

HPTLC Phytochemical Screening and Hydrophilic Antioxidant Activities of *Apium graveolens* L., *Cleome gynandra* L., and *Hibiscus sabdariffa* L. Used for Diabetes Management

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Abstract

Diabetes mellitus is a socially significant disease characterized by chronic hyperglycemia and metabolic disorders of proteins, carbohydrates, and lipids due to reduced function of insulin. Medicinal plants, rich in bioactive components that promote prevention and treatment, are inexpensive and no side effects. *Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa* from Burkina Faso were investigated for their phytochemical profile and antioxidant activities. The high-performance thin-layer chromatography profile revealed flavonoids, tannins, and sterols in these herbaceous. The *Hibiscus sabdariffa* methanolic extract exhibited the highest total phenolic (138.4 ± 0.5 mg GAE/g DW) and flavonoid (52.8 ± 0.6 mg RuE/g DW) contents comparatively to *Cleome gynandra* and *Apium graveolens*. *Hibiscus sabdariffa* methanolic extract also presented the highest antioxidant activity ($IC_{50} = 0.31 \pm 0.002$ mg/mL) using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay. A high correlation between flavonoid contents and hydrophilic antioxidant activities ($r = 0.99$) was observed, indicating that flavonoids contribute significantly to these herbaceous antioxidant properties. *Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa* constitute a natural source of phenolic compounds that could be exploited in diabetes mellitus management.

Keywords

Diabetes Mellitus, Medicinal Plants, Flavonoids Contents, Hydrophilic Antioxidant Activity, HPTLC Screening

1. Introduction

Free radicals are molecules or atoms that have one or more unpaired electrons on their outer layer. Playing the role of electron acceptor or donor, the free radicals are extremely reactive with other molecules. Derived from oxygen or nitrogen, the species' radicals can have beneficial or toxic effects on the human body [1] [2]. Due to their reactivity, they are involved in many cellular functions: phagocytosis, bactericide, cell signaling [2] [3]. However, their physiological effects are only observed when there is a balance between these substances and antioxidants, which protect the cellular system against reactive oxygen species (ROS) [1] [2]. Excess of ROS is known to be involved in many human diseases like diabetes, cardiovascular diseases, cancers [3] [4] [5] [6]. In addition to reducing agents (superoxide dismutase, catalase, glutathione peroxidase), the organism has a second defense line: "free radical scavengers". These compounds, mostly provided by food, play an essential role in neutralizing ROS's harmful effects [1] [3] [7]. Foods contain a wide range of micronutrients that can play a vital role in preventing diseases related to oxidative stress. Indeed, one of these microconstituents' common properties is their antioxidant power [4] [7]. Phytochemical antioxidants such as phenolic compounds and carotenoids have raised the interest among scientists, the food industry, and consumers for their role in human health benefits [4] [5] [6] [7] [8]. It is reported that frequent consumption of fruits and vegetables is correlated with a decrease in heart disease risk, diabetes, degenerative diseases, and cancers [1] [2] [3] [4]. Aware of the significant bioactive effects of polyphenols and carotenoids against reactive oxygen forms in the body, discovering new bioactive compounds in plant materials will increase antioxidant sources' potential.

Diabetes mellitus, a chronic metabolic disorder of the endocrine system, is characterized by high blood glucose content caused by insulin secretion or insufficiency [9]. Despite the appreciable progress in diabetes management through the use of conventional drugs, diabetes mellitus continues to be a significant health care problem in the world, and its prevalence is expected to rise from the current 382 - 471 million individuals by 2035 [10] [11]. Different approaches, such as insulin, pharmacotherapy, and diet therapy, are currently applied to manage diabetes. For centuries, herbs have been widely used to treat a variety of diseases. Today, these plants are still used as the first alternative to cure specific pathologies in developing countries due to the few side effects they present [12]. Recent studies showed that natural phenolic compounds and polysaccharides (such as tea, grapefruit, strawberries) inhibit the α -glucosidase enzyme and

making them a potential natural therapeutic agent for the treatment of diabetes mellitus [13] [14].

Apium graveolens L., *Cleome gynandra* L., and *Hibiscus sabdariffa* L. are three herbaceous vegetables used as culinary herbs and frequently cited for their antidiabetic activities. The present study aims to update the available scientific information on these plants' phytochemical profile and their hydrophilic antioxidant properties.

2. Materials and Methods

2.1. Ethnobotanical Surveys

An ethnobotanical survey was conducted by *Tipaalaga* (an association working for sustainable management of natural resources) members to determine plants used in the diet of people with diabetes. This study was carried out from November 22 to December 6, 2019, in Ouagadougou, Pabré, Gampela, and surrounding villages. The research was focused on people without distinction of age, religion, and sex. The interviews were based on a pre-tested questionnaire with specific questions about the informant, the vernacular name of the plant species, the edible parts, and the preparation [15]. The interviews were recorded using a dictaphone. A total of 145 people were interviewed, including 69 people with diabetes, with an average age of 45. Plant samples were collected and identified by the botanical team of Joseph KI-ZERBO University. A herbarium was made.

