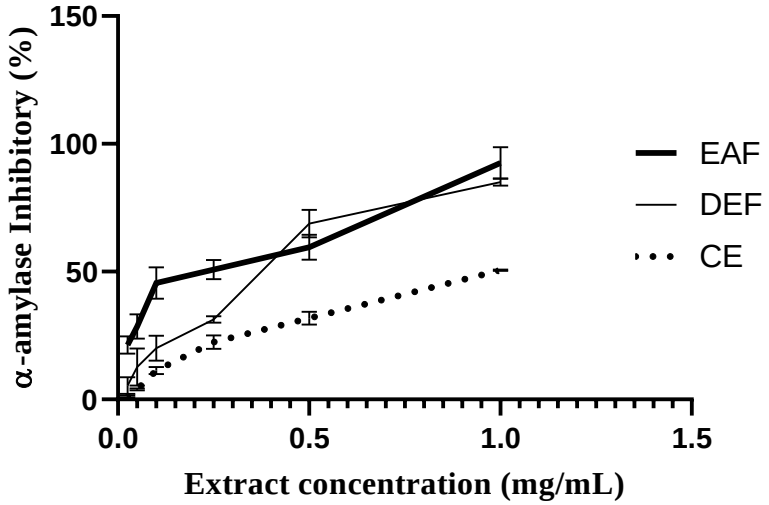


Figure 7: Alpha-amylase inhibitory activity of crudeextract and fraction of D.mespiliformis samples. Results are means ± SD (n=5) and expressed in Percentage Inhibition (% I). Significance was estimated at 5 %. CE: crude extract; DEF: diethylether fraction; EAF: ethylacetate fraction.

DISCUSSION

The variability of secondary metalites in plants is influenced by both biotic and abiotic factors (Pacifico et al. 2015). In the case of D. mespiliformis the phenolic content analysis indicates that neither season norecology had a significant impact on the production of these compounds in the plant. These results could be attributed to a similarity in soil type among the three sites, all characterized by Luvisolicsoils (Thiombiano and Kampmann, 2010), suggesting common nutrients. Studies have also shown that phenolic content variation is linked to soil nutrient content (Al Nasser, 2018).

The role that these compounds in the plant, particularly flavonoids, may explain their production. Flavonoids are widely present in the leaf cuticle and in the epidermal cells, likely

serving to protect tissues from the harmful effects of UV radiation(Macheixet al. 2005 ;Salem, 2009 ;Pacifico et al. 2015). The substantial increase i flavonoid production in November supports the influence of the season on the synthesis of these compounds. The physiological state during this period (NeglurandGajakosh, 2021), and its alignment with the fruit-ripening phase of D. mespiliformis (Nacoulma, 1996)could contribute to this heightened production, as flavonoids are known to be among the secondary metabolites most synthesized during this stage of a plant's development cycle(Jordánet al. 2013). Moreover, to cope with photo oxidative stress, the plant could synthesize these compounds to remedy the situation. Flavonoids, found, in the leafcuticle and the epidermalcells, likely protect tissues against UV radiation's harmful effects(Macheixet al. 2005 ;Salem, 2009 ;Pacifico et al. 2015). The results of $DPPH^{\bullet}$ free radical scavenging activity indicate seasonal and site-specific variability. These differences may be explained by variations in the phenolic compounds responsible for thisactivity, known for their role against oxidative stress by adsorbing, trapping, or inhibiting $DPPH^{\bullet}$ free radicals (Pacifico et al. 2015 ;Joshi et al. 2021). Studies suggest their synthesisis influenced by factorssuch as photo period, temperature, light, and rainfall, which promote photosynthesis (Radušienėet al. 2012).

About the capture of $ABTS^{+\bullet}$radicals, the study observed seasonal variations in activity at the same site. The high phenolic content noted in the most active extracts suggests that these compounds contribute to the observed activity. Studies have established correlations between $ABTS^{+\bullet}$ radical scavenging activity and phenolic compounds(Gonçaloet al. 2020).

Season and type of ecology influenced Fe^{3+}reduction activity in the plant. The climatic and pedological conditions of the harvest site could explain these results(Lakhdar et al. 2011). The synthesis of Fe^{3+}reducing compounds might be influenced by the plant's life state, as environmental factors lead to quantitative variations in compounds, predisposing a variation in their antioxidant potential(Tsaoet al. 2005). Phenolics, known for their antioxidant potential(Tsaoet al. 2005 ;Pettiand Scully, 2009 ;Pacifico et al. 2015)could play a role in this reduction process by chelating metal ions involved in free radical formation (Radušienėet al. 2012).

The anti-diabetic activity was assessed based on the extracts' ability to reduce postprandial glycemia. Leaf extracts were chosen due to their high phenolic compound content and significant antioxidant activity. High glucose levels can lead to diabetes, and one approachis to complex free glucose to mitigate metabolic imbalance. The studyshowedthat the extracts and fractions could trap glucose at above-normal blood glucose levels, with heightened activity observed in november, indicating a seasonal influence on compound production. Phenolic compounds, particularly flavonoids, were implicated in binding glucose to theirchains, forming glycosylated flavonoids(Rijkeet al. 2006). This conversion of free glucose reduces the amounttransported to the intestine, maintaining normal postprandial blood glucose levels(Rehmanet al. 2018). The decrease in adsorption maybeattributed to the extractsreachingtheir maximum adsorption capacity, possibly due to antagonisticeffectsbetween compounds.

In addition to complex free glucose, anotherstrategy for diabetes prevention involves reducing the activity of the digestive enzymes of carbohydrates(Dastjerdiet al. 2015 ;Punnagaiand Glory Josephine, 2018). Notably, moderate inhibitions obtained suggest that the crude extract and fractions could contribute to lowering postprandial glycemia. Achieving partial enzyme inhibition ispreferable in diabetes prevention(Yonemotoet al. 2014), as complete inhibition could lead to unabsorbed carbohydrate molecules undergoing fermentative processes, causing side effects like flatulence and diarrhea (Etxeberriaet al. 2012).The presence of compounds with α-amylase activity, including flavonoids, tannins, saponins, and terpenoids (Dastjerdiet al. 2015 ;Al Nasser, 2018), likely explains these results. Previous studies have also highlighted the involvement of these compounds in alpha-amylase inhibition (Pacifico et al. 2015 ;Dastjerdiet al. 2015). The results with ethylacetate fractions may be attributed to the solvent's polarity, extracting compounds like flavonoids that have higher affinity to inhibit enzyme activity.

CONCLUSION

This studyaimed to investigate both seasonal and ecological variations in phenolic and flavonoid contents, along with antioxidant activities. It foundthat the extracts were rich in phenolic compounds. Furthermore, the studyuncovered that season and ecology played a role in influencing both flavonoid contents and their antioxidant activities. Additionally, the crude extracts and fractions demonstrated antidiabetic activities. A future study will be

necessary to isolate phenolic compounds and assess their antidiabetic activities.

Authors' contributions

Pawendé Kabré: Investigation, Methodology, Experiment, Results analyses, and Writingthismanuscript
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Yacouba Sanou: Methodology, Experiment
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Paulin Ouoba: Plant identification, harvesting, and manuscriptreview
Martin Bienvenu Somda : Manuscriptreview
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All authors have read and agreedwith the publication of the manuscript.

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Conflicts of interest

The authors in this document declared no interests' conflict.

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