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Season and Ecotype Effects on Soluble Phenolic Compounds Content and Antioxidant Potential of *Tamarindus indica* and *Mitragyna inermis*

Relwendé Justin Ouédraogo[1,3], Lassina Ouattara[1,3], Pawendé Kabre[1,3], Yacouba Sanou[1,3], Martin Bienvenu Somda[2], Paulin Ouoba[1] and Georges Anicet Ouédraogo[3]

1. Département de Sciences Biologiques, Unité de Formation et de Recherche en Sciences de la Vie et de la Terre, Université Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso

2. Centre International de Recherche-Développement sur l'Elevage en zone Subhumide, Institut du Développement Rural, Université Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso

3. Laboratoire de Recherche et d'Enseignement en Santé et Biotechnologies Animales, Université Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso

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Abstract: *Tamarindus indica* and *Mitragyna inermis* are widely used by herbalists to cure diabetes. However, the suitable areas and season for harvesting these plants remain unexplored. Moreover, the adaptation to stress is mostly affected by an environmental factor. Also, the ingredients responsible of bioactivity remain unknown. Season and ecology effect on phenolic compounds content was carried out with Folin-Ciocalteu Reagent and their antioxidant potentials was checked by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Fe^{3+} chelation assays. Then, correlation between antioxidant activities and polyphenol contents of aqueous extract, phenolic-rich fractions, and flavonoid-rich fractions was established. For this purpose, sampling of stem, root barks, and leaves from three zones of Burkina Faso was done in the rainy season and the dry season. The results showed that antioxidant potential and properties vary from one area to another, from one plant to another and from one part to another for the same plant. Also, the dry season sounds favorable for the harvesting of these plants, as during this season plants produce high phenolic compounds with high anti-oxidant potential. The results obtained with these extracts suggest their plant collection and use as a basis for the research of improved traditional drugs following further investigations.

Key words: *T. indica, M. inermis*, ecotype, season, phenolic compounds, antioxidants.

1. Introduction

Traditional medicine has a wealth of therapeutic plants. Thus, *Mitragyna inermis* and *Tamarindus indica* are two plants traditionally used in the management of many diseases including metabolic diseases [1, 2].

Corresponding authors: Relwendé Justin Ouédraogo, Ph.D. candidate, research fields: Natural products and metabolic illnesses. Email: rjustino14@yahoo.com. Lassina Ouattara, Ph.D., research fields: Natural products and metabolic illnesses. Email: lasouatt@yahoo.fr.

On the one hand, Phytochemical studies conducted on *Mitragyna* (*M.*) *inermis* have shown that the aqueous decoction of the leaves contains sterols, triterpenes, polyphenols, flavonoids, catechic tannins, saponosides, quinines and alkaloids [3, 4]. Also, Pahaye et al noted the presence of flavonoids, alkaloids, tannins and anthraquinones in the aqueous decoction of leaves [5]. Therefore, this result supports the phytochemical data of the aqueous leaf decoction. Alowanou et al have highlighted the presence of condensed and gallic tannins, saponins, alkaloids,

flavonoids, anthraquinones, glycosides, terpenes and reducing compounds in the acetone decoction of leaves [6]. An estimation of the contents of the aqueous macerate revealed total polyphenols of (24.42 ± 0.91 mg GAE/100 mg extract) and total flavonoids of (0.95 ± 0.08 mg QE/100 mg extract) in the leaves [7]. Furthermore, a screening of chloroformic and ethanolic extracts of root and stem barks showed the presence of tannins, reducing compounds, sterols and triterpenes, alkaloids, emedols, carotenoids, steroidal and triterpenic saponosides, flavonoids, anthracenosides, leuco-anthocyanins, anthocyanosides and coumarins [8]. Also, in the barks, the presence of alkaloids, anthraquinones, flavonoids, glycosides, reducing sugars, saponins, tannins and terpenoids were revealed in the aqueous extract [9]. All these reported data show the important solubility of *M. inermis* parts metabolites in water and acetone. Regarding *T. indica*, tannins, saponins, sesquiterpenes, alkaloids, and phlobatanins were found in the aqueous and acetone extracts of stem barks and leaves [10]. Tannins and saponins were found in the aqueous macerate and decoctate of leaves and stem barks [11, 12]. An estimation of the leaves aqueous macerate contents revealed total polyphenols (20.24 ± 0.15 mg GAE/100 mg extract) and total flavonoids (9.90 ± 0.12 mg QE/100 mg extract) [7]. These results suggest water and acetone as suitable solvents for the extraction of compounds in leaves and stem barks. Nacoulma [1], Bhatia et al [13], Escalona-arranz et al [14] and Razali et al [15] have identified glycosides such as 8-C-glycosyl-apigenin; 6-C-glycosyl-apigenin; 8-C-glycosyl-luteolin; 6-C-glycosyl-luteolin; luteolin 7-o-glucoside; vitexin, isovitexin, orientin, iso-orientin, epicatechin, quercetin, and isorhamnetin in leaves.

On the other hand, studies have been conducted on *T. indica* and *M. inermis* antioxidant properties. With *M. inermis,* Pahaye et al have shown that decoction of the leaves increases the activity level of superoxide dismutase and catalase, but also reduces the reactivity of thiobarbituric acid after 8 consecutive days of treatment at 393 mg/kg body weight [5]. Also, from a study conducted on *M. inermis* leaves aqueous macerate, it was found that thisextract obviously reduces DPPH radicals ($DPPH^{\bullet}$) (522.17 ± 1.81 µmol AAE/g extract), 2,2′-azinobis-(3-ethylbenzothiaziline-6-sulfonate) radicals ($ABTS^{\bullet+}$) (6815.16 ± 51.00 µmol AAE/g extract) and Fe^{3+} (329.95 ± 49.11 µmol AAE/g extract) [7]. These results show a good improvement of organism antioxidant defense system and suggest the possible use of the aqueous extract as an antioxidant. Work on solvents for extraction of polyphenolic antioxidants from leaves has shown that their efficiency decreases from methanol to hexane through ethyl acetate [15]. Raghavendra et al observed antioxidant activities of methanolic extract of leaves with IC_{50} values of 35 µg/ml, 210 µg/ml and 72 µg/ml on $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ and total antioxidant activity test respectively [16]. Methanolic extracts of stem bark and leaves reduced $DPPH^{\bullet}$ by 23.4% and 16.8% at 2 mg/ml extract, and superoxide by 32.50 ± 1.51% and 31.86 ± 3.11% at 1 mg/ml extract respectively [17]. A recent study revealed that *T. indica* leaves aqueous extract reduced $DPPH^{\bullet}$ (360.02 ± 7.23 µmol AAE/g extract), $ABTS^{\bullet+}$ (7067.58 ± 0.00 µmol AAE/g extract) and Fe^{3+} (677.26 ± 24.53 µmol AAE/g extract) [7].

