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# Evaluation of Usefulness of Wild Plants Growing in Beni-Sueif Region, Upper Egypt

**Sameh AbouZid***

Department of Pharmacognosy, Faculty of Pharmacy, Beni-Sueif University, P. O. Box 62111, Beni-Sueif, Egypt

*Corresponding author*: * **safekry@bsu.edu.eg** or **wssmd3000@yahoo.com**

## ABSTRACT

Extracts prepared from 20 commonly occurring wild plants growing in Beni-Sueif governorate, Upper Egypt were tested for the presence of alkaloids, anthraquinones, cardenolides/bufadenolides, saponins, flavonoids and tannins. *In vitro* antioxidant activity was tested only for plant extracts rich in flavonoids and/or tannins, 10 in total: *Alhagi maurorum*, *Bidens pilosa*, *Conyza dioscoridis*, *Dolichos lablab*, *Kochia indica*, *Phragmites communis*, *Polygonum salcifolium*, *Solanum nigrum*, *Tamarix nilotica* and *Zygophyllum coccenium*. *In vitro* antioxidant activity was tested using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay. Extracts of *B. pilosa* and *T. nilotica* possessed good antioxidant activity having $IC_{50}$ values of 16.47 and 16.26 µg/mL, respectively.

**Keywords:** Antioxidant, flavonoids, *in vitro*, tannins
**Abbreviations: DPPH**, 1,1-diphenyl-2-picrylhydrazyl

## INTRODUCTION

Biodiversity conservation and sustainable economic development are among the major national concerns in developing countries. Drug discovery through biological and chemical screening of plants (Hostettmann 1999) can be a catalyst to achieve these high level requirements. Drug discovery can also disclose new sources of such economic materials to be used as pharmaceuticals and finding out precursors for biologically active constituents. There are several approaches for selecting plants as candidates for drug discovery (Fabricant *et al.* 2001), namely, random selection followed by chemical screening, random selection followed by one or more biologic assays, follow-up of biologic activity reports, follow-up of ethnomedical uses of plants and an approach based on the use of databases that correlate ethnomedical use with experimental biochemical or pharmacologic activities. Several investigators evaluated crude extracts prepared from wild plants collected either randomly or based on known ethnomedical use (Khafagi *et al.* 2000; Asase *et al.* 2005; Soltan *et al.* 2009).

This study was conducted in Beni-Sueif governorate, Upper Egypt. Beni-Sueif is situated 115 km south of Cairo, capital of Egypt. It occupies a land area of approximately 10954 $km^2$, having a cultivated land of 1203.59 $km^2$. The governorate has a total inhabitancy of 1369.41 $km^2$. Three phyto-geographical regions can be distinguished in Beni-Sueif (Täkholm 1974; Boulos 2000): the desert on the western side of the Nile River that is considered as an extension of the African Sahara region, the desert of the eastern side of the Nile that extends with the official border of the governorate to the Red Sea and the fertile land on both sides of the Nile including canal banks distributed throughout the governorate. The Nile valley and its canal banks have the greatest diversity in Egypt (Batanouny 2005). The present project focused on a systematic evaluation of a selection from commonly occurring wild plant species growing in Beni-Sueif. There is no previous study on wild plants growing in Beni-Sueif for the purpose of the present work.

Twenty plant species belonging to 11 families that are growing in Beni-Sueif were selected. The plants were screened for the presence of alkaloids, anthraquinones, cardenolides/bufadenolides, saponins, flavonoids and tannins. This was followed by testing the *in vitro* antioxidant activity for extracts prepared from selected plants.

## MATERIALS AND METHODS

### Chemicals, solvents and reagents

The following solvents were used in this work: petroleum ether (60-80ºC), *n*-hexane, ether, ethyl acetate, chloroform, methanol and ethyl alcohol 95%, these solvents were obtained from El Nasr Pharmaceutical Chemicals Co., Egypt. Authentic compounds were obtained from E-Merck, Co., Darmstadt, Germany. Sheets of Whatman filter paper No. 1 for paper chromatography (E. Merck). Chemicals used for phytochemical screening and *in vitro* antioxidant activity were obtained for Sigma Chemical Co. St. Louis, MO, USA.

