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Variation in phytochemical composition of *Chromolaena odorata* (L.) King and Robinson (Asteraceae) across climatic zone in Benin (West Africa)

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ABSTRACT

Chromolaena odorata (L.) King and Robinson (Asteraceae) is a tropical shrub with interesting chemical potential widely used in agriculture and medical science and which can be affected by several geographic and climatic conditions. Therefore, we investigated the phytochemical composition of this plant across climatic zones in Benin. The plant material collected from different locations was phytochemically screened by staining and precipitation tests. The total phenolic, flavonoid and tannin contents were determined using, the colorimetric method of Folin-Ciocalteu, the method of aluminum chloride and the method of vanillin, respectively, then the obtained data were subjected to analysis of variance. The phytochemical analysis revealed the presence of the main chemical groups such as alkaloids, free anthracene, coumarins, flavonoids, mucilage, tannins, reducing compounds, saponins, quinone derivatives, steroids. There was a significant difference ($P < 0.05$) in the phytochemical contents across geographical sites. In comparing the levels of phytochemicals among geographical locations, the raw material collected from the north climatic zone contained the highest phenolic and flavonoid contents, 147.59 ± 3.04 mg/g and 17.17 ±0.31 mg/g, respectively, compared to others. Overall, the study highlighted the potential of *C. odorata* as source of natural products. There was no difference in the phytochemical markers whereas the phytochemical contents vary across climatic zones. These results can be of use in the development of biopesticides from the raw material of *C. odorata*.

1. Introduction

Siam weed, botanically referred to as *Chromolaena odorata* (L.) R. M. King and Robinson, is an invasive plant native to South America and widely distributed in the tropical regions of the world [1]. It is an allelopathic species used in many fields of activities, such as agriculture and phytotherapy, due to its chemical potential [2]. Allelopathy is a biological process by which a plant produces and releases biochemicals that affect the growth, the development and the survival of other plants [3]. These chemical compounds are commonly called allelochemicals with either valuable or deleterious effects on target/receiver organisms [4]. Allelochemicals can be released by plants into environment by root exudates in soil, volatilization in air and decomposition of plant residues [5]. The Asian-West-African biotype of *C. odorata* is known to contain major allelochemicals such as alkaloids, tannins, flavonoids, saponins and phenolics [6]. These chemical

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compounds result from the secondary metabolism of the plants and are devoted to the plant self-defense against pests and to the competition for nutrients and other resources over other species around them [7]. Since its introduction in West Africa in 1930s [8], *C. odorata* is used by people in human and animal health in treatment of dozens of diseases, and in protection of crop and stored staple food from pest attacks [2]. Indeed, allelopathic species are potential sources of ecological pesticides as they exhibit repellent and/or inhibitory effects on pest organisms and neighboring plants [9]. Thousands of studies worldwide have focused on the exploitation of allelochemicals of plants as environmentally friendly and low-cost pesticides in agriculture for the sustainable management of crop pests [10]. Plant extract, powder, compost and mulch are some different ways of valorization resulting from research works on the use of the allelopathic species in ecological agriculture for pest and soil fertility management [2, 11]. Recent advances in agricultural chemistry and pesticide science make it easier for many farmers and small companies to produce different types of biopesticide from Siam weed plant material. However, differences in the chemical composition of the plant due to the geographical sites of collection may affect the efficacy of the organic inputs from the plant material [12]. As a master of fact, environmental factors such as climate variables and soil type are likely to influence the chemical composition of plants and therefore the efficacy of their extracts [13]. This may or not result in different chemotypes of plant within the same species across climatic regions [14]. Benin is divided into three climatic zones where *C. odorata* is reported to occur [15]. These climatic zones are different from one another regarding mainly rainfall, temperature, and altitude [16]. It is therefore essential to better understand about the phytochemical content of *C. odorata* across the different climatic regions for proper use of its allelopathic potential. To the best of our knowledge, no studies have been so far carried out on the variation in the phytochemistry of *C. odorata* in Benin although Avlessi et al. [17], Noudogbessi et al. [18] and Kossouoh et al. [19] have characterized the essential oil from the leaves of the plant collected from different locations of the southern region of the country. Therefore, the present research work aimed at investigating the difference in the phytochemical composition of *C. odorata* across climatic zones in Benin. It is a preliminary work focused on the main groups of chemical constituents to give insight into the effect of climate on the chemical composition of *C. odorata* in Benin.

