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Screening of Antioxidant and Radical Scavenging Activities of Some Omani Medicinal Plants

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الكشف عن أنشطة مضادات التأكسد وكبح الشوارد لبعض النباتات الطبية في عمان

صالح البوصافي، مبارك الريامي، خديجة العويسي و عبدالقادر هشام

خلاصة: لقد تم تقييم الأنشطه ألمضاده للأكسده وأنشطة كبح الشوارد لعشرة نباتات طبيه عمانيه. في هذه الدراسه تم استخدام نبات الريحان كمرجع لما عرف عنه من نشاط قوي لوقف التأكسد. لقد وجدنا أن نبات أكاسيا تورتيليس ونبات تفروسيا هوسكنشتي لها أنشطة مرتفعة لمضادات التأكسد وكبح الشوارد.

ABSTRACT: *n*-Butanol extracts of nine medicinal plants, *Cressa cretica, Ziziphus spina-christ, Acacia tortilis, Tephrosia haussknechti, Aristolochiae bracteolata, Citrullus colocynthis, Teucrium mascatense, Rhazya stricta* and *Nerium oleander,* found in Oman were screened for their antioxidant activity using phosphomolybdenum complex assays and their radical scavenging activity using DPPH assays. *Ocimumi basilicum,* a plant with well documented antioxidant activity, was used as a reference. *A. tortilis*, and *T. haussknechti* extracts possessed very high antioxidant activity (AOA) and high radical scavenging activity (RSA).

KEYWORDS: Antioxidant activities, radical scavenging, DPPH, phosphomolybdenum complex.

1. Introduction

Spices have been used since ancient times to improve taste and aroma of different types of food and some of them are well known for their antioxidant properties (Madsen and Bertelsen, 1995). Antioxidants are widely used as additives in food, pharmaceutical, and cosmetic industries to provide protection against oxidative degradation. Numerous degenerative diseases such as brain dysfunction, cancer, heart diseases and immune system decline could be the result of cellular damage caused by reactive oxygen species and free radicals present in human diet and antioxidants may play an important role in the prevention such diseases (Aruoma, 1998).

A great number of aromatic, spicy, medicinal and non-medicinal plants contain chemical compounds exhibiting antioxidant properties. Several studies were carried out on plants, such as rosemary and sage have led to the development of natural antioxidant formulations for food, cosmetic, and other applications (Cuvelier *et al*., 1996). However, scientific studies on the antioxidant properties of several plants used in Omani traditional

medicine are still rather scarce. Therefore, the evaluation of such properties seems to be a useful task with a view to find new sources of natural antioxidants.

The present work deals with preliminary studies on the antioxidant and radical scavenging activities of the n-butanol extracts prepared from the following plants: *Cressa cretica* (Convolvulaceae), *Ziziphus spina-christi* (Rhamnaceae), *Acacia tortilis* (Leguminosae), *Tephrosia haussknechtii* (Leguminosae), *Aristolochiae bracteolata* (Aristolochiacae), *Citrullus colocynthis* (Cucurbitaceae), *Teucrium mascatense* (Labiatae), *Rhazya stricta* (*Apocynaceae*) and *Nerium oleander* (Apocynaceae) which are readily available plants growing in northern Oman. To the best of our knowledge, only little information on the antioxidant and radical scavenging properties of these plants is available in the literature. *C. cretica* is an annual plant that grows in saline soils and is used to treat minor wounds. The aerial parts of *C. cretica* are source of flavonoids (Shahat *et al*., 2004), and terpenoids (Ramachandran and Ali, 2003). Antioxidant properties of the plant have not been reported. *Z. spina-christi* is one of the five popular trees in Oman. The fruits are edible. The crushed leaves are used as a natural cleaning as well as a conditioner for the hair. Hair washed with *Ziziphus* leaves becomes particularly lustrous and soft (Miller and Morris, 1988). It has been reported that the volatile fraction of flowers and leaves of *Z. spina-christi* possesses antimicrobial activity (El-Hamouly and Mohamed, 2001) and the plant is rich in flavoniod glycosides and triterpenoid saponins.

A. tortilis is the most commonly seen tree in Oman (Miller and Morris, 1988). The plentiful flowers of *A. tortilis* attract honey bees and these flowers are known to produce the best honey in Oman. Even though the reports on flavonoid isolation from *A. tortilis* (Muhaisen *et al*., 2002) are available in the literature, the information on antioxidant properties of this plant are not found.

T. haussknechti is a bright-flowered shrub of rocky, lower altitude hill-sides which is the most common *Tephrosia* species in northern Oman (Mandaville, 1978). The leaves of *T. haussknechti* are used to treat earache, swollen joints (Ghazanfar, 1994) but no antioxidant properties are reported .

A. bracteolata is a source of aristolochic acids (El Tahir, 1991), which is used to treat wounds, snakebites but no antioxidant properties are reported in the literature.