### Plant material

The selected plants (20 plants) were collected during the flowering stage from areas around Beni-Sueif region (boundaries of cultivated lands and Maydoum's desert) from March to October 2006. The plants were identified by Dr. M. Abdelhalim, Plant Taxonomy Department, Agricultural Research Center, Egypt. Voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Beni-Sueif University, Beni-Sueif, Egypt.

### Chemical screening

Aerial parts (vegetative material and flowers) of the 20 selected plants collected were dried in the shade then ground to a fine powder.

Ten g of the plant powder was extracted by 100 mL 70% ethanol for 45 min, filtered then divided into three portions. One portion of this extract was tested for alkaloids using Dragendorff's reagent (Kapoor *et al.* 1969). A second portion was tested for anthraquinones (Rizk 1982). Five mL of the filtrate was extracted by chloroform; the organic phase was then shaken with ammonia solution. Red color in aqueous layer indicated the presence of free

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Research Note

anthraquinone glycosides. Five mL of the filtrate was added to 1 mL concentrated HCL, boiled for 2 min, cooled, a few drops of hydrogen peroxide were added, and then extracted with chloroform. The organic layer was shaken with ammonia solution. Red color in the aqueous layer indicated the presence of combined anthraquinone glycosides. A third portion of the extract was tested for cardenolides/bufadienolides (Rizk 1982). Five mL of the filtrate was added to 5 mL of lead acetate, filtered then equal amount of disodium hydrogen phosphate was added, filtered then 2 mL of the filtrate was added to 2 mL of Baljet's reagent (9.5 mL picric acid + 0.5 mL 10% NaOH). Orange color developed after 20 min indicated the presence of cardiac glycosides.

Five g of the powdered plant material was added to 50 mL of water and warmed for 5 min then filtered. The first part of this extract was tested for tannins using $FeCl_3$. Another part of the aqueous extract was tested for saponins indicated by persistent froth.

One g of the powdered plant material was shaken with 10 mL 1% HCl for 5 min and filtered. This extract was tested for free flavonoids using 10% NaOH. Eight mL of the filtrate was shaken with 4 mL amyl alcohol. Yellow color developed in the organic layer indicates presence of free flavonoids. The aqueous layer was separated and added to equal amount of concentrated HCl, boiled for 2 min, cooled then extracted with 4 mL amyl alcohol. Yellow color developed in the organic layer indicated the presence of flavonoid glycosides (Segelman *et al.* 1969).

### *In vitro* antioxidant activity for plants rich in flavonoids and/or tannins

Measurement of DPPH radical scavenging activity was conducted on 11 species, which were rich in flavonoids and/or tannins, according to a previously reported method with a modification (Ichikawa *et al*. 2003). Ten g powdered plant material was extracted twice at room temperature with 100 mL methanol, then the extract was evaporated to dryness under reduced pressure using rotary evaporator. The residues left after drying (100 mg) were dissolved in 10 mL methanol. A 30 µl of each plant extract was added to 970 µl of DPPH solution (100 µM in methanol). After incubation at 25°C for 20 min in the dark, the absorbance at 517 nm was measured on UV-visible spectrophotometer (Shimadzu UV240 P/N 204-58000). Ascorbic acid was used as the positive control. The percentage inhibition of DPPH radical activity was calculated according to the following equation (Ichikawa *et al*. 2003):

% of inhibition of radical activity = $(A_0 - A_{20}) \times 100 / A_0$

$A_{20}$ = Absorbance after incubation for 20 min

$A_0$ = Initial Absorbance.