2.2. Plant Material

The plant material was composed of three herbaceous plants: *Apium graveolens* L. (Ag), *Cleome gynandra* L. (Cg), and *Hibiscus sabdariffa* L. (Hs). Plants samples were collected in December 2020 from the botanical garden of Gampela, located 15 km from Ouagadougou. Samples were dried separately at room temperature under ventilation. Samples were powdered into fine powder by an electrical grinder.

2.3. Chemicals and Standards

All the solvents used without acetonitrile and n-hexane (HPLC grade) were of analytical grade and purchased from Sigma-Aldrich (Taufkirchen, Germany). Water was purified by a Millipore instrument (MOLSHEIM France). Gallic acid, 2,2-diphenyl-1-picrylhydrazyl, Folin-Ciocalteu phenol reagent were obtained from Sigma Chemical Co. (St. Louis, MO). β -carotene (HPLC grade) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Buffer salts and all other chemicals were of analytical grade. Samples and standards solutions were filtered before chromatographic analysis using through Millipore membrane of 0.2 μ m.

2.4. Extraction

Twenty-five grams of powder of each herbaceous were extracted by maceration

at low temperature (4°C) for 24 hours with 200 mL of n-hexane. The experiment was repeated much time until the sample was colorless. The different filtrates were collected and concentrated at 40°C using a rotary evaporator (Buchi). The n-hexane extracts were utilized for non-polar compounds screening by the HPTLC method and quantification of carotenoid contents and lipophilic antioxidant capacity array. The dry residue was taken up with 200 mL of methanol at low temperature (4°C) for 24 hours. The process was repeated until a colorless sample. After filtration with filter paper, methanol extracts were collected and dried under vacuum on the rotatory evaporator at low temperature (<40°C). The dried extracts were re-dissolved to a minimum volume of methanol for polar compounds screening and hydrophilic antioxidant activity and phenolic compounds evaluation by spectrophotometer analysis.

2.5. High-Performance Thin-Layer Chromatography (HPTLC) Screening

2.5.1. Chromatography

Phytochemical screening of herbaceous extracts (*Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa*) was performed on 20 cm × 10 cm silica gel 60 F₂₅₄ HPTLC plates (Merck, Darmstadt, Germany). Sample solutions were applied on the HPTLC plates using a Linomat 5 applicator (CAMAG, Muttenz, Switzerland). Typically, 2 µL volumes of samples were applied as 5 mm bands with a semi-automatic plate spotter (CAMAG, Muttenz, Switzerland) Linomat 5 equipped with a microliter syringe. The distance between tracks was 10 mm. Distances from the left and right edge of the plate were 20 mm. A constant rate of application of 100 nL/s was used. Linear ascending development with a 10 mL mobile phase was performed in a filter paper-lined CAMAG twin-trough glass chamber previously saturated with mobile phase vapor for 30 min. The development distance was 80 mm approximately. Plates were dried after development through a hairdryer. In the twin trough chamber, the mobile phase was:

- Flavonoids: ethyl acetate-formic acid-water 80:10:10, v/v/v.
- Tannins: ethyl acetate-methanol-water-chloroform 18:2.4:2.1:6, v/v/v/v.
- Terpenoids: n-hexane-ethyl acetate 20:4, v/v.

2.5.2. Derivatization and Documentation

By immersion using the Immersion Device and the following reagents:

- **Flavonoids:** Natural Products reagent (0.5% diphenylbirinic acid aminoethyl ester in ethyl acetate) followed by Macrogol reagent (5% polyethylene glycol 400 in dichloromethane). The plate was heated at 110°C for 2 min and dipped in the NP reagent while hot, and dried in the fume hood. The plate was then immersed in the Macrogol reagent and dried in the fume hood. Flavonoids were detected under UV 366/>400 nm.
- **Tannins:** The plate was heated at 100°C for 2 min, then dipped in Fast Blue Salt B reagent while hot. The plate was then dried in a fume hood for 5 min after derivatization. Tannins were revealed under white light [16].

- Liebermann Burchard reagent was prepared by mixing acetic anhydride (5 mL) with concentrated sulfuric acid (5 mL), cooled 95% ethanol (50 mL) in that order. The developed plates were dried with cold air for 3 min, then immersed in the reagent. Finally, the developed plates were heated on the plate heater at 110°C for 3 - 5 min. The evaluation was performed immediately after that in white light [17].

