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Research Article

Phytochemical Study and Radical Scavenging Activity of Three Leafy Vegetables Grown in Zitenga Region of Burkina Faso

Benjamin Ouédraogo[1, 3], Alphonsine Ramdé Tiendrébogo[2, 3], Jules Yoda[2, *], Felix Kini[2]

[1]Research and Development Laboratory (LRD), University Ledea Bernard Ouedraogo (ULBO), Ouahigouya, Burkina Faso

[2]Department of Traditional Medicine, Pharmacopoeia – Pharmacy (MEPHATRA-PH), Health Sciences Research Institute (IRSS /CNRST), Ouagadougou, Burkina Faso

[3]International Research Laboratory IRL 3189 Environment, Health, Societies (CNRST, USTTB, UCAD, UGB, CNRS), Ouagadougou, Burkina Faso

Abstract

The aim of this study is to analyze the phytochemical composition and radical scavenging activity of three leafy vegetables from Burkina Faso: *Cleome gynandra, Hibiscus sabdariffa and Corchorus olitorius*. Samples of these plants were collected in Zitenga region, dried, powdered and extracted by decoction using water and a hydroethanolic solvent. Phytochemical screening was carried out by thin layer chromatography. Bioactive compounds, notably flavonoids and phenolic compounds, were measured respectively by the aluminum trichloride ($AlCl_3$) method and the Folin-Ciocalteu method. The radical scavenging activity of the extracts was also investigated using the DPPH method. Phytochemical analyzes revealed the presence of bioactive compounds such as flavonoids, tannins and saponosides in the three species studied. The highest total phenolic and total flavonoid contents were obtained respectively with the hydroethanolic extract of *Cleome gynandra* (223.54 ±0.18 mg TEA/g DM) and the aqueous extract of *Cleome gynandra* (69.09 ± 0.26 mg QE/g DM). The best antioxidant activity was obtained with the hydroethanolic extract of *Corchorus olitorius* (IC_{50}: 31.93 ± 1.95 µg /mL). The results showed that the extracts of *Cleome gynandra, Hibiscus sabdariffa and Corchorus olitorius* all exhibited radical scavenging activity, indicating their potential to reduce oxidative stress. These results suggest that *Cleome gynandra, Hibiscus sabdariffa and Corchorus olitorius* are rich sources of health-promoting phytochemicals. These food plants from Burkina Faso could therefore be transformed into nutraceuticals to promote health and prevent diseases linked to oxidative stress.

Keywords

Phytochemical Composition, *Cleome Gynandra*, *Hibiscus Sabdariffa*, *Corchorus Olitorius*, Radical Scavenging

*Corresponding author: jules_yoda@yahoo.fr (Jules Yoda)

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1. Introduction

Plants are widely used around the world for their therapeutic properties [1]. They constitute an important source of natural bioactive compounds exhibiting various biological activities. Indeed, plants contain active ingredients with various medicinal properties that can be involved in the treatment of many diseases.

A preliminary study carried out in the province of Oubritenga in the Central Plateau region of Burkina Faso made it possible to identify twenty-five (25) local plants adapted to drought, and presenting both nutritional and therapeutic virtues with high utilization values [2]. The present work focuses on three of these plants, namely *Cleome gynandra L. (Brassicaceae), Hibiscus sabdariffa L. (Malvaceae)* and *Corchorus olitorius. L. (Malvaceae*) respectively called Kiennebdo, Bito and Bulvanca in the local Moore language. These species are cultivated in a nutritious garden by a women's cooperative for family consumption but also for income-generating activities. In traditional medicine *Cleome gynandra*, is an herbaceous plant used to treat inflammation, pain, malaria and microbial infections [3]. *Hibiscus sabdariffa* is recognized for its antihypertensive, hypoglycemic and antioxidant properties [4-6]. As for *Corchorus olitorius*, it has anti-inflammatory, antidiabetic and antioxidant activities [7, 8].

Despite their well-established traditional uses, the phytochemical profiles and biological activities of these three plants have not been sufficiently elucidated. This study therefore aims to carry out a phytochemical screening, evaluate the contents of phenolic compounds, flavonoids, as well as the radical scavenging activity of extracts of *Cleome gynandra, Hibiscus sabdariffa* and *Corchorus olitorius*. The results obtained will provide scientific prerequisites for the transformation of these leafy vegetables into nutraceuticals in order to contribute to food and health security for populations.

