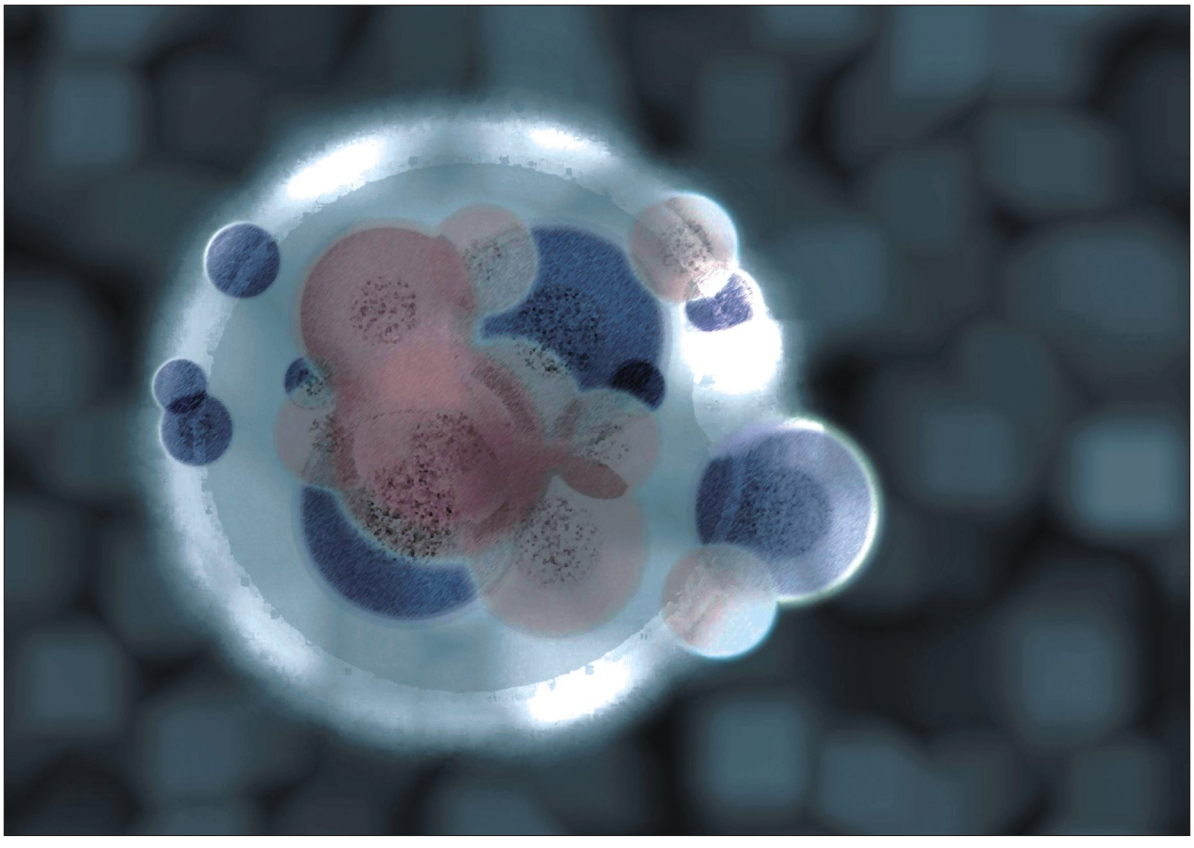

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Research Article
Polyphenolic Content Variability of *Solenostemon rotundifolius* [(Poir.) J. K. Morton] Accessions from Burkina Faso and Ghana

[1]Alphonse Tegawende Sawadogo, [2]Moussa Compaore, [1]Ignace Tonde and [1]Romaric Kiswendsida Nanema

[1]Genetics and Plant Breeding Team, Biosciences Laboratory, Sciences and Technologies Doctoral School, Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso
[2]Biochemistry and Chemistry of Natural Substances, Biochemistry and Chemistry Applied Laboratory (LABIOCA), Sciences and Technologies Doctoral School, Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso

Abstract

Background and Objective: *Solenostemon rotundifolius* was a well-known medicinal plant with a multi-use and limited knowledge in phytochemicals. For this purpose, it was carried out a phytochemical study for highlight the secondary metabolites variation including phenolics, flavonoids and flavonols contents. **Materials and Methods:** The plant material consisted of 121 accessions of *S. rotundifolius* including 40 from Burkina Faso and 81 from Ghana. One gram of leaf powder from each accession was extracted in 10 mL methanol. The polyphenolic, flavonoids and flavonols contents and the DPPH• inhibition effects were studied using spectrophotometric methods. Analysis of Variance (ANOVA) was carried out and the differences were verified using the Student-Newman-Keuls test. **Results:** The polyphenolic contents ranged from 174.09±3.70 mg GAE/g for accession UE52 to 0.48±0.07 mg GAE/g for accession UE134. Interestingly, despite the new edaphic and climatic conditions, there was a significant variability in the phytoconstituents production of the accessions according to their climatic zones of origin. Thus, they could be classified according to this order: Sahelian>Sudano-Sahelian>Sudanian>Sudan-Savanna>Guinea Savanna. Cluster analysis based on the presence of phenolic compounds revealed the medicinal potential of *S. rotundifolius* leaves. **Conclusion:** The study of phytochemical screening revealed the various secondary metabolites including polyphenols, flavonoids and flavonols in the leaves of *Solenostemon rotundifolius* and justified partially its use in traditional medicine.

Key words: *Solenostemon rotundifolius*, phytoconstituents, antiradical, Gampéla, polyphenolic content

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Corresponding Author: Alphonse Tegawende Sawadogo, Genetics and Plant Breeding Team, Biosciences Laboratory, Sciences and Technologies Doctoral School, Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso Tel: (+226)71374063/(+226)75103464
Moussa Compaore, Biochemistry and Chemistry of Natural Substances, Biochemistry and Chemistry Applied Laboratory (LABIOCA), Sciences and Technologies Doctoral School, Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso Tel: (+226)70064742

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The African continent was full of very diverse medicinal plants[1]. According to the World Health Organization, more than 80% of the population in Africa resorted to traditional medicine and pharmacopeia to deal with health problems[2]. Previous studies demonstrated that the use of plants rich in polyphenols reduced the burden of non-communicable diseases, including cancer, diabetes, obesity, cardiovascular diseases and neurodegenerative disease[3]. The Lamiaceae family is well known for their medicinal potential[4,5]. Many species of Lamiaceae are commonly used as medicinal plants. These included antioxidant substances, mainly flavonoids, saponins and anthraquinones[6]. Promoting such medicinal plants could significantly contribute to the sustainable use of plant diversity in many African regions.

Solenostemon rotundifolius (Lamiaceae) was currently cultivated in Asia and Africa as a tuber crop[7]. It is one of the most promising neglected species in the Sahelian Region of West Africa[1]. Due to the nutritional potential of its tubers, its adaptation to poor soil conditions and its tolerance to hydric stress, *S. rotundifolius* could be suggested as an ideal species for the development of resilient agriculture in the current context of climate change and land degradation.

As a member of Lamiaceae family, *S. rotundifolius* could be promoted as a tuber crop but also as a medicinal plant. The polyphenolic contents of leaves were widely used as an indicator of medicinal potential[8]. With the strong foliage development of *S. rotundifolius*, the medicinal use of the leaves and stems could contribute to sustaining the multipurpose use of this neglected species. Phytochemistry is also used to study geographic and seasonal variability of active compounds, particularly to implement good collection practices. The objective of the study was to analyze the phytochemical diversity of *S. rotundifolius* from different climatic zones of Burkina Faso and Ghana.