2.6. Total Phenolic and Flavonoid Contents

The total phenolic contents (TPC) in the herbaceous extracts were determined by the Folin-Ciocalteu colorimetric method [8] [18] with slight modifications. In brief, 0.1 mL of plant extract and gallic acid solution was mixed with 0.1 mL of Folin-Ciocalteu reagent previously diluted ten times with distilled water. After vortexing, the mixture was incubated for 8 min at room temperature, and 1.0 mL of 7.5% saturated sodium carbonate solution was added. The combinations were set at 37°C for 30 min in the dark. The absorbances of the resulting blue color were read at 760 nm with a SHIMADZU UV-Vis spectrophotometer. The phenolic contents of plant extracts were determined using the equation of the calibration curve ($y = 7.3194x + 0.1309$, $R^2 = 0.998$), and the results are expressed in mg of gallic acid equivalents (GAE) per gram of dry weight. All determinations were performed in triplicate ($n = 3$).

The determination of total flavonoid contents (TFC) in the herbaceous extracts was performed as reported previously [18] [19] using $AlCl_3$ colorimetric method. Rutin was used as standard, and the quantification was expressed by reporting the absorbance in the calibration curve of the rutin ($2.5608x + 0.0034$, $R^2 = 0.9995$). The total flavonoid contents were expressed as mg rutin equivalents/g dry weight. All determinations were performed in triplicate ($n = 3$).

2.7. HPLC-Diode Array Detector (DAD) Quantification of Carotenoids

HPLC-DAD analysis was carried on a Prominence UFCL system (Shimadzu, Kyoto, Japan) equipment with two Nexera X2 LC-30AD high-pressure gradient pumps, a Prominence DGU-20A5R degasser, a Nexera SIL-30AC Prominence autosampler (15°C, injection volume 10 μ L), a CTO-20AC Prominence column oven at 25°C and SPD-M20A Prominence diode array detector. Data acquisition and processing were performed by the LabSolutions software (Shimadzu, Kyoto, Japan).

Chromatograms were recorded at 450 nm, and carotenoids were quantified as β -carotene equivalents using an external calibration curve. The β -carotene solution was obtained by dissolving the standard in hexane containing 0.1% BHT. The β -carotene solution's concentration was measured at 450 nm by a spectrophotometer (Shimadzu, Kyoto, Japan) and calculated with the molar absorption coefficient of β -carotene in light hexane ($138,900 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$) [20]. The total carotenoid contents were expressed as mg β -carotene equivalents/g dry weight.

All the samples were analyzed in triplicate.

2.8. Hydrophilic and Lipophilic Antioxidant Activities Determined by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

Hydrophilic and lipophilic antioxidant activities were measured using the DPPH bleaching method [8] [21] [22]. The process is based on the capacity of plant extracts to scavenge compared to the DPPH radical (DPPH[•]) to the Trolox in a dose-response curve. DPPH radical absorbs in the visible at $\lambda_{\max} = 515$ nm and disappears with reduction by an antioxidant compound [8] [23]. Briefly 1.5 mL of the DPPH[•] solution (0.04 mg/mL in methanol) was mixed with 500 μ L of plant extract. After 8 min of incubation at 37°C in the dark, the absorbance decrease at 515 nm was measured spectrophotometrically (SHIMADZU). Briefly, new standard solutions (Trolox) of different concentrations from 1 to 5 μ g/mL in methanol were firstly prepared. 2 mL of each standard and sample were mixed with 2 mL of 0.01 mM DPPH solution, respectively. The mixtures were incubated in the dark at 37°C for 30 min. The radical scavenging rate of DPPH was calculated as follow:

$$\text{Radical scavenging rate} = 1 - (A_s - A_{s_0}) / A_0$$

A_s : absorbance of the sample; A_{s_0} : absorbance of the samples with 2 mL methanol; A_0 : absorbance of DPPH (0.01 Mm) without the sample.

2.9. Statistical Analysis

The importance of each plant is determined by calculating its Use Value species (UVs) according to the simplified formula of Cotton and Wilkie:

$$\text{UVs} = \frac{U}{N} \quad [24].$$

where U denotes the number of uses of the plant and N the number of informants. The data were processed and analyzed with the software SPSS version 15. Mean use values were compared using the One Way ANOVA (Analysis of Variance). Differences are considered statistically significant for a “p-value less than 0.05”.

3. Results and Discussion

3.1. Choice of Plants Used in the Diabetes Management

Leafy vegetables cited in the diet of diabetics are *Spinacia oleracea* (Chénopodiaceae), *Ipomoea batatas* (L.) Lam. (Convolvulaceae), *Ocimum gratissimum* L. (Lamiaceae), *Thymus vulgaris* (Lamiaceae), *Petroselinum crispum* L. (Apiaceae), *Corchorus olerius* L. (Malvaceae), *Cleome gynandra* L. (Brassicaceae), *Hibiscus sabdariffa* L. (Malvaceae), *Apium graveolens* L. (Apiaceae).

