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Phytochemical screening and antioxidant activity of ethanol leaf extract of *Aphania senegalensis* (Juss. ex Poir.) Radlk

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Abstract
Aphania senegalensis (Juss. ex Poir.) Redelk is a plant commonly used in Senegalese traditional medicine to treat different affections. The aims of this study were to investigate the phytochemical composition and the antioxidant potential of the ethanol leaf extract of *Aphania senegalensis*. Phytochemical investigation done with standard reactions had revealed that flavonoids, tannins, sterols and triterpenoids were the main constituents of the leaf extract. The phenol content was 151.63±10.92 mg equivalent tannic acid/g of ethanol dried extract. The extract exhibited ability to reduce ferric ion. From 1.95 to 31.25 µg/ml the absorbance values of the extract varied respectively from 0.024±0.009 to 0.36±0.31. On ABTS, nitric oxide and DPPH radicals reducing assays, the IC_{50} values of the extract were respectively 4.12± 0.11- 190.51±6.7- 2.77 ± 0.03 µg/ml.

Keywords: *Aphania senegalensis*, leaf, phytochemical screening, antioxidant.

1. Introduction
Aphania senegalensis (Juss. ex Poir.) Radlk. (synonyms: S*apindus senegalensis* Juss. Ex Poir.) is a common plant of West africa. *A. senegalensis* is used in Senegalese traditional medicine to treat various diseases such as bacterial and fungal infections, pain, inflammation and asthenia. The fruits of the plant are edible while the leaves are commonly used in traditional medicine. The anti-inflammatory and analgesic activities of leaf extracts of this plant have been previously established [1]. The leaf extract was found to be toxic at high doses [2]. However, there is paucity of scientific data on the phytochemical constituents of leaf extracts and its potential antioxidant activity. This study aimed therefore to assess phytochemical composition, the phenol content and the antioxidant activity of ethanol leaf extract of this plant.

2. Material and methods
2.1. Plant material
Leaves of *Aphania senegalensis* were collected at the Botanical Garden of Faculty of Medicine, Pharmacy and Odontology (Cheikh Anta DIOP University, Dakar, Senegal). Voucher specimen numbered K1613 were deposited at the herbarium of the botanical garden of this faculty. Plant leaves were dried at room temperature in a ventilated room and grounded to a fine powder. Powdered leaf of *Aphania senegalensis* (100 g) combined in a ratio of 1:10 with 95% ethanol was decocted for 30 minutes and filtered through Whatman No. 1 filter paper. Ethanol was removed under reduced pressure using a rotary evaporator.

2.2. Phytochemical screening
Standard phytochemical analyses were carried out to test for the presence of the phytoconstituents in the prepared extract. Chemical tests were carried out on ethanol and aqueous extracts of the powdered specimens using standard procedures for the detection of saponins (foaming index), steroids and triterpenoids (Liebermann-Buchard reaction), carotenoids (Carr and Price reaction) tannins (Stiasny test followed by ferric chloride test), flavonoids (Shibata's reaction), anthracene glycosides (Borntraeger test), cardiotonic glycosides (Baljet, Kedde and Raymond-Marthoud reagents tests), alkaloïds (Bouchardat, Valser-Mayer and Dragendorff's reagents tests), in order to identify the presence of phytochemical constituents [3].

2.3. Phenol content

Total phenol contents of samples were investigated using the reported method slightly modified [4].

A mass of dried sample (2.5 mg) was dissolved in 100 ml of distilled water. An aliquot (10 ml) of this solution was mixed with Folin Denis reagent (2 ml), 15% sodium carbonate (2 ml) and centrifuged for 4 minutes at 4000 rpm. Absorbance of the blue solution was measured at 760 nm.

A stock solution of tannic acid was prepared by dissolving 5 mg in 100 ml of distilled water. Then two-fold serial dilutions of tannic acid solution were made before adding Folin Denis reagent and 15% sodium carbonate. Absorbance was measured at 760 nm and plotted against concentrations. All experiments were done in triplicate and results were expressed as milligrams of tannic acid equivalents (TAE) per gram of sample (extract or fractions).

2.4. Antioxidant activity

2.4.1. Ferric reducing antioxidant power assay (FRAP)

The ferric reducing power was determined according to the described method [5]. An aliquot of 0.20 ml of each sample at appropriate concentration was mixed with 0.5 ml of phosphate buffered saline (0.2 M; pH 6.6) and 0.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$).

The mixture was incubated at 50°C for 30 min and 0.5 ml of 10% trichloroacetic acid was added. After centrifugation for 10 minutes at 3000 rpm, the supernatant (0.5 ml) was mixed with distilled water (0.5 ml) and 0.1% ferric chloride (0.1 ml). The experiments were done in triplicate. Absorbance was measured at 700 nm; ascorbic acid was used as positive control.