MATERIALS AND METHODS

Plant material: The plant material consisted of 121 accessions of *S. rotundifolius* including 40 from Burkina Faso and 81 from Ghana. Accessions from Burkina Faso came from the gene bank of the genetics and plant improvement team of the Biosciences Laboratory of Joseph KI-ZERBO University. They were collected in 9 provinces across the three agro-climatic zones of Burkina Faso: Sahelian Zone (annual rainfall <600 mm), the Sudano-Sahelian Zone (annual rainfall 600-900 mm) and the Sudanian Zone (annual rainfall >900 mm). Accessions from Ghana were provided by the Genbank of the Council for Scientific and Industrial Research (Savanna Agricultural Research Institute). They were collected in 9 districts covering two climatic zones in the North of Ghana: Sudan-Savanna (annual rainfall 900-1000 mm) and Guinea Savanna (annual rainfall 1100-1200 mm). The accessions were cultivated from July, 2020 to December, 2020 at Gampela (20 km from Ouagadougou). Biochemistry and Chemistry of Natural Substances, Biochemistry and Chemistry Applied Laboratory (LABIOCA), Sciences and Technologies Doctoral School, Université Joseph KI-ZERBO handling period from the beginning of October, 2021 to the beginning of January, 2022. The fresh leaves of each accession were collected and dried in the shade. The accessions with UE and UW codes were coming from Ghana and the accessions with E code from Burkina Faso.

Chemicals: The solvents (methanol, ethanol) used in this study were in analytical grade. The supplies like HCO_3Na, Folin-Ciocalteu Reagent, 2,2-diphényl-1-picrylhydrazyl, $AlCl_3$ and the standards (gallic acid, rutin, quercetin) were from Sigma-Aldrich (Germany). Greiner Bio-One F-Bottom 96-well Assay Microplate from Germany was used for all the tests.

Phytochemical and antiradical scavenging studies

Extraction: One gram of leaf powder from each accession was mixed in 10 mL methanol for 24 hrs under mechanical agitation. After this step, the filtrated extracts were centrifuged at 1000 rpm for 5 min. The methanol extracts were dried using a rotavapor apparatus coupled with a vacuum (Ibx Instruments, Belgium).

Total phenolic determination: The total polyphenols content of the extracts was determined by the method described by Checkouri *et al.*[9]. The samples in methanol (0.1 mg/mL) were mixed with Folin-Ciocalteu reagent and sodium carbonate according to this procedure. The absorbances were read at 760 nm using a spectrophotometer (BioTek Reader-Synergy LX, Switzerland). The polyphenolic content was determined using a reference curve with gallic acid (0-100 mg/L) as standard. The results were expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

Total flavonoid determination: Total flavonoid content was determined using the spectrophotometric method[10]. Briefly, $AlCl_3$ (2%) was mixed with extract (1 mg/mL) and the optical density was measured after 10 min of incubation at 415 nm by using a spectrophotometer (BioTek Reader-Synergy LX,

Switzerland). Quercetin (0-100 mg/L) was used for standard curve establishment. The results were expressed in milligrams of quercetin equivalent per gram of dry extract (mg QE/g).

Total flavonol determination: Total flavonols were quantified according to the method of Almaraz-Abarca *et al.*[11]. Samples were mixed with $AlCl_3$ (20%) and the absorbances were read after 15 min dark incubation at 425 nm. Total flavonol concentrations are expressed in mg rutin equivalent (mg RE/g) per gram of extract.

Anti-DPPH• evaluation: The anti-radical activity of plant extracts reflects their ability to trap free radicals in the organism. The spectrophotometric method with 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) described by Gerasimova *et al.*[12] was used with some modifications. About 100 µL of a methanolic DPPH solution (20 mg/L) was introduced into microwells previously containing 200 µL of extracts to test. A control not containing plant extract was also prepared. The absorbances were read at 517 nm. A range of concentrations of triplet extracts was used for the inhibiting 50% (IC_{50}) of DPPH radical graphically.

