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Research Article

BIOCHEMICAL EVALUATION OF FOUR MORPHOTYPES OF CHRYSANTHELLUM AMERICANUM L. VATKE IN THE SUDANO-SAHELIAN ZONE OF BURKINA FASO

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ABSTRACT

Chrysanthellum americanum L. VATKE is a herbaceous plant widely used in traditional medicine to treat a wide range of illnesses (kidney stones, anemia, malaria, etc.). Knowledge of the growth potential, vegetative development and phytochemical potential of a number of *Chrysanthellum americanum* L. VATKE morphotypes could help to enhance the value of the species. It is in this context that this study was initiated, with a view to gaining knowledge of the agro-morphological and biochemical characteristics of Chrysanthellum americanum L. VATKE. The study was carried out at the experimental station of the Institute of Environment and Agricultural Research Saria (INERA/Saria. Four morphotypes (MT1, MT2, MT3 and MT4) from a mixture of *Chrysanthellem americanum* seed collected from Bobo, Nandiala and Tema were grown in a Fisher design with three replications. A total of 27 agro-morphological parameters, including 8 qualitative and 19 quantitative, were observed and measured. Morphotypes showed variation in achene color and stem habit. Significant differences between morphotypes were observed for parameters related to growth, plant phenological cycle and yield. The MT3 morphotype was the best performing, with greater plant spread (22.34±1.02 cm), longer (10.78±0.57 cm) and wider leaves (6.28±0.21 cm), a high number of secondary branches (10.78±0.52), and higher yields of achenes (1.44±0.12 t/ha) and fresh tops (10.08±0.31 t/ha). Morphotype MT4 achieved the highest thousand achene weight (0.70 g), and morphotypes MT1 and MT4 recorded the highest fertile achene/flower frequencies (73%). For cycle length, morphotypes MT1 and MT2 were early with 71.33±2.40 JAS and 69.00±2.52 JAS. Respectively 9 biochemical parameters, 5 qualitative and 4 quantitative, were evaluated. All morphotypes showed the presence of saponosides, flavonoids, polyphenols, steroids and triterpenes, but no alkaloids. Analysis of variance revealed a significant difference between morphotypes for parameters related to phenolic compound content and antioxidant activities. Morphotype MT3 had the highest polyphenol content (110.66±1.97 mg EAG/g), flavonoid content (12.23±0.13 µg EQ/g) and iron reducing capacity (79±0.96 µmol/g). On the other hand, the MT2 morphotype performed best in DPPH antiradical activity, with 92.27±0.86% inhibition.

Keywords: *Chrysanthellum americanum*, *M*orphotypes, Biochemical parameters.

INTRODUCTION

Traditional medicine still remains the first resort for many Burkinabè due to the inaccessibility of conventional medicines (Cissé*and al.*, 2019). In Burkina Faso, over 80% of the population regularly turn to traditional medicine and medicinal plants for their healthcare (WHO, 2020). These medicinal plants are therefore an integral part of Burkina Faso's cultural heritage. These plants include several woody and herbaceous species, such as *Acacia macrostachya* R, *Adansonia digitata* L, *Azadirachta indica* A. JUSS,

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Chrysanthellum americanum (L.) VATKE or *Chrysanthellum indicum* var Afro-American B.L. TURNER.

Chrysanthellum americanum is a plant used in the wild. The whole plant is used. The leafy stems are used to treat yellow fever, hematuric icterus, dystonia, alcoholism, anuria, malaria, gallstones, salivary calculi, renal colic, urinary lithiasis, dyspepsia and intestinal fermentation (Nacoulma, 1996). However, in view of its medicinal use for human health, this species is under heavy anthropic pressure. Indeed, people use the whole plant (roots, stems and leaves). In order to meet local demand and contribute to the preservation of the plant in its natural habitat, one possibility would be to cultivate it. Consequently, knowledge of the biochemical characteristics in terms of compounds and content, as well as antioxidant or biological activity, of a number of *Chrysanthellum americanum* (L.) VATKE morphotypes under cultivation conditions would be a very important first step towards domesticating the species. The general aim of this study is to determine the agro-morphological and biochemical characteristics of *Chrysanthellum americanum* L. VATKE. Specifically, it aims to:(i) identify the phytochemical components of fourmorphotypes of *Chrysanthellum americanum* L. VATKE. (ii)evaluate the antioxidant activities of these morphotypes of *Chrysanthellum americanum* L. VATKE.

