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SHORT COMMUNICATION

Antioxidant activity and total phenolic and flavonoid contents of *Chrysopogon aucheri* (Boiss.) Stapf

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Summary

Introduction: *Chrysopogon aucheri* is a species native to the South of Iran. It is used for the treatment of some oxidative-based disorders.

Objective: Due to lack of biological research on *C. aucheri*, we were prompted to investigate the antioxidant activities of leaf total extract and different sub-fractions for the first time. The phenolic and flavonoid contents were also determined in the leaves as the interaction between these components and the antioxidant activity.

Methods: The antioxidant effects of total extract and sub-fractions were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging method. Total phenolic and flavonoid contents were also determined by Folin-Ciocalteu and aluminium chloride colorimetric methods, respectively.

Results: Total extract of *C. aucheri* leaves was found to possess desirable antioxidant activity *in vitro* in comparison with standard antioxidant BHT (butylated hydroxytoluene). The highest phenolic and flavonoid contents were observed in the methanol sub-fraction. Results showed weak and moderate correlations with phenolics and flavonoids, respectively, and therefore other metabolites of *C. aucheri* leaves might be responsible for antioxidant activities.

Conclusion: The results suggested that *C. aucheri* leaves possess desirable antioxidant activity when compared with BHT and support the ethnomedicinal claims of the use of the leaves in the management of some oxidative-based diseases.

Key words: ***Chrysopogon aucheri, Poaceae, antioxidant activity, phenolics, flavonoids***

INTRODUCTION

There is an increasing evidence indicating that reactive oxygen species (ROS) and free radical mediated reactions are involved in degenerative or pathological events such as aging, cancer, coronary heart and Alzheimer's diseases [1]. Epidemiological studies have consistently shown that there is a clear significant positive correlation between the intake of fruits and vegetables and a reduced rate of heart disease, mortality, common cancers and other degenerative diseases as well as ageing. This is attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants, fibres and other biotic compounds [2].

One of the largest genera of *Poaceae* family, the genus of *Chrysopogon*, belongs to the subfamily of Panicoideae and tribe Andropogoneae, which comprises about forty-eight dominant perennial bunchgrasses [3]. These grasses are widely distributed and cultivated in tropical and subtropical regions, especially in southeast of Asia. They are also found in Northern and Eastern parts of Africa, Egypt, Palestine, Afghanistan, Pakistan and India [4]. The genus of *Chrysopogon* is represented by two perennial species in flora of Iran, *C. aucheri* (Boiss.) Stapf and *C. gryllus* (L.) Trin. and distributed in tropical regions of Iran including Southern, Southeastern, North Western parts, respectively [4].

C. aucheri is locally known as 'Rish-Zard' [4] and its roots are used in Iranian folk medicine as antiseptic, repellent and treatment of stomach ache, colds and fever [5]. Literature survey revealed that there was no attempt to investigate biological activities of *C. aucheri*. Due to the widespread use of *C. aucheri* leaves in the Iranian traditional medicine for relief and treatment of some oxidative based disorders, such as diabetes, we were prompted to evaluate the antioxidant activities of the leaves and investigate the pharmacological basis for the folkloric use of it as a natural antioxidant agent. Since there was no evidence of phytochemical study of this plant and also due to the interaction between the antioxidant property and phenolic and flavonoid contents, the mentioned contents were determined for the first time.

MATERIAL AND METHODS

Plant material

Fresh leaves of *C. aucheri* were collected in April 2015 from Qotb-Abad village, Gohareh region, north of Bandar Abbas, Hormozgan Province, Iran (N 27° 73' 88.8", E 56° 54' 66.6", 600 m). The voucher was deposited in the Herbarium of the Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran, under code number GCAR/1482.

Extraction procedure

300 g air–dried ground leaves were extracted by percolator apparatus using 1 l of methanol (Merck). The extraction was repeated three times. The extracts were concentrated by rotary evaporator apparatus and the solvent produced a dark green gummy solid. The yield was 52 g total methanol extract. 10 g of the resulting extract was kept in a clean vial in a dark and cool place for further study.