2. Methodology

2.1. Plant Material

The plant material consists of the leaves of *Cleome gynandra, Hibiscus sabdariffa and Corchorus olitorius*, collected in a community garden in Zitenga (Figure 1). The harvested leaves were washed well and dried in a ventilated room, away from sunlight and dust (for 2 weeks) then reduced to powder using a blade crusher.

Cleome gynandra Hibiscus sabdariffa Corchorus olitorius

Figure 1. *Leafy vegetables in cultivation (Photo, Benjamin OUEDRAOGO, September 2022, Zitenga).*

2.2. Moisture Content

The residual moisture content of each vegetable powder was obtained using the thermogravimetric method, the principle of which is based on loss upon drying [9]. For this, one (1) g of weighed vegetable powder was placed in a previously tared watch glass. The assembly was placed at 105 ℃ for one (1) hour and thirty (30) minutes in a ventilated oven. After cooling in a desiccator, weighing was carried out. The operation was continued until a constant weight was obtained. The residual moisture content (R_{mc}) of the plant drug was determined according to the following formula:

$$\text{Rmc (\%)} = \frac{\text{Tp} - Taf}{\text{Tp (g)}} \text{ x } 100$$

Rmc: Residual moisture content
$T_{p:}$ Test portion of plant material (g);
T_{af}: Test portion after drying (g)

2.3. Extraction

The preparation of the extracts consisted of making an aqueous and hydroethanolic decoction of leaf powders of *Cleome gynandra, Hibiscus sabdariffa* and *Corchorus olitorius*. The aqueous decoction was obtained according to the

following procedure:

A test portion of 50 g of the plant powder was dispersed in 500 mL of distilled water. The whole thing was brought to the boil for 30 minutes. After cooling, the mixture was filtered through a fine mesh nylon screen. The filtrate was centrifuged at 2000 rpm for 10 minutes, then dried in a ventilated oven and preserved for further analyses.

The hydroethanolic decoction was also obtained according to this procedure:

A test portion of 50 g of the plant powder was dispersed in 500 mL of an ethanol-water mixture in the proportions 70/30 which constitutes the hydroethanolic solvent. The whole thing was brought to the boil for 30 minutes. After cooling, the mixture was filtered through a fine mesh nylon screen. The collected filtrate was centrifuged at 2000 rpm for 10 minutes, then dried in a ventilated oven and preserved for further analyses.

The extraction yield was determined by relating the mass of dry extract obtained to one hundred (100) grams of test portion of dry plant material.

2.4. Phytochemical Screening of Extracts

The phytochemical screening was carried out on chromatoplates (60 F_{254}, glass support 20 x 20 cm, Fluka –Silica gel) following the methods described in the literature [10]. The aim was to search for large chemical groups using thin layer chromatography (TLC) such as steroidal, terpene, phenolic and alkaloidal compounds.

Each dry extract was solubilized in its extraction solvent at a concentration of 10 mg/mL (10 mg in 1 mL of solvent) and 5 µL were deposited on the TLC plate. Chromatograms were developed over an 8 cm path in appropriate solvent systems.

Several specific reagents were used to reveal these groups of compounds. Sulfuric vanillin reagent and Libermann Burchard reagent for terpenes and sterols; 5% (V/V) methanolic KOH reagent for coumarins; NEU reagent for flavonoids; the $FeCl_3$ reagent for tannins and phenolic compounds and the sulfuric anisaldehyde reagent for saponosides.

2.5. Evaluation of Bioactive Compound Contents

2.5.1. Dosage of Phenolic Compounds

The determination of phenolic compounds was carried out following the Singleton method using the Folin-Ciocalteu reagent (FCR) [11]. The reaction mixture consists of 25 µL of extract at 0.1 mg/mL, 105 µL of 0.2 N FCR which was left to incubate for five minutes away from light. To this mixture were added 100 µL of a sodium carbonate solution (75 g/L in distilled water). It is left to incubate for one (1) hour away from light then the absorbance was measured at the wavelength of 760 nm on the spectrophotometer against a standard curve of tannic acid. The tests were carried out in triplicate and the total phenolic content of the extract expressed as tannic acid equivalent (TEA)/g. The total phenolic content of the extract was evaluated by the formula:

$$T_{pT} = (C_{tube} \times D) / Ci$$

C_{Tube}: Concentration in mg TEA/mL in the assay tube;
D: Dilution factor
Ci: Concentration in mg/mL in the stock solution.

2.5.2. Assay of Total Flavonoids

The determination of flavonoids was carried out according to the method of Kumaran [12], adapted by Abdel-Hamed [13]. To 100 µL of concentrated extract (1 mg/mL) were added 100 µL of aluminum trichloride (2% in methanol). The absorbance was read at λ = 415 nm after 40 min of incubation against a blank (100 µL of methanol and 100 µL of $AlCl_3$).