Statistical analysis: An analysis of variance was carried out based on the factor's accessions, country of collection and climatic zones and the differences between the mean values were verified using the Student-Newman-Keuls test at the significant level $p = 0.05$ and $p = 0.01$. Data are expressed as Mean±Standard deviation of 4 separate experiments. The test of Pearson correlations was carried out for polyphenols, flavonoids, flavonols and IC_{50}. A hierarchical ascending classification (HAC) with the average link as the aggregation criterion was performed and the group's differentiation from the HAC was analyzed using discriminant analysis.

RESULTS

Extraction yield: The average extract yield was 7.94%. The lowest yields were obtained with accessions E104 (5.3%), UE134 (5.3%) and UE23YT (4.6%) while the highest yields were registered for accessions UE44 (15.4%), UE156 (12%) and UE127 (11.9%). This observation could be indicative of phytoconstituent content potentiality.

Polyphenols content and DPPH• inhibition variabilities: Polyphenolic contents and percentage of DPPH• inhibition significantly differed at the 1% threshold ($p<0.0001$) within the accessions (Table 1). Polyphenolic contents varied from 28.96±1.5 to 174.09±3.70 mg GAE/g with an average of 107.13±2.14 mg GAE/g. The best contents were obtained with the accessions UE52, UE19YT and UW99HL and the lowest contents with UE109, UE134 and UE58 (Table 1). The flavonoid contents ranged from 6.74±0.47 to 100.55±3.87 mg QE/g with an average of 51.67±1.87 mg QE/g. The highest contents were registered with UE110, E80 and E30 and the lowest levels with E111, UE58 and UE134. The average flavonol content was 20.72±0.83 mg RE/g. It varied from 0.48±0.07 to 57.51±0.01 mg RE/g. The highest values were obtained with the extracts from accessions E140, E157 and UE66. The lowest flavonol content were recorded with accessions UW97CB, E182 and UE134. The antiradical capacities of the methanol extracts of the accessions varied from 1.31±0.07 to 58.40±0.01 µg/mL. The strongest antiradical capacities were recorded with the accessions E155, UW78JT and E78 while, the lowest antiradical capacities were obtained with E187, UE174 and UW99HL.

Polyphenols content and DPPH• inhibition variabilities according to the country: The analysis of variance based on country (Burkina Faso and Ghana) did not reveal significant differences between the accessions in the content of polyphenols, flavonoids, flavonols and IC_{50} (DPPH anti-radical activity) as shown in Table 2.

Polyphenols content and DPPH• inhibition variability according to the climatic zone of origin: The analysis of variance carried out between the accessions based on the climatic zone origin showed significant differences in free radical inhibitory activity (IC_{50}) and polyphenols,

Table 1: Polyphenolic content variation and radical inhibition

	Phenolic content (mg GAE/g)**	Flavonoid content (mg QE/g)**	Flavonol content (mg RE/g)**	IC_{50} (µg/mL)**
Minimum	28.96±1.5	6.74±0.47	0.48±0.07	1.31±0.07
Maximum	174.09±3.70	100.55±3.87	57.51±0.01	58.40±0.01
Mean	107.13±2.14	51.67±1.87	20.72±0.83	12.80±0.32
Accessions with low values	UE109, UE134, UE58	E111, UE58, UE134	UW97CB, E182, UE134	E155, UW78JT, E78
Accessions with high values	UE52, UE19YT, UW99HL	UE110, E80, E30	E140, E157, UE66	E187, UE174, UW99HL

**Variability was significant for $p<0.05$ in each column, GAE: Gallic acid equivalent, QE: Quercetin equivalent, RE: Rutin equivalent and ±: More or less than average

