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Phytochemical Screening, Polyphenol Content and Antioxidant Studies of Ethanol Leaf Extract of *Combretum aculeatum* vent.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ADF and EB planned all experiments. Authors ADF, MD and SIMD supported the phytochemical screening and phenol content determination. Authors ADF, ANS, JBHF and JONF carried out the antioxidant studies and provided the statistical analyses of data. Author ADF wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objectives: The present study aimed to identify the phytochemical groups of ethanol leaf extract of *Combretum aculeatum* Vent., and to evaluate the phenol content of extracts and their antioxidant activity.
Materials and Methods: Collected leaves were extracted with ethanol (95°). From the dried ethanol extract, 3 fractions were obtained after a liquid/liquid fractionation (hexane, ethyl acetate and water). Phytochemical screening of the raw extract was done using standard reactions. The phenol content was determined with Folin Denis reagent. Antioxidant activity was performed using 2, 2-diphenyl-picrylhydrazyl test (DPPH), ferric reducing antioxidant power (FRAP) and nitric oxide reducing tests.

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Results: Tannins, flavonoids and mucilage were the main phytochemical constituents of the leaf extract. Antioxidant assay revealed ethanol extract and its polar fractions were more active. In DPPH assay, the aqueous fraction (IC_{50}: 61.93±5.99 µg / ml) was more active than the ethanol extract, the ethyl acetate and hexane fractions with respective IC_{50} values 74.1±4.47 - 81.53±6.51 - 1710.33±71.15 µg/ml. In nitric oxide test the ethyl acetate fraction (IC_{50}: 56.66±3.09 µg/ml) had shown a better ability to inhibit nitric oxide radical than the ethanol extract, the hexane and aqueous fractions (IC_{50}: 84.33±9.80- 451.33±1.24- 101.33±4.10 µg/ml respectively). In FRAP assay the ethyl acetate and aqueous fractions had shown a better activity than the leaf extract.
Conclusion: Ethanol leaf extract had shown antioxidant activity in the different assays. This biological activity may be due to both polar and non polar compounds.

Keywords: Combretum aculeatum; leaf extract; fractions; phytochemical; antioxidant.

1. INTRODUCTION

Free radicals are naturally produced by the human body. The life in an oxygen rich environment depends on a vital balance between the physiological production of free radicals and the body's ability to eliminate it.

But overproduction of free radicals, beyond cellular needs, causes biological disorders that may lead to many diseases such as cancer, arthritis, cardiovascular diseases, etc. To protect itself from free radicals overproduction, human being has an endogenous antioxidant defence system. However, these systems could be overwhelmed or failing against increased production of free radicals. Then natural antioxidants supplementation from food may help to reduce deleterious effect due to these free radicals. Some plants such as green and black tea have strong antioxidant activity, which is due to their high polyphenol content [1-2]. Other phytochemical constituents (terpenoids, carotenoids, vitamins C and E) are also known for their antioxidant capacity. These make us to investigate the antioxidant potential of *Combretum aculeatum* Vent. (synonyms: *Combretum alternifolium* Spreng., *C. denhardtiorum* Engl. and Diels., *C. leuconili* Schweinf., *C. ovale* R.Br. ex G. Don.). *C. aculeatum* is a climbing shrub, common in the Sahelian and Sudano-Sahelian zones of Mauritania and Senegal to Eritrea. This plant is also present in Morocco, Saudi Arabia and Yemen [3]. In traditional medicine, the decoction of the root is used as purgative and antigonococcal [4]. Leaves extracts are prescribed against spasmodic colic, gonorrhea, leprosy, helminths, loss of appetite and weight loss [5].

The aim of our study was to determine the phytochemical composition, phenolic content and antioxidant activity of ethanol leaf extract of *C. aculeatum* and its hexane, ethyl acetate and water fractions.

2. MATERIALS AND METHODS

2.1 Plant Collection

Leaves of *Combretum aculeatum* were collected at the Botanical Garden of the Faculty of Medicine, Pharmacy and Odontology (Université Cheikh Anta DIOP de Dakar, Senegal). The plant was identified and authenticated by Dr W. Diatta (Herbarium of the Botanical Garden of the Faculty of Medicine, Pharmacy and Odontology of Dakar) ant its voucher number is K206/2013. Plant leaves were washed with distilled water and air dried at room temperature. Dried leaves were ground to a fine powder.

2.2 Extraction and Fractionation

Powdered leaves of *Combretum aculeatum* (75 g) were decocted for 30 minutes using 1 l of ethanol and filtered through Whatman No. 1 filter paper. Ethanol was removed under reduced pressure using a rotary evaporator.

