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Phenolic compounds and antioxidant activity of ethanolic leaf extracts of *Monanthotaxis sororia* (ANNONACEAE)

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Abstract

Medicinal plants are an important source of bioactive compounds, particularly phenolic compounds, which are recognized for their role in protecting against oxidative stress. The genus *Monanthotaxis* (ANNONACEAE) is known for its richness in secondary metabolites; however, *Monanthotaxis sororia* remains poorly studied. The present study aimed to characterize the phenolic compounds in the leaves of *Monanthotaxis sororia* and to evaluate their antioxidant activity. The ethanolic extraction was carried out by maceration at room temperature. Phytochemical screening revealed the presence of tannins, polyphenols, leucoanthocyanins, flavonoids, triterpenes, steroids, reducing compounds, monosaccharides, and holosides, indicating a significant diversity of primary and secondary metabolites. The results showed that the ethanolic extract has a high content of total phenolic compounds (1046,57 ± 164,45 mg PGE/100 g DW) as well as a substantial level of condensed tannins (441,32 ± 14,61 mg CE/100 g DW) and flavonoids (50,81 ± 0,08 mg QE/100 g DW). The evaluation of antioxidant activity revealed significant activity, with an IC_{50} of 0,077 mg/mL. These results suggest that *Monanthotaxis sororia* represents a promising source of phenolic compounds with high antioxidant potential. This study provides new data on the phytochemical profile of this species and opens perspectives for further investigations aimed at identifying the active molecules.

Keywords: *Monanthotaxis sororia*; Phenolic Compounds; Tannins; Flavonoids; Antioxidant activity

1. Introduction

Medicinal plants have long held an essential place in traditional healthcare systems. Today, they continue to generate increasing scientific interest due to the diversity of bioactive molecules they contain [1]. Among these molecules, phenolic compounds are particularly noteworthy because of their role in protection against oxidative stress [2-3].

The genus *Monanthotaxis* (ANNONACEAE) comprises nearly one hundred species distributed across the tropical regions of Africa and is traditionally used to treat various ailments (fever, digestive disorders, infections) [4]. Phytopharmaceutical studies conducted on several species of this genus have highlighted the diversity of their secondary metabolites, including flavonoids, terpenoids, polyphenols, and other bioactive derivatives exhibiting various biological activities [5-6].

However, despite the ethnobotanical and pharmacological interest in the genus *Monanthotaxis*, few studies have systematically explored both the profile of phenolic compounds and the antioxidant activity of extracts from certain species. To our knowledge, no detailed work has yet been devoted to *Monanthotaxis sororia*. In this context, the present

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study aims to evaluate the phenolic compounds of the ethanolic extract of *Monanthotaxis sororia* leaves as well as its antioxidant activity. It thus seeks to provide new scientific data on the antioxidant potential of this species and to establish a foundation for future phytochemical and pharmacological studies.

2. Materials and methods

2.1. Plant material

Leaves of *Monanthotaxis sororia* were collected in Andavakoera, Antsiranana II District, DIANA Region, Madagascar, in September 2022. This species is endemic to Madagascar. The identification of this species was carried out by a botanist at the Tsimbazaza Botanical and Zoological Park (PBZT). The leaves were sorted, washed, dried, and then ground to extract the metabolites present.

2.2. Preparation of the extract

An ethanolic extraction was performed on the leaves of *Monanthotaxis sororia*. Ten grams of leaf powder were macerated in 100 mL of 90 % ethanol. The mixture was stirred for 3 hours at room temperature, then macerated for 72 hours at 4 °C. After filtration through a fine mesh, the filtrate was centrifuged at 5500 rpm for 10 minutes. The supernatant was then dried at 40 °C to obtain a dry extract ready for analysis.