The seeds of *C. colocynthis* are used to treat tumors and the green flesh of the immature fruit is taken in small quantities as a purgative (Miller and Morris, 1988). The fruits of *C. colocynthis* contain flavones (Maatooq, *et al*., 1997), triterpenes glycosides (Hatam, 1989) but antioxidant properties of the plant have not been reported.

T. mascatense is an aromatic perennial, a native of Oman which is used in traditional medicine for fever, diabetes and stomach pain (Mandaville, 1978; Ghazanfar, 1994). The composition and antimicrobial activity of this plant was recently reported (Hisham *et al*, 2006) no studies on the antioxidant properties of this plant is available.

R. stricta, known as '*harmal'* in Arabic is used in Arabian folk medicine as a remedy for respiratory ailments and fevers (Mandaville, 1978). Alkaloids have been isolated from the leaves of *R. stricta* (Atta-ur-Rahman and Habib-ur-Rahman, 1996) and antioxidant action of the leaves extract has been reported (Ali *et al*., 2000)

In traditional medicine, various parts of *N. oleander* are used for treatment of cough, swellings, eyes and skin diseases. Methanolic extract of the fresh leaves of *N. oleander* showed central nervous system (CNS) depressant effect in mice (Begum *et al*., 1999). Flavonoids were present in all organs of the plant (Duret and Paris, 1977), but roots and the leaves of *N. oleander* contain triterpenoids (Begum *et al*., 1997) with no antioxidant properties reported in the literature.

It was noticed that the antioxidant activity of the above selected plants were either not investigated or poorly investigated and therefore testing of their antioxidant and antiradical properties is of interest, primarily to find new promising sources of natural antioxidants.

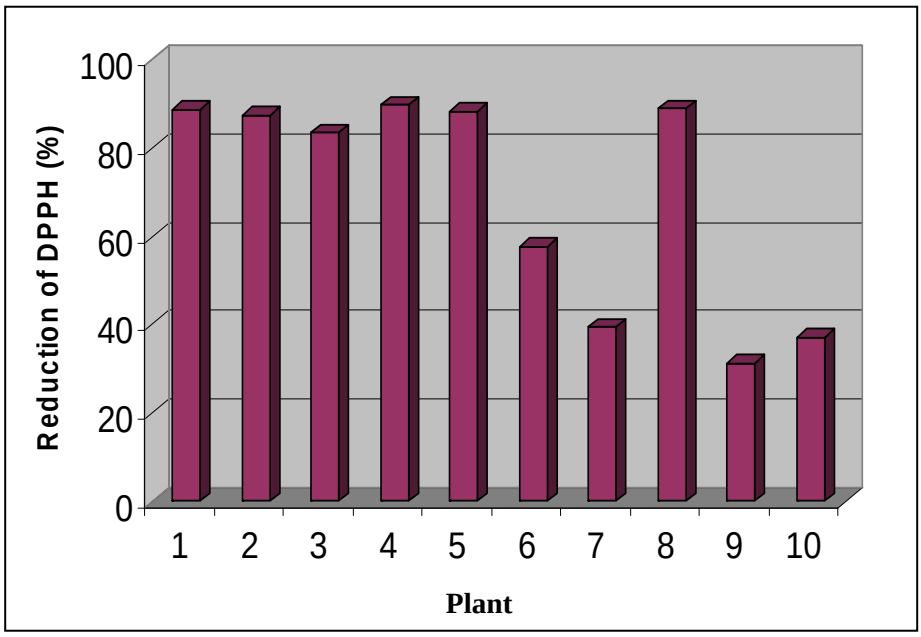

Figure-1: DPPH radical scavenging activities of *n*-BuOH extracts.

1. *Oscimum basilicum*; 2. *Cressa cretica*; 3. *Ziziphus spina-christi*; 4. *Acasia tortilis;* 5. *Tephrosia hausknechti*; 6 *Aristolochia bracteolate*;7 *Citrullus colocynthis*; 8.*Teucrium mascatense;* 9 *Rhazya stricta* ;10. *Nerium Oleander*

2. Results and discussion

2.1 DPPH radical-scavenging activities

The n-BuOH extracts of the plants were prepared as described in section 3 and their free radical scavenging activity was determined by DPPH free radical method (Brand-Williams *et al*., 1995). The n-BuOH extract of *Ocimumi basilicum* (Labiatae), a plant well known for its antioxidant and radical scavenging properties, was used as a reference.

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 nm. When $DPPH^{\bullet}$ reacts with an antioxidant compound, which can donate hydrogen, it is reduced and the color changes from deep-violet to light-yellow. The results of DPPH radical-scavenging activity of *n*-butanol extracts are shown in Figure 1.

