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Phytochemical screening and antiradical activity of a hydroethanolic extract of the calyxes of *Hibiscus sabdariffa* L. (Malvaceae), a species of food and pharmacological interest in Togolese flora

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Abstract
Hibiscus sabdariffa L. is a food plant used in traditional medicine in Togo for its biological activities due to the many bioactive molecules it contains. Several previous studies have confirmed the biological activities of this species. However, few data are available in Togo. It is therefore necessary to investigate this plant in Togo, known as the impact of climatic and edaphic variations on its phytochemical constitution. The aim of this study is to contribute to the development of this species by determining its phytochemical compounds and assessing the antioxidant activity of its calyxes. A hydroethanol extraction was performed on *H. sabdariffa* calyxes harvested in Lomé. Phytochemical tests were carried out on the dry extract obtained, using characterization reactions based on coloring and/or precipitation principles. Antiradical activity was determined by DPPH• radical inhibition and ferric ion reduction tests of the $[(Fe(III)TPTZ)_2]$ complex. Phytochemical screening revealed the presence of alkaloids, reducing carbohydrates, flavonoids, tannins, saponins, total carbohydrates and total polyphenols in the extract under consideration. The results also showed that the calyx hydroethanol extract analyzed contained high levels of phenolic compounds, i.e. 32.596 ± 1.121 mg EAG/g, 31.537 ± 1.161 mg EAG/g and 64.461 ± 2.718 mg ER/g for total phenols, tannins and flavonoids respectively. These compounds were probably responsible for the anti-free radical activity observed, with an IC_{50} of 247.00 ± 2.88 µg/ml for inhibition of the DPPH• radical, and a reducing power for Fe^{3+} ferric ions, with an EC_{50} of 10.153 ± 0.123 µg/ml. *H. sabdariffa* calyxes from the Togolese flora thus possess an interesting antioxidant activity concomitant with their phytochemical constitution. These results also justify the therapeutic and dietary uses of these calyxes, which should be considered as an endogenous source of healthy, sustainable food security in Togo.

Keywords: *Hibiscus sabdariffa* L., calyxes, phytochemical screening, antioxidant activity, Togo

Introduction
Natural plant substances are sought after for their numerous biological activities that promote positive health effects. These activities explain the use of these substances in the agri-food and pharmaceutical sectors [1] They have long been an important source of therapeutic agents [2] Currently, a great deal of research is being carried out on these natural substances for the health benefits they provide, principally their antioxidant power and their possible role in the prevention, protection and treatment of several diseases such as cardiovascular disease and cancer. Some of these compounds also help boost the immune system. For these reasons, people around the world in general, and in Africa in particular, turn to plants for medical care. Around 80% of the world's population depends in fact, on traditional medicine where the efficacy of plants has been paramount [3]. Several studies are therefore interested in medicinal plants due to their wealth of secondary metabolites, which have the advantage of a wide diversity of chemical structures and biological activities [4]. Phytochemical analysis and identification of secondary metabolites have revealed that they underpin biological properties such as antiviral, antibacterial, antifungal, insecticidal, antimalarial, antioxidant, anticancer, anti-inflammatory and enzyme-inhibiting activities of medicinal plants [1, 5, 6]. These secondary metabolites represent an important source of molecules such as alkaloids, flavonoids, phenolic