Three plants were distinguished from the others by their high use value (UVs ≥ 0.60): *Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa*. The leaves of these plants are usually boiled, wrung out, and eaten or mixed with flour in the form of couscous. Some leaves can be eaten as a salad.

3.2. High-Performance Thin-Layer Chromatography (HPTLC) Screening

Phytochemical screening of different plant extracts was performed on HPTLC plates (glass) silica gel F₅₄ (Merck), and the results were presented in **Figures 1-3**. The different colors of spots (orange, yellow, blue, green, pink, purple) observed on the chromatogram under UV/366 nm, and white light may correspond to several secondary metabolite groups. To specify the nature of the compounds revealed at UV/366 nm, Natural Products reagent (flavonoids), Fast Blue Salt B reagent (tannins), and Liebermann Burchard reagent (sterols) were used.

3.2.1. Sterols Detection

Under UV/366 nm, sterols color blue, yellow, and green; and terpenes in blue, yellow, green, and purple [25]. The HPTLC plate showed blue, yellow-green, purple, and red spots under UV/366 nm, after heating at 110°C and spraying with the Liebermann Burchard reagent (**Figure 1(a)**). These stains were characteristic of sterols and triterpenes under UV/366 nm. In the visible light, the spots of triterpenes (genins) were blue and violet after heating at 110°C and spraying with the Liebermann Burchard reagent (**Figure 1(b)**). As for triterpenes of type oleanane and ursane, the spots were red. The triterpenes of type lupine were colored in yellow-orange fluorescence by Liebermann Burchard reagent after heating at 110°C in the visible light [25]. This information from the literature has allowed non-polar compound identification on the chromatogram (**Figure 1**). The reagent of Liebermann Burchard revealed in all plant extracts (Ag, Cg, and Hs) the presence of sterols (spots of R_f = 0.89; 0.7; 0.6) and triterpenes of type lupine (R_f = 0.8; 0.3).

3.2.2. Flavonoids Detection

Figure 2 presented the chromatographic profile of flavonoids of herbaceous extracts (Ag, Cg, and Hs) obtained by the high-performance thin-layer chromatography method. Viewing the derivatized plate under 366 nm revealed, in all plant samples, blue, green, yellow, yellow-orange, greenish-yellow, and fluorescent spots. These different strains on the chromatogram were characterized by the presence of flavonoids in the herbaceous methanol extracts. Indeed, flavonoids form with several specific reagents (Natural Product, aluminum chloride) well-colored complexes under UV/366 nm or visible light [25]. Flavonols were identified on the plate by greenish-yellow and yellow spots (**Figure 2**). The blue areas were revealed by the flavones, methylated flavone, isoflavones, flavanones, and chalcones. The appearance of green zones identified flavanols and aurones. The characterization of flavonoids in crude extracts showed that these phenolic compounds are strongly present in the herbaceous samples, particularly the *Hibiscus sabdariffa* (Hs). In sum, the presence of flavonoids in herbaceous plants studied has been confirmed by Neu's reagent, making them appear visible as yellow and brown spots. These colors become intense and diversified under UV/366 nm [26].

3.2.3. Tannins Detection

Phenolic compounds, particularly tannins, were revealed on the HPTLC plate using the high-performance thin-layer chromatography method. The plate showed the fingerprint of herbaceous samples (Ag, Cg, and Hs) after heating at 110°C and spraying with 2% FeCl₃ reagent. Tannins appeared in brown and blue-blackish in visible light on the chromatogram (Figure 3). All plant samples showed the presence of tannins, particularly the *Hibiscus sabdariffa* (Hs).

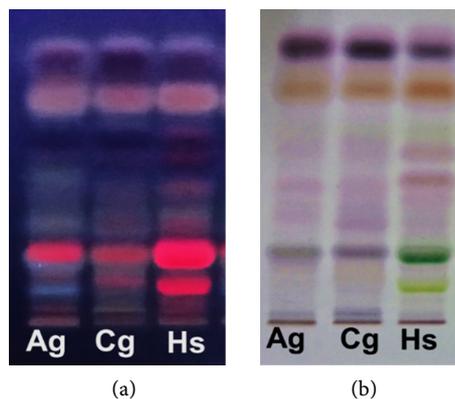


Figure 1. Chromatogram for detection of sterols and polyterpenes. (a): UV/366 nm; (b) Visible.

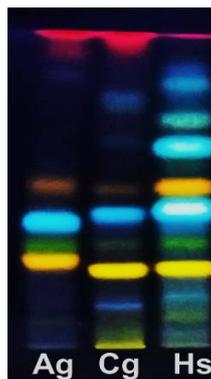


Figure 2. Chromatogram for detection of flavonoids.