All these studies highlight solvent polarity importance in obtaining antioxidant compounds from these plants. Therefore, the different parts of these plants could be used either in the improvement of the natural antioxidant defense system or in the prevention of the radical propagation whose consequences can be the cellular apoptosis and the alteration of the biomolecules as well as their receptor. The high number of repeat studies on the same solvents with the same parts undoubtedly shows a variability of composition and properties which imperatively requires verification. So, the plant pharmacological activities depend on the quantity and quality of the active ingredients. However, these

parameters are strongly impacted by the season and the place of harvest [18, 19]. Little data exists on the real influence of the season and ecological type on these different plant parts phenolic compounds content and antioxidant activities around the herbalists' activity zone. It is the reason why, this study was initiated on *T. indica* and *M. inermis* different parts; two plants widely marketed by herbalists for their anti-diabetic and antihypertensive virtues.

T. indica flowers at the end of the dry season after leafing out, generally from June to September, and its fruiting takes place from October to February. Leaf fall occurs from January to June; leafing occurs during the rainy season and flowering occurs from May to September [2]. The average annual rainfall is 1100-1200 mm in Dindéresso and Banfora while it is 1000-1100 mm in Boromo. In September, Dindéresso and Banfora are on the same isohyet but in November, Dindéresso and Boromo are on the same isohyet [20]. September is part of the rainy season and November, the beginning of the dry season. The luvisol type (soils with clay and organic matter accumulation in the lower layers, high activity clays and a high percentage of base saturation) is the soil common to all three zones, only ferric in Dindéresso and Banfora but gleyed in Boromo. Concerning the geological formations, Dindéresso and Banfora are formed at the base of Sandstone but of Migmatites and Syenites for Boromo. According to the phytogeography, Boromo is part of the East Mouhoun district characterized by forest galleries constituted in major part by sudanian deciduous species. Dindéresso belongs to the West Mouhoun district characterized by large forest galleries with mostly Sempervirente vegetation made up of Guinean species. As for Banfora, it is part of the Comoé district whose vegetation is made up of clear forests populated essentially by *Isoberlinia doka*. [20-22]. The present study aimed at verifying the effect of season and ecological type on the phenolic compounds content and anti-free radical and antioxidant activity of both plants leaves, stem barks and roots.

2. Materials and Methods

2.1 Plant Materials

The plant material consisted of leaves, stem barks and roots of *M. inermis* (voucher specimen UNB 939) and *T. indica* (voucher specimen UNB 938). The voucher specimens are deposited at the Herbarium of Université Nazi Boni. *T. indica* samples were collected in Boromo (N11°45'57.94"; W2°55'40.986") and in Dindéresso (N11°19'43.464"; W4°23'42.726"). *M. inermis* samples were collected in addition to the same areas, from Banfora (N10°28'2.616"; W4°46'18.018"). Harvesting was done in the rainy season (September 2020) and in the dry season (November 2020). The samples were washed, dried and then ground to powder using a mortar and pestle. The resulting powder was stored in a bag and the moisture measured by a moisture determinator (KERN MLS 50-3C, Germany) before any use.

2.2 Preparation of Extracts and Fractions

2.2.1 Aqueous decoction

Five grams of each powder was placed in a filter Erlenmeyer flask and 100 mL of distilled water was added. The whole mixture was boiled with the help of a hot plate (701546-Economy hot plate, 1500 W; 230 V) for 30 min. At the end of the time, the decoction was cooled and filtered with Wathman N°2 into 100 mL flask. The residue was wrung out with distilled water to complete the volume to 100 mL. The filtrate was then centrifuged at 6530 rpm using a centrifuge (Hettich MIKRO 220R; Germany) for 30 min at 4 °C. The supernatants were collected; the pH of the extracts corrected to 6-8 and stored at 4 °C for immediate use *in vitro* assays [23].

2.2.2 The fraction rich in polyphenols

A mass of 2 g of plant powder was macerated with 20 mL of acetone-water 70% (V/V) (CARLO ERBA, RE, V8N076129B, France) for 90 min under regular manual stirring. At the end of the time, a filtration

with a Wathman paper N°1 was performed. The resulting filtrate was stored at 4 ℃ for immediate use *in vitro* assays [24].

2.2.3 The flavonoid-rich fraction

A mass of 2.5 g of each powder was placed in an Erlenmeyer flask and 50 mL of distilled water was added. The mixture was boiled on a hot plate for 30 min. At the end of the time, the decoction was cooled and filtered into 50 mL flask. The residue was wrung out with distilled water to make up the volume to 50 mL. The filtrate was then centrifuged at 6530 rpm for 30 min at 4 ℃. Then, 25 mL of the collected supernatants were delipidated with 10 mL of petroleum ether (CARLO ERBA, RPE, V7H621087I, France) for 15 min and, after decantation, the aqueous phase was retained. This aqueous phase underwent two successive extractions with 25 mL of diethyl ether (CARLO ERBA, RPE, V8E450228E, France) (free flavonoids fraction) and then with 25 mL of ethyl acetate (CARLO ERBA, RPE, P0C119200D, France) (bound flavonoids fraction). After decantation, 1 mL of sulfuric acid (CARLO ERBA, RPE, V6F420016F, France) was added to the ethyl acetate phase for hydrolysis during 2 h. At the end of the time, the aqueous precipitate containing free flavonoids was separated. After neutralization of acidity with distilled water, further extraction with diethyl ether was performed and then decanted. The two diethyl ether fractions were dried, solubilized in 1 mL of 95% ethanol (CARLO ERBA, RE, V0I137050L, France) and the total volume was made up to 6 mL with distilled water. The latter solution was stored at 4 ℃ for immediate use *in vitro* tests [25].