2.3. Phytochemical screening

A phytochemical screening was conducted on the extracts to identify the metabolites present. Tannins and other polyphenols were detected through the ferric chloride test, revealing characteristic colorations [7]. Leucoanthocyanins were identified using the Bate-Smith test, which produces a red coloration after acid heating [8]. Flavonoids were detected by the cyanidin reaction, with a pink to violet coloration indicating a positive result [9]. Alkaloids were revealed by the Mayer and Wagner tests, showing characteristic precipitation [10]. Steroids, unsaturated sterols, and triterpenes were identified using the Salkowski and Liebermann-Burchard tests, based on characteristic colorations [11]. Deoxy sugars were detected by the Keller-Kiliani test [12], while reducing compounds were revealed by Fehling's test [13]. Monosaccharides and holosides were identified using the thymol test, indicated by a red coloration [14].

2.4. Determination of total phenolic compounds

The total phenolic content was determined according to the method of Singleton & Rossi [15] using the Folin-Ciocalteu reagent. A pyrogallol standard range (0–50 mg/L) was used for calibration. For each analysis, 500 µL of sample were mixed with 100 µL of Folin-Ciocalteu reagent and 4,4 mL of 15 % sodium carbonate solution. After incubation for 5 minutes in the dark, the absorbance was measured at 710 nm. The results were expressed in mg pyrogallol equivalent per 100 g of dry weight (mg PGE/100 g DW), based on the equation of the calibration curve.

2.5. Determination of total flavonoids

The total flavonoid content was determined using the colorimetric method with aluminum trichloride [16]. A quercetin standard range (0–20 µg/mL) was prepared. For each standard solution in the range, 5 mL of 2 % aluminum trichloride solution was added, then incubated for 15 minutes before reading at 425 nm to establish the calibration curve. A 0,1 % solution of the crude extract was analyzed under the same conditions, using a blank tube without the addition of aluminum trichloride solution. After incubation, the absorbance was measured at 425 nm. The total flavonoid content was calculated from the regression equation and expressed as mg quercetin equivalent per 100 g of dry weight (mg QE/100 g DW).

2.6. Determination of condensed tannins

The content of condensed tannins was determined using the colorimetric vanillin method in an acidic medium [17]. The extract to be assayed was obtained by mixing 0,5 g of dry residue in 15 mL of a methanol-hydrochloric acid solution for 20 minutes, followed by collection of the supernatant after centrifugation. A catechin standard curve was prepared with concentrations ranging from 0 to 100 µg/mL. For the assay, 50 µL of each sample was mixed with 1,5 mL of 4 % vanillin/methanol, after which 750 µL of concentrated hydrochloric acid was added. The mixture was incubated at 30 °C for 20 minutes in the dark. After incubation, absorbance was measured at 500 nm. The content of condensed tannins was expressed in mg catechin equivalent per 100 g of dry weight (mg CE/100 g DW).

2.7. Evaluation of antioxidant activity

The antioxidant capacity of the extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The measurement of radical-scavenging activity was performed following the protocol of Yamaguchi *et al* [18]. Samples (at concentrations ranging from 0,1 to 10 mg/mL) were added to 1 mL of 0,1 mM methanolic DPPH. After 30 minutes, the decrease in absorbance was measured at 517 nm. Quercetin was used as a positive control.

3. Results

3.1. Extraction yield

The ethanolic extraction of *Monanthotaxis sororia* leaves was carried out by maceration at room temperature. The extraction yield was 19,48 ± 0,05 %.

3.2. Phytochemical screening

Phytochemical screening of the ethanolic extract of *Monanthotaxis sororia* leaves revealed the presence of several secondary and primary metabolites. The tests indicated the presence of alkaloids, tannins, polyphenols, leucoanthocyanins, deoxy sugars, triterpenes, steroids, reducing compounds, monosaccharides, holosides, and flavonoids. These results demonstrate that the extract contains numerous chemical compounds potentially exhibiting biological activity. The results of each test are presented in Table 1.

