INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

# PHARMACOGNOSTICAL, ANTIMICROBIAL AND LAXATIVE STUDY OF *SCORZONERA UNDULATA* IN LIBYA

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Article Received on: 16/02/13 Revised on: 01/03/13 Approved for publication: 21/04/13

**DOI: 10.7897/2230-8407.04415**

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**ABSTRACT**

This work describes the microscopical, phytochemical, antimicrobial and laxative studies of methanolic extracts of both aerial and root parts of *Scorzonera undulata* (Asteracea) which is commonly known in Libya as Elgiz. The results indicate the presence of some secondary metabolites; coumarines, flavonoids, tannins and different types of glycosides; phenolic, anthraquinone and cardiac glycosides. The aerial part extract also exhibited antimicrobial activities against three standard strains of bacteria; *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* with a reasonable low minimum inhibitory concentration (MIC) against *Escherichia coli* of 25 mg/ml while the root part extract showed antibacterial activity only against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with a minimum inhibitory concentration of 25 mg/ml and 100 mg/ml respectively. Both extracts of *Scorzonera undulate* didn't show any anti-fungal effect against the fungus *Candida albicans*. The laxative study was applied only on the aerial part of *Scorzonera undulata* (200 and 400 mg/kg) using gastro-intestinal transit of a charcoal meal method where it exhibited a high significant dose dependent increase in the percentage of total length of the intestine. These findings proved the folk use of *Scorzonera undulate* as a laxative plant.

**Keywords:** *Scorzonera undulata*, Asteracea, Laxative study, Antimicrobial effect

## INTRODUCTION

The Scorzonera is a genus belonging to the family Asteraceae. It grows mainly in dry areas of Europe and Asia. It involves about 90 species distributed throughout Europe, Asia and Africa[1]. In Libya, it consists of 3 species: *Scorzonera laciniata, Scorzonera hispanica* and *Scorzonera undulata*[2].

*Scorzonera undulata* is a perennial species which is a diploid and a highly polymorphic plant. It grows in the pastures, hills and sandy clay alluvium. *Scrozonera undulata* is mainly used as food, however in Tunisia, the roots are appreciated for their sweetness; they are either eaten raw or cooked in water. They are also used to prepare a decoction for its benefits as depurative. The ashes of burned roots are said to be effective in the treatment of burns[3]. In Libya *Scrozonera undulata* is commonly named as Elgiz and the leaves are used traditionally as laxative. The chemical composition and antimicrobial activities of volatile components from capitula and aerial parts of *Scrozonera undulata* have been described[4]. In fact 36 constituents were identified in the oil and the main components of them were methyl hexadecanoate 30.4%, methyl linolenate 23.9% and heneicosane 12.2%. The *Scrozonera undulata* oil exhibited an interesting antibacterial activity against gram-positive and gram-negative bacteria but no antifungal activity was detected.

The aerial part ethyl acetate extract of *Scrozonera undulata* seemed to be more active than the petroleum one. It showed an antibacterial activity against all bacteria strains tested *Staphylococcus aureus, Enterococcus fecalis, Proteus mirabilis, Pseudomonas aeruginosa* and *Citrobacter freundeï* except for *E. coli*. For antifungal activity, the petroleum ether and ethyl acetate extracts of the aerial part inhibited strong action against all tested fungi. The roots extracts from *S.undulata* showed weak antimicrobial activities compared with the aerial parts extracts of the same plant[3]. The antioxidant activity was investigated with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method using Blois method modified by Brand-Williams *et al*.[5] and Molyneux[6]. It was found that the methanol extract showed significant antioxidant activity compared to the reference antioxidant trolox in a dose dependent manner. IC50 value of the extract was found to be 0.6 µg/ml while the IC50 value of the positive control (trolox) was found to be 0.87 µg/ml.

Under normal circumstances constipation is due to an inappropriate diet, lack of physical activity and/or psychological factors[7]. It is characterized by reduced and difficult bowel movements. Generally, the reasons are functional and not serious in nature, but continuous irregularity in bowel movements should always be investigated in case there is a risk of malignant disease. The subjective symptoms (straining heavily, hard stool, painful defecation and a feeling of insufficient evacuation) make it one of the most commonly reported health problems. Various types of plant-derived laxative are used; stimulant and more drastically acting laxatives (purgatives), which act mainly via physiochemical effects within the bowel lumen. These are anthraquinones such as emodin, aloe-emodin, related anthrones and anthranols, they are products of acetate biosynthetic pathway and are commonly found as glycosides in the living plants[7].