2.3 Estimation of Total Phenolic Compounds Content

Estimation of phenolic contents was done using the Folin-Ciocalteu Reagent with some modifications [23, 26]. To 0.250 mL of extract or fraction, 0.250 mL of 95% ethanol, 1.250 mL of distilled water and 0.125 mL of Folin-Ciocalteu Reagent 50% (V/V) (MERCK KGaA, HC9059050I, Germany) were added successively. The mixture was left to incubate at room temperature for 5 min. At the end of the time, 0.250 mL of Na_2CO_3 5% (CARLO ERBA, RPE, V7I654128G, France) was added to the test tubes. The latter mixture was incubated in the dark for 60 min, and then optical densities were taken at 725 nm using a spectrophotometer (Spectrumlab23A, No. 23A11096) against a blank prepared under the same conditions with a substitution of Na_2CO_3 with distilled water. A standard curve was constructed using gallic acid (Sigma-Aldrich Chemie, Steinheim, China) (0-200 mg/L) (y = 10.546 x; R^2 = 0.995). Optical densities were taken at least in triplicate and results were expressed as mg Gallic Acid Equivalent (GAE)/g Dry Matter (DM).

2.4 Anti-free Radical Activity: DPPH$^\bullet$ Scavenging Test

The modified method of Brand-Williams et al [27] was used. DPPH (Thermo Fisher, P19F002, Germany) 60 μM in 95% ethanol was prepared and the optical density adjusted to 0.760 ± 0.2. Thus, 2 mL of this $DPPH_o$ (stock solution) was added to 400 μL of each extract or fraction for the assay and with only 2 mL of ethanol for the blank. After incubation for 1 min at laboratory room temperature, the absorbances were read against the blank at 517 nm. The assay was performed in triplicate. Ascorbic acid (Thechno Pharmchem Haryana, India) was used as reference [28]. The results were expressed as inhibition rates:

$$Inhibition(\%) = \frac{OD_o - OD}{OD_o} \times 100$$

Where ODo = optical density of DPPH$^\bullet$ in the absence of the extract

OD = optical density of DPPH$^\bullet$ in the presence of the extract.

2.5 Fe^{3+} Chelation

The method adapted from Benzie and Strain [29] was used. Therefore, 0.250 mL of each extract or fraction was mixed with 0.625 mL of phosphate buffer (0.2 M; pH 6.6) and 0.625 mL of aqueous potassium hexacyanoferrate $[K_3Fe(CN)_6]$ (1%) (CARLO ERBA,

EPR, V4L501144L, France). After 30 min of incubation at 50 ℃, 0.625 mL of trichloroacetic acid (10%) (CARLO ERBA, EPR, V9C099200A, France) was added and the mixture was centrifuged at 3000 rpm for 10 min. Then, 0.625 mL of the supernatant was mixed with 0.625 mL of distilled water and 0.125 mL of $FeCl_3$ solution (0.1%) (CARLO ERBA, EPR, V7D589039A, France). Optical densities were read at 700 nm against a blank in which $FeCl_3$ was substituted with distilled water. A standard curve was produced using Ascorbic acid (0-200 mg/L) (y = 6.123 x + 0.2462; R^2 = 0.9985). Optical densities were measured in triplicate and results were expressed as micromoles of Ascorbic Acid Equivalent (AAE)/g dry matter.

2.6 Statistical Analysis

A single extraction was performed on each sample and *in vitro* analyses in five replicates (n = 5) except for the fractions where three replicates were performed (n = 3). Results were expressed as mean ± standard deviation. Data were subject to a one-factor ANOVA and comparison of means by Ducan multiple series tests (p < 0.05). Pearson's correlation was calculated using Microsoft Excel 2016.

3. Results and Discussion

3.1 Phenolic Compounds Content, Iron Chelation and Free Radical Scavenging Activities of Aqueous Decoctions

The estimation of total phenolic compounds soluble in the water was done with the Folin-Ciocalteu Reagent. The phenolic compounds content varied from 47.05-107.67 mg GAE/g dry matter.

In this regard, at the level of the stem, root and leaves of the *M. inermis*, the ecotype harvested in Dindéresso on the dry season (November) showed a high content (100.1, 95.96 and 102.27 mg GAE/g DM respectively). This phenolic compounds content remains similar to that of Banfora ecotype on the same season at the root level and to those of the Banfora ecotype harvested in both seasons at the leaf level ($p > 0.05$). These results suggest little influence of harvest area on metabolites content. So, this would be explained by the same rainfall range of Dindéresso and Banfora. Furthermore, an evolutionary trend in phenolic compounds content from September to November was observed with the parts of the different ecotypes. Brahmiet al checked phenolic compounds content in leaves and fruits, then observed this trend from October to January [30]. Indeed, these authors stressed that this variation is well observed during the vegetative cycle in response to defense needs. As for antioxidant activity, at the root level, the Dindéresso ecotype harvested on September presented the strong reduction (2852.27 µmol AAE/g DM). There was no significant difference in antioxidant activity from one season to the next with the roots harvested in Banfora and with the roots harvested in the dry season in Dindéresso. Regarding stem bark, no significant difference in activity was observed between seasons with the Banfora and Dindéresso ecotypes ($p < 0.05$). This could denote the lack of influence of season and ecology on stem bark activity. The Dindéresso ecotype harvested in dry season showed the strong reduction of Fe^{3+} at leaf level (1954.28 µmol AAE/g DM) (Figure 1). The influence of season and ecological type is noticeable at the leaf level. Following the harvesting seasons, a correlation in soluble polyphenol content was observed with the Dindéresso ecotype (r = 0.9980; $p < 0.05$) but weakly with the Banfora ecotype. This could show the influence of the season on the genesis of phenolic compounds in Dindéresso and Banfora ecotypes. A noticeable gradual evolution of phenolic compounds content from roots to leaves was observed on all ecotypes harvested in dry season. So, dose-dependent activities were observed between total phenol content and Fe^{3+} reduction in samples harvested in Boromo (r = 0.9990; $p < 0.05$) and Banfora (r = 0.9979; $p < 0.05$) respectively in dry and rainy seasons (Table 1).