2. Results and Discussion

2.1. Phytochemical constituents of Chromolaena odorata

The phytochemical analysis revealed, regardless of the climatic zone, 11 main chemical groups identified out of a total of the existing 19 phytochemicals in the aqueous extract of *Chromolaena odorata* (Table 2). The identified phytochemical constituents were alkaloids, tannins (gallic and catechic), flavonoids, quinone derivatives, saponins, steroids, coumarins, mucilage, reducing compounds, and free anthracenes. This is consistent with many other studies having previously reported these secondary metabolites in the Asian and West African biotype of *C. odorata* [6, 20, 21].

Table 1. Phytochemical profile of *C. odorata* across climatic zones

Phytochemical	Guinean zone	Sudano-guinean zone	Sudanian zone
Alkaloids	+	+	+
Gallic tannins	+	+	+
Catechic tannins	+	+	+
Flavonoids	+	+	+
Anthocyanins	-	-	-
Leuco-anthocyanins	-	-	-
Saponins	+	+	+
Triterpenoids	-	-	-
Steroids	+	+	+
Quinone derivatives	+	+	+
Cardenolids	-	-	-
Cyanogenic derivatives	-	-	-
Mucilage	+	+	+
Coumarins	+	+	+
Reducing compounds	+	+	+
Free anthracene	+	+	+
C- anthracene	-	-	-
O-heterosids	-	-	-
C-heterosids	-	-	-

+: Presence, **-:** Absence

On the other hand, anthocyanins, leuco-anthocyanins, triterpenoids and cyanogenic derivatives were not identified in this study. The absence of anthocyanins in *C. odorata* considering different types of extract (aqueous, ethanolic, methanolic etc.) was already reported [21, 22].

No difference was found in the chemical composition of the aqueous extract of *C. odorata* across the climatic zones of the study area as the same number and type of phytochemical markers was identified in the plant no matter how the sample location was. That indicates that the variation in the environmental factors across the climatic zones in Benin did not affect the qualitative phytochemical composition of *C. odorata* considering the main chemical groups of secondary metabolites. These findings are in accordance with those of Ghasemzadeh et al. [23] who reported no variation in phytochemical markers of Parkia speciosa Hassk. from three different locations of Malaysia. It is also similar to the results of Wijaya Kusuma et al. [24] who reported no difference in the phytochemicals of Hyptis capitate grown in different locations of East Kalimantan (Indonesia), representing typically different environments. Indeed, rather than the environmental factors, phytochemical markers are mainly influenced by the genetic factors of the plant species/variety.

Chemical groups such as alkaloids, tannins, flavonoids, coumarins and steroids are considered as major plant allelochemicals with increasing interest in sustainable pest management in agriculture [4]. The presence of these major allelochemical constituents in the plant from all the three climatic zones of the study area, indicating that *C. odorata* plant material across the entire study area is potential source of naturel products.

3.2. Quantification of total polyphenols (TPC), flavonoids (TFC) and tannins (CTC)

Many factors are likely to influence the phytochemical contents of a given plant species. The statistical analysis revealed that the total polyphenolic and flavonoid contents vary significantly ($P < 0.05$) and highly significantly ($P < 0.001$), respectively, across the climatic zones demonstrating that geographical location of the sample site influenced the phytochemical contents of *C. odorata*. In contrast, there was no significant difference ($P > 0.05$) in condensed tannin content irrespective of climatic zone even though a trend of increase in values was noticed in the southern (guinean) zone of the study area.

These results are consistent with those of Irakli et al. [25] who reported significant difference in the phytochemical contents of Lentil (Lens culinaris L.) as influenced by the climate conditions of different locations in Greece. Many studies agree that the environmental variables such as temperature, humidity, rainfall, sunlight, altitude and soil type are important drivers of the concentration of the phytochemical compounds in plants [13, 26, 27].

The highest values of TPC (147.59 ± 3.04 mg/g) and TFC (17.17 ±0.31 mg/g) were recorded in the samples collected from the sudanian zone, while the lowest 128.61 ± 6.72 mg/g and 11.20 ±1.13 mg/g, respectively, were recorded in the samples from the guinean climatic zone (Table 2).

Table 2. Variation in phytochemical contents of *C. odorata* across geographical zones

Climatic zone	**TPC (mg/g DW GAE)[a]**	**TFC (mg/g DW QE)[b]**	**CTC (mg/g DW CAE)[c]**
Guinean zone	128.61 ± 6.72**b**	11.20 ±1.13**b**	24.93 ±0.72 **a**
Sudano-guinean zone	133.52 ± 6.34**ab**	11.69 ±1.36**b**	21.02 ±3.50 **a**
Sudanian zone	147.59 ± 3.04**a**	17.17 ±0.31**a**	21.08 ±3.47 **a**

In the same column, values with different letters are significantly different ($P < 0.05$).