Although the aerial parts of *Scrozonera undulata* present in Libya have been traditionally used in the treatment of constipation, there is no extensive laxative study of this valuable plant. To prove the ethno-medical claims, the present study was designed to evaluate the laxative activities of its methanolic extract in mice model. Microscopical study of the powdered *Scrozonera undulata* aerial parts and roots were done, the methanolic extracts of both parts of *S. undulata* were screened for phytochemical and in vitro antimicrobial activities.

## MATERIALS AND METHODS

### Plant material and extraction

*Scorzonera undulata* was gathered at the flowering stage in April 2012 in the area of Zentan (West Mountain in Libya). The Plant material was identified by Botany department,

Faculty of Sciences, Tripoli University, Libya and a voucher specimen was deposited at the Herbarium. The aerial parts and roots of *Scrozonera undulata* were air-dried for several weeks, powdered separately and examined under microscope for detection of key elements.

The powdered aerial parts and roots of *Scrozonera undulata* have been macerated separately in methanol for 3 days at room temperature, The combined methanolic extracts were concentrated by means of rotatory evaporator at a temperature not exceeding 60° C under reduced pressure, the concentrated extracts left uncovered in glass containers for few days to assure complete drying then stored at 4°C until tested.

**Phytochemical investigation**

The methanolic extracts of both the aerial parts and roots of *Scorzonera undulata* were subjected for detection of secondary metabolites such as alkaloids, glycosides, flavonoids, coumarins, tannins…etc.[8].

**Antimicrobial tests**

The methanolic extracts of both the aerial parts and roots of *Scorzonera undulata* were tested against three bacterial species including one gram positive species (*Staphylococcus aureus* ATCC 29213), two gram negative species (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* NCTC 6749) and one fungal species (*Candida albicans*) obtained from Liofilchem, Italy.

The antimicrobial activity was performed using the agar cup cut diffusion method[9]. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures (18 hours culture of each microbial species) to sterilized Mueller-Hinton agar for bacteria species and Sabouraud dextrose for Candida species. The medium was punched with 8 mm diameter wells and filled with 100 µl of the test sample. Positive control (Phenol) was included in the test. The plates were incubated without agitation for 24 hours at 37°C and inhibition zones formed around the wells were measured (mm) using a caliper. All tests were done in duplicate and the results were recorded as the mean diameter of the zones of growth inhibition surrounding the discs.

The Minimum Inhibitory Concentration (MIC) was determined by broth microdilution technique[10], the 96-well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculum into each well. 100 µl of the stock solution of *Scrozonera undulata* at the concentration of 5 mg/ml was added into the first wells. Then, 100 µl of their serial dilutions was transferred into 6 consecutive wells. The last well containing 195 µl of nutrient broth without the compound and 5 µl of the inoculum from each strip was used as negative control. The final volume in each well was 200 µl. The plate was covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker at 300 rpm for 20 seconds and then incubated at appropriate temperatures for 24 hours. Microbial growth in each medium was determined by reading the respective absorbance (Abs) at 600 nm and was confirmed by plating 5 µl samples from clear wells on the nutrient agar medium. The extracts tested in this study were screened two times against each organism. The MIC was defined as the lowest concentration of the respective compound able to inhibit the growth of microorganisms.

**Animals**

Albino female mice weighing between (20-30 g) were used throughout this study for both test and control groups. The animals were obtained from the local animal house of the Pharmacology Department, Faculty of Pharmacy, Tripoli University, Tripoli-Libya. The animals were overnight fastened before the experiment but allowed access to water.

**Gastro-Intestinal Transit of a Charcoal Meal Method**

This method involves measuring the transit time of charcoal meal which is easily visible through the gastrointestinal tract of mice[11]. The methanolic extracts, charcoal meals and tween 80 as a control were administrated orally by means of intra-gastric feeding syringe.