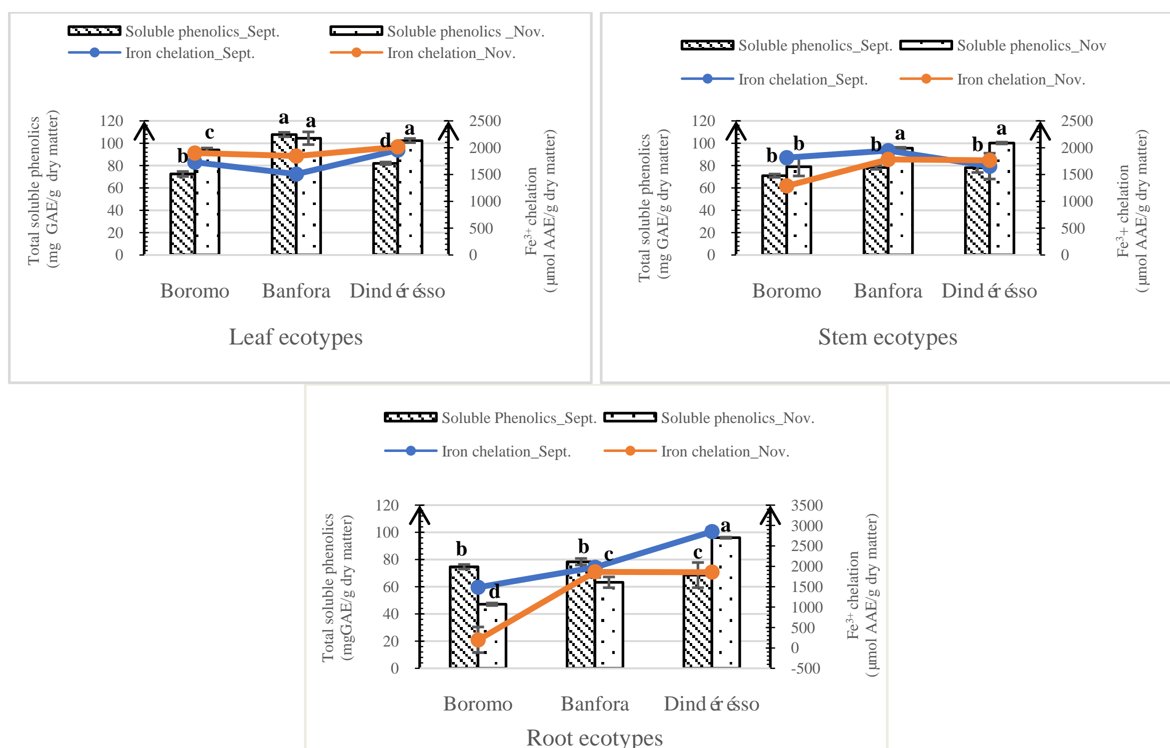

Fig. 1 Soluble phenolic compounds content and Fe^{3+} chelation of *M. inermis* aqueous decocts from different ecotypes: Total soluble polyphenols (milligram Gallic Acid Equivalent/gram dry matter) and Fe^{3+} reduction activity (micromoles Ascorbic Acid Equivalent/gram dry matter) are represented. Data are mean ± SD values of five test replicates for total phenolics and Fe^{3+} reduction. Bars with the same letter are not phenolic content significantly different ($P > 0.05$) on same plant part. The Pearson correlation coefficient of Fe^{3+} reduction activity between samples collected in November and September in Boromo, Banfora and Dindérésso are 0.8145; -0.2156, 0.1018 respectively. The Pearson correlation coefficient of total polyphenol content between the samples collected in November and September in Boromo, Banfora and Dindérésso are respectively -0.7457; 0.6649, 0.9980.

Table 1 Pearson's correlation between iron chelation, DPPH• reduction activity and total phenolic compounds content of *M. inermis* samples ($p < 0.05$).

Total soluble phenolics		DPPH• scavenging	Fe^{3+} chelation
September	Boromo	0.2867	-0.9881
	Dindérésso	0.5588	-0.8645
	Banfora	0.9996	-0.9979
November	Boromo	0.9705	0.9990
	Dindérésso	0.7971	0.4681
	Banfora	0.9276	-0.5323

NB: Pearson correlation measures the strength of the linear relationship between two variables. Positive values mean direct relationship between both variables. Negative values mean indirect relationship between both variables.

Ranilla et al have observed on a study a significant correlation between antioxidant activity and phenolic compounds content [23]. A direct correlation of antioxidant activity following the harvest season was observed with the Boromo ecotype ($r = 0.8145$; $p < 0.05$). These results could support the influence of season on antioxidant activity of Boromo ecotypes. It has been reported that antioxidant activity is influenced by the climatic factor; the organ and its harvesting stage which define the antioxidant compounds present [30].

Following the DPPH test for free radical scavenging activities, among *M. inermis* root and stem bark samples, the Banfora ecotype harvested in the dry season showed the highest reductions in DPPH• (86.05% and 92.21% respectively). As for the leaves,

all dry season ecotypes reduced totally DPPH•. These results showed that the best activities came from the samples harvested in dry season. Thus, the effect of ecology on the radical scavenging activity of the plants was not noticeable in the leaves. All the dry season samples showed a high correlation between phenolic compounds content and free radical scavenging activity. Concerning the rainy season, only the Banfora ecotype showed a good correlation between phenolic compounds content and DPPH• reduction (r = 0.9996; $p < 0.05$). The observations show that for these samples, the antiradical activity would be related to the high phenolic compounds content. Studies have shown correlation between phenolic compounds content and antiradical activity [23, 31]. The DPPH• reduction of Banfora and Dindéresso ecotypes varied with harvesting season (r = 0.9073 and r = 0.9645 respectively; $p < 0.05$) (Figure 2). The difference in phenolic compounds production would be related to the defense mechanism against environmental stress. This production is influenced by the duration of photoperiod, temperature, duration and intensity of sunlight as well as rainfall which promotes photosynthesis [18]. As for the antioxidant activity, it would be attributed to a difference in the quantity and quality of active ingredients [19, 32].

