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Evaluation of antioxidant and antifungal activities of methanolic aerial part extract of *Iris unguicularis* Poiret

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ABSTRACT

Evaluation of antioxidant and antifungal capacities of methanolic extract of Iris unguicularis Poiret was studied for the development of the medicinal values, leading to the investigation of the properties of areal part of this plant, which growth in the mountain of Megriss Setif Algeria. The antioxidant study has been carried out by two methods: radical scavenging activity (DPPH) and the reducing power. However, the antifungal activity was tested with three fungi including yeast (Aspergillus niger 2CA936, Aspergillus flavius NRRL3357 and Candida albicans ATCC1024). Our results exhibit a high antioxidant activity and strong antifungal capacities.

Keywords: *Iris unguicularis Poiret*; methanolic extract; Antioxidant; Antifungal.

INTRODUCTION

Iris unguicularis Poiret belong to the family of *Iridaceae*[1], which contains 92 genera and more than 1800 species [2] [3], mostly perennial herbs with underground storage organs called rhizomes (bulbs). Some genera are important in traditional medicines, especially *Iris* and *Gladiolus*. The genus Iris comprises over 300 species [4] [1].*Iris unguicularis* Poiret namely called Iris of Algiers have a large clumps of bright green leaves exceeded throughout in the winter with pretty blue flowers, which is characteristic of this species, native in rock and woods of North Africa, Syria, Greece and Crete [1] (figure 1).

Figure 1: *Iris unguicularis* Poiret

The compounds obtained from different species of Iris were reported to have piscicidal, anti-neoplastic, anti-oxidant, anti- inflammatory, antiplasmodial, antibiotic and anti-tuberculosis properties [5] [6] [7] [8].

From plants which belong to genus Iris were isolated isoflavonoids[9][10], quinones[11] and iridal type triterpenoids[12].Williams *et al*[13],confirmed presence of flavonoids, xanthones and isoflavones in leaf, flower and rhizomes of several cultivars of bearded irises.

The aims of this study is to develop the phytochemicals and therapeutics characteristics of this beautiful North African endemic species.

MATERIALS AND METHODS

Plant material

The areal part of *Iris unguicularis* Poiret were collected from the mountain of Megriss Setif -Algeria in May 2014 and determined by GAAMOUNE Sofiane (National Institute of Agriculture Research –Setif –Algeria).

Preparation of methanol extracts

Air-dried herbs were powdered and macerated in 80 % methanol for 24h, 48 h and 72 h at room temperature (1:10 w/v, 100 g dried herb). After maceration, the extracts were collected, filtered and evaporated to dryness under vacuum [14]. The dry extract was stored at a temperature of-18 C ° for later use.

Determination of Total Phenolic Content

For total polyphenol determination, the Foline Ciocalteu method was used [15]. The sample (0.2 ml) is mixed with 1 ml of the Folin-Ciocalteu reagent previously diluted with 10 ml of deionized water. The solution is allowed to stand for 4 min at 25 C° before 0.2 ml of a saturated sodium carbonate solution (75 mg/ml) is added. The mixed solution is allowed to stand for another 120 min before the absorbance at 765nm is measured. Gallic acid is used as a standard for the calibration curve. The total phenolics content is expressed as mg equivalent gallic acid per gram of extract (mg EAG/GE)

Determination of total flavonoids contents

The flavonoids contents in ours extracts were estimated by the Aluminum chloride solution according to the method described by Bahorun et al., (1996) [16]. Briefly, 1 ml of the methanol solution of each extract was added to 1 ml of 2 % $AlCl_3$ in methanol. After 10 min, the absorbance was determined at 430 nm. Quercetin and rutin (0 - 40ug/ml) were used as a standard. Results were expressed as mg equivalent Quercetin and rutin per gram of extract (mg EQ/GE)

Determination of total tannins contents

Tanin content was evaluated using the hemoglobin precipitation assay. An aliquot of 0.5 ml of each extracts is mixed with 0,5ml of hemolysis bovine blood to reach a final concentration of 1 mg/ml. the mixture was centrifuged at 480g for 20 min and the absorbance was measured at 578nm [17].

In same time, tannic acid at various concentrations (100-600 µg / ml) is used in the same manner as the samples. Result are expressed as mg equivalent of tannic acid / gram of extract E TA/GE.

Quantitative Estimation of Alkaloids:

To 1ml of methanolic extract 5 ml phosphate Buffer (pH 4.7)and 5 ml BCG solution was added. The mixture was shook with 4 ml of chloroform. The extract was collected in a 10-ml volumetric flask and diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents [18].