MATERIALS AND METHODS

Plant material

The plant material used consisted of four morphotypes of *Chrysanthellum americanum* from a mixture of seeds collected in the localities of Tema, Nandiala and Bobo Dioulasso. *Chrysanthellum americanum* seeds were collected in August 2021.This mixture of collected seeds was sown in bulk in a trial at Saria during September 2021 to observe plant growth and development. The four morphotypes were selected on the basis of leaf size, plant height and stem habit within the plants obtained in this trial. Seed multiplication of these four morphotypes was carried out in June 2022, and the harvested seeds were used for this study.

Biochemical characterization of *Chrysanthellum americanum* L. morphotypes.

Field sampling

On each replicate, two plants of each morphotype were randomly selected and uprooted at the flowering stage (Table I). The four morphotypes did not reach the flowering stage at the same time. Samples were labelled (replicate number and morphotype number) and then shade-dried at room temperature for twenty days. They were then placed in different envelopes, labelled as above, and sent to the Laboratoire de Biochimie, Biotechnologie, Technologie Alimentaire et Nutrition (LABIOTAN) for biochemical analysis.

Extraction of phytochemical compounds

Phenolic compounds were extracted by decoction. This extraction method followed the protocol described by Konkon*and al*. (2006), with a few modifications. It was carried out by first crushing the various samples. For each sample, 10 g of plant powder was weighed using an electronic balance and then placed in a flask containing 150 mL of distilled water (Figure 1). The flask containing the mixture was gently stirred by hand. It was then heated in a reflux set-up and left to boil for 30 min. The hot flask was removed from the reflux set-up with forceps and cooled to room temperature for 3 min. After cooling the flask, the mixture was filtered through coffee filter paper into a jar. Finally, the filtered solution (or aqueous extract) was divided between two Petri dishes and evaporated in the oven at 50°C for 48 hours. After evaporation, compounds adhering to the walls of the Petri dishes were scraped off with a scalpel and collected in labelled bags. These were used to assay phenolic compounds (total phenolics, total flavonoids), to assess antioxidant activity (DPPH; FRAP) and to screen phytochemical compounds (tannins and polyphenols, flavonoids, alkaloids, saponosides, terpenes and steroids).

Table 1. Dates on which morphotypes were uprooted for drying.

Morphotypes	Dates	Number of Days After Sowing (DAS) and Re-Sowing (DRS)
Morphotype 1	25/09/2022	47 JAS
Morphotype 2	16/09/2022	38 JAS
Morphotype 3	09/10/2020	48 JAR
Morphotype 4	21/10/2022	60 JAR

