In Vitro Antioxidant Property and Phytochemical Constituents of *Senna alata* Leaves Aqueous Extract Collected in Ngaoundéré (Cameroon)

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Abstract

Leaves extract of *Senna alata* L. is a plant used as indigenous medicine to treat various types of disease like ulcers, stomach, pain and fever. The present study was undertaken to study the phytochemical screening, total phenolic compounds (TPC), total flavonoid compounds (TFC) and in vitro antioxidant of *Senna alata* leaves' extract growing in Ngaoundéré town (Cameroon). Respective bioactivities of the phytochemicals were determined. Quantitative analysis of the total phenolic content was determined by using the Folin-Ciocalteu method while total flavonoid was estimated using aluminium trichloride (AlCl_3). The antioxidant capacities in the forms of DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) were evaluated by spectrophotometric methods. For these purposes, aqueous extract were prepared. Now the experimental screening of phytochemicals showed negative results for the absence of reducing compounds, steroids/triterpenes, and tannins. The results showed that TPC, TFC values were higher: 14.768 ± 0.26 mg GAE per g of extract, 4.32 ± 0.12 mg Ru per g of extract, respectively. The *Senna alata* Leaves' extract (SALE) exhibited the best DPPH inhibition concentration 50% ($\text{IC}_{50} = 12.05$ mg. mL^{-1}), and FRAP method ($\text{IC}_{50} = 2.79$ mg. mL^{-1}) compared to that of the positive control, ascorbic acid ($\text{IC}_{50} = 17.69$ mg/mL); Hence, extract from the leaves of *Senna alata* contains high secondary metabolites which accounts for its strong antioxidant ability thus justifying its use as natural occurring antioxidants in folkloric medicine.

1 Introduction

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Living organisms are equipped with a defense system to neutralize free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals ($\text{HO}^\bullet$), and non-free-radical species, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$)¹. If the production of free radicals exceeds the antioxidant capacity of a living system, free radicals are capable of attacking the healthy cells of the body to cause oxidative stress leading to the pathogenesis of several human's degenerative diseases like cancer, inflammation, atherosclerosis, neurological disease, cardiovascular disease and rheumatoid arthritis².

During oxidative stress, free radicals not detoxified by the antioxidant system cause damage to biological molecules

contained in cells, including the oxidation of DNA, proteins, lipids, and carbohydrates, but also secondary lesions due to the cytotoxic and mutagenic nature of the released metabolites lipid oxidation³. Antioxidants are natural or chemical products which are defined as chemicals which, more specifically, delay the deterioration or discoloration caused by the oxidation and neutralization of free radicals but can also have toxicological effects and suspected carcinogenic potential⁵. In the search for alternatives antioxidant sources, the present research is focused on leaves of *Senna alata*.

S. alata L., (Synonym *Cassia alata* (L.) Roxb.,) belonging to the Leguminosae family and Fabaceae subfamily, is a herbaceous plant with thick downy branches, which is distributed in the many tropical countries including Cameroon. *S. alata*, commonly named ringworm cassia as the plant leaves were used for

treatment of ringworm. It is recognizable by its dense and bright orange yellow flowers that are arranged spirally on its rachides, an average height of between 1 and 5 meters and has horizontally spread branches. The inflorescence looks like a yellow candle. It is often cultivated for medicinal purposes, and also as a pantropical ornamental shrub to warm temperate areas. The fruit is a pod, while the seeds are small and square in shape. The plant has been shown several pharmacological virtues. Its leaves are used against yellow fever or malaria, and as antiasthmatics, or antidiabetics⁶. The leaves are also specific for the treatment of ringworm and eczema⁷ (Co, 1989), scabies, athlete's foot⁸, herpes⁹ and insect bites¹⁰. Various extracts and different parts of *S. alata* have been reported to own many pharmacological activities such as laxative¹¹, wound healing¹², hypoglycaemic¹³, anti-bacterial¹⁴, analgesic¹⁵ and anti-inflammatory¹⁶.

In this work, the present study was designed to determine the qualitative screening of phytochemicals, the total phenolic compounds (TPC), total flavonoid compounds (TFC) and to examine the *in vitro* antioxidant of *S. alata* leaves extract to ascertain its acclaimed use as natural occurring antioxidants in folkloric medicine.

2 Materials and methods

2.1 Chemicals and reagents

Ethanol, Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na_2CO_3), gallic acid, aluminum chloride (AlCl_3), crystalline sodium acetate, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, Butylated Hydroxy Toluene (BHT), ascorbic acid were obtained from Sigma-Aldrich Chemicals (Yaoundé, Cameroun). All solvents and reagents used for the investigation were of analytical grade.

