EVALUATION OF ANTIOXIDANT ACTIVITIES OF THE METHANOLIC EXTRACT OF *CYMBOPOGON SCHOENANTHUS* L. (SPRENG) OF ALGERIA

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Abstract

The main Objective of this study is to investigate the Phytochemical Screening and In-vitro evaluation of antioxidant of*Cymbopogon schoenanthus* L.(spreng) methanolic extract of south Algeria. The methanol extracts were subjected to phytochemical tests for secondary metabolites, tannins, saponins, steroid, alkaloids and glycosides in accordance with Trease and Evans (1987) and Harborne (1998) with little modification.
The phytochemical screening investigations on of *Cymbopogon schoenanthus* L. plant extracts was revealed the presence of steroid, tannins, Reducing sugars, alkaloids, essential oil and flavonoids. These compounds are known to be biologically active.Theantioxidant activity of methanolic extracts has been done by using DPPH essay. The IC_{50} values observed for DPPH essay were 167,92±3,18µg/ml for methanolic extract of the aerial part, 78,15±1,19µg/ml of the root part.

Keywords: *Cymbopogon schoenanthus* L. - Phytochemical Screening - Antioxidant activities - Algeria.

Evaluation de l'activiste antioxydants de l'extrait méthanoïque de *Cymbopogon schoenanthus* l. (spreng) d'Algérie

Résumé :

Le principal objectif de cette étude est consisté à faire un criblage phytochimiqueet l'activité antioxydant de l'extrait méthanoliquede *Cymbopogonschoenanthus* L.(speng) du sud Algérien.L'extraitméthanoliquea été soumis pour un test phytochimiques des métabolites secondaires, les tanins, les saponines, les stéroïdes, les alcaloïdes et les glycosides.Le criblagephytochimiquedel'extrait de *Cymbopogonschoenanthus* L. a révélé la présence de stéroïdes, de tanins, de sucres réducteurs, d'alcaloïdes, d'huile essentielle et de flavonoïdes.Ces composés sont connue biologiquement actifs.L'activité antioxydant de l'extrait a été réalisée par le test DPPH .Les valeurs IC50 observées pour le dosage DPPH éteint de 167,92±3,18µg/ml pour l'extrait méthanolique de la partie aérienne, 78,15±1,19µg/ml de la partie racinaire.

Mots clés : *Cymbopogonschoenanthus* L. - Criblage phytochimique - Activités antioxydantes - Algérie.

Introduction

Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. However, oxidative stress also has a useful role in physiologic adaptation and in the regulation of intracellular signal transduction. (1). Oxidative stress is defined by an imbalance between increased

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levels of reactive oxygen species (ROS) and a low activity of antioxidant (2).

An imbalance between free radicals and antioxidants leads to oxidative damage of proteins, fat, nucleic acids, and carbohydrates. Antioxidants have protected the body from the harmful effect of the free radicals (3).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule.Antioxidant means "against oxidation". Any substance at low concentrations compared to that of an oxidizable substrate that significantly delays or prevents oxidation of that substrate is called as antioxidant. Antioxidants play vital role in preserving the quality of food and maintaining health of human being. (4).

Plant-derived antioxidants, especially, the phenolics have gained considerable importance due to their potential health benefits. Epidemiological studies have shown that consumption of plant-based food containing antioxidants is beneficial to health because it down-regulates many degenerative processes and can effectively lower the incidence of cancer and cardio-vascular diseases(5). Nature has provided an entire store-house of remedies to treat all ailments of mankind. Plants and plant products use as medicines could be traced as far back as the foundation of human civilization.Bioactive compounds" are extra nutritional constituents that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health (6). These bioactive compounds are actually combinations of secondary products present in the plant. They have been used as food preservatives, pharmaceuticals, alternative medicines and natural therapies for centuries. These compounds are mostly alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins and fatty acids. These compounds are odorous, complex, volatile compounds produced by special cells or groups of cells and concentrated in one particular region of plant such as the leaves, bark and stems (7).

In Sahara of Algeria, the flora is very rich in medicinal plants which produce valuable natural substances. As part of the study evaluation of the biological effectiveness of the medicinal plants, *Cymbopogon schoenanthus*L. This plant is a sub-spontaneous grass, tropical-Afro-asiatic, which is used as traditional medicines (8) to treat digestive diseases: aerophagia, flatulence and urinary decrease, analeptic drink for new mother after childbirth, bad breath, gumboils and urinary incontinence (9). The leaves of this herb, when fresh and young are consumed in salads and also used for preparation of traditional meat recipes. The white Centre of the leaves is used to impart a flavor to curries (10). In addition, it is used as a source of essential oil (11; 12). As well as the role of oil in lambs experimentally infected with *Haemonchus contortus* (13). Activities antimicrobial (14;15). Activies antioxidant (16)**.**

The aim of this study was to assess, using the DPPH assay, the antioxidant activity associated with the chemical composition the methanolic extract from *Cymbopogon schoenanthus* (Poaceae)**.**

2. MATERIAL AND METHODS

Plant material

Cymbopogon schoenanthus L. was collected during the flowering phase (July 2015) from Ghardaia is located within the Sahara Desert in northern-central of south Algeria (32°29□ 25.35□ □ N; 3°40□ 25.87□ □ E). The plant material was cleaned chopped into pieces and derided in air.