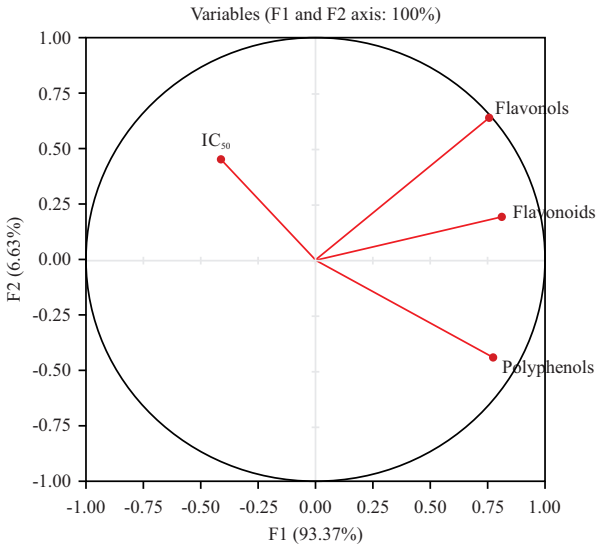

Fig. 1: Association of phytoconstituents and anti-radicals activity

Table 2: Polyphenolics content and DPPH• inhibition variabilities according to the origin

Country origin	Phenolic content* (mg GAE/g)	Flavonoid content* (mg QE/g)	Flavonol content* (mg RE/g)	IC_{50} (µg/mL)*
Burkina Faso (n = 40)	107.93±0.75	50.33±0.60	21.63±0.37	13.88±0.09
Ghana (n = 81)	108.71±0.81	52.33±0.45	20.26±0.21	12.27±0.02

n: Number of accessions, GAE: Equivalent gallic acid, QE: Quercetin equivalent, RE: Rutin equivalent, *Variability was unsignificant for p<0.05 in each column and ±: More or less than average

Table 3: Polyphenolics content and DPPH• inhibition variability according to the climatic zone

Climatic zones origin	Phenolic content (mg GAE/g)	Flavonoid content (mg QE/g)	Flavonol content (mg RE/g)	IC_{50} (µg/mL)
Sahelian (<600 mm) (n = 5)	114.14±1.75^{a}	55.29±1.04^{a}	25.35±0.94^{a}	0.50±0.001^{e}
Sudano-Sahelian (600-900 mm) (n = 26)	103.48±0.53^{b}	50.27±0.64^{b}	23.4b±1.91^{b}	4.12±0.81^{d}
Sudanian (>900 mm) (n = 9)	95.24±1.06^{c}	45.31±0.83^{c}	18.49±0.51^{c}	6.023±0.84^{c}
Sudan-Savanna (900-1000 mm) (n = 58)	88.08±0.95^{c}	30.39±1.03^{d}	10.11±0.63^{d}	11.95±1.06^{b}
Guinea Savanna (1100-1200 mm) (n = 23)	78.19±0.65^{d}	26.79±0.83^{d}	8.99±0.41^{d}	15.15±0.39^{a}

n: Number of accessions, data in the same column with the same letter were not different significantly for p<0.001, GAE: Equivalent gallic acid, QE: Quercetin equivalent, RE: Rutin equivalent and ±: More or less than average

flavonoids and flavonols contents (Table 3). The highest contents were observed for accessions from less watered areas (<600 mm) (114.14±1.75 mg GAE/g; 55.29±1.04 mg QE/g and 25.35±0.94 mg RE/g). The polyphenols, flavonoids and flavonol contents of the accessions from the Sudano-Sahelian Zone (600 to 900 mm) were 103.48±0.53 mg GAE/g, 50.27±0.64 mg QE/g and 23.4±1.91 mg RE/g, respectively. Accessions from the Sudanian Zone of Burkina Faso, Sudan-Savanna of Ghana and the Guinea Savanna Zone from Ghana (>900 mm) recorded the lowest levels of polyphenolics content. It varied from 78.19±0.65 to 95.24±1.06 mg GAE/g, from 26.79±0.83 to 45.31±0.83 mg QE/g and from 8.99±0.41 to 18.49±0.51 mg RE/g. Accessions from the Sahelian Zone (< 600 mm) recorded the highest antioxidant activities. The IC_{50} of these accessions was 0.50±0.001 µg/mL. The antioxidant activity of accessions from the Sudano-Sahelian Zone (600 at 900 mm) was intermediate between the Sahelian zone and other climatic zones (>900 mm). Accessions from the Sudanian Zone of Burkina Faso, Sudan-Savanna of Ghana and the Guinea Savanna Zone (>900 mm) had lower antioxidant activity than the accessions of other climatic zones. The IC_{50} of these accessions varied from 6.023±0.84 to 15.15±0.39 µg/mL.