For liquid/liquid fractionation, 2 g of dried ethanol extract was dissolved in a mixture (distilled water/ hexane; 1:1). After decantation in a separatory funnel, the aqueous solution obtained was extracted twice with hexane. The hexane solutions were combined to give the hexane fraction. The aqueous solution was again subjected to liquid-liquid extraction with ethyl acetate under the same conditions as above. The ethyl acetate and aqueous solutions obtained were evaporated separately and lead to the corresponding fractions.

2.3 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), tannins (Stiasny test followed by ferric chloride test), flavonoids (Shibata's test), anthracene glycosides (Borntraeger test), cardiotonic glycosides (Baljet, Kedde and Raymond-Marthoud reagents tests), reducing sugars (Fehling's test), mucilage (Ruthenium red test), steroids and triterpenoids (Liebermann-Buchard test), carotenoids (antimony chloride/chloroform test), alkaloïds (Bouchardat, Valser-Mayer and Dragendorff's reagents tests), in order to identify the presence of phytochemical constituents [6,7].

2.4 Phenol Content (PC)

Total phenol contents of samples were investigated using the reported method slightly modified [8].

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).

2.5 Antioxidant Activity

2.5.1 DPPH assay

The determination of the DPPH free radical scavenging activity of samples was done using the described method [9]. 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 Combretum aculeatum, its fractions 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).

2.5.2 Nitric Oxide (NO) radical reduction test

Reduction of free radical NO was determined using the colorimetric assay described [10]. 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. Ascorbic acid was used as positive control and each concentration of sample was 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.

2.5.3 Ferric Reducing Antioxidant Power assay (FRAP)

The ferric reducing power was determined according to the described method [11]. 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). 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.

2.5.4 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

3.1 Extraction and Phytochemical Screening

After extraction of 75 g of powdered leaf material with ethanol, a mass of 5.06 g of dried extract was obtained representing a yield of 5.06 % w/w. Hexane, ethyl acetate and water fractions represented respectively 24.16- 25.42- 47.08% w/w of the ethanol leaf extract.

Phytochemical screening revealed that tannins, flavonoids, reducing sugars and mucilages were the main constituents of the ethanol leaf extract of *Combretum aculeatum*. Negative reactions were obtained for the presence of carotenoids, steroids, triterpenoids, alkaloids, saponins (foaming index less than 100), anthracenic derivatives and cardiac glycosides (Table 1).

Table 1. Phytochemical groups identified in leaf extract of *Combretum aculeatum* Vent.

Phytochemical groups	Results
Tannins	**+**
Carotenoids	-
Steroids and triterpenoids	-
Flavonoids	**+**
Alkaloids	**-**
Anthracenic glycosides	**-**
Cardiac glycosides	**-**
Reducing sugars	**+**
Saponins	**-**
Mucilages	**+**

+: presence; - : absence

3.2 Phenol Content

The aqueous and ethyl acetate fractions (PC: 78.89±3.48 and 67.69±4.34 mg ETA/g respectively) had higher phenol content than the ethanol extract (63.42±4.08 mg ETA/g). The hexane fraction (34.04±3.53 mg ETA/g) was found to have the smallest phenol content (Table 2).

3.3 DPPH Assay

Scavenging activity showed that the aqueous fraction had a lower IC_{50} (61.93±5.99 µg / ml) than the ethanol extract (74.1±4.47 µg/ ml). The ethyl acetate and hexane fractions with respective IC_{50} values 81.53±6.51 and 1710.33±71.15 µg/ml were found to be less active than the ethanol leaf extract. Ascorbic acid had the lowest IC_{50} (0.58±0.63 µg/ml) (Fig. 1).

Table 2. Phenol content of ethanol leaf extract of *Combretum aculeatum* and its fractions

Sample	Phenol content (mg Equivalent tannic acid/g)
Ethanol extract	63.42±4.08
Hexane fraction	34.04±3.53
Ethyl acetate fraction	67.69±4.34
Aqueous fraction	78.89±3.48

3.4 Nitric Oxide Reduction

The ethyl acetate fraction had shown a better ability to inhibit nitric oxide radical than the ethanol extract (IC_{50}: 56.66±3.09 µg/ml *versus* 84.33±9.80 µg/ml respectively). Hexane and aqueous fractions (IC_{50}: 451.33±1.24 µg/ml and 101.33±4.10 µg/ml respectively) were less active than ethanol leaf extract while ascorbic acid (IC_{50}: 11.5±0.71 µg/ml) had shown the better nitric oxide reducing activity (see Fig. 1).