The remained crude extract was partitioned between water, chloroform, ethyl acetate and methanol by liquid-liquid fractionation method [6]. These sub-fractions were also kept separately in clean vials, in a dark and cool place for further test studies. The yields were 8, 19, 11 and 14 g aqueous, chloroform, ethyl acetate and methanol sub-fractions, respectively.

DPPH assay

The antioxidant activities of total extract, sub-fractions and standard were assessed on the basis of radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity by modified method [7]. Diluted working solutions of the extract and sub-fractions were prepared in methanol. BHT (butylated hydroxytoluene) was used as standard in 60 to 1000 µg/ml solution. 0.1 mM of DPPH was prepared in methanol and 3 ml of this solution was mixed with 1 ml of different concentrations of sample solutions

(1000 to 5000 μg/ml) and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Shimadzu spectrophotometer. Methanol (1 ml) with DPPH solution (0.1mM, 3 ml) was used as control. The optical density was recorded and % inhibition was calculated using the formula given below [9]:

$$\text{inhibition of DPPH activity (\%)} = \\ = (A - B / A) \times 100$$

where A is the optical density of control and B is the optical density of sample. The evaluation of the antioxidant activity was preformed three times for each sample.

Total phenolic and flavonoid contents

The content of total phenolic compounds in the methanol extract was determined by Folin-Ciocalteu method [7]. 1 ml of 0.01 g/ml methanol extract was mixed with 5 ml Folin-Ciocalteu reagent (diluted tenfold with distilled water) and 4 ml (7.5 g/100 ml) sodium carbonate. After 1 h at room temperature, the absorption of clear solutions was read at 765 nm. For the preparation of calibration curve, different concentrations of methanol gallic acid solutions were mixed with the same reagents as described above, and after 30 min, the absorption of clear solutions was measured. The amount of total phenolic compounds was expressed as gallic acid equivalent (GAE) in milligrams per gram of dried extract. The experiment was repeated three times and the mean value was reported.

The content of flavonoids was determined using the aluminum chloride colorimetric method. 1 ml of 0.01 g/ml methanol extract was mixed with 1 ml of 2% $AlCl_3$ ethanol solution. After 1 h at a room temperature, the absorbance was measured at 420 nm. The results were expressed in mg rutin in g of dry matter by comparison with standard rutin treated with the same conditions [10].

Statistical analysis

Data are presented as mean ± standard deviation (SD) of each triplicate or more tests using MS Excel software.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

Total extract of *C. aucheri* leaves and related aqueous, methanol, ethyl acetate and chloroform sub-fractions were assayed for DPPH radical scavenging activity to obtain their concentrations to scavenge 50% DPPH (IC_{50}). As it was shown in table 1, the highest DPPH radical scavenging activity (the lowest IC_{50}) was shown by total extract (315.55±17.98 μg/ml), and the lowest activity was shown by the ethyl acetate sub-fraction (1124.79±22.12 μg/ml).

The phenolic content (mg/g) in plant samples were determined from regression equation of calibration curve (y=5.0121x, R^2=0.99) and expressed in gallic acid equivalents (GAE), varied between 13.50 and 80.21 (tab. 1). The highest and lowest contents were found in the methanol sub-fraction (80.21±0.58) and the ethyl acetate sub-fraction (13.50±2.4), respectively.

The content of flavonoids in plant samples (mg/g) was expressed in rutin equivalents (regression equation of calibration curve, y=5.3267x, R^2=0.99). The

Table 1.

Radical scavenging activity by DPPH assay and the phenolic and flavonoid contents in *C. aucheri* total extract and sub-fractions

Plant materials	DPPH activity[a]	Phenolic content[b]	Flavonoid content[c]
Total extract	315.55±17.98	33.68±0.13	31.30±0.42
Chloroform sub-fraction	973.655±18.73	57.35±0.70	31.72±1.32
Ethyl acetate sub-fraction	1124.79±22.12	13.50±2.4	12.01±5.91
Methanol sub-fraction	490.48±15.86	80.21±0.58	64.62±0.19
Aqueous sub-fraction	580.23±52.94	38.83±1.69	34.48±6.59
BHT	78.24±2.89	–	–

[a] The concentration [μg/ml] of the plant extracts in inhibition of 50% DPPH Radical (IC_{50})
[b] mg/g plant extracts in gallic acid equivalent
[c] mg/g plant extract in rutin equivalent

highest contents were found in the methanol sub-fraction (64.62±0.19), and the lowest in the ethyl acetate sub-fraction (12.01±5.91).