Polyphenolic content and antiradical correlation: The correlations between the contents of polyphenolic compounds were significant. The highest significant correlations (p<0.05) were observed between flavonoids and flavonol contents (r = 0.72) and between total polyphenols and total flavonoids with r = 0.55. Antioxidant activity is negatively correlated to polyphenolic compounds (r = -0.20; p<0.05). This data was confirmed by principal compounds analysis (Fig. 1).

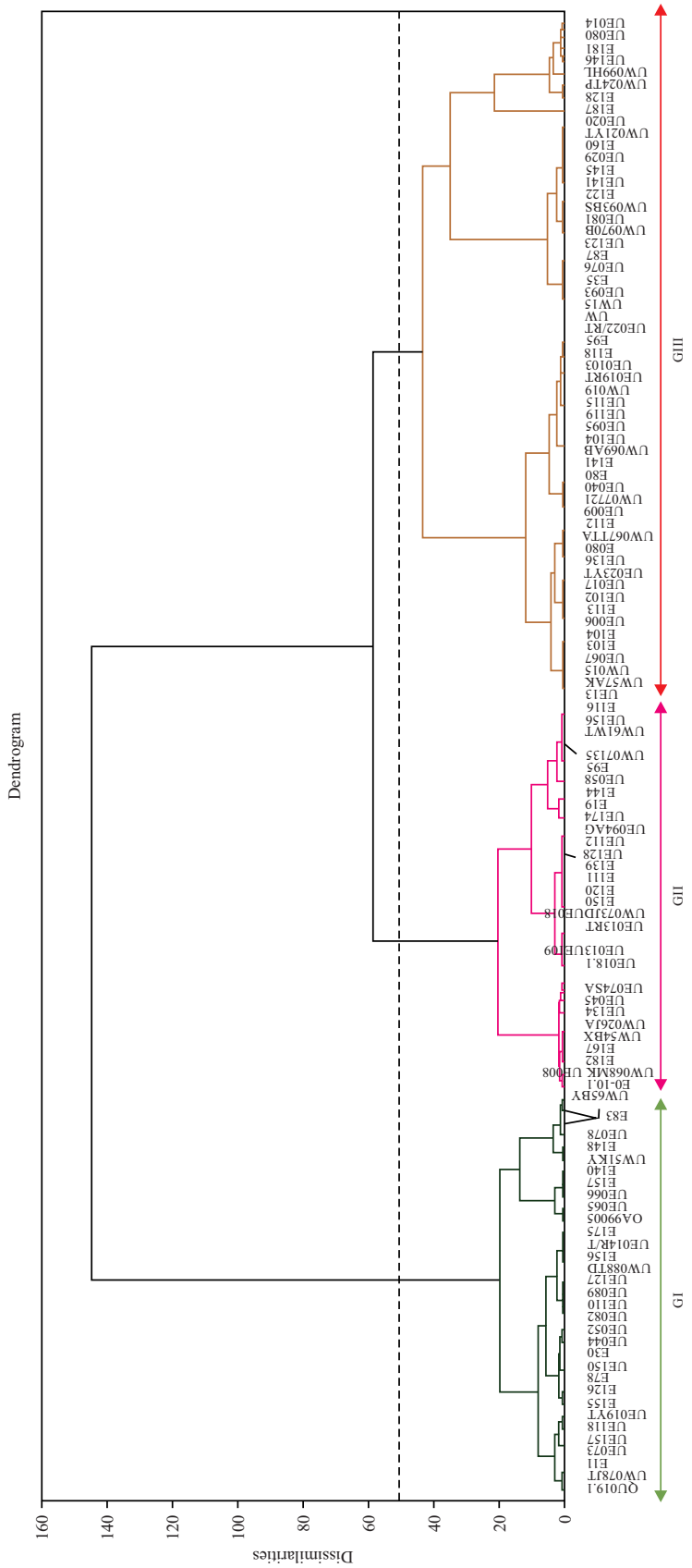

Fig. 2: Hierarchical ascending classification of accessions according to polyphenolic compounds and their antioxidant activity