2.2 Collection of plant material

The freshly collected aerial part of the *S. alata* was gathered in September, 2019 from local area of Ngaoundéré town, Adamaoua Region of Cameroon. The sample was collected from its natural habitat. The plant was subsequently identified by Professor Pierre-Marie Maïongmetsem, a botanist in the Biological Sciences, Faculty of Science, University of Ngaoundéré, Cameroon. The voucher specimen was deposited under the reference number at the Cameroon National Herbarium at Yaoundé, Cameroun.

2.3 Preparation of plant extract

The *S. alata* leaves were prepared according to traditional use. Briefly, the freshly collected leaves part of the plant was cleaned and washed properly with tap water under running water, and dried in the shade at room temperature (22-25 °C) until constant weight for two weeks. Then, the dried plant materials was ground to a fine powder into a coarse with the help of a suitable grinder

and passed through a 22 mesh sieve. The dry powder of the sample (10 g) was decocted with 100 mL of distiller water (1: 10) for 20 minutes and cooled. After decantation, the supernatant was filtered through a Whatman filter paper No. 1, concentrated at 55 °C by evaporating in a vacuum rotary evaporator (HEILDOLPH®) and dried to a constant weight in an oven set at 40 °C. The dried extract was stored in at room temperature in a dry place prior to use refrigerator.

2.4 Ethics statement

For the collection of plant, no specific permits were required for the described field studies. For any locations/activities, no specific permissions were required. The location where the plant was collected was not privately-owned or protected in any way and the field studies did not involve endangered or protected species. This study was approved by the University of Cameroon's institutional review board.

2.5 Qualitative phytochemical screening

Preliminary qualitative phytochemical screening for the determination of secondary metabolites was carried out according to standard methods¹⁷⁻¹⁹ to detect the presence or absence of bioactive compounds.

2.5.1 Test for flavonoids

In a test tube, introduce 1 mL of extract to be tested, add 1 mL of hydrochloric acid (HCl) and 3 magnesium shavings. The appearance of a red or yellow coloration reveals the presence of flavonoids.

2.5.2 Test for tannins

To 1 mL of extract to be analyzed, add 0.5 mL of a 1% aqueous solution of FeCl_3 . The presence of tannins is indicated by a greenish or blue-blackish coloration.

2.5.3 Test for Coumarins

Put 5 mL of extract in a tube, add 0.5mL of 10% NH_4OH , mix and observe under UV at 366 nm. Intense fluorescence indicates the presence of coumarins.

2.5.4 Test for Alkaloids

The tests are carried out by precipitation reactions with the reagents of Mayer and Wagner.

1 mL of each extract is divided into two equal volumes. One volume is treated with 0.5 mL of

Mayer's reagent, the other with 0.5 mL of Wagner's reagent. The appearance of a white or brown precipitate, respectively, reveals the presence of the alkaloids.

2.5.5 Test for Terpenoides/steroids

5 mL of extract is added to 2 mL of chloroform and 3 mL of concentrated sulfuric acid. The formation of two phases and a brown color at the interphase of two liquids and a purple

coloration of the supernatant layer reveal the presence of terpenoids and sterols.

2.5.6 Test for saponins: Foam test

In a test tube, introduce 10 mL of extract to be tested and stirred for a few seconds then left to stand for 15 min. A height of persistent foam indicates the presence saponins.

2.5.7 Test for reducing compounds

Put 1 mL of extract in a test tube, add 2 mL of Fehling's liquor (1 mL reagent A and 1 mL reagent B), and incubate the whole for 8 min in a boiling water bath. The appearance of a brick-red precipitate indicates the presence of the reducing compounds.

2.6 Total phenolics content determination

For the determination of phenolic compounds present by Folin-Ciocalteu reagent, the method described by Singleton and Rossi²⁰ was used. This method provided the total phenolic content of a given sample. Briefly speaking, 0.1 mL (in the concentration of 1 mg/ mL) of plant extract solution was prepared in methanol and 0.5 mL of sample was introduced into test tubes. After this, 2.5 mL of 10 % (v/v) Folin- Ciocalteu reagent and 2 mL of 20 % Na₂CO₃ solution was added to the mixture and well shaken for 15 seconds. A set of gallic acid standard solutions (10, 20, 40, 60, 80 and 100 µg/ mL) were prepared as earlier in methanol. The tubes were covered with parafilm and incubated for 30 minutes at room temperature in the dark and the absorbance of the reaction mixtures was determined by UV-2100 SPECTROPHOTOMETER (UNICO®) at 765 nm. TPC was estimated from a standard curve of gallic acid (0.2-1 mg. mL⁻¹). All measurements were repeated three times and results were expressed as mg gallic acid equivalent (GAE) per grams of extract.