## RESULTS AND DISCUSSION

Two approaches were used for selection of higher plants as candidates for drug discovery. The first approach was random selection of plants followed by chemical screening. The procedure involved collection, identification, extraction and chemical screening of extracts derived from commonly occurring native plants. Extracts prepared from those plants were tested for the presence of alkaloids, anthraquinones, cardenolides/bufadienolides, saponins, flavonoids and tannins. Of the 22 plants investigated 13 plants contained saponins, 4 plants contained alkaloids, 2 plants contained anthraquinones, 2 plants contained cardiac glycosides and all plants contained flavonoids and tannins. Results of the chemical screening are shown in **Table 1**. Random selection of plants followed by chemical screening has been used for a long time in drug discovery programs especially in developing countries (Fabricant *et al.* 2001). The main advantage of this approach is its simplicity. However, false-positive or false-negative results and inability to relate one class of phytochemicals to specific biological action are the main disadvantages.

The second approach was biological assay for a selection of plants from the earlier chemical screening step. We selected plant extracts that were rich in flavonoids and/or tannins. The selected plants were: *Alhagi maurorum*, *Bidens pilosa*, *Conyza dioscoridis*, *Dolichos lablab*, *Kochia indica*, *Phragmites communis*, *Polygonum salcifolium*, *Solanum nigrum*, *Tamarix nilotica* and *Zygophyllum coccineum*. Extracts of these plants were tested for their *in vitro* antioxidant activity. A simple, rapid and sensetive method for antioxidant screening of plant extracts using DPPH radical was used. Free radicals are involved in a number of pathological conditions such as inflammatory diseases, atherosclerosis, cerebral ischemia, AIDS, and cancer (Thomas *et al.* 1997). These free radicals are induced in the human body due to environmental pollutants, chemicals, physical stress, radiation, etc. Consumption of antioxidants or free radical scavengers is necessary to compensate depletion of antioxidants of the immune system. There is an increasing interest in the use of medicinal plants as antioxidants. In our experiments, extracts of *B. pilosa* and *T. nilotica* were found to possess a good antioxidant activity with $IC_{50}$ values of 16.47 ± 0.33 and 16.26 ± 0.31 µg/mL, respectively (**Table 2**).

*B. pilosa* is an annual herb 0.2-1.2 m, growing on canal banks, road sides and waste ground in Egypt (Boulos 2000). Literature review has indicated that *B. pilosa* contains fla-

**Table 1** Chemical screening of investigated plants growing in Beni-Sueif, Upper Egypt.

| Plants | Family | Voucher No. | Phytochemical class a | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Alk. | Anth. | Card. | Sap. | Flav. | Tan. |
| *Alhagi maurorum* Medik. | Leguminoseae | BSP101 | - | - | - | ++ | + | ++ |
| *Sesbania sesban* (L.) | Leguminoseae | BSP116 | - | - | - | ++ | + | + |
| *Dolichos lablab* (L.) | Leguminoseae | BSP109 | - | - | - | + | ++ | + |
| *Conyza dioscoridis* (L.) | Asteraceae | BSP104 | - | - | - | traces | ++ | + |
| *Conyza linifolia* (Willd.) | Asteraceae | BSP105 | - | - | - | + | + | + |
| *Bidens pilosa* (L.) | Asteraceae | BSP103 | _ | + | + | + | + | ++ |
| *Amaranthus chlorostachys* (L.) | Amaranthaceae | BSP102 | - | - | + | + | + | + |
| *Cynodon dactylon* (L.) Pers. | Gramineae | BSP106 | - | - | - | traces | - | - |
| *Echinochloa colona* (L.) | Gramineae | BSP110 | - | - | - | traces | + | + |
| *Phragmites communis (L.)* | Gramineae | BSP114 | + | - | - | + | + | ++ |
| *Cyperus rotundus* (L.) | Cyperaceae | BSP108 | - | - | - | traces | + | - |
| *Cyperus laevigatus* (L.) | Cyperaceae | BSP107 | - | - | - | traces | + | - |
| *Polygonum salcifolium* (L.) | Polygonaceae | BSP113 | - | - | - | traces | ++ | ++ |
| *Rumex dentatus* (L.) | Polygonaceae | BSP115 | - | + | - | + | + | + |
| *Zygophyllum coccenium* (L.) | Zygophyllaceae | BSP120 | - | - | + | ++ | + | ++ |
| *Solanum nigrum* (L.) | Solanaceae | BSP117 | + | _ | _ | ++ | ++ | + |
| *Withania somnifera* (L.) Dunal. | Solanaceae | BSP119 | + | - | - | + | + | + |
| *Mentha longifolia* (L.) Huds. | Labiateae | BSP112 | + | - | - | traces | + | + |
| *Kochia indica* (Wight) | Chenopodiaceae | BSP111 | - | - | - | + | ++ | + |
| *Tamarix nilotica* (L.) | Tamaricaceae | BSP118 | - | - | - | + | + | ++ |