Result are expressed as mg equivalent of atropine / gram of extract E ATR/GE.

Determination of carotenoids

Total carotenoid contents in the extracts were determined by a spectrophotometric assay described by Youngmin Choi et al (2006) [19]. Approximately, 5 ml of extract were mixed with equal volume of distilled water and 15 ml of hexane/acetone/methanol (50/25/25, v/v) solution. The mixture was then homogenized with a Polytron and centrifuged at 3000 rpm (940g) for 10 min. The absorbance of the top layer of hexane was measured at 450 nm using a spectrophotometer. Total carotenoid contents of the samples were calculated as l g β-carotene per 100 g of

sample using an extinction coefficient of$E\ _{1cm}^{1\%}$=2505 [20].Result are expressed asmg equivalent of β-carotene / gram of extract (E β-carotene/GE)

DPPH Assay

The donation capacity of extracts were measured by bleaching of the purple-colored solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato [21]. One milliliter of the extracts at different concentrations was added to 0.5 mL of a DPPH-methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (micrograms per milliliter), the antiradical dose required to cause a 50 % inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = [(A_0 - A_1)/ A_0] \times 100$$

Where A_0 is the absorbance of the control at 30 min and A_1 is the absorbance of the sample at 30 min. BHT was used as a positive control. Samples were analyzed in triplicate [22].

Reducing power

The reducing power was determined according to the method of Oyaizu (1986) [23]. Each extract (0.5–10 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 10 mg/ml potassium ferricyanide, and the mixture was incubated at 50 C° for 20 min. After 2.5 ml of 100 mg/ml trichloroacetic acid were added, the mixture was centrifuged at 200g for 10 min. The upper layer (5 ml) was mixed with 5ml of deionized water and 1ml of 1 mg/ml ferric chloride, and the absorbance was measured at 700nm against a blank.

A higher absorbance indicates a higher reducing power. IC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Ascorbic acid, BHT was used for comparison [24].

Antifungal activity

Test strains and culture media

Two fungi: *Aspergillus niger* 2CA936 and *Aspergillus flavus* NRRL 3357; and one yeast: *Candida albicans* ATCC1024 were used in the experiment. The potato dextrose agar was used for fungi culture and Sabouraud for yeast.

Antifungal activity

The antifungal activity was tested by disc diffusion method with modifications [25]. The potato dextrose agarplates were inoculated with each fungal culture, (*Aspergillus niger* 2CA936, *Aspergillus flavus NRRL 3357*), 8 days old by point inoculation. The spore suspension was prepared in an emulsion of 0,5 % tween and adjusted to a concentration of 2-3 × 10^6 spores/ml, corresponding to 0.15 to 0.17 absorption at 530 nm [26].

However, *Candida albicans* ATCC1024 suspension is obtained in physiological saline 0.9 % adjusted to 10^5CFU / ml. The culture was incubated for 24 h at 37 C°.

One hundred microliter of suspension was placed over agar in Petri dishes and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 10 µl of each sample at different concentrations.

Statistical analysis

Results were expressed as the mean ± standard deviation. Data was statistically analyzed using t test of Student with the criterion of P values < 0.05 to determine whether there were any significant differences between methanol extract of *Iris unguicularis* Poiret and standards, using Graphpad prism 5 Demo Software.

RESULTS AND DISCUSSION

The yield of methanolic extract gave 9,6 % of total secondary metabolites. The quantities of polyphenols, flavonoids, tannins, alkaloids and carotenoids reach 180,47±2,02mg EAG/GE , 58,09±3,79mg EQ/GE,25,87±11,15 EAT/GE, 8,81±1,87 mg E ATR/GE and18,43±0,70mg E β-carotene/GE respectively.

Although it does not represent similar conditions to the processes that occur in vivo, the colorimetric method to evaluate the ability of scavenging the stable free radical DPPH represents a simple and rapid method to detect the

presence of substances with antioxidant properties in crude plant extracts in order to become targets of bioguided assays, aiming the isolation of such substances. Figure 1 demonstrate DPPH activity of the extract and BHT. Both the samples showed same activity.