acids, vitamins, tannins, carotenoids and so on. These metabolites provide bio-inspiration for drug development, thanks to their numerous biological activities, which promote positive health effects. They have therapeutic applications in the biomedical field and enter into the constitution of drugs [7, 8, 9]. For all these reasons, Newman and Cragg [10] have indicated that the role of natural substances in drug development is crucial. These substances are used in the treatment of pathologies, often thanks to their antioxidant properties [11]. Indeed, the causes of many pathologies in general and specifically in humans are multifactorial, including oxidative stress, due to reactive oxygen species (ROS) [12]. The main danger posed by free radicals is the damage they cause when they react with important cellular compounds (DNA and cell membrane). Exposure to free radicals causes abnormal cell proliferation and multiplication, leading to cancer, cell dysfunction or cell death [13, 14]. Oxidative stress is thus due to a profound imbalance in the balance between prooxidants and antioxidants, in favor of the former. These free radicals come in various forms of reactive oxygen species (ROS), such as: the hydroxyl radical ($OH^{\bullet}$), the superoxide ion ($O^{2\bullet}$), the peroxide radical ($ROO^{\bullet}$), non-radical species including singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and certain transition metals such as copper and iron [16, 17]. However, this damage can be counteracted by antioxidants. An antioxidant is any substance which, at low concentration relative to the oxidizable substrate, significantly slows or prevents the oxidation of such a substrate [18]. It is a bioactive molecule capable of trapping reactive oxygen species, even at low concentrations [19]. Many secondary metabolites are molecules with active redox potential, capable of modulating the intracellular redox balance in living cells. They may also be acidic compounds, which can be used in foods and donate an electron or an atom. Most of these metabolites then act as antioxidants [20]. The antioxidant network is also complex, with antioxidants synthesized by the body's own cells and others supplied by the diet. It is in this context that antioxidant phytochemicals from plants remain important. Indeed, the use of synthetic molecules is being questioned because of the potential toxicological risks they may pose to the body. Sources of natural antioxidant molecules are therefore actively sought [21]. Medicinal plants are an important source of these antioxidant molecules, as well as proteins, fibers, carbohydrates and, above all, vitamins A, B and C [22]. As such, they contribute to the prevention and healing of pathologies [23]. The leafy vegetables of the Togolese flora, like those of other countries, constitute an excellent contribution to enrichment and diversification of the human diet [24]. This is why it is so important to research the medicinal and nutritional potential of these food plants. The aim is to ensure food security and the production of improved traditional medicines that are effective and accessible to the population. *Hibiscus sabdariffa* L. is one of the plants used for its antioxidant properties in Togo. The calyxes and leaves of this plant are eaten by the Togolese population. This species, also known as Roselle, is a member of the Malvaceae family. It is widely recognized for its many therapeutic virtues, including antiradical [25], antihypertensive and cardioprotective[1], liver anti-fibrosis [26], hepatoprotective[27], anti-atherosclerotic, anti-leukemic and analgesic effects [28, 29, 30]. However, this plant is still little used in Togo, due to the lack of data available on its antioxidant potential and the phytochemical composition of its organs, such as the calyxes, to guide the population. It is in this context that the current study was carried out, with the aim of contributing to the development of this species of food and pharmacological interest in the Togolese flora, by determining the phytochemical composition and antioxidant activity of the hydroethanol extract of the calyxes.

Material and Methods

Plant Material

Hibiscus sabdariffa calyxes (Figure 1) constituted the plant material for this study. They were collected from a field in Lomé in October 2022. The plant (Figure 2) was identified and registered under Togo number 15894 at the herbarium of the University of Lomé. The fresh calyxes thus obtained were dried and then pulverized to obtain a powder.

Fig 1: Calyxes of *H. sabdariffa* L.

Fig 2: Plant of *Hibiscus sabdariffa* L.

Methods

Preparation of Extract

The hydroethanol extract was prepared by dissolving 500 g of *Hibiscus sabdariffa* calyxes powder in 5000 ml of 50:50 water-ethanol solvent mixture for 72 hours with regular manual agitation. The resulting macerate was then filtered through cotton and n°4 filter paper. The filtrate was then evaporated with a Heidolph 2 Rotavapor to obtain a dry

extract. The resulting extract was stored in the refrigerator at -4 °C until tested.

Phytochemical Screening

Screening is a qualitative chemical analysis based on differential staining and/or precipitation reactions of the main groups of chemical compounds contained in a substance.

The different chemical groups such as alkaloid, condensed tannins, flavonoid, saponosides, and reducing sugars in the extract of the calyxes of *H. sabdariffa* have been characterized with reference to the techniques described by the previous studies [31, 32, 33]. In addition, the contents of total phenols and tannin were determined using the Folin-Ciocalteu test, and that of flavonoid was determined using aluminum chloride test as described previously [32, 33].