a alk. = alkaloid, anth. = anthraquinones, card. = cardenolides/bufadienolides, sap. = saponins, flav. = flavonoids, tan. = tannins. +: present, -: absent, ++: present in large amount.

**Table 2** Free radical scavenging activity of investigated plants.

| Plant name | $IC_{50}$ ± S.D. (µg/mL) |
|---|---|
| *Alhagi maurorum* | 26.98 ± 1.88 |
| *Bidens pilosa* | 16.47 ± 0.33 |
| *Conyza dioscoridis* | 21.87 ± 1.80 |
| *Dolichos lablab* | 70.09 ± 1.15 |
| *Kochia indica* | 66.67 ± 0.94 |
| *Phragmites communis* | 81.52 ± 1.42 |
| *Polygonum salcifolium* | 25.77 ± 1.02 |
| *Solanum nigrum* | 60.00 ± 1.82 |
| *Tamarix nilotica* | 16.26 ± 0.31 |
| *Zygophyllum coccenium* | 394.74 ± 2.85 |
| Ascorbic acid (reference) | 10.06 ± 0.42 |

vonoids, centaurein and centaureidin, that have immunomodulatory activity (Changa *et al*. 2007).

*T. nilotica* is a tree 2-5 m; wide spread in Egypt, growing in saline sandy soils, edges of salt marshes, coastal and inland sandy plains and Nile banks (Boulos 2000). *T. nilotica* has been used in the Egyptian traditional medicine as antiseptic agent. This plant is known since the pharaonic time and has been mentioned in medical papyri to expel fever, relieve headache, to draw out inflammation and as an aphrodisiac (Kamal 1967). Literature review has indicated that *T. nilotica* contains many phenolic and flavonoid constituents. Ethyl ester of kaempferol 3-*O*-*β*-D-glucuronide, the methyl and ethyl esters of quercetin3-*O*-*β*-D-glucuronide are the main flavonoids isolated from the flowers of the plant. Methyl ester of ferulic acid 3-*O*-sulphate, coniferyl alcohol 4-*O*-sulphate, kampferol 4′-methyl ether, kampferol 3-*O*-*β*-D-glucupyranuronide, tamarixetin, tamarixetin 3-*O*-*β*-D-glucupyranuronide and quercetin 3-*O*-*β*-D-glucupyranuronide were isolated from the leaves of the plant (El-Sisi *et al*. 1973; Nawwar *et al*. 1982, 1984a, 1984b; Barakat *et al.* 1987; AbouZid *et al.* 2009; Orabi *et al.* 2009, 2010). Therefore, there is a possibility that the proven *in vitro* antioxidant activity of *T. nilotica* is due to its phenolic constituents.

## CONCLUDING REMARKS

The combined chemical and biological screenings provided information about antioxidant activities of wild plants growing in Beni-Sueif governorate. Significant *in vitro* antioxidant activity was demonstrated in *B. pilosa* and *T. nilotica*. Our results may serve as a useful strategy in the search for natural products with potential biological activity.

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