2.7 Flavonoid content determination

Colorimetric assay was used to determine the content of flavonoid according to known procedure²¹. For the reaction, 1 mL of the plant extract at the concentration of 1mg/ mL was added with 0.3 mL of 10 % aluminum solution dissolved in methanol and the mixture was incubated for 15 minutes at room temperature. A set of standard solutions of Rutin (20, 40, 60, 80 and 100 µg/ mL) were prepared as mentioned earlier. The absorbance was measured for test and standard solutions using reagent blank at 510 nm using the UV-2100 SPECTROPHOTOMETER (UNICO®). The total content of flavonoid was denoted as mg of RU/ g of extract.

2.8 DPPH radical scavenging activity assay

Radical-scavenging activity was determined by bleaching a purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, followed by the change from a violet (DPPH•) to a yellow (DPPH-H) color, using a method developed by Blois²². DPPH stable free radical method is an easy, rapid and sensitive way to survey the

antioxidant activity of a specific compound or plant extracts²³. Herein, 3 ml of crude extract solution in extract at different concentrations (0, 50, 100, 150 and 200 µg/mL) was pipetted and put into the microplate and then mixed with 1 mL DPPH at a concentration of 0.1 mM dissolved in methanol. The reaction mixture were shaken vigorously and incubated for 15 min in the darkness place at room temperature. Then, the absorbance of resulting solution was measured at 517 nm with a microplate reader. The inhibition of DPPH radical was calculated by comparing the results of the test (with extract) to those of the control (without extract) using the following formula as follows²⁴: % Inhibition = $[(A_0 - A_1) / A_0] \times 100$, where: A₀ was the absorbance for the reference sample (just DPPH solution) and A₁ was the absorbance for the tested sample. The result was expressed as IC₅₀ value (the concentration which corresponded to 50 % of the initial DPPH concentration) obtained from the separate linear regression plots of the mean percentage of the antioxidant activity against concentration of the test extract (mg. mL⁻¹). All the run were triplicate.

2.9 Ferric reducing antioxidant power (FRAP) activity

The Ferric reducing antioxidant power procedure was followed to a modified method described by Bougandoura and Bendimerad²⁵ with minor modifications as detailed in the following. The principle of this method is based on the reduction of the ferric-tripyridyl triazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the aqueous extract was taken in various concentrations (50 to 1000 µg per mL) from the stock solution and was mixed with 2 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2 mL of aqueous potassium ferricyanide (1 %) in a test tube. After incubation in water bath at 50 °C for 20 min, 2 mL of 10 % trichloroacetic acid was added to the mixture to stop the reaction, and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2 mL) was mixed with 2 mL distilled water and 0.5 mL of 1 % ferric chloride (FeCl₃) was prepared freshly in some distilled water and then the absorbance of reaction mixture was measured at 700 nm using the UV-2100 SPECTROPHOTOMETER (UNICO®). Higher absorbance of the reaction mixture indicates an important reducing power. As positive control, ascorbic acid was used and results of antioxidant activity were expressed as absorbance reading. The iron (III) reducing activity determination was determined in triplicate and expressed in mg Ascorbic Acid Equivalent per mL of extract.

2.10 Statistical analysis

Data were reported as mean ± standard deviation from three repetitions, except for IC₅₀ values computed from linear regression of triplicates of each concentration tested. One-Way analysis of variance (ANOVA) followed by Dunnett's post hoc using SPSS version 16.0 were carried out to determine statistical significance between the means and p-values < 0.05 were considered statistically significant.

3 Results and discussion

3.1 Phytochemical Screening

One of the essential goals of a phytochemical test is the detection of the different families of secondary metabolites existing in the studied part of the plant by qualitative characterization reactions. The result of phytochemical test is summarized in Table 1. It appears from this table that the tests for alkaloids, phenolic compounds, saponins, and flavonoids were positive. In contrast, the tannins, steroids / terpenoids, and Reducing compounds were not detected. This result is contrary to a team of researchers who indicated the presence of alkaloids, phenols, tannins, flavonoids, saponins, steroids and sugar in the aqueous extract of the leaves of *S. alata*²⁶. The qualitative chemicals are an indication of an antioxidant and anti-inflammatory values of this plant and may be responsible for the traditional uses of *S. alata* leaves.