Thus, the appearance of a stable yellow color makes it possible to evaluate by UV spectrophotometry using a BioRad spectrophotometer (model 680Japan) the flavonoid content of the sample compared to a reference solution of Quercetin (0-70 µg/mL). The tests were carried out in triplicate and the results were expressed in milligrams of Quercetin Equivalent per gram of dry extract (mg QE/g).

The flavonoid content of the extract was obtained by the formula:

$$T_{Flav} = (A.\ m_0)/ (A_0.\ m)$$

T_{Flav}: flavonoid content of the extract expressed in mg EQ /mg;
A: Absorbance of the extract; A_0: Absorbance of Quercetin
m: mass of the extract in mg; m_0: mass of Quercetin in mg

2.5.3. Evaluation of Radical Scavenging Activity by the DPPH Method

The method for measuring antioxidant power using the DPPH (2,2-Diphenyl-picrylhydrazine) method is based on the ability of a compound to reduce the DPPH °radical (Figure 2).

Reduction results in an immediate color change from violet to yellow in the presence of an anti-free radical compound.

The reaction is then quantified by measuring the absorbance of the solution spectrophotometrically at a wavelength of 490 nm. The change in color from purple to yellow is proportional to the antioxidant power.

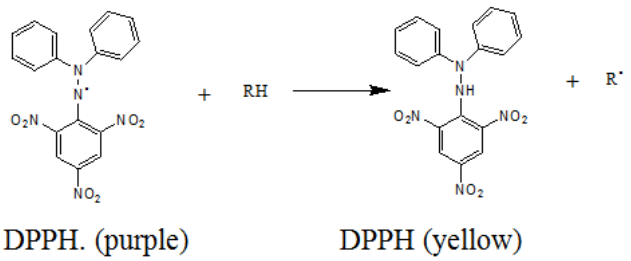


Figure 2. *Reduction of DPPH by an antioxidant.*

The ability of the extracts to reduce DPPH free radicals was determined by the method of KIM et al [14]. Thus, a series of concentrations was carried out from the initial concentration (1 mg/mL) of the samples and Trolox used as the reference substance. On a 96-well microplate, filling for each concentration was done with 200 µL of DPPH solution (0.04 mg/ml) and 20 µL of the diluted extract or reference. After 30 minutes of incubation, the absorbance was read at the wavelength λ = 490 nm using a BioRad spectrophotometer (model 680 Japan). The blank (without sample) was prepared under the same conditions and is composed of 200 µL of DPPH and 20 µL of ethanol. A curve of the percentage of DPPH inhibition was plotted as a function of the sample concentration (Table 1). On the curve, the concentration necessary to degrade 50% DPPH (IC_{50}) was determined.

Table 1. *Composition of the reaction mixture for the DPPH reduction test in solution.*

Reagent	Test (µL)	negative Control (µL)	Blanc of test (µL)
DPPH	200	200	
Extract/Reference	20		20
Ethanol		20	200
Total	220	220	220

3. Results and Discussion

3.1. Moisture Content

The Moisture Content of plant raw materials are recorded in the following table:

Table 2. *Residual moisture content of the plant raw material.*

	Cleome gynandra	*Hibiscus sabdariffa*	*Corchorus olitorius*
Moisture Content (%)	4.26 ±0.1	3.80 ±0.15	5.38 ±0.15

The Moisture Content are all below 10%. Indeed, certain factors such as humidity, heat, radiation, microorganisms and enzymes often constitute causes of degradation of plant material. The content allowed in dry plant material for its proper conservation must not exceed 10%. The above results show that our samples can be preserved with less risk of contamination and/or alteration of chemical principles [15].

3.2. Extraction Yield

The different extraction operations of plant material gave variable yields which are recorded in the following table (table 3).

Table 3. *Extraction yields.*

Plants	*Cleome gynandra*		*Hibiscus sabdariffa*		*Corchorus olitorius*	
	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
Yields (%)	28.07	17.63	30.01	34.14	9.41	18.84

Extraction yields ranged from 9.41% to 34.14%. The best extraction yield was obtained with the hydroethanolic extract of *Hibiscus sabdariffa*; while the lowest yield was obtained with the aqueous extract of *Corchorus olitorius*.