Six out of nine *n*-BuOH extracts used in the present study showed high level of DPPH scavenging activities ie., *C.cretica*-87.7%, *Z. spina-christi*-83.5%, *A. tortilis*-89.8 %, *T. hausknechti*-88.4% and *T. mascatense*-89.1%. In fact the extracts of *A. tortilis* and *T. mascatense* are having remarkably higher level of radical scavenging activity compared to *O. Basilicum*. On the other hand, the extracts of *A. bracteolate*-57.8%, *C. colocynthis*-39.6%, *R. stricta*-31.3%, and *N. oleander*-37.2% showed only moderate or weaker activities.

2.2 Antioxidant activity evaluation by the phosphomolybdenum method

The antioxidant activity of the extracts was evaluated by phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound(s) and the formation of a green

phosphate/Mo(V) complex with a maximal absorption at 695 nm. The results of antioxidant activity exhibited by different plant extracts are summarized in Table 1.

Table 1. Antioxidant activities of *n*-BuOH extracts

Plant	A_w [a]
Oscimum basilicum	1.0
Cressa cretica	0.78
Ziziphus spina-christi	1.0
Acasia tortilis	1.4
Tephrosia hausknechti	1.0
Aristolochia bracteolata	0.88
Citrullus colocynthis	0.82
Teucrium mascatense	0.97
Rhazya stricta	0.75
Nerium oleander	0.99

[a] A_w, activity relative to *Oscimum basilicum* on a weight basis

The extract of *A. tortilis* exhibited higher antioxidant activity than *O. basilicum*, while extracts of *Z. spina-christi, T. hausknechti* and *N. oleander* showed antioxidant activities, similar to the reference extract. The extracts of *A. bracteolate, T. mascatense* and *C. colocynthis* showed moderate antioxidant activities, whereas *C. cretica* and *R. stricta* exhibited only weak activities compared to the reference plant extract.

3. Experimental

3.1 Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium phosphate and ammonium molybdate were purchased from Sigma-Aldrich and all solvents were of analytical grade.

3.2 Plant material

Ocimum basilicum (leaves and stems), *Cressa cretica* (leaves and stems), *Ziziphus spina-christ* (leaves), *Acacia tortilis* (leaves), *Tephrosia haussknechti* (leaves and stems), *Aristolochiae bracteolata* (leaves and stems), *Citrullus colocynthis* (fruits), *Teucrium mascatense* (leaves and stems), *Rhazya stricta* (leaves and stems) and *Nerium oleander* (leaves) were collected from Northern Oman during 2003 and were authenticated by Department of Biology, Sultan Qaboos University. The freshly cut plants were dried in the drying room with ventilation at ambient temperature prior to use for investigation.

3.3 Extraction

The dried, powdered plant materials were extracted with methanol (500 mL)at room temperature and the methanol extracts were concentrated under reduced pressure to get greenish viscous materials which were first partitioned with hexane/H_2O, then with EtOAc/H_2O and finally with *n*-Butanol/H_2O to give hexane extracts,

ethyl acetate extracts and *n*-Butanol extracts. The concentrated *n*-Butanol extracts were used for the evaluation of the antioxidant activities.

3.4 DPPH radical-scavenging activity

The free radical scavenging activity of the plant extracts was determined by DPPH free radical method (Brand-Williams *et al*., 1995). A 2.0 ml methanolic solution of DPPH (0.1 mM) was mixed with 0.1 ml of extract solution (0.1 mg/ml) in methanol and, after 60 min standing; the absorbance of the mixture was measured at 517 nm against methanol as the blank on a UV/visible light spectrophotometer (Shimadzu UV 1650 PC, Japan). Triplicate measurements were made and the radical scavenging activity was calculated by the percentage of DPPH that was scavenged using the following formula:

% Reduction = $[(A_B - A_A)/A_B] \times 100$

Where: A_B-absorption of blank sample; A_A- absorption of tested extract solution.

3.5 Evaluation of antioxidant activity

The antioxidant activity of plant extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*., (1999). An aliquot of 0.3 ml of sample solution (1 mM in methanol) was combined in a 4-ml vial with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of *Ocimumi basilicum* (Labiatae).

4. Conclusions

The *n*-BuOH extracts *A. tortilis*, *T. mascatense* and *T. hausknechti* were found to be the strongest radical scavengers in DPPH radical assay and the strongest antioxidants in phosphomolybdenum assay among the plants screened. They are promising plants for further investigation of antioxidant properties particularly *A. tortilis* of which antiradical and antioxidant activities were higher than that of *O. basilicum*.

In a previous work, we showed that aristolochic acid and aristolochic acid-D isolated from *A. bracteolata*, is as effective as vitamin C in antioxidant activity on a molar basis (Al-Busafi *et al*., 2004). The results in Table-1 suggests that the plants *A. tortilis*, *T. hausknechtii* and *Z. spina-christi* may have better antioxidants than vitamin C.

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