Figure 3. Chromatogram for detection of tannins.

Phytochemical screening of the herbaceous plants (*Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa*) using the high-performance thin-layer method revealed secondary metabolites such as sterols, triterpenes, flavonoids, and tannins. Secondary metabolites are bioactive compounds. Some of them in the plant extracts (Ag, Cg, and Hs) would explain the therapeutic properties *Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa*. In light of recent scientific development, plants' medicinal properties have been investigated worldwide due to their potential pharmacological activities and economic viability [27]. Many aromatic and medicinal plants contain chemical compounds (flavonoids, tannins, carotenoids), exhibiting antioxidant properties [8] [27] [28]. Phenolic compounds have been reported to inhibit α -amylase and α -glucosidase associated with lipid peroxidation and type 2 diabetes [29] [30].

3.3. Total Phenolic, Flavonoid and Carotenoid Contents

The total phenolic, flavonoid, and carotenoid contents of *Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa* extracts measured in methanol (TPC and TFC) and n-hexane (TCC) were presented in **Table 1**. Phenolic contents varied from 86.5 ± 0.7 for *Apium graveolens* (Ag) to 138.4 ± 0.5 mg Gallic Acid Equivalents/g dry weight for *Hibiscus sabdariffa* (Hs). The methanol extract of *Hibiscus sabdariffa* showed the highest total phenolic compound than *Cleome gynandra* and *Apium graveolens* respectively. The three species' total phenolic contents were increased in the following order: *Apium graveolens* < *Cleome gynandra* < *Hibiscus sabdariffa*. Significant differences between the results were linked to genotypic and environmental differences within species, time of taking samples, and determination methods [31]. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. The total phenolic content measured by the Folin-Ciocalteu method does not give a full picture of the quality or quantity of phenolic constituents. The total flavonoid contents (TFC) showed a similar trend, varying from 29.2 ± 0.6 for *Apium graveolens* to 52.8 ± 0.6 mg Rutin Equivalents/g dry weight for *Hibiscus sabdariffa*. As presented in **Table 1**, the total phenolic and flavonoid contents are ranked in the following order: *Apium graveolens* < *Cleome gynandra* < *Hibiscus sabdariffa*. The total flavonoid contents showed a linear correlation with the total phenolic contents ($r = 0.91$) (**Table 2**).

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and total carotenoid content (TCC) in herbaceous extracts.

Plants	TPC (mg GAE/g DW)	TFC (mg RuE/g DW)	TCC (mg β C/g DW)	IC ₅₀ (mg/mL)	
				Hydrophilic antioxidant	Lipophilic antioxidant
<i>Apium graveolens</i> (Ag)	86.5 ± 0.7^c	29.2 ± 0.6^c	5.2 ± 0.004^c	1.10 ± 0.002^b	1.5 ± 0.002^b
<i>Cleome gynandra</i> (Cg)	96.9 ± 0.8^b	42.8 ± 0.03^b	24.4 ± 0.09^a	0.55 ± 0.001^c	1.6 ± 0.002^c
<i>Hibiscus sabdariffa</i> (Hs)	138.4 ± 0.5^a	52.8 ± 0.6^a	14.4 ± 0.3^b	0.31 ± 0.002^a	0.402 ± 0.001^a

Data are expressed as mean \pm SE of triplicate experiments. The means in each column, followed by a different letter, are significantly different ($P < 0.05$).

Table 2. Correlation coefficient (R^2) between phenolic and flavonoid contents and hydrophilic antioxidant activities.

	DPPH	TCP
TCP	0.86	-
TFT	0.99	0.91

In addition to phenolic compounds, carotenoids play an essential role in preventing human diseases and maintaining good health as part of a balanced diet [32]. The total carotenoid contents (TCC) were measured in n-hexane by the high-performance liquid chromatography method. TCC values ranged from 5.2 ± 0.004 for *Apium graveolens* to 24.4 ± 0.09 mg β -carotene Equivalents/g dry weight for *Cleome gynandra* (Table 1). As shown in Table 1, the hexane crude extract of *Cleome gynandra* presents the high content of total carotenoid, followed by *Hibiscus sabdariffa*, and *Apium graveolens*, respectively.