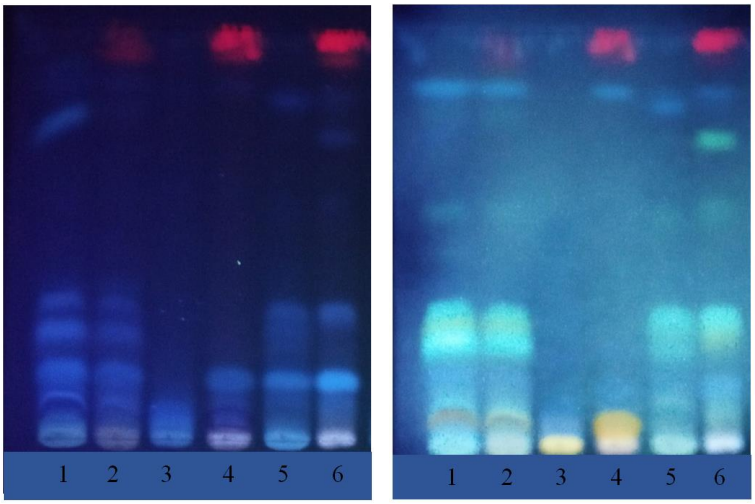
As far as extraction solvents go, water was best for *Cleome gynandra*. This high yield could be explained by the hydrophilic nature of the constituents present in the plant extracts. Indeed, water, the extraction solvent, carries away the polar compounds. Thus, the more hydrophilic a molecule is, the more easily it will pass through a decoction and/or maceration [16]. For the other two plants, namely *Hibiscus sabdariffa* and *Corchorus olitorius*, ethanol was the best extraction solvent. Water-alcohol mixtures are known to be good solvents for extracting polar compounds [17].

3.3. Chemical Groups

The different fractions were characterized by TLC in order to highlight the chemical groups of interest (figure 3):

1) Chromatographic fingerprint: Solvent system: ethyl acetate-formic acid-water (8-1-1 V/V/V) Observation: UV 366;
2) Highlighting flavonoids: Solvent system: ethyl acetate-formic acid-water (8-1-1 V/V/V) Observation: UV 366 nm, Sprayed with NEU reagent.

The results obtained are illustrated by thin layer chromatography (TLC) and recorded in the following table (table 4).



Chromatographic fingerprint Highlighting flavonoids

Figure 3. *TLC for detection of secondary metabolites.*

Hibiscus sabdariffa. aqueous (1); Hibiscus sabdariffa ethanolic (2); Cleome gynandra aqueous (3); Cleome gynandra ethanolic (4); Corchorus olitorius aqueous (5); Corchorus olitorius ethanolic (6).

Table 4. *Results of phytochemical screening by TLC.*

Plants	Compounds				
	Flavonoids	Tannins	Sterols	Triterpenes	Saponosides
Cleome gynandra	+	+	-	-	+
Hibiscus sabdariffa	+	+	-	-	+
Corchorus olitorius	+	+	-	-	+

Legend: presence (+); not detected (-)

Phytochemical screening revealed the presence of chemical groups of interest in the extracts, namely tannins, flavonoids and saponosides. These chemical groups are endowed with several biological properties, notably their antioxidant capacity [18].

The fingerprint shows that all three plants contain many chemical groups. Different combinations of these plants in cooking could be a great source of secondary metabolites for our body.

3.4. Contents of Bioactive Compounds and Antioxidant Activity

The contents of total phenolics, total flavonoids and antioxidant activity were successively determined with the aqueous and hydroethanolic decoctions. The results obtained are presented in Table 5.

Table 5. *Contents of total phenolics, total flavonoids and antioxidant activities of the extracts.*

Plants Extracts	Total phenolics contents (mg TEA/g DM)		Total flavonoids (mg QE/g DM)	DPPH scavenging activity IC_{50} (µg/mL)
Cleome gynandra	Aqueous	98.34 ±0.01	69.09 ±0.26	331 ±0.52
	Ethanolic	223.54 ±0.18	31.31 ±0.73	71.67 ±3.92

Plants Extracts	Total phenolics contents (mg TEA/g DM)		Total flavonoids (mg QE/g DM)	DPPH scavenging activity IC_{50} (µg/mL)
Corchorus olitorius	Aqueous	164.84 ±0.45	37.97 ±0.73	120.70 ±5.11
	Ethanolic	56.31 ±0.028	35.30 ±0.49	31.93 ±1.95
Hibiscus sabdariffa	Aqueous	23.75 ±0.001	16.23 ±0.18	229.49 ±1.27
	Ethanolic	99.093±0.07	13.97 ±0.53	137.57 ±0.47

The total phenolic contents of the extracts vary from 23.75 ±0.001 mg TEA/g to 223.54 ±0.18 mg TEA/g of dry matter. The highest content was obtained with the hydroethanolic extract of *Cleome gynandra* for a content of 223.54 ±0.18 mg TEA/g DM, and the lowest content with the aqueous extract of *Hibiscus sabdariffa* for a content of 23.75 ± 0.001 mg TEA/g.