3.5 FRAP Assay

For all samples increasing absorbance were noticed relatively to the concentration variations. Hexane, ethyl acetate and aqueous fractions exhibited highest ferric reducing power than the ethanol extract of *C. aculeatum*. At all tested concentrations, polar fractions (ethyl acetate and aqueous fractions) had better ability to reduce ferric ion than the hexane fraction while ascorbic acid was more active among all samples (Fig. 2). The absorbance values of the ethyl acetate, aqueous and hexane fractions varied respectively from 0.231±0.025 - 0.278±0.026 - 0.198±0.047 at 3.9 µg/ml to 0.689±0.036 - 0.553±0.031 - 0.508±0.026 at 62.5 µg/ml.

4. DISCUSSION

The polar constituents were quantitatively more present than the non polar compounds. The polar fractions (ethyl acetate and aqueous fractions) represented 72.5% of the mass of the ethanol extract. Flavonol glycosides, hydrolyzed tannins, reducing sugars and mucilage identified on phytochemical screening are known for their

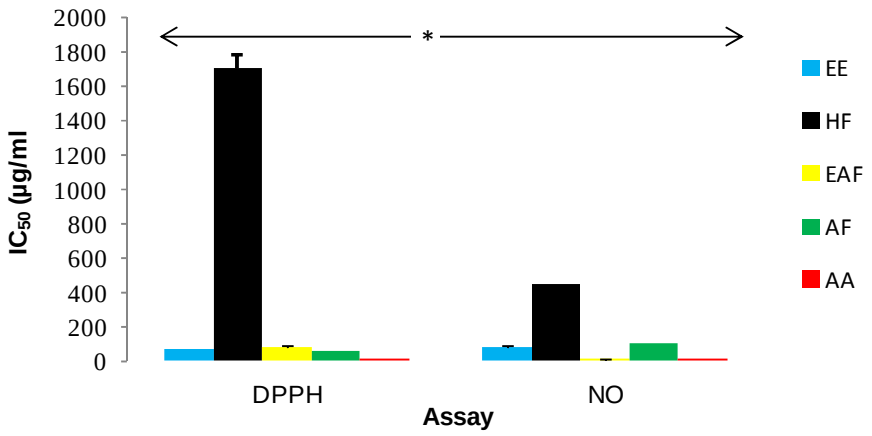

Fig. 1. IC_{50} (µg/ml) of different samples on DPPH and NO reduction assays
*EE: ethanol extract; HF: hexane fraction; EAF: ethyl acetate fraction; AF: aqueous fraction; AA: ascorbic acid; *: significant difference ($p<0.05$)*

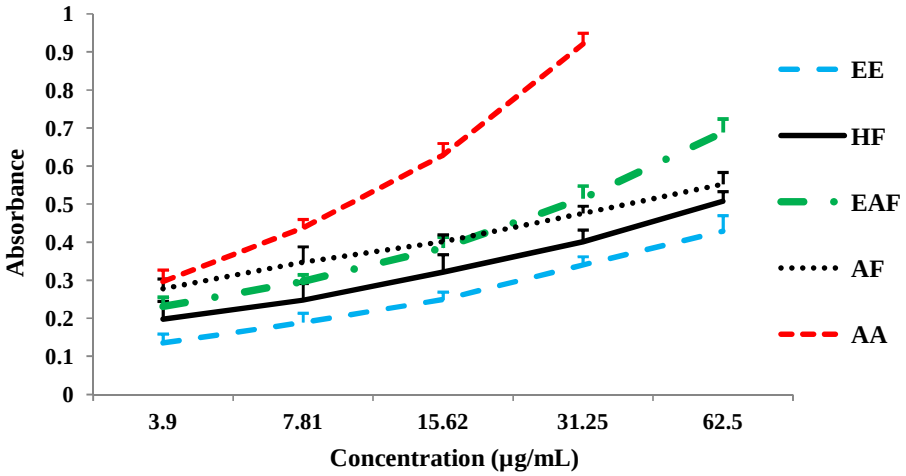

Fig. 2. Ferric reducing power of ethanol extract of *Combretum aculeatum* and its fractions
EE: ethanol extract; HF: hexane fraction; EAF: ethyl acetate fraction; AF: aqueous fraction; AA: ascorbic acid

polarity. This suggested that condensed tannins, less polar than the other chemical groups may be quantitatively extracted by the less polar solvent (hexane fraction).

Aqueous fraction had shown a better activity than the ethanol extract on DPPH assay (IC_{50}: 61.93±5.99 µg / ml versus 74.1±4.47 µg/ ml respectively) while on NO reducing assay the ethyl acetate fraction had the highest inhibition effect. FRAP assay had shown that the polar fractions had better ferric reducing capacity than the crude extract. The hexane fraction which revealed the lowest phenol content had shown a higher ferric reducing ability than the crude extract.