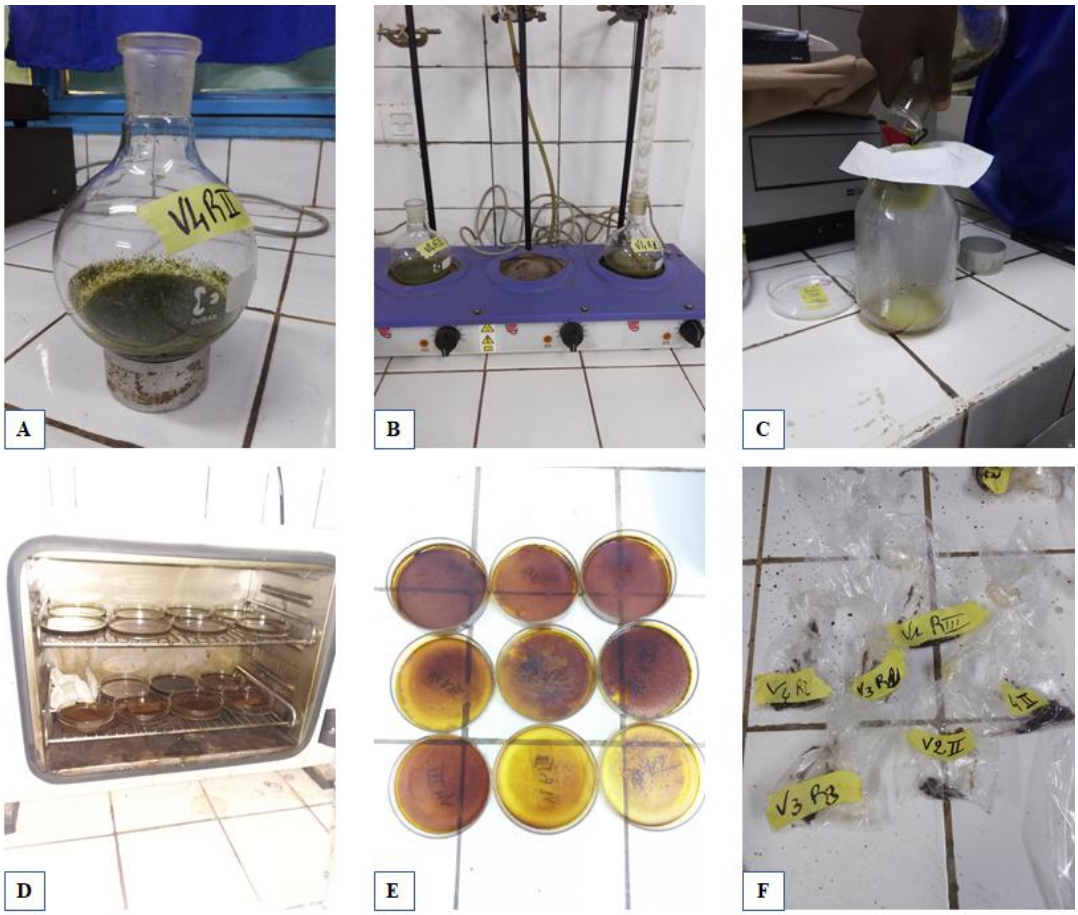

Figure 1. extraction of phytochemical compounds
Legend: A: flask containing the mixture of distilled water and plant powder; B: reflux set-up; C: filtering the boiled mixture; D: placing the Petri dishes in the oven and evaporating them; E: scraping the compounds from the walls of the Petri dishes; F: recovery of compounds in labelled bags.

Determination of phenolic compounds

Determination of total phenolics

Total phenolics were determined using the procedure described by Singleton (1999). It is based on the high oxidizability of phenolic compounds. The reagent used is a mixture of phosphomolybdate and sodium tungstate, which is reduced to a mixture of tungsten blue and molybdenum during phenol oxidation in an alkaline medium. Folin-Ciocalteu Reagent (FCR) has its colorimetric properties modified when complexed with certain molecules. It reacts with the OH function of phenols. A calibration curve was first performed using gallic acid at 2 mg/mL. Thus, 1000 µL of gallic acid were taken and placed in T0 and T-1. A cascade dilution was performed with ethanol 80% of a volume of 1000 µL from T-1 to T-10 followed by homogenization from one tube to another. These solutions were used in place of the extracts (1 mg/mL) for the calibration curve assay. To each vial containing 125 µL of sample solution (gallic acid or extract), 625 µL of FCR (0.2N) were added. After waiting 5 min, 500 µL of sodium carbonate solution (75 g/L) was added and, after shaking, the various solutions were left to stand, protected from light, for 1h30 min. Readings were taken at 760 nm wavelength using a spectrophotometer (Epoch; BioTeK), against a blank for each sample consisting of a mixture of 500 µL sodium carbonate, 625 µL 80% ethanol and 125 µL gallic acid or extract (Table II). Readings were taken in triplicate per sample extract, and total phenolic content was expressed as mg Gallic Acid Equivalent per gram (mg GAE/g) of dry matter (dry plants).

$$T = \frac{C \times D}{Ci} \times 10$$

T = total phenolic concentration in mg EAG/g dry matter;

C = sample concentration read off the standard curve;

D = dilution factor of the sample to be assayed;

Ci = initial concentration of the sample solution to be assayed.

Table 2. Composition and quantification of the total phenolics assay.

Addition/Tube	Sample (Test)	White
Sample solution(µL)	125	125
FCR 0,2N (µL)	625	---
Ethanol 80% (µL)	---	625
Waiting time	5 mn	5 mn
HCO3N à 75g/l (µL)	500	500
Incubation time	1h30	1h30

Determination of total flavonoids

Total flavonoid content was determined using the DOWD colorimetric method adapted by ARVOUET-GRAND et al. (1994): 750 µL of 2% AlCl3 in pure ethanol was mixed with 750 µL of 1 mg/mL extract. For each sample, a blank was made by mixing 750 µL of extract and 750 µL of 80% ethanol (Table III). After 15 min incubation in the dark at room temperature, optical densities were read at 415 nm using a spectrophotometer against a previously plotted calibration curve. This calibration curve was plotted using successive dilutions of quercetin solution (2 mg/mL) with 80% ethanol from T-1 to T-10 in the range of 3.5 mL (quercetin) for T0 and 7 mL (quercetin plus ethanol) for T-1 to T-10. Readings were taken in triplicate per sample extract, and total flavonoid content is expressed as mg Quercetin Equivalent (QE) per gram (mg QE/g) of dry matter.

$$T = \frac{C \text{ x } D}{Ci} \text{X } 1000$$

T = total flavonoid concentration in mg EQ/g dry matter;

C = sample concentration read off the standard curve;

D = dilution factor of the sample to be assayed;

Ci = initial concentration of the sample solution to be assayed.