The fastened animals were pretreated with the test plant extract and 15 min later a charcoal meal (0.5 ml of an aqueous suspension of charcoal in 5% gum acacia) was given orally. The animals were killed by ether inhalation 20 minutes after the meal; the intestine and stomach were then removed. The pylorus is attached to a glass rod and the intestine suspended for 20 seconds with a weight of 3 g attached to the ileo-caecal junction, to straighten it out. The mean distance travelled by the charcoal was then measured and compared with the control group, expressing results as a percentage of the total length of intestine obtained from the formula (total length of small intestine=distance moved by charcoal (cm)/ intestinal length (cm) x 100).. .

**Statistical analysis**

Data generated from the above studies were statistically analyzed with "SPSS", a computerized statistical program (version 13.0). Results were expressed as Mean ± S.E. The results were analyzed for normality of distribution (i.e. if the results obtained are parametric or non-parametric), using Kolmogorove-Smirnov test. The one-way analysis of variance (ANOVA) was used for comparing more than 2 means of a parametric data followed by the LSD`s post hoc multiple comparisons to determine which population means were different. The differences between data are considered to be significant if the $P<0.05$ and a highly significant if $P<0.01$.

**RESULTS AND DISCUSSION**

Microscopical examination of powdered aerial parts and roots of *Scorzonera undulata* showed the following features:

A. A unicellular, non-glandular trichomes branched (stelatte), B. Non-glandular unicellular trichomes with large lumen, C. Fragments of fiber compact with no lumen, D. Anomocytic stomata from 4-5 subsidiary cells, E. Parenchyma cells with intercellular spaces, F. Elongated parenchyma cells with diosmin flavonoid and G. Starch grains round shape with no hilum (Figure 1).

*Scorzonera undulata* revealed the presence of some secondary metabolites; coumarines, flavonoids, tannins and different types of glycosides; phenolic, anthraquinone and cardiac glycosides (Table 1). Both phenolics and essential oils are thought to be responsible for antimicrobial effects which include tannins and flavonoids[7].

**TABLE 1: PHYTOCHEMICAL SCREENING OF *SCORZONERA UNDULATA***

| Test | Observation | Result |
|---|---|---|
| Anthraquinone glycosides | Rose color | +ve |
| phenolic glycosides | Red Brown precipitate | +ve |
| Flavonoids | Pale yellow color | +ve |
| Coumarins | Fluorescence | +ve |
| Saponins | No formation of froth | -ve |
| Alkaloids | No precipitate | -ve |
| Tannins | Blue green | +ve |
| cardiac glycoside | Brown ring | +ve |

+ve=present, -ve= absent.

**TABLE 2: DIAMETER OF INHIBITION ZONES (MM) OF METHANOLIC EXTRACT OF *SCORZONERA UNDULATA***

| Microorganism | Aerial part (mm) | Root part (mm) | Phenol (mm) |
|---|---|---|---|
| *Escherichia coli* | 12 | Zero | 25 |
| *Pseudomonas aeruginosa* | 18 | 14 | 30 |
| *Staphylococcus aureus* | 12 | 14 | 37 |
| *Candida albicans* | Zero | Zero | 31 |

(mm) Diameter of Inhibition zone.

**TABLE 3: MINIMUM INHIBITORY CONCENTRATION ACTIVITY OF *SCORZONERA UNDULATA***

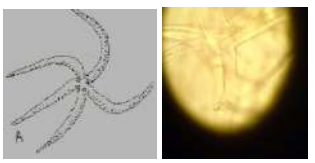
| Plant organ | Microorganism | MIC (mg/ml) |
|---|---|---|
| Root part | *Staphylococcus aureus* | 25 |
| | *Pseudomonas aeruginosa* | 100 |
| Aerial part | *Escherichia coli* | 25 |

**(**MIC) Minimum Inhibitory Concentration

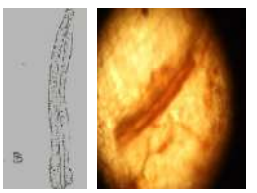
**TABLE 4: THE LAXATIVE EFFECT OF *SCORZONERA UNDULATA* AERIAL PART BY GASTRO-INTESTINAL TRANSIT OF A CHARCOAL MEAL METHOD**

| Treatment (mg/kg) | Percentage of total length of intestine (%) |
|---|---|
| Control (Tween 80) | 60.8 |
| *Scorzonera undulata* 200 | 72.8** |
| *Scorzonera undulata* 400 | 96.8** |

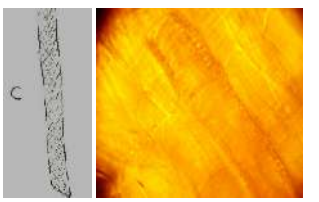
The values are the % of total length of intestine; ** highly significant different from the control group at $p<0.01$, (n=7).