Preparation of extracts

Total methanol extract of *Cymbopogon schoenanthus* was prepared by maceration technique, the dried and powder of plant (aerial or roots parts) (5g) were macerated with (20 ml) of methanol at room temperature3 time (24 hours ×3). After filtration, the extract was concentrated using a rotary evaporator at a maximum temperature of 45°C, the residuals obtained were divided, a half part was stored in a freezer at – 4°C until further study (17).

Extraction yield

The extraction yield is calculated by the formula given by (18)

$$R (\%) = 100\ M/\ M'.$$

R: is the yield in%;

M: the mass of the extract after evaporation of the solvent in mg

M': is the dry mass of the plant sample in mg.

Phytochemical screening

The methanol extracts were subjected to phytochemical tests for secondary metabolites, tannins, saponins, steroid, alkaloids and glycosides in accordance with (19) and (20) with little modification.

Tests for Flavonoids

Pieces of magnesium ribbon and1ml HCl concentrated were mixed with 5 ml methanolic extract after few minutes and pink or orange color showed the presence of flavonoid. (21).

Test for Saponins

5 ml of distilled water was mixed with methanolic extract in a test tube and it was mixed vigorously. The frothing was mixed, the foam appearance showed the presence of saponins.

Test for reducing sugars (Fehling's test)

The methanol extract (2ml) was added to boiling Fehling's solution
(A and B) in a test tube. Obtaining a brick-red precipitate indicates the presence of the reducing compounds (22).

Test for tannins

To 2ml of methanolic solution of each extract, 2 drops of ferric chloride (Fecl3 solution diluted to 1%) were added (23). The appearance of a dark green color indicates the presence of catechic tannins.
The appearance of a blue-green color indicates the presence of gallic tannins

Test for Sterols and triterpenes

Sterols and terpenes were sought after by the Liebermann reaction. 5ml of the methanolic extract were evaporated. The residue is dissolved hot in 1 ml acetic anhydride and 1 ml of chloroform; we added 1 ml of concentrated sulfuric acid. The appearance, at the interphase, purple ring, turning blue then green, said a positive reaction.

Test for Alkaloids

2 ml of the methanolic extract, were treated separately with both reagents (Maeyer's , Hager's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.The appearance orange, brown or white precipitate indicates the presence of Alkaloids Respectively.

Test for essential oil

Essential oil was extracted from air-dried parts of *Cymbopogon schoenanthus* by

hydrodistillation for (3h) using a Clevenger apparatus type

Statistical analysis

Comparative analyses were conducted by one-way ANOVA analysis followed by the post hoc Tukey's test, and $p<0.05$ was considered as being significant.

Antioxidant activity

The free radical scavenging capacity methanol extract of *Cymbopogon schoenanthus* was evaluated with the methodology described by (24) as elaborated by (25). The solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mmol), was prepared and 800 µl of DPPH solution was added to 200µl of the solution of methanolic extract at different concentrations, absorption was measured at 517 nm up to 30 min or until it remained constant. The scavenging capacity of DPPH radical was calculated using the following formula.

Inhibition % = $\frac{AC-AS}{AC} \times 100$

Where, A control (Ac) is the absorbance of the control reaction and A sample (AS), is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged Ascorbic acid was used as standards.

The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extracts concentrations (26).

3. RESULTS AND DISCUSSION

Extraction yield

Table 1. extract methanolic Yields of *Cymbopogon schoenantuhs*

Extract	Color	Aspect	Yield%
MCA	Dark green	Powder	6,057±1[a]
MCR	Brown	hygroscopic	2,917±0,13[b]
P= 0,006 ***			

MCA : methanolic extract of Cymbopogon *schoenanthus* the aerial part
MCR: methanolic extract of *Cymbopogon schoenanthus* the root part
Values are averages ± standard deviation of triplicate analysis. values in the same row for each test followed by a different letter (a-b) are significantly different ($p < 0.05$)

The preparation of extracts from the aerial and root part of *Cymbopogon schoenanthus*was carried out by maceration methods at room temperature, was obtained with a yield of 6,057±1% (w/w) of root part, 2,917±0,13% of aerial part **(Table1)**

Phytochemical screening

Investigations on the phytochemical screening of *Cymbopogon schoenanthus* plant extracts revealed the presence of steroid, tannins, reducing sugars, alkaloids, essential oil and flavonoids (**Table 2**). These compounds are known to be

biologically active. Our results are in close agreement with that reported by (27) and (28).