Table 3. Composition and quantification of the total flavonoids assay.

Addition/Tube	Sample (Test)	White
Sample solution (µL)	750	750
Ethanol 80 %(µL)	----	750
$AlCl_3$ 2%(µL)	750	----
Incubation time	15 mn	15 mn

Study of antioxidant activity

Two methods were used to assess antioxidant activity: DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power).

DPPH anti-free radical activity

This method is based on the decrease in absorbance at 517 nm when the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) reacts with an antiradical (Figure 2). In the presence of the free-radical scavengers, the violet DPPH (2,2-Diphenyl-1-picrylhydrazyl) is reduced to the yellow 2,2 -diphenyl-1-picrylhydrazyl (Maataoui *al.*, 2006). The DPPH method is studied following the model of Velazquez *al.* (2003). 500 µL of each sample (1 mg/mL) were mixed with 1000 µL of DPPH (20 mg/L). Ethanol 80% was used as a blank (1000 µL). The absorbance of the mixture was read after 15 min at 517 nm, using a spectrophotometer. Table IV shows the composition and quantification for the evaluation of DPPH free radical scavenging activity.

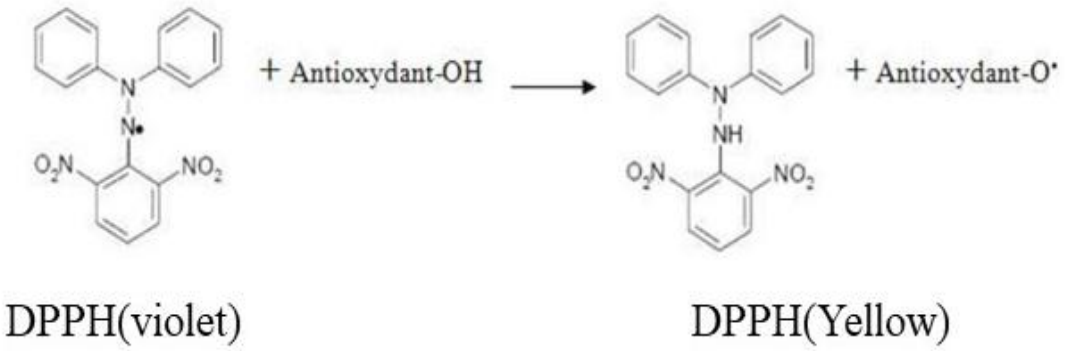

Figure 2. Reaction of an antioxidant with the DPPH radical Source: Congo (2012).

Table 4. Composition and quantification for assessing DPPH free radical scavenging activity.

Addition/Tube	Sample (Test)	White
Sample solution (µL)	500	---
DPPH (µL)	1000	1000
Ethanol (80%) (µL)	---	500
Incubation times	15 mn	15 mn

Free radical scavenging activity was expressed as percentage inhibition (% inhibition) using the following formula:

$$AAR(\%) = \frac{Abs\ (blc) - Abs\ (samp)}{\mathbf{Abs\ (blc)}}\ X\ 100$$

Ferric Reducing Antioxidant Power (FRAP)

The FRAP (Ferric Reducing Antioxidant Power) method is based on the reduction of ferric ion (Fe3+) to ferrous ion (Fe2+). The reducing power of compounds was assessed using this method. The variant of Hinneburgand al. (2006) was used to assess the iron-reducing power of *Chrysanthellum americanum* extracts. In a test tube containing 0.5 mL sample solution, 1.25 mL phosphate buffer (0.2 M; pH 6.6) was added, followed by 1.25 mL 1% potassium hexacyanoferrate [K3Fe (CN)6] in water. The mixture was heated to 50°C in a water bath for 30 minutes. Next, 1.25 mL trichloroacetic acid (0.1%) was added and the mixture centrifuged at 2000 rpm for 10 minutes. Three 625 µL aliquots were made on a plate, to which were added 625 µL of distilled water and 125 µL of freshly prepared 0.1% FeCl3 in water (Table V). A blank without sample was prepared under the same conditions. Readings were taken at 700 nm against a previously prepared ascorbic acid calibration curve, concentration 2 mg/mL. The iron-reducing potential of *Chrysanthellum americanum* extracts is expressed in mmol Ascorbic Acid Equivalent per gram (mmol EAA/g) of dry extract according to the following formula:

$$C = \frac{C\ \mathbf{x}\ D}{\mathbf{M\ x\ Ci}}\ X\ 100$$

C = concentration of reducing compounds in mmol EAA/g dry matter;
c = sample concentration read;
D = dilution factor of extract stock solution;
Ci = concentration of extract stock solution;
M = molar mass of ascorbic acid (176.1 g/mol).

Table 5. Composition and quantification for assessing iron's reducing power.

Addition/Tube	Sample (Test)	White
Sample solution (µL)	625	---
$FeCl_3$ (0,1 %) (µL)	125	125
Distilled water (µL)	625	1250
Incubation times	30 mn	30 mn

Phytochemical screening

Tube tests were carried out for phytochemical characterization in order to detect the main families of secondary metabolites present in *Chrysanthellum americanum* L. VATKE. The qualitative methods described by Ciulei in 1982 were used to highlight the presence of the chemical compounds present in the plants. The extract solution was prepared by mixing 10 mg of extract in 10 mL of distilled water.

Detection of flavonoids: Shibata test

A volume of 1 mL extract solution was withdrawn and added to 1 mL distilled water plus fragments of magnesium turnings with 4 drops of concentrated hydrochloric acid (HCl) in test tubes. Control tubes were also made.

The appearance of red or orange coloration indicates the presence of flavonoids.

Detection of saponosides: foam test

In test tubes, 1 mL extract solution was diluted with 1 mL distilled water, then shaken vigorously for at least 15 min. The appearance of a column of foam at least 1 cm high, persisting for 15 min, indicates the presence of saponosides.

Detection of tannins and polyphenols: FeCl3 test

Two to three drops of 1% FeCl3 were added to 1 mL extract solution diluted in 1 mL distilled water. The appearance of a blue-black or blackish-green coloration indicates the presence of tannins.

Detection of terpenes and steroids: Libermann-Burchard test

A 1 mL volume of each extract solution was introduced into test tubes. Next, 1 mL acetic anhydride and 1 mL chloroform were added successively to 1 mL extract solution in each test tube. Finally, 1 mL of concentrated sulfuric acid (H2SO4) was gently added to the mixture. The appearance of a red-brown ring at the interface zone of the two liquids marks the presence of terpenes and steroids.

Data analysis

All the data collected was entered and saved on Excel 2016 spreadsheets. A descriptive analysis was carried out for the agronomic qualitative parameters and the biochemical qualitative parameters observed. Quantitative parameters were analyzed using Minitab 18 software. Analyses of variance (ANOVA) were carried out for parameters relating to plant growth, parameters relating to the phenological cycle of the plants and parameters relating to yield and its components, in order to determine significant differences within morphotypes. They were also used for biochemical parameters relating to phenolic compound content and antioxidant activity. Mean values were compared using the Fisher test at the 5% threshold. In addition to the analysis of variance (ANOVA) performed for parameters related to yield components, including 1000 achene weight, frequency of fertile achenes per flower and frequency of sterile achenes per flower, a descriptive and comparative analysis based on morphotypes was performed using histograms. The Pearson correlation matrix was used to observe relationships between agronomic parameters relating to plant growth, the phenological cycle of plants, yield and its components, and biochemical parameters relating to phenological compound content and antioxidant activity.

RESULTS AND DISCUSSION

Tube tests carried out on all samples revealed positive results for saponosides, flavonoids, tannins and polyphenols, steroids and triterpenes (Figures 3; 4 and 5). However, all samples were negative for alkaloids. Table VI shows the results of tube tests.