Assessing Antioxidant Activity with the FRAP Test

The FRAP assay uses antioxidants as reductants in a redox couple-related colorimetric method that employs an easily reduced oxidant, F_e^{3+}. The reduction of a ferric tripyridyltriazine complex (F_e^{3+}-TPTZ) to F_e^{2+}-TPTZ, i.e. F_e^{3+} [colorless] to F_e^{2+} [blue], was monitored by measuring absorbance at 593 nm. Absorbance is linked to the reducing power of the electron-donating antioxidants present in the extract tested. The FRAP assay was carried out using the method described previously by Liu *et al*. [34].

Statistical Analysis of Data

Data were typed using Microsoft Office Excel 2010 and analyzed using Graph Pad prism 9 software. Analysis of variance (ANOVA) followed by the TwoWay test was performed, as well as the Tukey test for comparison of means with the control. Results were expressed as mean (n = 3) plus or minus standard error on the mean (M ± SEM). Differences were considered statistically significant at the 5% level ($p < 0.05$).

Results

***In vitro* Test Results**

Phytochemical Screening Results

Table 1 below presents the results of phytochemical screening of the hydroethanolic extract of *Hibiscus sabdariffa* calyxes.

Table 1: Results of Phytochemical Screening of *H. sabdariffa* Calyxes Extract

Phytochemical Compounds Considered	Results
Flavonoids	+
Alcaloids	+
Tanins	+
Saponins	+
Reducing carbohydrates	+
Total phénols	+

+: presence

Phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, saponins, reducing carbohydrates and total phenols.

Quantitative assay results

The results of quantitative assays for polyphenolic compounds, tannins and flavonoids using rutin and gallic acid standards respectively are shown in Table 2.

Table 2: Phytochemical Content of *H. sabdariffa* Calyxes Extract

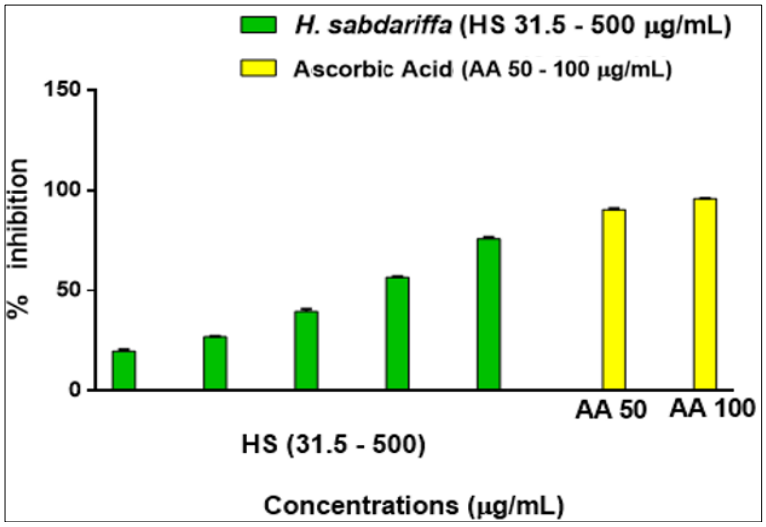
Phytochemical Compounds Considered	Content	Reference Molecules Considered
total Phenols	32,596 ± 1,121 mg GAE /g	Gallic Acide
Tanins	31,537 ± 1,161 mg GAE /g	Gallic Acide
Flavonoids	64,461 ± 2,718 mgRE/g	Rutin

GAE: Gallic Acid Equivalent; RE: Rutin Equivalent

Quantitative assay shows that the extract contains a higher quantity of flavonoids than tannins.

Anti-free radical activity of *H. sabdariffa* extract

Figure 3 shows the percentages of DPPH radical inhibition and Table 3 presents the percentage of 50% free radical hinibition. It may be noted that the hydroethanol extract expressed a free radical scavenging power (50%) at a concentration of 247.00 ± 2.88 µg/ml (IC_{50}), which is lower than that expressed by ascorbic acid with an IC_{50} of 29.456 ± 0.133 µg/ml (Table 3).