DISCUSSION

Owing to the variety of their phytochemicals, plants have been used for different medical purposes. They are also of great importance as antioxidant agents in food and pharmaceutical industries. The chemical composition of the volatile oil extracted from *C. aucheri* roots were investigate in 2016.

The literature survey revealed that the chemical composition of the volatile oil extracted from *C. aucheri* roots was investigated [11] but there was no biological evaluation of the antioxidant effects of *C. aucheri* leaves. The present study was considered as a first investigation performed on the antioxidant effects of total extract and different sub-fractions of the leaves by DPPH assay. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The findings of the study on the antioxidant activities of the crude extract and sub-fractions from *C. aucheri* leaves indicated that crude extract possessed the most significant activity and is accepted to have DPPH radical's reduction property as compared to standard BHT characterized as a well-known antioxidant. Regarding the best antioxidant activity and the interaction between the antioxidant property and phenolic and flavonoid contents, total extract was expected to contain the higher content of phenolics and flavonoids. However, the methanol sub-fraction was of the highest content. The correlation coefficient (r) between phenolic and flavonoid content and the data of DPPH scavenging activity of the plant extract and its fractions calculated by MS Excel showed a weak correlation with phenolics (-0.35) and moderate correlation with flavonoids (-0.59). Therefore, other metabolites of *C. aucheri* leaves might be responsible for antioxidant activities.

These final results justify the ethnomedicinal use of *C. aucheri* leaves and the leaves could be a potential candidate as a natural antioxidant agent.

Previous studies on other *Chrysopogon* species also showed significant antioxidant activities. Subhadradevi *et al.* showed significant antioxidant activities of *Vetiveria zizanoides* (Syn: *C. zizanioides*) roots extract by various *in vitro* antioxidant assays [12]. The antioxidant activities of *C. zizanoides* essential oil were also reported in 2012. Chou *et al.* showed that the significant antiinflammatory effect of the oil was associated with the significant antioxidant properties of *C. zizanoides* essential oil [13].

These results show that the genus of *Chrysopogon* including *C. aucheri* can be a potential alternative of natural antioxidant. In this regard and considering that *C. aucheri* is adapted to dry lands and is applied in livestock production and animal nutrition [14], it could be a potent choice to prevent desertification in deserts, wadies and dry lands.

CONCLUSION

In conclusion, the present study revealed that leaves of *C. aucheri* growing wild in South Iran could become a new potential natural antioxidant agent. The final results justified the ethnomedicinal use of *C. aucheri* leaves in management of some oxidative-based disorders. Further phytochemical investigations are suggested to elucidate the crude extract molecular structure for antioxidant activity.

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Conflict of interest: Authors declare no conflict of interest.

REFERENCES

1. Sun C, Wang J, Fang L, Gao X, Tan R. Free radical scavenging and antioxidant activities of EPS2, an exopolysaccharide produced by a marine filamentous fungus *Keissleriella* sp. YS 4108. Life Sci 2004; 75:1063-1073. doi: http://dx.doi.org/10.1016/j.lfs.2004.02.015

2. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables – the millennium's health. Int J Food Sci Technol 2001; 36:703-725. doi: http://dx.doi.org/10.1111/j.1365-2621.2001.00513.x

3. Ahmad S, Call CA, Schupp EW. Regeneration ecology of *Chrysopogon aucheri* and *Cymbopogon jwarancusa* in upland Balochistan. Morphology,

viability and movement of seeds (Spikelets). Pak J Biol Sci 2000; 3(10):1583-1587.