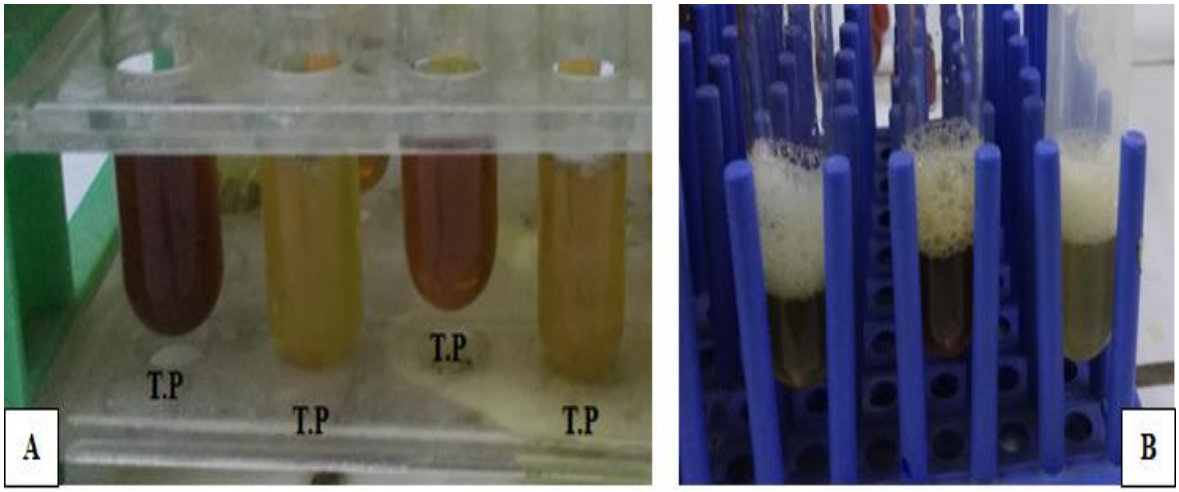

Figure 3. test for flavonoids and saponosides in tubes
Legend: A: test for flavonoids; B: test for saponosides. **P.T:** Positive test**; C.T:** Control tube.

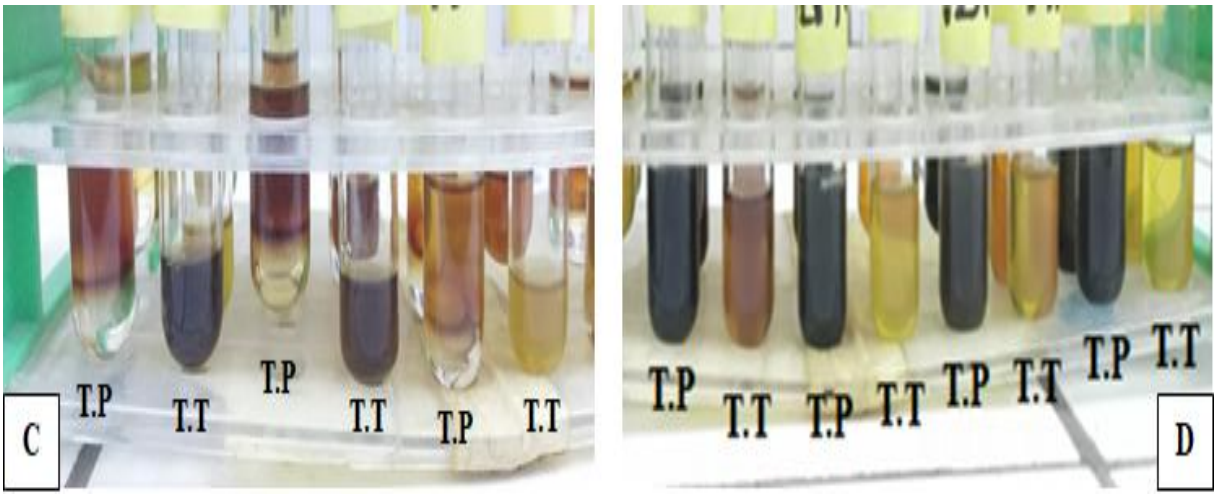

Figure 4. Test for terpenes/steroids, tannins and polyphenols in tubes Legend: C: test for terpenes/steroids; D: test for tannins and polyphenols': positive test; T.T: control tube.