Absorbance increasing relatively to that of concentration represented the reducing capacity of tested sample.

2.4.2. ABTS assay

Reduction of free radical ABTS (2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) was investigated using the described method [6]. Two stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were prepared and mixed in equal volumes before allowing them to react for 12 h at room temperature in darkness. This mixture was diluted by adding ethanol, in order to obtain an absorbance 0.7 at 734 nm. Samples (2 ml) were mixed with 2 ml of ABTS solution and the mixture was left at room temperature for 2 h in darkness. The absorbance of each sample was measured at 734 nm after 30 min. Each experiment was done in triplicate. Experiments were done in triplicate and the antioxidant activity related to the ABTS free radical scavenging effect was expressed as IC_{50} (concentration of sample required to scavenge 50% of free radicals).

2.4.3. Nitric oxide (NO) radical reduction assay

Reduction of nitric oxide (NO) free radical was determined using the colorimetric assay described [7]. A 13.8 mM nitroprusside solution (1 ml) was added to distilled water (250 µl) and an aliquot of each sample (250 µl) at appropriate concentration. The mixture was incubated at room temperature for 150 min.

Then, 1 ml of sulfanilic acid solution (0.5% in 20% glacial acetic acid) was added to 500 µl of the solution cited above. The resulting mixture was incubated again for 5 minutes.

Finally, 1 ml of aqueous solution of N-(1-Naphtyl) ethylenediamine dihydrochloride (0.038 mM) was added to the above solution; after incubation for 30 minutes the absorbance was measured at 540 nm. The ethanol leaf extract of *A. senegalensis* and ascorbic acid were tested in triplicate.

The antioxidant activity related to the nitric oxide free radical scavenging effect was expressed as IC_{50} (µg/ ml) representing the concentration of the sample that caused 50% inhibition.

2.4.4. DPPH radical scavenging assay

The determination of the DPPH free radical scavenging activity of samples was done using the described method [8]. An ethanol solution of DPPH was prepared by dissolving 4 mg in 100 ml of ethanol. An aliquot of each sample (0.8 ml) at appropriate concentration was added to 3 ml of ethanol solution of DPPH.

The ethanol leaf extract of *A. senegalensis* and ascorbic acid were tested at different concentrations. The absorbance of each sample was measured at 517 nm after 30 min. Each experiment was done in triplicate and the absorbance of the initial ethanol DPPH solution did not change after 30 min. The antioxidant activity related to the DPPH free radical scavenging effect was expressed as IC_{50} (concentration of sample required to scavenge 50% of free radicals).

2.4.5. Statistical analyses

Data were expressed as mean± SD. Analyses of variance (ANOVA) were done for the comparison of results using Fischer's test. Statistical significance was set at $p<0.05$.

3. Results and discussion

3.1. Extraction yield

From 100 g of powdered leaf material of *Aphania senegalensis*, 13.51 g of dried ethanol extract were obtained after extraction with ethanol representing a yield of 13.51%.

3.2. Phytochemical screening

Phytochemical screening had revealed that tannins, flavonoids, steroids and triterpenoids were the main phytoconstituents of the ethanol leaf extract of *Aphania senegalensis*. Flavonoids, tannins and triterpenoids from plants extracts were found to have antioxidant ability [9-10]. Negative reactions were obtained for the presence of saponins (foaming index less than 100), carotenoids, anthracene derivatives, cardiotonic glycosides and alkaloids (Table 1).

Table 1: Phytochemical groups identified in leaf extract of *Aphania senegalensis*.

Phytochemical groups	Results
Saponins	-
Steroids and triterpenoids	+
Carotenoids	-
Tanins	+
Flavonoids	+
Anthracene glycosides	-
Cardiotonic glycosides	-
Alkaloids	-

+: presence - : absence

3.3. Phenol content

The leaf extract of *Aphania senegalensis* had a high phenol content 151.63±10.92 mg equivalent tannic acid/g of ethanol dried extract. This phenol content was more important than that of six plants of the same *Sapindaceae* family where the highest content was related for *Allophylus africanus* P. Beauv. (21.046mg tannic acid/g) [11].