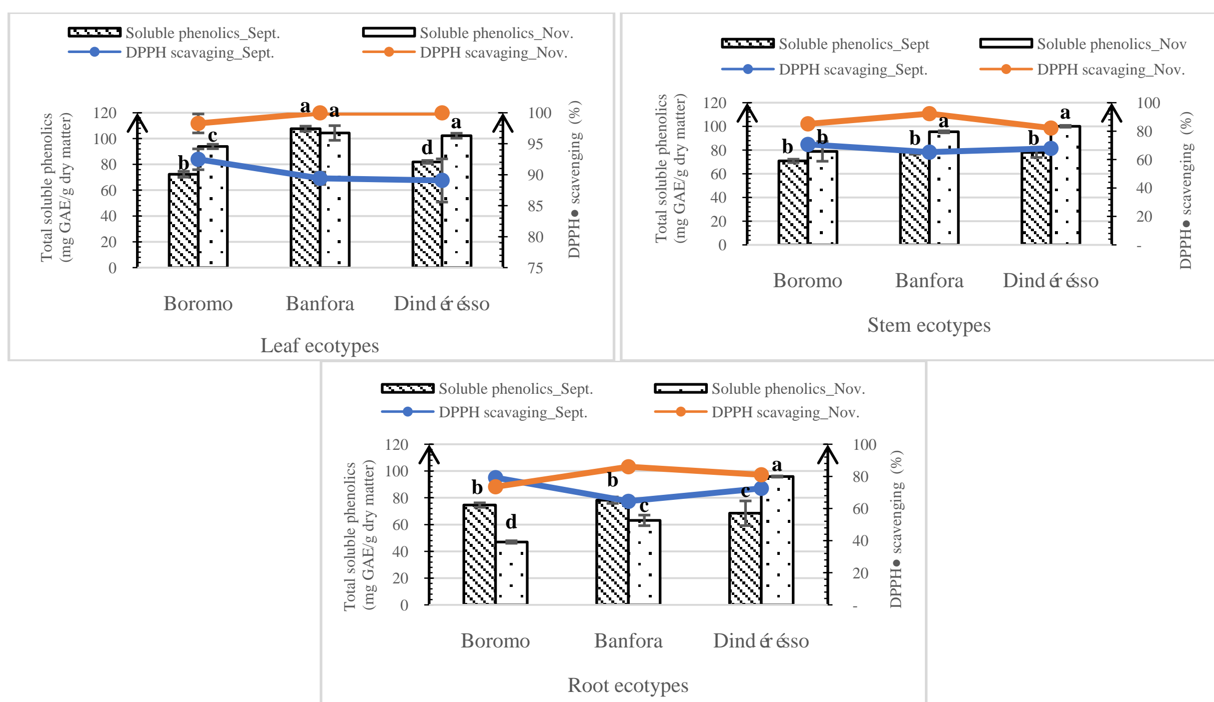

Fig. 2 DPPH• scavenging as a function of phenolic compounds content in *M. inermis* samples according to seasons: Total soluble phenolic compounds (milligram Gallic Acid Equivalent/gram dry matter) and DPPH• scavenging activity (%) are shown. Data are mean ± SD values from five test replicates for total phenolics and DPPH• reduction. The Pearson's correlation coefficient of DPPH• scavenging activity between the samples collected in November and September in Boromo, Banfora and Dindéresso are 0.6302; 0.9073, 0.9645 respectively. The Pearson correlation coefficient of the total phenolics content between the samples harvested in November and September in Boromo, Banfora and Dindéresso are respectively -0.7457; 0.6649, 0.9980.

With *T. indica*, phenolic compounds content varied from 75.49 to 130.37 mg GAE/g dry matter. Concerning stem and root barks, Boromo ecotype harvested in the dry season had the highest phenolic compounds contents (130.37 and 111.41 mg GAE/g DM respectively). As for the leaves, the Dindéresso and Boromo ecotypes harvested in the same season had the highest phenolics content without a significant difference (96.24 and 92.29 mg GAE/g DM respectively). A correlation was observed on phenolic

compounds content following seasons with Boromo samples (r = 0.8724; $p < 0.05$). This could show a seasonal variation in total phenolic compounds biosynthesis in this area. Therefore, from September to November, as in the case of *M. inermis*, the phenolic compounds content generally increased. This could show the climatic influence on the biosynthesis of this plant in relation to the need for vegetative growth and resistance to stress. In the antioxidant test with Fe^{3+} chelation, the activities varied from 1617.93 to 2601.30 µmol AAE/g dry matter. Indeed, a strong reduction of Fe^{3+} in the stem bark extracts was observed with the Boromo and Dindéréssо ecotypes harvested in dry season. A lack of significant difference in antioxidant activity was observed between the same ecotypes in the rainy season. This could justify the absence of ecological effects on the stem barksantioxidant activity. At the root bark level, both samples from Boromo and Dindéréssо reduced Fe^{3+} without significant difference ($p > 0.05$). This could show the lack of effect of season and environment on the antioxidant activity of root barks from Boromo and Dindéréssо ecotype. The highest antioxidant activity at the leaf level was observed with the Dindéréssо ecotypes without a significant difference with the Boromo ecotype harvested in the rainy season (Figure 3). A correlation between Fe^{3+} chelation activity and total phenolic compounds content was observed with Boromo ecotype for both seasons (r = 0.9765 and 0.9929, rainy and dry month respectively; $p < 0.05$) but on only rainy season with Dindéréssо ecotype (r = 0.9575; $p < 0.05$) (Table 2). Also, a relationship between antioxidant activity and harvesting season was observed with the Dindéréssо ecotype (r = 0.9029; $p < 0.05$). This could show the ability of Boromo ecotypes to express them face to climatic stress.