Table 1 Result of the phytochemical screening

Metabolites present	Results
Alkaloids	+
Tannins and other polyphenols	+
Leucoanthocyanins	+
Iridoids	-
Deoxy sugars	+
Triterpenes and steroids	+
Reducing compounds	+
Monosaccharides and holosides	+
Flavonoids	+

(+) = presence ; (–) = absence

3.3. Contents of total phenolic compounds, total flavonoids, and condensed tannins

Colorimetric assays allowed the quantification of total phenolic compounds, total flavonoids, and condensed tannins present in the ethanolic leaf extract. The corresponding values are presented in Table 2.

Table 2 Content of total phenolic compounds, total flavonoids and condensed tannins

Phytochemical compounds	Content
Total phenolic compounds	1046.57 ± 164.45 mg PGE/100 g DW
Total flavonoids	50.81 ± 0.08 mg QE/100 g DW
Condensed tannins	441.32 ± 14.61 mg CE/100 g DW

The quantifications carried out demonstrate that the ethanolic extract of *Monanthotaxis sororia* leaves exhibits a high content of phenolic compounds (1046,57 ± 164,45 mg PGE/100 g DW). Condensed tannins are also well represented (441,32 ± 14,61 mg CE/100 g DW). In contrast, the flavonoid content is relatively low (50,81 ± 0,08 mg QE/100 g DW) but still indicative of a measurable contribution to the overall bioactive activity of the extract.

3.4. Antioxidant capacity

The DPPH radical-scavenging test was conducted to characterize the antioxidant capacity of the ethanolic leaf extract. Figure 1 shows the DPPH radical-scavenging activities obtained.

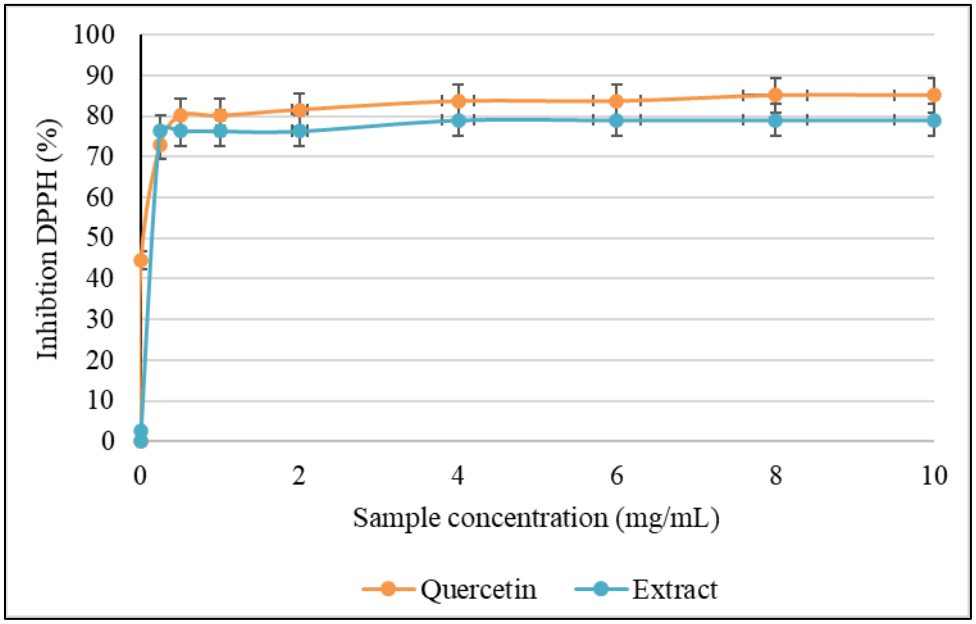

Figure 1 DPPH antiradical activity curves of quercetin and the ethanol extract of *Monanthotaxis sororia*

Based on the measured antiradical activities, the inhibitory concentrations required to reduce the free radical content by 50 % (IC_{50}) were determined and are presented in Table 3.