3.4. Hydrophilic and Lipophilic Antioxidant Activities, as Determined by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

Plant samples' antioxidant properties have been attributed to their bioactive compounds, being the most important carotenoid compounds and phenolic compounds [21]. The antioxidant activity of three herbaceous samples (*Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa*) was determined using the established assay that uses the radical DPPH \cdot as chromogen. This method permits the assay of hydrophilic and lipophilic antioxidant activities in the same sample. Table 1 showed the hydrophilic and lipophilic antioxidant activities in the three herbaceous plant studies. As illustrated in Table 1, the three herbaceous samples' hydrophilic antioxidant activities represented a significant contribution in all cases. In opposite, the lipophilic antioxidant capacities were less important. From the results, it could be concluded that the antioxidant activity level depends on the herbaceous variety. The correlations of hydrophilic antioxidant activity with total phenolic ($r = 0.86$) and flavonoid ($r = 0.99$) contents were significant. Among herbaceous plant studies, *Hibiscus sabdariffa* has exceptionally high antioxidant activity, as demonstrated by DPPH assay. *Hibiscus sabdariffa* also presented high phenolic and flavonoid contents (Table 1), two primary natural hydrophilic antioxidants [33]. Some epidemiological reports showed that antioxidant consumption could significantly influence health [33]. It was reported in the literature that natural plant products could prevent and management of non-transmissible diseases with minimal side effects and toxicity [29] [34]. Most often, the diabetic person is currently stressed. This situation disrupts hormonal function and causes a rise in cortisol levels, which leads to an increase in blood sugar levels.

However, there is no correlation between carotenoid contents and lipophilic antioxidant activities, which can be due to the low carotenoid contents observed in the herbaceous plants. It was reported that the β -carotene could not be the

significant lipophilic antioxidant in certain plant samples explaining the no correlation with the lipophilic antioxidant activity. The significant correlation of hydrophilic antioxidant capacity with the phenolic compounds and flavonoid contents indicated that the Folin-Ciocalteu could be used to determine the hydrophilic antioxidant activity of herbaceous samples.

4. Conclusion

Three herbaceous plants, potentially antidiabetic, were subjected to phytochemical screening by high-performance thin-layer chromatography (HPTLC). This study revealed phenolic compounds such as flavonoids and tannins in the *Apium graveolens*, *Cleome gynandra*, and *Hibiscus sabdariffa* samples. The non-polar compounds like sterols and triterpenes were also identified. Notwithstanding the biological and medicinal importance of these herbaceous plants studied, their hydrophilic and lipophilic antioxidant activities were determined spectrophotometrically by the radical DPPH[•]. These herbaceous extracts showed a significant contribution of the hydrophilic antioxidants (86%) to the global antioxidant activity than lipophilic antioxidants. It was also established a good correlation between hydrophilic antioxidant activities and flavonoid contents (99%). However, no correlation was revealed between lipophilic antioxidant activities and carotenoid contents in these herbaceous. The role of free radicals in metabolic diseases like diabetes, liver diseases, and hypertension is now known. So, these herbaceous can be introduced into the diet of patients suffering from chronic diseases (diabetes, hypertension, etc.). *Hibiscus sabdariffa* contained the highest content of phytochemicals (phenolic compounds) and is therefore of high value for the development of nutraceuticals and functional food, particularly for diabetes management.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Papaioannou, E.H., Stoforos, N.G. and Liakopoulou-Kyriakides, M. (2011) Substrate Contribution on Free Radical Scavenging Capacity of Carotenoid Extracts Produced from *Blakeslea trispora* Cultures. *World Journal of Microbiology and Biotechnology*, **27**, 851-858. <https://doi.org/10.1007/s11274-010-0527-z>
- [2] Prior, R.L., Wu, X.L. and Schaich, K. (2005) Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural Food and Chemistry*, **53**, 4290-4302. <https://doi.org/10.1021/jf0502698>