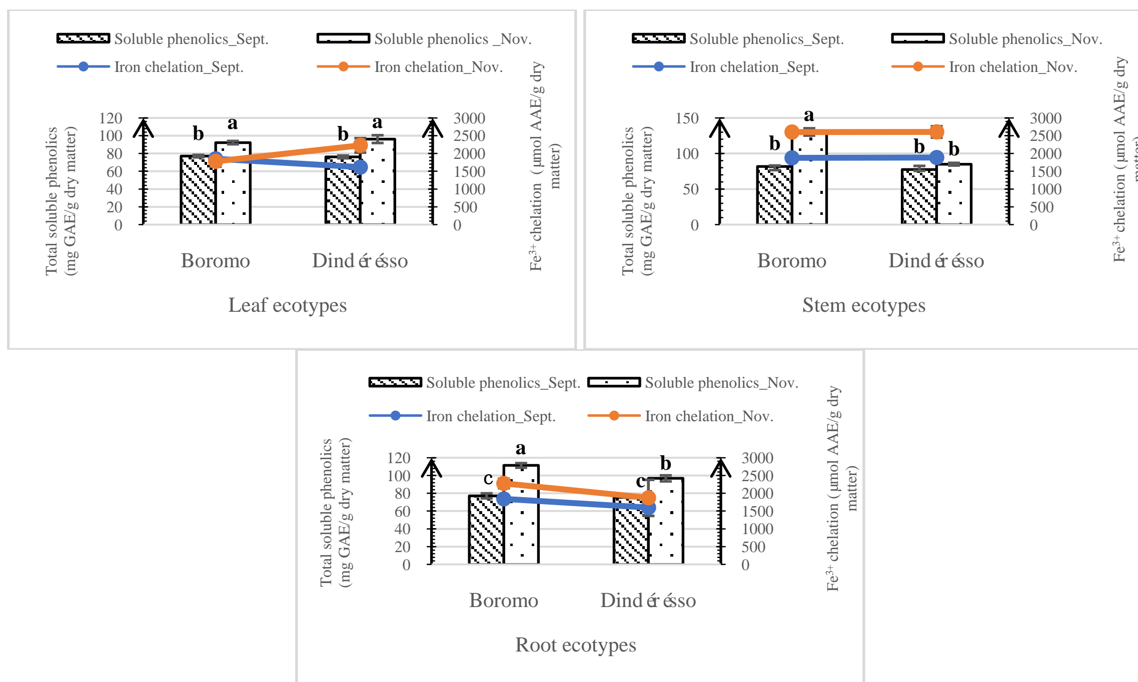

Fig. 3 Phenolic compounds content and Fe^{3+}chelation of *T. indica* aqueous decocts from different ecotypes: Total soluble phenolic compounds (milligram Gallic Acid Equivalent/gram dry matter) and Fe^{3+} chelation activity (micromoles Ascorbic Acid Equivalent/gram dry matter) are shown. Data are mean ± SD values of five test replicates for total phenolic compounds and Fe^{3+} chelation. Bars with the same letter are not phenolic content significantly different (P > 0.05) on same plant part. The Pearson's correlation coefficient of Fe^{3+} chelation activity between the samples collected in November and September in Boromo and Dindéréssо are 0.6624; 0.9029 respectively. The Pearson correlation coefficient of the total phenolic compounds content between the samples harvested in November and September in Boromo and Dindéréssо are respectively 0.8724; -0.9524.

Table 2 The Pearson correlation between Fe^{3+} chelation, $DPPH^{\bullet}$ scavenging activity and total phenolic compounds content of *T. indica* samples ($p < 0.05$).

Total soluble phenolics		$DPPH^{\bullet}$ scavenging	Reduction of Fe^{3+}
September	Boromo	0.3259	0.9765
	Dindérésso	-0.0173	0.9575
November	Boromo	-0.3003	0.9929
	Dindérésso	-0.9993	-0.8954

NB: Pearson correlation measures the strength of the linear relationship between two variables. Positive values mean direct relationship between both variables. Negative values mean indirect relationship between both variables.

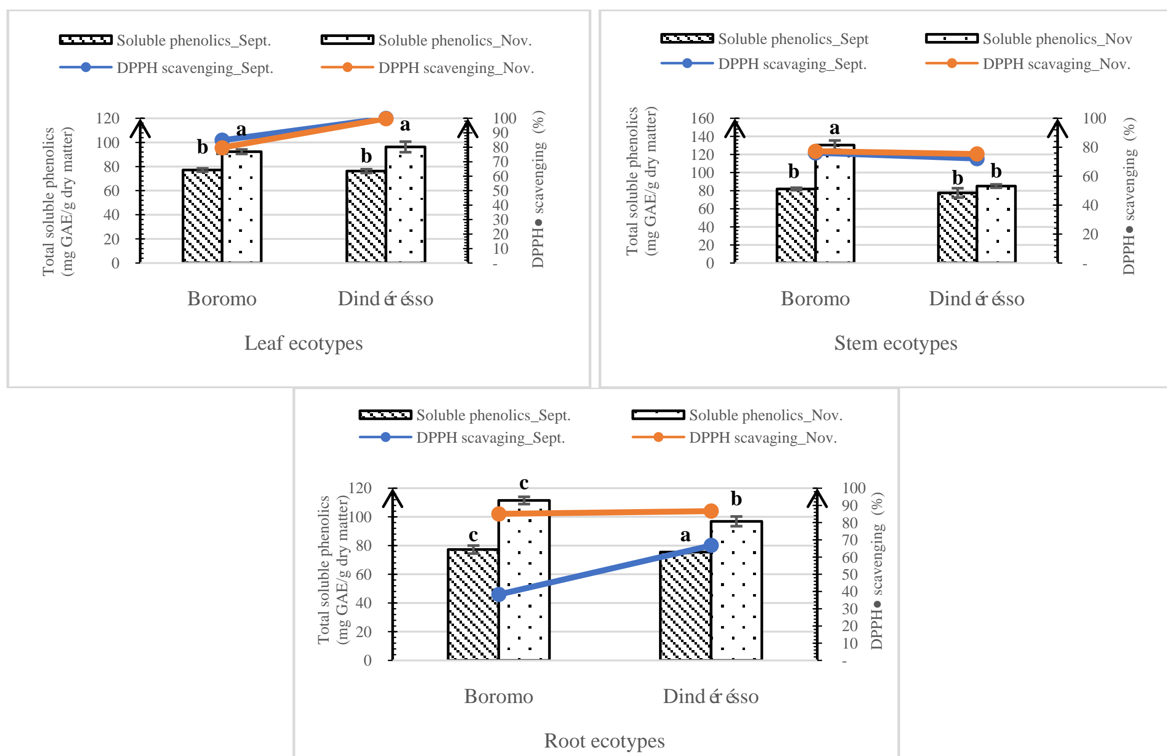

Fig. 4 Reduction of $DPPH^{\bullet}$ as a function of phenolic compounds content in *T. indica* samples according to seasons: Total soluble phenolic compounds (milligram Gallic Acid Equivalent/gram dry matter) and $DPPH^{\bullet}$ scavenging activity (%) are shown. Data are mean ± SD values from five test replicates for total phenolic compounds and $DPPH^{\bullet}$ reduction. The Pearson's correlation coefficient of $DPPH^{\bullet}$ reduction activity between the samples collected in November and September in Boromo and Dindérésso are -0.8852; 0.8047 respectively. The Pearson correlation coefficient of the total phenolic compounds content between the samples harvested in November and September in Boromo and Dindérésso are respectively 0.8724; -0.9524.