Table 3 IC_{50} of the samples

Samples	IC_{50} (mg/mL)
Quercetin	0.028
Extract	0.077

Table 3 shows that the ethanolic extract of *Monanthotaxis sororia* leaves exhibits significant antioxidant activity with an IC_{50} of 0,077 mg/mL. This concentration is slightly higher than that of quercetin (IC_{50} of 0,028 mg/mL), which is used as a positive control.

4. Discussion

4.1. Extraction yield

The ethanolic extraction yield of *Monanthotaxis sororia* leaves obtained by cold maceration is 19,48 ± 0,05 %. This relatively high value suggests that ethanol, a solvent with intermediate polarity, is effective in extracting a wide range of compounds from this plant matrix. Similar yields have been reported for plants in the ANNONACEAE family, with ethanolic extractions of 17,86 % for *Uvaria rufa* and 21 % for *Annona muricata* [19-20]. This good yield can be explained by ethanol's ability to dissolve both hydrophilic and moderately lipophilic compounds, thereby maximizing the recovery of leaf constituents.

4.2. Phytochemical screening

Phytochemical screening revealed a remarkable metabolic richness of the extract. The simultaneous presence of alkaloids, tannins, polyphenols, flavonoids, and triterpenes is particularly noteworthy. These families of secondary metabolites are recognized for their wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties [21-23]. The detection of leucoanthocyanins as well as steroids further reinforces the diverse

chemical profile of this plant. The identification of primary metabolites (Monosaccharides, holosides, deoxy sugars) and reducing compounds, although less specific to targeted biological activity, confirms that the extraction successfully recovered a substantial portion of the plant material. These compounds may play a synergistic role or modulate the bioavailability of active secondary metabolites [24].

4.3. Phenolic compound content

The ethanolic extract of *Monanthotaxis sororia* leaves exhibits a high content of total phenolic compounds. Analysis shows that this richness is dominated by condensed tannins. Within the ANNONACEAE family, several studies have shown that polyphenols, flavonoids, and tannins constitute major groups responsible for the observed biological activities, notably in *Monodora myristica*, *Uvaria chamae*, and *Annona squamosa* [6, 25-27]. The profile observed in *Monanthotaxis sororia* is broadly consistent with the chemical characteristics of this family.

4.4. Antioxidant activity

The evaluation of radical-scavenging activity using the DPPH assay revealed an IC_{50} of 0,077 mg/mL, indicating a significant antioxidant activity of the ethanolic extract of *Monanthotaxis sororia*. Numerous studies have shown that a high content of phenolic compounds is generally associated with strong antioxidant activity [28-29].

The remarkable activity observed for the extract of *Monanthotaxis sororia* can therefore be consistently explained by its high total phenolic content, and particularly by the abundance of condensed tannins, which are the main contributors to radical-scavenging activity. Flavonoids, although present in modest amounts, also contribute to this activity through additive or synergistic effects.

The antioxidant activity of phenolic compounds primarily relies on their ability to neutralize free radicals through the transfer of electrons or hydrogen atoms, as well as their capacity to stabilize reactive oxygen species via electronic delocalization [30-31].

5. Conclusion

This study provides new data on the phenolic composition and antioxidant activity of *Monanthotaxis sororia* leaves. Ethanol extraction yielded a satisfactory output. Phytochemical screening revealed the presence of tannins, polyphenols, flavonoids, triterpenes, and other primary and secondary metabolites, confirming the chemical richness of this species. Quantification of phenolic compounds showed a high content, correlated with significant antioxidant activity, highlighting the central role of polyphenols, condensed tannins, and flavonoids in the extract's antioxidant capacity.

These results confirm that *Monanthotaxis sororia* represents a promising source of bioactive compounds and justify its potential for therapeutic and cosmetic applications. They also provide a solid foundation for further studies aimed at identifying the responsible molecules and exploring their specific biological effects, in order to fully harness the potential of this species.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

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