- [3] Tibiri, A., Sawadogo, W.R., Ouedraogo, N., Banzouzi, J.T., Guissou, I.P. and Nacoulma, O.G. (2010) Evaluation of Antioxidant Activity, Total Phenolic and Flavonoid Contents of *Antada Africana* Guill. et Perr. (Mimosaceae) Organ Extracts. *Research Journal of Medical Sciences*, **4**, 81-87. <https://doi.org/10.3923/rjmsci.2010.81.87>
- [4] Hema, A., Palé, E., Sérémé, A. and Nacro, M. (2016) Comparison of Total Antioxidant Capacity, Polyphenolic and Anthocyanins Contents from Some Varieties of Dry Beans of Burkina. *ChemXpress*, **9**, 1-9.
- [5] Seeram, N.P. (2008) Berry Fruits for Cancer Prevention: Current Status and Future Prospects. *Journal of Agricultural Food and Chemistry*, **56**, 630-635. <https://doi.org/10.1021/jf072504n>
- [6] Johnsen, S.P., Overvad, K., Stripp, C., et al. (2003) Intake of Fruit and Vegetables and the Risk of Ischemic Stroke in a Cohort of Danish Men and Women. *The American Journal of Clinical Nutrition*, **78**, 57-64. <https://doi.org/10.1093/ajcn/78.1.57>
- [7] Jasprica, I., Bojic, M., Mornar, A., Besic, E., Bucan, K. and Medic-Saric, M. (2007) Evaluation of Antioxidative Activity of Croatian Propolis Samples Using DPPH- and ABTS⁺ Stable Free Radical Assays. *Molecules*, **12**, 1006-1021. <https://doi.org/10.3390/12051006>
- [8] Noba, A., Koala, M., Hema, A., Bationo, R.K., Dabiré, C.M., Palé, E. and Nacro, M. (2020) Carotenoids Identification by HPTLC-MS and Vitamin C Content of Fruits of *Saba senegalensis* (A. DC) Pichon. *African Journal of Pure and Applied Chemistry*, **14**, 60-68.
- [9] Begum, S.A.S., Rao, M. and Ramachandra, M.S. (2019) Evaluation of Antidiabetic Activity of *Swertia chirayita* and *Panax ginseng*. *European Journal of Pharmaceutical and Medical Research*, **7**, 516-522.
- [10] Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N.H., et al. (2017) Global Estimates for the Prevalence of Diabetes for 2015 and 2040. *IDF Diabetes Atlas*, **128**, 40-50. <https://doi.org/10.1016/j.diabres.2017.03.024>
- [11] Bi, X., Lim, J. and Henry, C.J. (2017) Spices in the Management of Diabetes Mellitus. *Food Chemistry*, **217**, 281-293. <https://doi.org/10.1016/j.foodchem.2016.08.111>
- [12] Muñiz-Ramirez, A., Perez, R.M., Garcia, E. and Garcia, F.E. (2020) Antidiabetic Activity of *Aloe vera* Leaves. *Evidence-Based Complementary and Alternative Medicine*, **2020**, Article ID: 6371201. <https://doi.org/10.1155/2020/6371201>
- [13] Purbowatingrum, Ngadiwiyana, Fachriyah, E., Ismiyanto, Ariestiani, B. and Khikmah (2018) Antidiabetic Activity from Cinnamaldyde Encapsulated by Nanochitosan. *IOP Conference Series: Materials Science and Engineering*, **349**, 012048. <https://doi.org/10.1088/1757-899X/349/1/012048>
- [14] Dewi, U.K. and Tyas, R.S. (2009) Efek rebusan daun tapak dara pada dosis dan frekuensi yang berbeda terhadap kerusakan dan akumulasi glikogen pada hepar mencit (*Mus musculus*). *BIOMA*, **11**, 1-5.
- [15] Martin, G. (1995) Ethnobotany. A Methods Manual. Kew, Royaume-Uni, Royal Botanic Gardens. In: Londres, Chapman and Hall. <https://fr.scribd.com/document/311438001/Martin-1995-Ethnobotany-A-Methods-Manual-pdf>
- [16] Reich, E., Schibli, A., Widmer, V., Jorns, R., Wolfram, E. and DeBatt, A. (2006) HPTLC Methods for Identification of Green Tea and Green Tea Extract. *Journal of Liquid Chromatography & Related Technologies*, **29**, 2141-2151.