3.4. Antioxidant activity

3.4.1. FRAP assay

For ethanol leaf extract of *Aphania senegalensis* and ascorbic acid, increasing absorbance were noticed relatively to the concentration variations. Ascorbic acid had shown better ability to reduce ferric ion than the ethanol leaf extract at all tested concentrations (Fig. 1). At 1.95-3.9-7.81-15.62-31.25 µg/ml the absorbance values of the extract varied respectively from 0.024±0.009- 0.042±0.015- 0.113±0.035- 0.217±0.33- 0.36±0.31 while those of ascorbic acid were 0.18±0.030- 0.297±0.030- 0.438±0.022- 0.629±0.031- 0.921±0.029 at the same concentrations. It has been reported that ferric reducing antioxidant power may reflect antioxidant activity [12]. But this point of view is discussed. However FRAP assay provides a very useful total antioxidant concentration and can be applied to plant extracts [13].

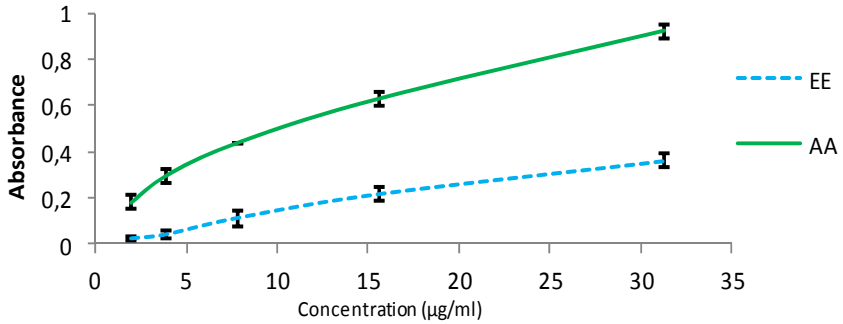

Fig.1. Ferric reducingpower of ethanol leaf extract of *A. senegalensis* (EE) and ascorbic acid (AA).

3.4.2. ABTS assay

The ethanol leaf extract of *A. senegalensis* and ascorbic acid had shown ability to scavenge the free radical ABTS with respective IC_{50} values 4.12± 0.11 and 2.45± 0.07 µg/ml. The capacity of hydrogen-donating constituents to scavenge $ABTS^{\bullet+}$ produced can be estimated spectrophotometrically, by measurement at 734 nm. What minimized interference from other absorbing compounds and from sample turbidity [13]. Flavonoids which had been identified on phytochemical screening of the ethanol leaf extract are known to be good hydrogen donors [9].

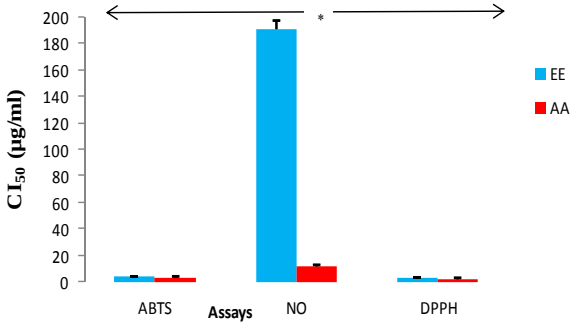

Fig.2.IC_{50} (µg/ml) of ethanol leaf extract (EE) and ascorbic acid (AA) on ABTS , No and DPPH tests.

*: significant difference ($p<0.05$)

3.4.3. Nitric oxide reduction

The ethanol leaf extract (IC_{50}: 190.51±6.7 µg/ml) and ascorbic acid (IC_{50}: 11.5±0.71 µg/ml) had shown nitric oxide reducing activity (see Fig. 2). Meanwhile the IC_{50} value of the ethanol extract was very high comparatively to those of DPPH (2.77 ± 0.03µg/ml) and ABTS (4.12± 0.11 µg/ml) tests and make us to suggest that the extract was less active on nitric oxide reducing assay.

3.4.4. DPPH assay

The ethanol leaf extract of *A. senegalensis* and ascorbic acid with respective IC_{50} values 2.77 ± 0.03 and 1.38±0.012 µg/ml were found to have good ability to scavenge the free radical DPPH (see Fig. 2). The molecule DPPH is considered as a stable free radical in virtue of the delocalization of the spare electron over the molecule as a whole. Then the molecule does not dimerize. When the DPPH solution is mixed with a substrate hydrogen donor, the original violet color changes [14]. Studies on DPPH scavenging ability of extracts of *Oenothera biennis* [15] and *Citrus* essential oils [16] had revealed a relationship between potential and DPPH scavenging for phenolic acids but not for flavonoids. What suggested that tannins identified on leaf extract of *Aphania senegalensis* may be responsible of DPPH scavenging effect. Meanwhile flavonoids are among the best electron or hydrogen donor and may inhibit the free radical DPPH [9].

4. Conclusion

The ethanol leaf extract of *Aphania senegalensis* had shown antioxidant activity and high phenol content. Further studies may be done on isolation of active compounds.

5. References

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