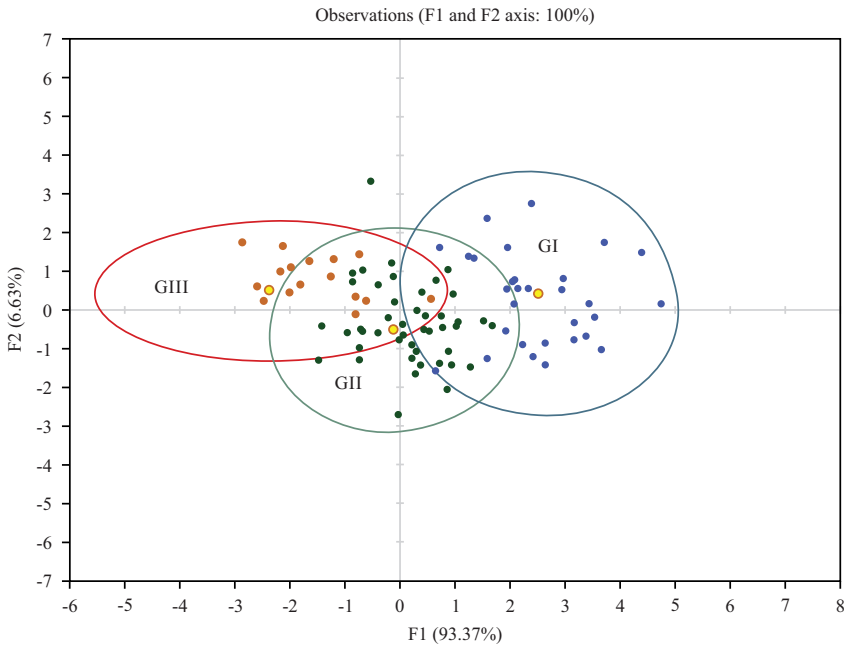

Fig. 3: Discrimination of groups of accessions based on polyphenolic content and radial inhibitory activity of *S. rotundifolius*
GI, GII and GIII: Different groups from the HAC

Table 4: Phytoconstituent contents and free radical inhibitory activity of the groups

Groups	Polyphenols (mg GAE/g)	Flavonoids (mg QE/g)	Flavonols (mg RE/g)	IC_{50} (µg/mL)
I (n = 33)	133.13±3.15^{a}	74.51±1.65^{a}	35.23±2.94^{a}	5.06±0.64^{c}
II (n = 32)	112.43±1.05^{b}	48.70±1.08^{b}	16.42±1.08^{b}	11.29±0.85^{b}
III (n = 56)	71.04±0.83^{c}	33.29±1.93^{c}	13.25±1.05^{c}	18.24±1.01^{a}

n: Number of accessions, Data in the same column with the same letter were not different significantly for p<0.001, GAE: Gallic acid equivalent, QE: Quercetin equivalent, RE: Rutin equivalent and ±More or less than average

Groups of accessions based on polyphenolic content and antiradical correlation: The hierarchical ascending classification (HAC) was made based on the content of polyphenols, flavonoids, flavonols and antioxidant activity. The dendrogram from the HAC revealed a distribution of the 121 accessions into three groups (Fig. 2). Group I is made up of 33 accessions including 22 from Ghana and 11 from Burkina Faso. Group II is made up of 32 accessions including 20 from Ghana and 12 from Burkina Faso. Group III is made up of 56 accessions including 39 accessions from Ghana and 17 accessions from Burkina Faso.