- <https://doi.org/10.1080/15512160600760293>
- [17] Reich, E., Blatter, A., Jorns, R., Kreuter, M. and Thiekötter, K. (2002) An AOAC Peer-Verified Method for Identification of *Echinacea* Species by HPTLC. *Journal of Planar Chromatography*, **15**, 244-251. <https://doi.org/10.1556/JPC.15.2002.4.1>
- [18] Ouédraogo, J.C.W., Koala, M., Ouédraogo, W., Noufou, O., Kini, F., Gerbaux, P. and Bonzi-Coulibaly, Y.L. (2017) Total Phenolics and Total Flavonoid Contents, Antioxidant Activity and Flavonoids Identification by High-Performance Liquid Chromatography-Tandem Mass Spectrometry of *Odontonema strictum* (Acanthaceae) Leaves. *Asian Journal of Plant Science and Research*, **7**, 54-63.
- [19] Brito, A., Areche, C., Sepúlveda, B., Kennelly, E.J. and Simirgiotis, M.J. (2014) Anthocyanin Characterization, Total Phenolic Quantification and Antioxidant Features of Some Chilean Edible Berry Extracts. *Molecules*, **19**, 10936-10955. <https://doi.org/10.3390/molecules190810936>
- [20] Eitzbach, L., Pfeiffer, A., Weber, F. and Schieber, A. (2018) Characterization of Carotenoid Profiles in Goldenberry (*Physalis peruviana* L.) Fruits at Various Ripening Stages and in Different Plant Tissues by HPLC-DAD-APCI-MSⁿ. *Food Chemistry*, **245**, 508-517. <https://doi.org/10.1016/j.foodchem.2017.10.120>
- [21] Lizárraga-Velázquez, C.E., Hernández, C., González-Aguilar, G.A. and Heredia, J.B. (2018) Effect of Hydrophilic and Lipophilic Antioxidants from Mango Peel (*Mangifera indica* L. cv. Ataulfo) on Lipid Peroxidation in Fish Oil. *CyTA—Journal of Food*, **16**, 1095-1101. <https://doi.org/10.1080/19476337.2018.1513425>
- [22] Zhao, Z.G., Yan, H.F., Zheng, R., Khan, Saeed, M., Fu, X., Tao, Z. and Zhang, Z.Y. (2018) Anthocyanins Characterization and Antioxidant Activities of Sugarcane (*Saccharum officinarum* L.) Rind Extracts. *Industrial Crops & Products*, **113**, 38-45. <https://doi.org/10.1016/j.indcrop.2018.01.015>
- [23] Dudonné, S., Vitrac, X., Courtiere, P., Woillez, M. and Mérillon, J.M. (2009) Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. *Journal of Agricultural and Food Chemistry*, **57**, 1768-1774. <https://doi.org/10.1021/jf803011r>
- [24] Cotton, C.M. and Wilkie, P. (1996) *Ethnobotany: Principles and Applications*. John Wiley & Sons, Chichester. <https://pubs.acs.org/doi/abs/10.1021/jm9701841>
- [25] Brou, K.G., Mamyrbekova-Bekro, J.A., Dogbo, D.O., Gogbeu, S.J. and Bekro, Y.A. (2010) On the Qualitative Phytochemical Composition of Crude Hydromethanolic Extracts of the Leaves of 6 Varieties of *Manihot Esculenta* Crantz of Côte d'Ivoire. *European Journal of Scientific Research*, **45**, 200-211.
- [26] Wagner, H., Bladt, S. and Zgainski, E. (1996) *Plant Drug Analysis: A Thin Layer, Chromatography Atlas*. 2nd Edition, Springer Verlag, Berlin Heidelberg, New York, 320 p. <https://doi.org/10.1007/978-3-642-00574-9>
- [27] Ye, J., Feng, L.L., Xiong, J. and Xiong, Y.D. (2011) Ultrasound-Assisted Extraction of Corn Carotenoids in Ethanol. *International Journal of Food Science and Technology*, **46**, 2131-2136. <https://doi.org/10.1111/j.1365-2621.2011.02727.x>
- [28] Nitiéma, M., Koala, M., Belemnaba, L., Ouédraogo, J.C.W., Ouédraogo, S., Kini, B.F., Ouédraogo, S. and Guissou, I.P. (2019) Endothelium-Independent Vasorelaxant Effects of Anthocyanins-Enriched Extract from *Odontonema strictum* (Nees) Kuntze (Acanthaceae) Flowers: Ca²⁺ Channels Involvement. *European Journal of Medicinal Plants*, **29**, 1-11. <https://doi.org/10.9734/ejmp/2019/v29i330155>
- [29] Nnenna, A.O., Chidi, U.S., Azuka, A.B., Kelechi, A.K. and Udeh, E.K. (2020) Inhibitory Potential and Antidiabetic Activity of Leaf Extracts of *Vitex doniana*. *African*

Journal of Biochemistry Research, **14**, 72-80.

<https://doi.org/10.5897/AJBR2020.1098>

- [30] Reddy, N.V., Anarthe, S.J. and Raghavendra, N.M. (2010) *In Vitro* Antioxidant and Antidiabetic Activity of *Asystasia gangetica* (Chinese Violet) Linn. (Acanthaceae). *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **1**, 72-75.
- [31] Wojdyło, A., Oszmiański, J. and Czemerys, R. (2007) Antioxidant Activity and Phenolic Compounds in 32 Selected Herbs. *Food Chemistry*, **105**, 940-949.
<https://doi.org/10.1016/j.foodchem.2007.04.038>
- [32] Cano, M.P., Gómez-Maqueo, A., García-Cayuela, T. and Welti-Chanes, J. (2017) Characterization of Carotenoid Profile of Spanish Sanguinos and Verdal Prickly Pear (*Opuntia ficus-indica*, spp.) Tissues. *Food Chemistry*, **237**, 612-622.
<https://doi.org/10.1016/j.foodchem.2017.05.135>
- [33] Thaipong, K., Boonprakob, U., Cisneros-Zevallos, L. and Byrne, D.H. (2005) Hydrophilic and Lipophilic Antioxidant Activities of Guava Fruits. *The Southeast Asian Journal of Tropical Medicine and Public Health*, **4**, 254-257.
- [34] Bhatia, A., Singh, B., Arora, R. and Arora, S. (2019) *In Vitro* Evaluation of the α -Glucosidase Inhibitory Potential of Methanolic Extracts of Traditionally Used Antidiabetic Plants. *BMC Complementary and Alternative Medicine*, **19**, Article No. 74. <https://doi.org/10.1186/s12906-019-2482-z>