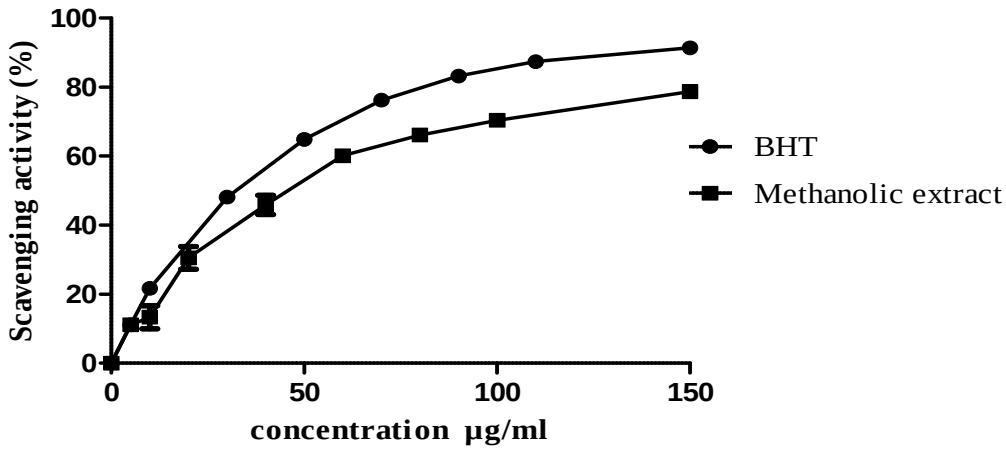

Figure 1: scavenging activity on the DPPH radical of methanolic extract of Iris unguicularis Poiret

The DPPH radical scavenging activity of the extract(IC_{50}41,370±1,635 µg/ml **) was weaker than that of BHT(IC_{50}34,01±1,1µg/ml).

The reducing capacity of the extract is another significant indicator of antioxidant activity. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability [27].

Figure 2 shows the dose response curves for the reducing powers of our extract(20 - 150 µg/ml). It was found that the reducing power increased with concentration of each sample.

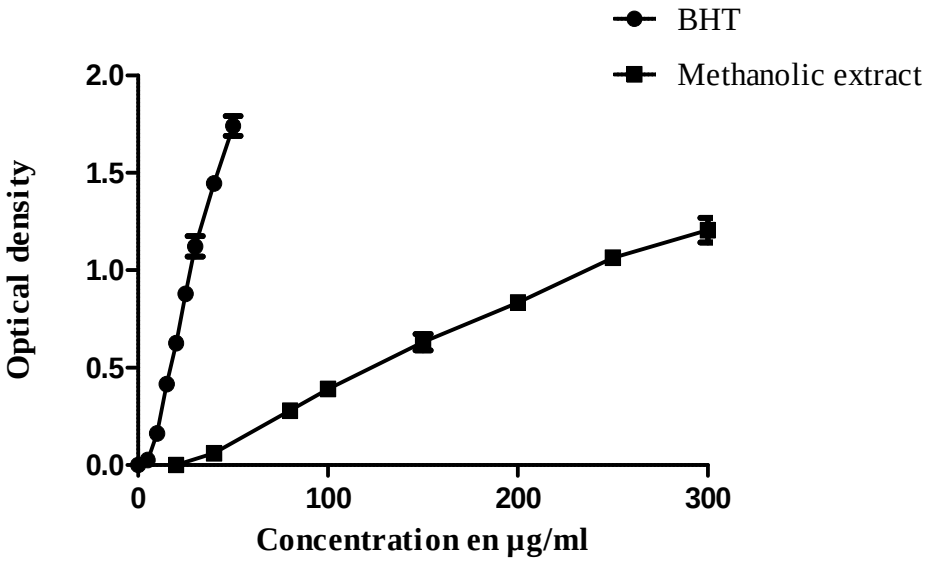

Figure 2: reducing power of methanolic extract of *Iris unguicularis* Poiret

The results of this research indicated that the reducing power of methanolic extract of *Iris unguicularis Poiret* (EC_{50}129,45±2,40µg/ml***)was less than standard (EC_{50} 16,06±0,18 µg/ml).

The results of antifungal activity of metanolic extract of *Iris unguicularis Poiret* were expressed in table 1:

Table 1: Antifungal activity of methanolic extract of *Iris unguicularis* Poiret, standard and control

	Aspergillus flavius NRRL3357	*Aspergillus niger 2CA936*	*Candida albicans ATCC1024*
Nystatin	15,53±0,79[a]	9,40±0,22[c]	9,29±0,19
Clotrimazon	23,86±1,15	15,85±0,32[d]	44,28±0,49
Amphotericin	16,20±1,19[b]	17,55±0,14[e]	15,58±0,12
Methanolicextract	25,50±1,23[a,b]	*100% inhibition of spores formation* [c,d,e]	–
Control	*No inhibition*	*No inhibition*	*No inhibition*

The similars letters in different columns indicate a very significant difference.