[a]**TPC:** Total Phenolic Content; [b]**TFC:** Total Flavonoid Content; [c]**CTC:** Condensed Tannins Content

The main differences in the environmental conditions between the collection sites of the raw material are temperature, altitude and sun exposure, rainfall and rainfall modal. The sudanian (north) zone had the highest altitude and temperature which might favor the highest concentration of polyphenols and flavonoids in the plant. This corroborates the results of Hayat et al. [28] who reported that *Marrubium vulgare* collected from Oulad Daoud Zkhanine (26.5 °C, 438 m above sea level, 57% humidity) had higher polyphenols and flavonoids contents than the one from Cape Three Forks (18 °C, 390 m above sea level, 73% humidity) in Morocco

.3. Experimental

3.1. Study area

This study was carried out in the Republic of Benin. Benin is a country located in West Africa ranging from latitude 6°30' to 12°30' North and longitude 1° to 3°40' East and covers 112,622 km². The country is divided into three main climatic zones namely the guinean, sudano-guinean and sudanian zones (Figure 1). The geographical location and characteristic of these climatic zones are summarized in Table 3.

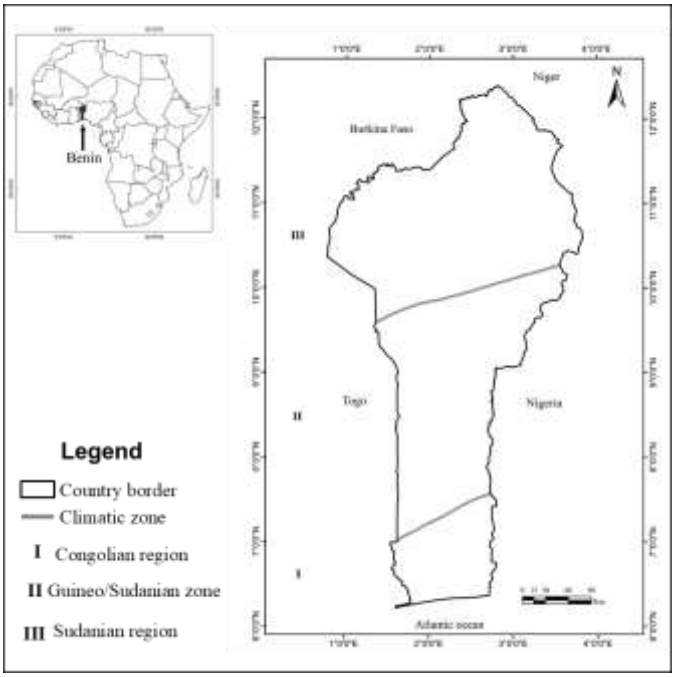

Figure 1: Location of the study area and its different climatic zones

3.2. Plant material collection and extraction

The samples of fresh leaves of *Chromolaena odorata* were collected from each climatic zone in the study area during wet season in October 2020. The plant samples were washed with distilled water and air-dried at room temperature in the laboratory for two weeks. Dry plant materials were powdered and boiled for 30 minutes in distilled water (1: 5 w/v). The aqueous extracts obtained were filtered using Wattman filter paper and then dried in a rotary evaporator under reduced pressure at a temperature of 50 ° C. The dry plant extracts were stored in dark bottles and kept at 4° C for later use.

Table 3. Characteristics of the climatic zones of the study area

Variable	Guinean zone	Sudano-guinean zone	Sudanian zone
Location (latitude)	6°25' - 7°30' N	7°30' - 9°45' N	9°45' - 12°25' N
Altitude (m)	56–223	153–308	214–609
Rainfall regime	Bimodal	Unimodal	Unimodal
Rainfall (mm/year)	1200	900 – 1100	600 - 900
Temperature (° C)	25 – 29	21.2 – 32.5	24 – 31
Relative humidity (%)	69 – 97	45.5 – 87.1	18 – 99
Climatic characteristics	Sub-humid humid	Sub-humid dry	Semi-arid

3.3. Phytochemical screening

The phytochemical screening was carried out according to the differential staining and precipitation reactions described by Houghton and Raman [29] to identify the chemical groups contained in the aqueous extract of the plant. The alkaloids were highlighted by the Meyer test, tannins by Stiasny's test, flavonoids by the cyanidine test, anthocyanins by the hydrochloric acid and ammonia test at 50%, Leuco anthocyanins by the Shinoda test, quinone derivatives by the Born-Trager test, saponins by the foam index test, triterpenoids by the Liebermann-Buchard test, steroids by the Kedde test, cyanogenic derivatives by the Gugnard test, mucilage by the absolute alcohol test, the reducing compounds by the hot Fehling Liquor test, coumarins by the ammonia test at 25% and revealed at UV 365 nm. All tests were done in triplicate.