On the DPPH test, antioxidants gave hydrogen radical to stabilize it and form the DPPH-H [12]. In the nitric oxide reducing test, antioxidant

compounds trapped nitric oxide in competition with oxygen and reduced production of nitric oxide which has a pro-oxidant effect [13]. Polyphenol compounds are known for their antioxidant effect [14] and could then be partly responsible for the antioxidant activity of the extract.

The higher efficacy of polar fractions in FRAP assay may be related to phenolic compounds. Generally plant phenolic constituents exhibited significant scavenging effect against free radical DPPH and hydrolysable tannins had been accounted for the higher antioxidant effect of some fruit juices [15-16]. Furthermore flavonoids were among the best electron or hydrogen donor [16].

Higher amount of mucilage had been detected by phytochemical analysis. Reducing antioxidant power of some plants had been related to their mucilage content [17]. Then polysaccharides such as glucuronoxylans isolated from aerial parts of *Althaea officinalis* var. *robusta* and *Plantago lanceleota* var. *libor* had shown antioxidant activity accounted for 69% of the activity of the reference alpha-tocopherol [18-19].

Antioxidants agents are known for the major role they play in scavenging and inhibiting free radicals, preventing some diseases such as infections, Alzheimer's disease, cancer, diabetes, cardiovascular disease. Traditional uses of the leaf extracts to treat loss of appetite and weight loss might be due to the ability of antioxidant to strengthen human body defense capabilities against infections such as gonorrhea, leprosy and helminthiasis which are among indications of that plant organ in folk medicine [5]. Different antioxidant effect observed for fractions suggested that different compounds may be related to these said activity.

5. CONCLUSION

In this study, ethanol leaf extract of *Combretum aculeatum* which contained tannins, flavonoids, mucilage and reducing sugars, exhibited antioxidant activity. The ethyl acetate and water fractions had shown better antioxidant ability than the hexane fraction. Further studies for isolation of active compounds are needed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rechner AR, Wagner E, Van Buren L, Van DE Put F, Wiseman S, Rice- Evans CA. Black tea represents a major source of dietary phenolics among regular tea drinkers. Free Radic. Res. 2002;36:1127-1135.
2. Campenella L, Bonanni A, Tomassetti M. Determination of the antioxydant capacity of samples of different types of tea, or of beverages based on tea or other herbal products using a superoxide dismutase biosensor. J. Pharm. Biomed. Anal. 2003; 32:725-736.
3. Brink M, Achigan-Dako EG. Plant resources of Tropical Africa 16. Fibres. Economic Botany. 2012; 66(3):312-313.
4. Berhaut J. Flore illustrée du Sénégal, Tome 2. Ed. Clairafrique, Dakar, Sénégal; 1974.
5. Kerharo J. L'aromathérapie et la gemmothérapie dans la pharmacopée sénégalaise traditionnelle. J. Agric. Trop. Bot. Appl. 1971;18:109-141.
6. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. Springer Science & Business Media; 1998.
7. Sepulveda E, Saenz C, Aliaga E. Extraction and characterization of mucilage in *Opuntia* Spp. J. of Arid Enviro. 2007; 534-545.
8. Joslyn MA. Methods in food analysis. Physical, chemical and instrumental methods of analysis. Academic Press, London and New York; 1970.
9. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Technol. 2004; 26:211–219.
10. Garratt DC. The quantitative analysis of Drugs. Chapman and Hall Ltd., Tokyo; 1964.
11. Bassène E. Initiation à la recherche sur les substances naturelles: Extraction,

analyses, essais biologiques. Presses Universitaires, Dakar; 2012.
12. Dangle O, Fargeix G, Dufour C. One electron oxidation of quercetin derivatives in protic and non protic media. J. Chem. Soc. Perkin Trans. 1999;2:1387-1396.
13. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-1008.
14. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997;2(4):152-159.
15. Lee MW, Lee YA, Park HM, Toh SH, Lee EJ, Jang HD, Kim YH. Antioxidative phenolic compounds from the roots of *Rhodiola sachalinensis* A. Bor. Arch. Pharmacol. Res. 2000;23(5):455-458.
16. Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. 2000; 48:4581-89.
17. Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kundo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J. Nutr. Sci. Vitaminol. 2001;47(5):357-62.
18. Nguimbou RM, Boudjeko T, Njintang NY, Himeda M, Scher J, Mbofung CM. Mucilage chemical profile and antioxidant activity of giant swamp taro tubers. J. Food Sci. Techn. 2014;51(12):3559-67.
19. Kardosova A, Machova E. Antioxidant activity of medicinal plant polysaccharides. Fitoterapia. 2006;77(5):367-373.

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