Following the $DPPH^{\bullet}$ test, the reduction rate varied from 38.34 to 100% of the radicals. On all the parts, the ecotype of Dindérésso harvested in dry season presented the greatest antiradical activity. This strong $DPPH^{\bullet}$ reduction was shared with the Boromo ecotype at the stem level and the Dindérésso ecotype at the leaf level harvested in the rainy season ($p < 0.05$). These activities could be due to other non-phenolic antiradical compounds (Table 2). A relationship between season and $DPPH^{\bullet}$ reduction was observed with the Dindérésso ecotype (r = 0.8047; $p < 0.05$) (Figure 4). This could mean a variation in activity with the harvesting season. Some studies while underlining the impact of the environment and the season on the content of polyphenols and their antioxidant activity have also noted, this variation is not generalizable. It would be dependent on the plant, the part and the stage of harvest [18, 19, 30, 32].

3.2 Phenolic Compounds Content, Fe^{3+} Chelation and DPPH• Scavenging Activities of Acetone and Ethyl Acetate Fractions

The results on the aqueous extracts phenolic compounds content, the stem and root barks of *T. indica* from the Boromo ecotype were found to be better. As for the leaves, the Dindérésso ecotype was the most represented. Concerning the parts of *M. inermis*, the ecotype of Dindérésso was most represented.

Moreover, acetone fractions of ecotypes selected for their phenolic compounds content were tested *in vitro*. The contents varied from 87.77 to 112.31 mg GAE/g DM. Indeed, these fractions supposed to be rich in phenolic compounds presented similar phenolic compounds contents to those of the aqueous extract at the root level. The aqueous extract was found to be richer in phenolic compounds on the stem of *T. indica* and on the leaves of *M. inermis*. Concerning the stem bark and leaves of *M. inermis* and *T. indica* respectively, the acetone fraction revealed significantly higher content ($p < 0.05$). Kwon et al by assay have shown heat promotes the extraction quantitatively of polyphenols [28]. To this effect, estimation conducted on the macerated leaves of both plants showed low phenolic compounds content compared to this study [7]. This could justify the ability of the aqueous decoction to extract more phenolic compounds than the acetone-water macerate. There was no correlation in phenolic compounds content between the aqueous and acetone extracts.

All the results showed that the acetone extracts are more antioxidant than the aqueous extracts (Figure 5). Indeed, the antioxidant activities of the acetone fractions varied from 2653.44 to 2849.47 µmol AAE/g DM. Moreover, a correlation of antioxidant activity between the two extracts was observed ($r = 0.7658$; $p < 0.05$). The antiradical tests of the acetone fractions on double DPPH• concentration than that submitted to the aqueous extracts revealed in general that the acetone fractions are more antiradical (Figure 6). In fact, the antiradical activities of the acetone fractions varied from 81 to 96.9% of the DPPH•. However, the antioxidant and free radical scavenging activity was not dependent on total phenolic compounds. Tannins, saponins, sesquiterpenes, alkaloids, and phlobatanins were found in the aqueous and acetone extracts of *T. indica* stem barks and leaves [10].

Alowanou et al have highlighted the presence of condensed and gallic tannins, saponins, alkaloids, flavonoids, anthraquinones, glycosides, terpenes and reducing compounds in the acetone decoction of *M. inermis* leaves [6]. This could show the observed antioxidant and free radical scavenging activities are not only due to the quantity of phenolic compounds (Table 3).

Table 3 The Pearson's correlation between iron chelation, DPPH• scavenging activity and total phenolics content of different extracts of *T. indica* and *M. inermis* ($p < 0.05$).

Total soluble phenolics		DPPH• scavenging	Fe^{3+} chelation
Aqueous extract	*T. indica*	-0.9715	0.9422
	M. inermis	0.7969	0.4682
Acetone extract	*T. indica*	-0.9803	-0.3145
	M. inermis	-0.7135	-0.4838
Flavonoid rich fraction	*T. indica*	0.3986	0.9997
	M. inermis	0.9398	0.9942

NB: Pearson correlation measures the strength of the linear relationship between two variables. Positive values mean direct relationship between both variables. Negative values mean indirect relationship between both variables.