Regarding the contents of total flavonoids, variations from 13.97 ±0.53 mg QE/g to 69.09 ±0.26 mg QE/g of dry matter are recorded. The best content was obtained with the aqueous extract of *Cleome gynandra* (at 69.09 ±0.26 mg QE/g DM) and the low content with the hydroethanolic extract of *Hibiscus sabdariffa* (13.97 ±0.53 mg QE/g). High contents of total phenolics and total flavonoids are therefore obtained on the one hand with water as extraction solvent, and on the other hand with a water-ethanol mixture. The use of water for cooking these plants is significant, which will provide a considerable quantity of phytonutrients to the body.

Evaluation of the antioxidant activity of the extracts is necessary. Indeed, the body naturally generates free radicals. However, some come from exogenous factors such as diet, exposure to the sun and pollution. These are, for example, superoxide ($O_2^{\cdot}$-), hydroxyl ($HO^{\cdot}$), hydroperoxyl ($HO_2^{\cdot}$) and nitrogen monoxide ($NO^{\cdot}$). At high levels, these free radicals and oxidants present in the body generate oxidative stress which corresponds to a state of imbalance between free radicals and antioxidants. It is a deleterious process that can damage cellular structures, lipids, proteins and DNA. Antioxidants protect the body against the harmful action of free radicals. Some antioxidants are produced by our own body and others such as vitamins C, E and β-carotene are ingested. The DPPH method was used during our work.

The 50% inhibitory concentration (IC_{50}), that is to say the concentration of the extract capable of causing 50% inhibition of the DPPH radical, was presented in Table 5.

By the DPPH method, hydroethanolic extracts appear more active than aqueous extracts. Previous studies have shown the correlation between the presence of phenolic compounds in an extract and its antioxidant activity [19-22].

Indeed, phenolic compounds, particularly flavonoids, are recognized as powerful antioxidants against free radicals due to their property of providing hydrogen atoms available in the hydroxyl substituents of their phenolic groups [23]. Their protective effects in biological systems are linked to their ability to transfer electrons to free radicals, to chelate metals, to activate antioxidant enzymes or to inhibit oxidases [4]. Plant flavonoids are known for their effectiveness against fever, edema and inflammation of the mucous membranes [24, 25]. Regular consumption of these three leafy vegetables helps fight oxidative stress [26] and prevent certain chronic diseases.

4. Conclusion

This study made it possible to evaluate the phytochemical profile and the radical scavenging activity of three leafy vegetables, *Cleome gynandra*, *Hibiscus sabdariffa* and *Corchorus olitorius*.

Phytochemical screening revealed the presence of chemical groups of interest in the extracts, namely tannins, flavonoids and saponosides. The results also demonstrated that these three plants are rich sources of phenolic compounds and flavonoids, secondary metabolites known for their therapeutic properties. However, *Cleome gynandra* presented the best contents of total phenolics and flavonoids. The best antioxidant power was obtained with the hydroethanolic extract of *Corchorus olitorius*. This antioxidant property is likely to contribute to the beneficial effects of these plants on health. The results of this study constitute important scientific prerequisites for the development and promotion of new nutraceuticals and/or phytomedicines for better nutrition and good population health. An evaluation of the nutritional potential of these three plants is therefore essential.

Abbreviations

ULBO	University Ledea Bernard Ouedraogo
LRD	Research and Development Laboratory
IRSS	Health Sciences Research Institute
CNRST	National Center for Scientific and Technological Research
USTTB	University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali
UCAD	University Cheikh Anta Diop, Dakar, Senegal
UGB	University Gaston Berger, Saint Louis, Senegal
CNRS	National center for Scientific Research, Paris, France

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Author Contributions

Benjamin Ouédraogo: Data curation, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft

Alphonsine Ramdé Tiendrébéogo: Conceptualization, Formal Analysis, Funding acquisition, Project administration, Supervision

Jules Yoda: Formal Analysis, Methodology, Software, Supervision, Validation, Visualization

Felix Kini: Supervision, Validation, Visualization

Conflicts of Interest

The authors declare no conflicts of interest.

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