Results are expressed as mean ± MSE (n = 3)

Fig 3: DPPH Radical Inhibition by Extract

Table 3: DPPH Free Radical Scavenging Activity Expressed as CI_{50}

Samples	CI_{50} (µg/ml)
Ascorbic acide	29,46 ± 0,133
Hibiscus sabdariffa	247,00 ± 2,88

The concentration of extract inhibiting 50% of free radicals was 247.00 ± 2.88 (Table 3).

Antioxidant Activity of *H. sabdariffa* Calyxes Extract

Table 4 shows that the extract has a higher reducing power for F_e^{3+} ferric ions than the reference molecule (Quercetin), with an EC_{50} of 10.153 ± 0.123 µg/ml.

Table 4: FRAP test results expressed as EC50

Samples	EC_{50} (µg/ml)
Quercetin	22.326 ± 0.003
Hibiscus sabdariffa	10.153 ± 0.123

Values are expressed as mean ± MSE (n = 3)

The concentration of extract providing 50% F_e^{2+} ion protection (EC_{50}) was 10.153 ± 0.123 µg/ml (Table 4).

Discussion

Evaluation of the anti-free radical activity of *Hibiscus sabdariffa* calyxes showed that this activity is dose-dependent and not significantly different from that obtained with ascorbic acid used as the reference molecule. The results obtained show the effectiveness of the calyx extract in reducing free radicals, with an IC_{50} evaluated at 247.00 ± 2.88 µg/ml. In addition, evaluation of antioxidant power by reduction of ferric ions in the $[(F_e(III)TPTZ)_2]^{3+}$ complex yielded a ferrous ion content of 10.153 ± 0.123 µg/ml. Taken together, these results show that the antioxidant and free radical scavenging capacity of the hydroethanol extract of Hibiscus sabdarifa calyxes is significant. This activity was confirmed by the presence, in the extract analyzed, of antioxidant molecules that can intervene via these two types of mechanism of action, such as total phenols, flavonoids and tannins, known for their antioxidant activity [35, 36]. According to Kumaran and Karunakaran [37], the reducing power of a compound can indeed serve as a significant indicator for its potential antioxidant activity. Work carried out by Hinneburg *et al*. [38] has also shown that the reduction of DPPH radicals, molybdenum ions and ferric ions is correlated with the phenolic compound content of the material tested. It has also been reported that phenolic compounds, generally flavonoids, stilbenes, phenolic acids and tannins, possess remarkable antioxidant properties due to the existence of conjugated double bonds within their intrinsic structures. This enables them to stabilize free radicals by acquiring several resonance-stabilized mesomeric forms [39]. The interesting levels of total phenols, flavonoids and tannins in natural substances also support a number of biological activities. According to Manach *et al*. [40], phenolic compounds are able to modulate the activity of certain enzymes and modify the behavior of several cellular systems, suggesting that they may exert a multitude of biological activities that are effectively linked to significant antioxidant properties. The *Hibiscus sabdariffa* calyxes analysed therefore have proven biological activities, as demonstrated by the numerous pharmacological uses made of them by populations in Togo and elsewhere. Indeed, previous studies have reported that this species has antihypertensive, antispasmodic, antimicrobial, antiulcer, antifungal, nephroprotective, hepatoprotective, tonic and antianemic activities [41, 42]. Hibiscus sabdarifa therefore represents a food species of the Togolese flora of interest in the search for endogenous avenues of healthy, sustainable nutrition.

Conclusion

The results of the present study show that *Hibiscus sabdariffa* calyxes exhibit significant free radical scavenging activity concomitant with their content of phytochemicals such as total phenols, tannins and flavonoids. These data confirm those of previous studies and justify the food and pharmacological uses of this species. In addition, this study shows the need for in-depth studies for a better valorization of this species, which constitutes an endogenous source of sustainable food and nutritional security.

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