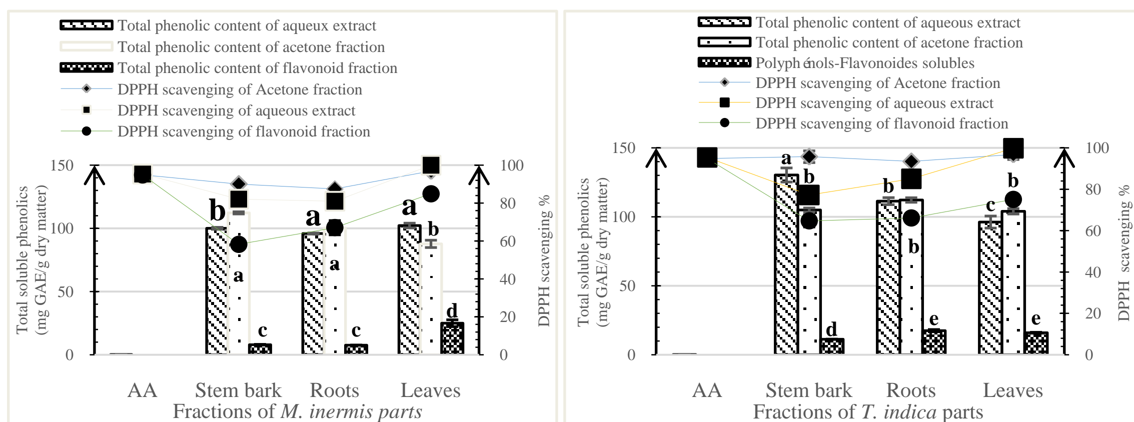

Fig. 5 DPPH• scavenging as a function of phenolics content in aqueous, flavonoid-rich and acetonic plant fractions: Total soluble phenolic compounds (milligram Gallic Acid Equivalent/gram dry matter) and DPPH• scavenging activity (%) are represented. Data are mean ± SD values from three test replicates for total phenolics and DPPH• reduction. Bars with the same letter are not phenolic content significantly different (P > 0.05) on same plant part. Pearson's correlation coefficient of DPPH• reduction activity between aqueous and acetone extract is 0.6125. The Pearson's correlation coefficient of total polyphenol content between aqueous extract and flavonoid rich fraction is -0.0183. The Pearson's correlation coefficient of total polyphenol content between the acetone extract and the flavonoid-rich fraction is -0.5894. The Pearson's correlation coefficient of DPPH• reduction activity between the acetone fraction and the flavonoid-rich fraction is 0.6272. The Pearson's correlation coefficient of DPPH• reduction activity between aqueous extract and flavonoid rich fraction is 0.8561.

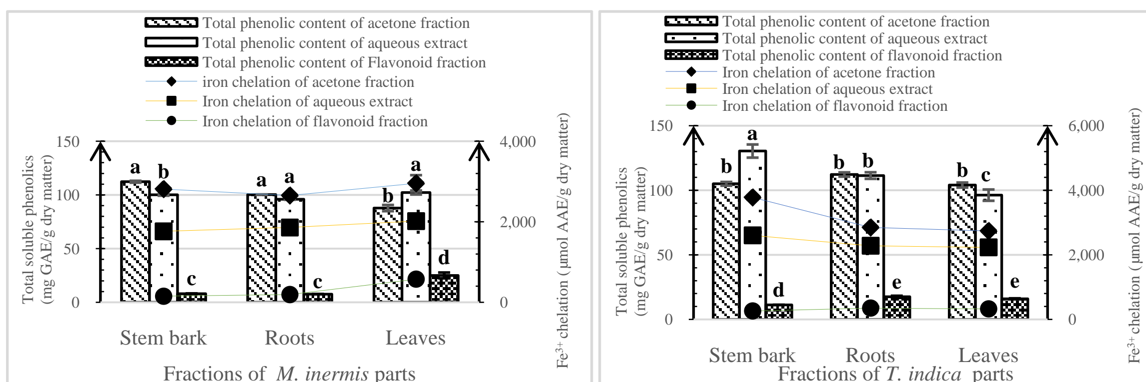

Fig. 6 Fe^{3+} Chelation as a function of polyphenol concentration in aqueous, acetonic and flavonoid-rich fractions of plants: Total soluble phenolics (milligram Gallic Acid Equivalent/gram dry matter) and Fe^{3+} chelation activity (micromoles Ascorbic Acid Equivalent/gram dry matter) are shown. Data are mean ± SD values from three test replicates for total phenolics and Fe^{3+} chelation. Pearson's correlation coefficient of total phenolics content between aqueous and acetone extracts is 0.1951. The Pearson's correlation coefficient of Fe^{3+} chelation activity between aqueous and acetone extract is 0.7658. The Pearson's correlation coefficient of Fe^{3+} chelation activity between aqueous extract and flavonoid rich fraction is 0.1939. The Pearson's correlation coefficient of Fe^{3+} chelation activity between the acetone fraction and the flavonoid-rich fraction is 0.0328.

Flavonoid-rich fractions exhibited free radical scavenging (58.23-84.91% DPPH•) and antioxidant properties (149.87-273.28 µmol AAE/g DM) (Figures 5 & 6). The part of phenolics in flavonoid rich fraction varied from 8.65-24.45% of the aqueous extract. The activities due to flavonoids remained low compared to those of the aqueous decoction and the phenolic-rich fraction ($p < 0.05$). The low antioxidant activity could support the fact that the high antioxidant activity of the aqueous decoction was the result of a synergistic action of compounds especially polyphenols but also a possible contribution of non-flavonoid compounds such as carotenoids and xanthophylls [33]. Indeed, phytochemical studies conducted on *M. inermis*

showed that the aqueous decoction of the leaves contains sterols, triterpenes, polyphenols, flavonoids, catechic tannins, saponosides, quinones and alkaloids [3, 4]. There is no correlation of total phenolics content and antioxidant activity between aqueous, acetone extracts and flavonoid rich fractions. But, a correlation of DPPH• reduction between aqueous extract and flavonoid rich fraction was observed (r = 0.8561; $p < 0.05$). This could mean a contribution of flavonoids on the anti-free radical activity of aqueous extracts. Soluble polyphenol-flavonoid dependent antioxidant activities of *T. indica* and *M. inermis* were observed (r = 0.9997 and 0.9942 respectively; $p < 0.05$) but only with *M. inermis* on DPPH• (r = 0.9398; $p < 0.05$) (Table 3). Flavonoids such as 8-C-glycosyl-apigenin; 6-C-glycosyl-apigenin; 8-C-glycosyl-luteolin; 6-C-glycosyl-luteolin; luteolin 7-o-glucoside; vitexin, isovitexin, orientin, iso-orientin, epicatechin, quercetin, and isorhamnetin were found in *T. indica* leaves [1, 13, 14]. Indeed, Lamien-Meda et al [34] and Mentreddy et al [35] have also found that flavonoids are endowed with antioxidant activities with strong correlation between antioxidant activity and flavonoid content. Thus, the observed activities would be due to flavonoids synergic action.

4. Conclusions

The study showed that the season generally influences the content of active ingredients and antioxidant and anti-free radical properties. The optimal harvesting season would be the dry season, namely November. The ecological influence in general was not sufficiently noticeable. Therefore, harvesting could be done on any of the three positions on the dry season. The bioactivity of the plants would be due to a synergy of action or to the individual action of the compounds of the extracts. Polyphenols are not the singles responsible for the antioxidant and anti-free radical activities of the traditional potion. But, the general dependence observed between the phenolic content and the activities underlines an important contribution of activities of polyphenols. On future studies it would be necessary to identify the ingredients especially polyphenols and explore their activity share. All the potentials observed below show that these plants in traditional medicine could reduce the incidences of diseases associated with oxidative stress like diabetes and hypertension.

Conflicts of Interest

The authors declare no conflicts of interest.

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