3.4. Quantification of the major phytochemicals

Determination of total polyphenols

The total polyphenols compounds (TPC) was determined by the method of Folin-Ciocalteu with some modifications. 125 µL of each extract (2 mg/mL) were added to 625 µL of Folin-Ciocalteu reagent (10%). 500 µL of sodium carbonate solution (Na_2CO_3, 75 g/L) were added to this solution after 5 min of incubation under

dark condition. Then the mixture was incubated also in dark at room temperature for 2 hours and the absorbance was measured at 765 nm by a spectrophotometer (UV/VIS). The calibration curve from gallic acid standard range methanolic solution (0–250 µg/ml) was used to obtained the concentration. The TPC expressed in milligram of gallic acid equivalent per gram of extract (mg GAE/g) was calculated as follows:

$TPC = C_1 \times V/m$

where: C_1 – the concentration of gallic acid established from the calibration curve, mg/mL;

V – the volume of extract, mL;

m – the weight of pure plant extract, g.

Determination of total flavonoids

The determination of the total flavonoid compounds (TFC) was carried out according to the aluminum trichloride ($AlCl_3$) method. 500 µL of each plant extract (2 mg/mL) was added to 500 µL of methanolic solution of $AlCl_3$ (2%) and 3 mL of methanol. The mixture was stirred and incubated in dark at room temperature for 10 min. The absorbance was measured at 415 nm using a spectrophotometer (UV/VIS). We used the calibration curve from quercetin standard range methanolic solution (0–125 µg/mL) to obtain the concentration. The TFC expressed in milligram of quercetin equivalent per gram of extract (mg QE/g) was calculated as follows:

$TFC = C_2 \times V/m$

where: C_2 – the concentration of quercetin established from the calibration curve, mg/mL;

V – the volume of extract, mL;

m – the weight of pure plant extract, g.

Determination of the condensed tannins

The quantification of the condensed tannins content (CTC) was done using the vanillin method with some modifications. 3 mL of ethanolic vanillin solution (4%) was mixed with 500 µL of each plant extract, then 1.5 mL of concentrated chlorhydric acid (HCl) and 2 mL of methanol were added. The mixture was stirred and incubated in dark at room temperature for 15 min and then the absorbance was measured at 500 nm against a blank. The CTC was calculated by the formula below and expressed in milligram of catechin equivalent per gram of extract (mg CE/g) with reference to the catechin calibration curve (0 - 250 µg/mL).

$CTC = C_3 \times V/m$

where: C_3 – the concentration of catechin established from the calibration curve, mg/mL;

V – the volume of extract, mL;

m – the weight of pure plant extract, g.

3.5. Statistical analysis

All experimental assays were performed in triplicate and the data were expressed as means ± standard error. The total polyphenol, flavonoid and condensed tannin contents were subjected to the analysis of variance (ANOVA) and Student-Newman-Keuls (SNK) test was used for mean separation. The statistical analysis was performed at 95% confidence interval using R software version 4.0.4 [30].

4. Conclusion

The chemical potential of plant species is of great value in agriculture and human health. This is the reason it was investigated in *Chromolaena odorata* across climatic zones in Benin. The proper valorization of a given plant species natural products necessitates its chemical profiling. Our results suggested that Even even though no difference was found in the phytochemical markers across climatic zone of the study area, the phytochemical contents differed significantly from zone to another. The sudanian zone situated in the Northern part of the study area had the highest concentrations of polyphenols and flavonoids while the lowest amounts was were recorded in the guinean zone located in the southern part of the country. The severe environmental conditions prevailing in the Northern climatic zone might have induced this. We therefore concluded that the environmental factors affect the phytochemical composition of *C. odorata* in Benin as the large variation in temperature and highest altitude seem to favor the content of the phytochemicals detected. These findings can be of a paramount important in the production of biopesticides from raw materials of *C. odorata*. Nevertheless, further studies are needed to deeply investigate the chemical composition of this plant so as to highlight the allelochemical compounds present and to identify the probable chemical races in Benin.

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