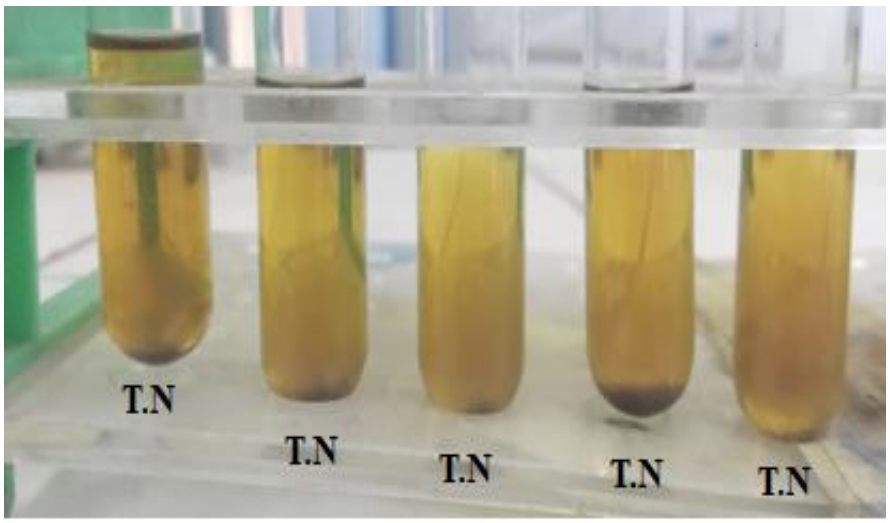

Figure 5. Alkaloid detection test in tubes Legend: T.N: negative test.

Table 6. Tube test results for chemical groups by sample.

Samples	Chemical groups				
	Saponosides	Flavonoïds	Tanins et polyphenols	Steroïds /Triterpenes	Alkaloids
MT1	+	+	+	+	-
MT2	+	+	+	+	-
MT3	+	+	+	+	-
MT4	+	+	+	+	-

Legend: +: present; -: absent.

The results of the analysis of variance (ANOVA) showed a highly significant difference (P=0.000) between morphotypes for polyphenol content and iron-reducing capacity, followed by a highly significant difference (P=0.009) for flavonoid content and a significant difference (P=0.025) for DPPH free radical scavenging activity (Table VII). Thus, morphotype MT3 recorded the highest polyphenol content (110.66±1.97 mg EAG/g), flavonoid content (12.23±0.13 mg EQ/g) and iron reducing capacity (79±0.96 mmol/g), while morphotype MT2 recorded the highest DPPH free radical scavenging activity (92.27±0.86% inhibition), but low polyphenol content (73.59±1.51 mg EAG/g). In contrast, the MT4 morphotype had the lowest polyphenol content (6.94±1.45 mg EAG/g), followed by the MT2 morphotype with the lowest flavonoid content (8.26±0.22 mg EQ/g) and the lowest iron-reducing capacity (14.28±1.30 mmol/g). Morphotype MT1 also recorded the lowest DPPH free radical scavenging activity (79.37±5.65%). The MT1 morphotype had an intermediate polyphenol content (92.08±7.06 mg EAG/g) and the MT1 and MT4 morphotypes also had intermediate flavonoid contents (9.98±1.48 mg EQ/g and 9.93±0.24 mg EQ/g) and intermediate iron reduction capacities (55.97±9.40 mmol/g and 57.85±4.21 mmol/g). With regard to DPPH free radical scavenging activity, morphotypes MT3 and MT4 recorded intermediate values (88.84 ± 0.93% and 88.03±0.56% inhibition). The calibration curves for gallic acid (y=10.276x + 0.0614; R2: 0.9976), quercetin (y=16.248x + 0.0625; R2: 0.9912) and ascorbic acid (y=15.317x + 0.0607; R2: 0.9892) served as positive references for the determination of polyphenol and flavonoid content and iron reducing power (Appendix 1).

Table 7. Results for phenolic compounds and antioxidant activity.

Samples	Polyphenols (mg EAG/g)	Flavonoids (mg EQ/g)	DPPH (% Inhibition)	FRAP (mmol/g)
MT1	92,08±7,06^{b}	9,98±1,48^{ab}	79,37±5,65^{b}	55,97±9,40^{b}
MT2	73,59±1,51^{c}	8,26±0,22^{b}	92,27±0,86^{a}	14,28±1,30^{c}
MT3	110,66±1,97^{a}	12,23±0,13^{a}	88,84±0,93^{ab}	79±0,96^{a}
MT4	6,94±1,45^{d}	9,93±0,24^{ab}	88,03±0,56^{ab}	57,85±4,21^{b}
Pr > F	0,000	0,009	0,025	0,000

Legend: DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reduccing antioxidant power, EAG: Gallic Acid equivalent, EQ: Quercetin equivalent.