Table 1: Phytochemical screening of aqueous extract of *Senna alata*

Phytochemicals	Presence/absence
Alkaloids	+
Phenolic compounds	+
Flavonoids	+
Tannins	-
Saponins	+
Triterpenes or steroids	-
reducing compounds	-

Presence of phytochemical is denoted by (+) sign and absent of phytochemical is (-)

3.2 Determination of total phenolic contents

The presence of phenolic compounds identified in the leaves extract of *S. alata* in the current study could be responsible for its folkloric therapeutic usage for animals. The total phenolic content in the aqueous extract was 14.768 ± 0.26 mg/ gram GAE. Calibration curve from gallic acid showed maximum absorbance at 765 nm wavelength (equation $y = 3.8747x + 0.0418$, $R^2 = 0.9815$). These results are inferior to other studies which have presented a phenol content of 59.21 ± 3.02 in the hydro-ethanolic extract of the leaves of *Cassia alata*²⁷. The total phenol contents of four crude extracts determined by Folin-Ciocalteu method were reported as gallic acid equivalents. Phenolics help to prevent cell damage caused by oxidative stress because they exhibit strong antioxidant activities against free radicals (Fig. 1)²⁵.

3.3 Determination of total flavonoid contents

The result of total flavonoid contents of the extract was 4.32 ± 0.12 mg/ gram of extract. Equation of calibration curve of rutin standard was $y = 3.0495x - 0.0093$, $R^2 = 0.9833$. The Fig. 2

showed the calibration curve of standard (Rutin). These results are lower than those found by other researchers who revealed that the content of total flavonoids in the methanolic extract of *Cassia alata* was 24.37 ± 0.25 mg UK / g dry extract²⁸. Flavonoids, for instance, are known to be very potent water-soluble antioxidant.

Fig 1: Calibration curve of gallic acid

Fig 2: Calibration curve of Rutin

3.4 DPPH radical scavenging activity

The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. Radical DPPH is often used as an indicator to test the ability of the extract to give a hydrogen atom or an electron and therefore antioxidant activity²⁹. Extract reduces the color of DPPH due to the power of hydrogen donating ability. DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. Antioxidants may guard against reactive oxygen species (ROS) toxicities by scavenging reactive metabolites and converting them to less reactive molecules. For example, the antioxidant activity of phenols is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers³⁰. DPPH assay is widely used methods for screening antioxidant activity of plant extract. The aqueous extract of *S. alata* were subjected to antioxidant assay against 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and IC₅₀ value of 12.79 mg. mL⁻¹, was reported. The result of free radical scavenging activity is summarized in Fig 3. A dose-response curve of DPPH radicals scavenging activities was observed that the DPPH radical-

scavenging activity decreased as the concentration of the extract increased.

Fig 3: Free Radical Scavenging Activity

3.5 Ferric-reducing antioxidant power (FRAP) assay

To better assess the antioxidant effect of aqueous extract of *S. alata*, and alongside its antioxidant power by trapping free radicals, its ability to inhibit lipid peroxidation and its chelation power of iron ions, another aspect was targeted; the reductive power of extract from plants through the FRAP technique. This technique measures the reduction of Fe^{3+} (iron iron) in Fe^{2+} (ferrous iron) in the presence of an antioxidant²⁸. FRAP assay is the only antioxidants assay that measure antioxidants or reductants in a sample directly and can thus be used to quantify the amounts of total antioxidants or reductants in foods³¹.

The scavenging activity of aqueous extract of *S. alata* toward to ferric-reducing antioxidant decreased from 0.2 to 1.0 mg/ mL. It showed the lowest antioxidant activity of 87.82 % at a concentration of 0.1 mg. mL while IC_{50} value of 2.79 mg. mL⁻¹ was reported. This likely indicates that in an extract the bioactive molecules that provide the trapping power of free radicals (DPPH) are themselves responsible for the reducing power of iron. Some authors report that the antioxidant capacity of natural substances may have() a reciprocal correlation with their reducing capacity³² and that the latter may, in turn, serve as an important indicator of their antioxidant activity (Fig 4)³³.

Fig 4: Ferric reducing antioxidant power (FRAP) activity

4 Conclusions

The production of free radicals is regulated by our body which has developed antioxidant defenses to protect against the potentially destructive effects of free radicals. The present study however showed that the aqueous extract of *S. alata* is strong antioxidant activity, high total phenolic and total flavonoids content. This extract can be considered as good sources of natural antioxidants for side dishes and medicinal uses. Further in details phytochemical analysis should be done to identify to elucidate the exact bioactive compound which is responsible for the antioxidant action.

5 Acknowledgements

Nil

6 Authors' contributions

JV designed hypothesis, conceived, wrote the paper, analyzed the data and critically reviewed the manuscript. SDS performed the experiments in this study, assisted with analyzing the data, confirmed the results, and contributed to the writing of the article. All authors reviewed and approved the final manuscript.

7 Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

8 Ethics approval and consent to participate

This information is not relevant since our study does involve neither animals nor humans.

9 Consent for publication

Not applicable since our manuscript does not contain any individual person's data in any form.

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