DISCUSSION

The antioxidant activity of plant extract cannot be evaluated by only a single method due to the complex nature of phytochemicals; therefore, it is important to employ commonly accepted assays to evaluate the antioxidant activity of plant extract. Numerous antioxidant methods have been developed to evaluate antioxidant activity and to explain how antioxidants function. Of these, reducing power, DPPH assay is the most commonly accepted assay to evaluate antioxidant activity [28].

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [29].The degree of color change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test [30].The results of radical scavenging showed that, methanolic extract possessed strong radical scavenging effect in spite of their low value against BHT.

The reducing power property of a compound indicates that it is electron donor, and can reduce the oxidized intermediates of lipid peroxidation processes and convert them to more stable products and consequently terminate radical chain reactions [31]

Antioxidant capacity may be associated with a high phenol content, as Mansouri et al. [32] reported that most of the antioxidant activity of plants is derived from phenols. Structurally, phenols comprise an aromatic ring bearing one or more hydroxyl substituents. The antioxidant activity of this type of molecule is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons or chelate metal cations [33]. The methanolic extract of *Iris unguicularis* Poiret, contain (180, 47±2, 02 mg EAG/GE) of polyphenols considered as important quantity, thus can't explain the low chelating iron power in comparison with BHT. Our explanation is the presence of other chemicals groups which may intermediate to weakenthe used extract.

Iris unguicularis Poiret methanolic extract demonstrate a broad-spectrum antifungal activity against *Aspergillus flavius* and *Aspergillus niger* in using 1mg/disc of extract.

The antifungal activity of the phenolic compounds has been attributed to their lipophilic properties, which determine their ability to penetrate into the plasma membrane and induce changes in the physico-chemical properties of the cell wall, cell membrane, and cellular organelles [34].

Previous investigators have also documented the antibacterial and antifungal properties of flavonoids [35] [36] [37] [38]. This activity might be explained by their ability to complex with cell wall and induce the formation of pseudomulticellular aggregates, inhibiting microbial growth [39] [40]. Thus, may explain the high antifungal activity of *Iris unguicularis* Poiret.

CONCLUSION

Iris unguicularis Poiret namely called Iris of Algiers is a beautiful endemic species; their secondary metabolites, therapeutic and pesticide capacities constitute an enigmatic until know. Our study show an interesting scavenger power and relatively moderate chelating iron capacity but a very important antifungal activity against the fungi used. However, exhaustive researches are needed to isolate the active constituents responsible for the observed effect.

REFERENCES

[1] Hoffer-Massard F. (**2009**) Premier printemps sur les quais de Montreux – le 15 mars 2008. *Bulletin du Cercle vaudois de botanique*, 38: 11-24.
[2] Ali SI, Mathew B. Flora of Pakistan, 202, 1. Karachi: Karachi University Press; **2000**.p. 4-29.
[3] Hooker JD. Flora of British India. Bishman, S. M. P. S., 2. ; **1982**. p. 665.