Table 8. Pearson correlation matrix between biochemical parameters linked to phenolic compound content and antioxidant activity.

Characters	Polyphénols	Flavonoids	DPPH	FRAP
Polyphénols	1			
Flavonoïdes	**0,414**	1		
DPPH	-0,310	**-0,726**	1	
FRAP	0,223	**0,681**	**-0,430**	1

Values in bold are significant at the 5% level.
Legend: DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power.

The correlation test was used to highlight the relationships between the biochemical parameters linked to the content of phenolic compounds and antioxidant activity. The level of correlation was observed through correlation coefficients in Pearson's correlation matrix with a significance threshold of 5% (Table VIII). ➢Positive correlations were observed between: polyphenol content and flavonoid content (r=0.414); flavonoid content and iron reducing power (FRAP) (r=0.681). ➢Negative correlations were revealed between: flavonoid content and DPPH antiradical activity (r=-0.726); DPPH antiradical activity and iron reducing power (FRAP) (r=-0.430).

For qualitative biochemical parameters carried out with the aqueous extract, only alkaloids were absent in all morphotypes. Guenne *al.* (2011) observed the absence of alkaloids in the methanoic extract of *Chrysanthellum americanum*. Cisséand *al.*, 2019 observed only the absence of alkaloids with aqueous extract of *Chrysanthellum americanum* and KONE et al (2022) observed the presence of alkaloids with aqueous extract of *Chrysanthellum americanum*. The results of these qualitative biochemical parameters are thought to be due to the genetic character of the species and the environmental conditions of the environment. According to Cissé *and al* (2019), the plant's richness in secondary metabolites, namely tannins and polyphenols, flavonoids and saponosides, is responsible for its pharmacological activities. However, among the quantitative parameters, analysis of variance showed a significant difference between morphotypes.

The MT3 morphotype was very rich in polyphenols (110.66±1.97 mg EAG/g) and flavonoids (12.23±0.13 mg EQ/g). On the other hand, the MT2 morphotype had the highest DPPH antiradical activity (92.27±0.86% inhibition) and the MT3 morphotype had the highest iron reducing capacity (FRAP) (79±0.96 mmol/g). The previous study by Guenne *al*. (2011) revealed low average levels of total phenolics (13.2±0.1 mg EAG/100 mg), total flavonoids (4.22±0.06 mg EQ/100mg) and iron reducing power (0.99±0.01 mmol EAA/g) with the *Chrysanthellum américanum* species. Also, Benhammou (2012) obtained with the methanoic extract of *Cotula cinerea* from the Asteraceae family, a polyphenol content of 22.220.41 mg EAG/g DM and a flavonoid content of 3.9310.061 mg EC/g DM (mg catechin equivalent/g dry matter). This significant difference can be explained by genotypic factors, biotic conditions (plant height and spread, number of secondary branches) and abiotic conditions (soil fertility level, soil type, temperature, sunshine) and the field sampling period. The variation in polyphenol content is thought to be due to biotic conditions (species, organ and physiological stage) and abiotic conditions (edaphic factors) (Ksouri et al., 2008), the nature of the soil and the type of microclimate (Atmaniand *et al*., 2009). The Pearson correlation matrix showed that flavonoid content was positively correlated with iron reducing power (FRAP). This relationship reflects the fact that the morphotypes with the highest flavonoid content had the highest FRAP. In contrast, flavonoid content was negatively correlated with DPPH free radical scavenging activity. This correlation can be explained by the fact that the morphotypes that obtained plants richer in flavonoids recorded the lowest DPPH antiradical activity.

CONCLUSION

The results obtained enabled phytochemical compounds to be identified and antioxidant activity to be assessed. The MT3 morphotype was richer in biochemical compounds than the others. It had a good content of phenolic compounds (polyphenols and flavonoids) and high antioxidant activity (DPPH and FRAP). The MT3 morphotype could be recommended for agricultural production as well as in the treatment of diseases linked to oxidative stress in order to meet the needs of the population. This study could be considered as an important source of basic information for scientific studies. For the continuation of this study, the following perspectives can be envisaged: Repeat the trial in the same area or in other climatic zones to reinforce our results obtained; continue the study with a larger number of morphotypes in order to gain a better understanding of the diversity within the species; integrate other biochemical parameters such as vitamins, mineral elements and the screening of other chemical groups (Mucilages, Carbohydrates, Coumarins).

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

No AI tools or associated technologies were used in the writing or generation of scientific content in this article

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