Table 2. Phytochemicals found in methanolic extract of *Cymbopogon schoenanthus*

Phytochemicals	Aerial part	Root part
Flavonoids	+	++
Saponin	-	-
Steroids	+	+
Reducing sugars	+++	+++
Tannins	++	++
Alkaloids-wagner's-reagents	+	+
Alkaloids-Draghandroff's-reagents	+	+
Alkaloids-Mayer's-reagents	-	-
Volatile oils	+	+

+++: Strong intensity reaction, ++: Medium intensity reaction
+: Weak intensity reaction, −: Nondetected

Antioxidant activity

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (29). Radical scavenging activities are very important to prevent the deleterious role of free radical in different diseases including cancer. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. The linear regressions for antioxidant power of different extracts and control were used to calculate the IC_{50} values. The results of radical scavenging assays and IC_{50} values are given in **Fig. 1** and **Table 3**.

The results indicated the proton donating ability of the extractives which could serve as free radical inhibitors or scavengers and can also be served as primary antioxidants.

IC_{50} for DPPH radical-scavenging activity was 167,92±3,18µg/ml for methanolic extract of the aerial part, 78,15±1,19µg/ml of the root part. The IC_{50} values for Ascorbic acid, 62µg/ml. While another study indicated that the antioxidant activity estimated that IC_{50} for DPPH radical-scavenging activity was 16,4±8,8 to 26,4±4,8 µg/ml. (30) but (31), showed that the DPPH free radical scavenging activity of *C. schoenanthus methanolic*extract ranged from 56,83±1,51 µg/ml. The difference results might be the existing of polyphenol (Phenolic, Flavonoids compounds…) contents of the extractives and its anti-oxidant properties (32). (33).

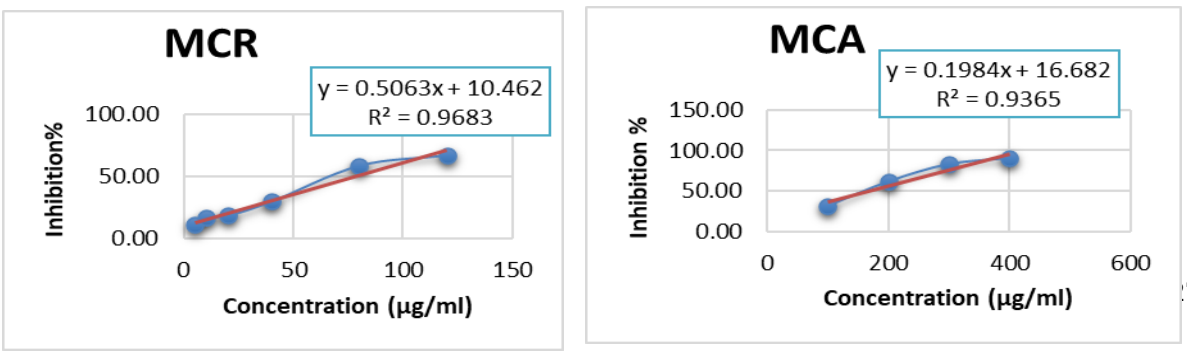

Fig. 1: Reducing power of antioxidant activity methanolic extracts of *Cymbopogonschoenanthus*

Table 3. The IC_{50} values of DPPH scavenging effect of *C. schoenanthus* extract

Extract	**IC_{50} ±SD (DPPH)(µg/mL).**
MCA	**167,92±3,18[a]**
MCR	**78,15±1,19[b]**
A. ascorbic	**2,89±0,06[c]**
P	**≤0,000 *****

MCA : methanolic extract of Cymbopogon *schoenanthus* the aerial part
MCR: methanolic extract of Cymbopogon schoenanthus the root part

Conclusion

The phytochemical screening of *Cymbopogon schoenanthus*revealed the presence of steroids, tannins, reducing sugars, alkaloids and flavonoids. large groups of secondary metabolites that are able to neutralize or quench the free radicals. And their derivatives are the largest group of polyphenols found in plants that possess strong antioxidant activities due to the scavenging reactive oxygen species and inhibition of oxidative stress, the IC_{50} values observed for DPPH essay were 167,92±3,18µg/ml for methanolic extract of the aerial part, 78,15±1,19µg/ml of the root part. *Cymbopogon schoenanthus*, are traditionally used for the treatment of various diseases. *C. schoenanthus* is currently, thought to have medicinal value

5. ACKNOWLEDGEMENTS

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