4. Mozaffarian V. A Dictionary of Iranian Plants Name. Tehran 2006.

5. Zargari A. Medicinal Plants. Vol. 4, Tehran 1991.

6. Houghton PJ, Raman A. Laboratory handbook for the fractionation of natural extracts. London 1998:54.

7. Blois MS. Antioxidant determination by the use of a stable free radical. Nature 1958; 181:1198-1200. doi: http://dx.doi.org/10.1038/1811199a

8. Bors W, Saran M, Elstner EF. Screening for plant antioxidants. In: Modern methods of plant analysis. vol. 13, Berlin 1992:277-295.

9. Folin O, Ciocalteu V. On tyrosine and tryptophane determination in proteins. J Biol Chem 1927; 27:627-650.

10. Kumazawa S, Hamasaka T, Nakayama T. Antioxidant activity of propolis of various geographic origins. Food Chem 2004; 84:329-339. doi: http://dx.doi.org/10.1016/S0308-8146(03)00216-4

11. Sadoughinia M, Asgarpanah J. Terpene constituents of *Chrysopogon aucheri* (Boiss.) Stapf. roots. J Essnt Oil Res 2016; 28(3):260-263. doi: http://dx.doi.org/10.1080/10412905.2015.1129991

12. Subhadradevi V, Asokkumar K, Umamaheswari M, Sivashanmugam A, Sankaranand R. *In vitro* antioxidant activity of *Vetiveria zizanioides* root extract. Tanzan J Health Res 2010; 12(4):274-279.

13. Chou ST, Lai CP, Lin CC, Shih Y. Study of the chemical composition, antioxidant activity and anti-inflammatory activity of essential oil from *Vetiveria zizanioides*. Food Chem 2012; 134(1):262-268. doi: http://dx.doi.org/10.1016/j.foodchem.2012.02.131

14. Banu G, Islam M, Ahmad S, Aslam S, Koukab S. Seasonal variation in nutritive value of *Chrysopogon aucheri* (Boiss) Stapf., and *Cymbopogon jwarancusa* (Jones) Schult., in highland Balochistan, Pakistan. Pak J Bot 2009; 41(2):511-517.

Aktywność antyoksydacyjna oraz całkowita zawartość fenoli i flawonoidów w *Chrysopogon aucheri* (Boiss.) Stapf.

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Streszczenie

Wstęp: *Chrysopogon aucheri* jest gatunkiem rodzimym dla południowego Iranu. Stosuje się go w leczeniu różnych schorzeń spowodowanych stresem oksydacyjnym**.**

Cel: Ze względu na brak badań biologicznych dotyczących *C. aucheri*, postanowiono zbadać właściwości antyoksydacyjne całkowitego wyciągu z liści tego gatunku i poszczególnych frakcji. Celem pracy było także określenie całkowitej zawartości fenoli i flawonoidów i korelacji tego z aktywnością przeciwutleniającą wyciągów.

Metody: Działanie antyoksydacyjne całkowitego wyciągu i jego frakcji zostało określone metodą DPPH. Całkowitą zawartość fenoli i flawonoidów oznaczono spektrofotometycznie metodą Folina-Ciocalteu i z użyciem chlorku glinu.

Wyniki: Całkowity wyciąg z liści *C. aucheri* wykazał spodziewaną aktywność antyoksydacyjną *in vitro* w porównaniu ze standardową substancją przeciwutleniającą BHT (butylohydroksytoluen). Największą zawartość fenoli i flawonoidów zanotowano we frakcji metanolowej. Wyniki pokazują słabą i średnią korelację odpowiednio z fenolami i flawonoidami, i co za tym idzie inne metabolity liści *C. aucheri* mogą być odpowiedzialne za aktywność antyoksydacyjną.

Wnioski: Wyniki sugerują, że liście *C. aucheri* mają spodziewane właściwości antyoksydacyjne w porównaniu z BHT i potwierdzają zasadność stosowania badanego surowca w tradycyjnym leczeniu niektórych schorzeń spowodowanych stresem oksydacyjnym.

Słowa kluczowe: ***Chrysopogon aucheri, Poaceae, aktywność antyoksydacyjna, fenole, flawonoidy***