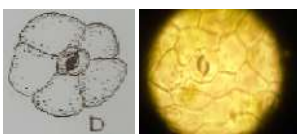
A.unicellular,non-glanddular Trichome branched(stelatte).



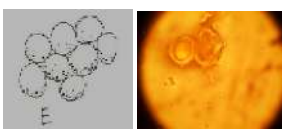
B.Non-glandular,unicellular Trichome,with large lumen.



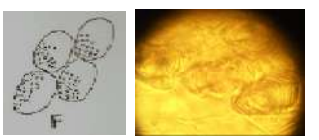
C.fragment of fiber compact with no lumen.



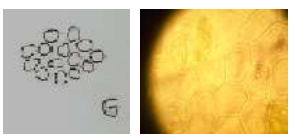
D.Anomocytic stomata, from 4-5 subsidary cell.



E.parenchyma cells with intercellular spaces.



F.Elongated parenchyma cells with diosmin (flavonoid) KOH→ Yellow.



G.starch grains round shape with no hilum

**Figure 1: Powdered aerial parts and roots of *Scorzonera undulata* with magnification power of X10, X40.**

The antimicrobial study of the methanolic extracts of *S. undulata* by agar cup cut diffusion method showed antibacterial activity of the aerial part against three strains of bacteria which were *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* while that the root part showed antibacrterial activity against only *P. aeruginosa* and *S. aureus*. The antifungal activity against *Candida albicans* was negative for both the aerial and root parts of *S. undulata* (Table 2). The aerial part of *S. undulata* exhibited reasonable low MIC against *Escherichia coli* 25 mg/ml, while the *S. undulata* root part showed a higher MIC against *P. aeruginosa* 100 mg/ml and low *MIC against S. aureus* which was 25 mg/ml (Table 3). The pharmacological effectiveness of glycosides is dependent on the aglycones, but the sugars render the compounds more soluble and increase the power of fixation of the glycosides[12]. Plants containing anthraquinone glycosides have been known to possess laxative activities, amongst which are senna leaves, frangula and Cascara barks[7]. The laxative effect of anthranoid containing drugs is due to increased peristalsis of the colon. The result is a reduced transit time and consequent reduction in reabsorption of water from the colon. Additionally, the stimulation of active chloride secretion results in an inversion of normal physiological conditions and a subsequent increased secretion of water. Overall, this result in an increase of the faecal volume with an increase in the GI pressure[7]. The effects of graded doses of the methanolic extract of *S. undulata* on gastrointestinal motility showed a high significant increase ($P< 0.01$) in the percentage movement of charcoal plug in a dose dependent manner

(Table 4). It could therefore be suggested that the secondary plant metabolites present in *S. undulata* are responsible for the biological activities observed and the increase of the intestinal motility could be useful actions in the treatment of constipation. The presence of tannins in the plant which has an astringent property may decrease the purgative action of anthraquinone glycosides rendering its laxative effect mild.

## CONCLUSION

Extracts of *Scrozonera undulata* have been found to possess antimicrobial and laxative activities which make it a good candidate for further works in constipation management. However, it is advised that herbal practitioners should be aware of the unlikely side effects that may occur, especially on prolonged usage. Furthermore, the presence of cardiac glycosides in the plant should be taken into consideration. Further pharmacological studies on the plant are needed.

**ACKNOWLEDGEMENTS**

The authors would like to thank all people who supported and helped in performing this research Dr. Suhair Aburawi, Mrs. Laila Sharef Gashoot, Mr. Musa Nouh, Mr. Abdusalam Swaiah, Miss Najat Elmegrh and Miss Aida galboon, Faculty of Pharmacy Tripoli University.

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**Cite this article as:**

Kargol H. S., Elgadi H. M., Gadamsi M. T, Shubar H. M. and Geroushi A. M.. Pharmacognostical, antimicrobial and laxative study of Scorzonera undulata in Libya. Int. Res. J. Pharm. 2013; 4(4):96-99

Source of support: Nil, Conflict of interest: None Declared