[4] Krishnan MS. In: Sastri BN, editor. The Wealth of India: A Dictionary of Raw Material and Industrial Products, 2. New Delhi: Council of Scientific and Industrial Research; **2001**. p. 254–6.
[5] Hanawa F., Tahara S., Mizutani J., **1991**: *Phytochemistry*, 30: 2197–2198.
[6] Hideyuki, I., Miyake, Y., Yoshida, T., **1995**: *Chemical Pharmaceutical Bulletin*, 43: 1260–1262.
[7]Miyake, Y., Ito, H., Yoshida, T., **1997**: *Canadian Journal of Chemistry*, 75: 734–741.
[8] Papendorf O, König GM, Wright AD (**1998**) *Phytochemistry* 49: 2383–2386.
[9] WU, Y. X., XU, L. X., **1992**: *Acta Pharmateutica Sinica*, 27: 64–68.ISSN 0513-4870.
[10] Morita, N., Shimokoriyama, M., Shimizu,M., **1972**: *YakugakuZasshi*, 92 (8): 1052–1054.
[11] Seki, K., Tomihari, T., Haga, K., **1994**: *Phytochemistry*, 37 (3):807–815.
[12] Krick, W., Marner, F. J., Jaenicke, L., **1983**: *ZeitschriftfűrNaturforschung*, 38: 179–184.
[13] WILLIAMS, CH. A., HARBORNE, J. B.,COLASANTE, M., **1997**: *BiochemicalSystematics and Ecology*, 25 (4): 309–325.
[14]Neda S L., Neda M.M.D., Jelena M I ., Biljana N B ., **2010**. *Cent. Eur. J. Biol* , 331–337.
[15] Li WD., Wei C L., White P J., Beta T., **2007**. *Journal of Agricultural and Food Chemistry*, 55: 291-298.
[16]Bahorun T., Gressier B., Trotin F., Brunete C., Dine T ., Vasseur J., Gazin J C., Pinkas M., Luycky M., Gazin M., **1996** . Oxigen species scavenging activity of phenolic extract from howthorn fresh plant organs and pharmaceutical preparation. ArzneimForsch / Drug Res, 1-6.
[17] Hagerman A E., Butler L G. **1978**. *J Agr Food Chem*, 809-812.
[18] Hemachakradhar K., PavankumarRaju N., **2013**. *International journal of science inventions today*., 2(1): 31-39
[19] Youngmin C., Heon-Sang J., Junsoo L., **2006**. *Food Chemistry*, 103 : 130–138.
[20] De Ritter E., Purcell A E.,**1981**. Carotenoid analytical methods,pp 815–923 . In. Bauernfeind J C., Carotenoid as colorants and vitamin A precuisorsed. Academic Press, London, U.K.
[21] Hanato T., Kagawa H., Yasuhara T., Okuda T., **1998**. *Chemical & Pharmaceutical Bulletin*, 2090–2097.
[22] Bettaieb R I., Bourgou S., Ben SlimenDebez I., JabriKaroui I., HamrouniSellami. I, Msaada K., Limam F., Marzouk B., **2011**. *Food Bioprocess Techno* , 1007
[23] Oyaizu M., **1986**. *Japanese Journal of Nutrition*, pp 307–315.
[24] Huang S J., Mau J L., **2006**. *Swiss Society of Food Science and Technology* 39 :707–716.
[25] NCCLS (National Committee for Clinical Laboratory Standards)., **1999**. Performance standards for antimicrobial susceptibility testing. Wayne Pa. 9th International Supplement, M100-S9.
[26] Yazdani D., ZainalAbidin M A., Tan Y H., Kamaruzaman S., Jaganath I B., **2012**. *Journal of Medicinal Plants Research*, 6(42): 5464-5468
[27] Olayinka A, Aiyegoro AI, Okoh. Preliminary phytochemical screening and In vitro antioxidant activities of the aqueous extract of Helichrysumlongifolium DC. BMC Complementary and Alternative Medicine, Oxford University Press. **2010**; 10:21.
[28]Zou Y, Lu Y, and Wei D. *J Agr Food Chem* **2004**; 52: 5032-5039.
[29] Soares, JR, Dinis, TCP, Cunha, AP, Almeida, LM. *Free Radical Res*. **1997**; 26: 469–478.
[30] Krishnaiah D, Sarbatly R, Nithyanandam RR: *Food Bioprod Process* **2011**,89:217–233.
[31] Yen GC, Chen HY (**1995**). *J. Agric. Food Chem*. 43: 27-37.
[32] A. Mansouri, G. Embared, E. Kokkalou, P. Kefalas, *Food Chem*. 89 (**2005**)411–420.
[33] R. Amarowicz, R.B. Pegg, M. Rahimi, B. Barl, J.A. Weil, *Food Chem*. 84 (**2004**)551–562
[34]Dambolena, J.S.; Zygadlo, J.A.; Rubinstein, H.R. *Int. J. Food Microbiol*. **2011**, 145,140-146.
[35] Fang, J.J.; Ye, G.; Chen, W.L.; Zhao, W.M. *Phytochemistry* **2008**, 69, 1279-1286.
[36] Hernández, N.E.; Tereschuk, M.L.; Abdala, L.R. *J. Ethnopharmacol*. **2000**, 73, 317-322.
[37]Kuete, V.; Ngameni, B.; Simo, C.C.F.; Tankeu, R.K.; Ngadjui, B.T.; Meyer, J.J.M.; Lall, N.;Kuiate, R.R. J. *Ethnopharmacol*. **2008**, 120, 17-24.
[38]Mbaveng, A.T.; Ngameni, B.; Kuete, V.; Simo, I.K.; Ambassa, T.; Roy, R.; Bezabih, M.; Etoa, F.X.;Ngadjui, B.T.; Abegaz, B.M.; et al. *J. Ethnopharmacol*. **2008**, 116, 483-489.
[39]Cushnie, T.P.T.; Lamb, A.J. *Int. J. Antimicrob. Agents* **2011**, 38, 99-107.
[40]Cushnie, T.P.T.; Lamb, A.J. *Int. J. Antimicrob. Agents* **2005**,26, 343-356.