Characteristics of groups of accessions from the HAC: The characterization of the groups from the HAC was carried out using discriminant analysis (Fig. 3). It was carried out based on the content of polyphenols, flavonoids, flavonols and antioxidant activity. The discriminant analysis, with 100% of the total inertia, combines, on the one hand, the content of polyphenols, flavonoids and flavonols at the F1 axis (93.37% of the total inertia) and on the other hand, combines the antioxidant activity (IC_{50}) to the F2 axis (6.63% of the total inertia). Analysis of variance revealed significant differences between the groups of accessions for the content of polyphenolic compounds of accessions and radical inhibitory activity (Table 4). Interestingly, according to this data, it was found their order: GI>GII>GIII.

DISCUSSION

The results of this study revealed the existence of a great variability of accessions of *S. rotundifolius* from Burkina Faso and Ghana for polyphenolic content. This variability is related to the phytoconstituent contents and the antiradical activity of leaf extracts of *S. rotundifolius*.

This variability could be justified by the genetic potential of the accessions in the mass where the accessions were cultivated in the same field. At the vegetative stage, the green and red colors that it was observed were also mentioned by Romaric *et al.*[13] in the description of the morphotypes of *S. rotundifolius*. Żbik *et al.*[14] explain the appearance of reddish colors to the presence of anthocyanins in Lamiaceae.

Polyphenols are associated with numerous physiological processes involved in food quality and plant reaction to traumatism[15]. They are regarded as powerful natural antioxidants with various biological and pharmacological properties, including antioxidant, cytotoxic, anti-inflammatory, antihypertensive and anti-diabetic activities[16].

The capacity of a plant species to resist insects and microorganisms is also often correlated with its content of phenolic compounds[17]. They are produced as secondary metabolites, in response to environmental stress or to provide a defense mechanism against attacks causing diseases in plants[18]. Polyphenols, as one of the most significant active components of plants, are produced by the secondary metabolism and are important for protecting plants from degradation after harvest[19].

In general, the contents of polyphenols vary qualitatively and quantitatively from one plant to the other, this can be attributed to several factors such as climatic factors environmental factors and geographical area[20]. The genetic, farming conditions and stage of development impact the synthesis of polyphenols in the plant[21]. Previously, the chemical analysis of *S. rotundifolius* leaves showed the existence of antioxidants properties revealed by the DPPH test and the richness of *S. rotundifolius* in polyphenols especially in flavonoids[22]. The anti-radical activity of extracts from accessions from the Sahelian climatic zone is thirty times higher than that of extracts from accessions from the climatic Guinea Savanna Zone from Ghana. According to Trifan *et al.*[23] these are the flavanols that exhibit better anti-DPPH activity. Cluster analysis revealed the existence of a group (group I) made up of accessions with high polyphenols, flavonoids and total phenols correlated with antioxidant capacity. The correlation between phenols total and flavonoids probably finds its explanation in the fact that flavonoids are the majority polyphenolic compounds.

CONCLUSION

The determination of the polyphenolic compounds of the 121 accessions of *S. rotundifolius* made it possible to highlight the presence of total polyphenols, total flavonoids and flavonols totals. The methanolic extracts of the accessions have very high antioxidant activity, thanks to their constituents (polyphenolic compounds). Knowledge and use of medicinal plants constitute a true heritage of human beings. Their importance in the field of public health has been much accentuated in recent years thanks to the therapeutics they provide. This renewed interest comes partly from the fact that plants' medicinal products represent an inexhaustible source of bioactive natural substances.

SIGNIFICANCE STATEMENT

In West Africa, *S. rotundifolius* has been a basic food plant for certain regions' populations. The valorization of *S. rotundifolius* required better knowledge of its phytochemical variability on the scale of its main cultivation area. Hence there is a need for phytochemical characterization of leaves through the evaluation of the content of total polyphenols, total flavonoids, total flavonols and their antioxidant capacity. Interestingly, despite the new edaphic and climatic conditions, there was a significant variability in the phytoconstituents production of the accessions according to their climatic zones of origin. Cluster analysis based on the presence of phenolic compounds revealed the medicinal potential of *S. rotundifolius* leaves.

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