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Biodetermination of Plant Saponins Content by Using *Trichoderma viride*

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ABSTRACT

The saponins contents of six Egyptian plants; *Alhagi maurorum*, *Lippia nodiflora*, *Pergularia tomentosa*, *Spergularia marina*, *Tribulus terrestris* and *Zygophyllum album* were biologically determined by using *Trichoderma viride* Pers. a sensitive fungus to saponins. Potato-glucose medium was used in culturing the fungus. The experiment revealed that the high value of saponins contents was recorded in *Spergularia marina* (6.06 mg/100 ml). Whereas, the minimum value was recorded in *Lippia nodiflora* and *Alhagi maurorum* (1.14 and 1.88 mg/100 ml, respectively).

Key words: *Trichoderma viride*, *Pergularia tomentosa*, *Spergularia marina*, *Alhagi maurorum*, *Lippia nodiflora*, *Zygophyllum album*, saponins.

INTRODUCTION

Saponins, group of secondary metabolites, are found in large number of plant species. They are the large group of glycosides that characterize by the surface-active properties. They dissolve in water forming colloidal solutions and soap like foams after shaking (Tyler *et al*., 1981). Saponins were used as soap and many trivial names of saponins-rich species are derived from this feature, for example, soapwort (*Saponaria officinalis*), Soaproot (*Chlorogalum pomeridianum*), soapbark (*Guillaju saponaria*), soapberry (*Sapindus saponaria)* and soapnut (*Sapindus mukurossi*) (Hostettman and Marston, 1995).

Saponins can be classified into two major groups based on the nature of their aglycones skeleton. The first group consists of the steroidal saponins, while the second consists of the triterpenoidal saponins. The steroidal saponins almost present in the monocotyledonous angiosperms, while the triterpeniod saponins is mainly found in the dicotyledonous angiosperms. Some authors distinguish a third group called steroidal amines, which are classified by others as steroidal alkalloids. The sugars can be attached to the aglycone either as one, two or three side chains. This attachment causes great natural diversity of saponins structures (Bruneton, 1995).

Saponins are believed to form the main constituents of many drugs and folk medicines. Many saponins have pharmacological properties and are used in phytotherapy and in the cosmetic industry (Estrada *et al.,* 2000). The fungi exhibited a high susceptibility for both triterpenes and steroidal saponins. Their antifungal activity has been widely investigated, predominantly for possible pharmaceutical and agriculture purposes. Some saponins exhibit fungicidal activity at very low concentration. Medicagenic acid provides a good inhibition effect to *Trichoderma viride* growth at low concentrations (Oleszek, 1999). Biological and pharmacological properties of saponins have been reported in several reviews. Most saponins have hemolytic properties that they have the ability to rupture erythrocytes (Ahn *et al.,* 1998; Baumann *et al*., 2000; Oda *et al.,* 2000). Saponins are also highly toxic to cold-blood species. The toxicity towards these species has lead to the use of drugs containing saponins to catch fish (Bruneton, 1995). Also, saponins have been isolated from the plant sources exhibited an anti-inflammatory effect (Just *et al.,* 1998) and antitumer activity (Itabashi *et al.,* 1999).

The present study was performed to determine the saponins contents of six Egyptian plants by using a quick biological method. This method depends upon the inhibitory effect of saponins on the mycelial growth of *Trichoderma viride* Pers.

MATERIALS AND METHODS

Plant materials

The plants under investigation were collected from different habitats in Egypt (Table 1). The plant species were identified according to Täckholm (1974) and Boulos (2000) and confirmed by matching with (ASW) herbarium specimens.

Preparation of extract

The dry powder of each plant was extracted with 80% EtOH by maceration until exhaustion. The alcohol extract was concentrated into a syrupy consistency (Hamed *et al.,* 2004; Hamed *et al.,* 2005).

Preparation of culture medium

Potato-glucose solution was prepared by boiling 200g plied potatoes in one litre distilled water for 20 min. After straining of potatoes out, 20 gm of glucose were added to the filtrate and completed into one litre by distilled water.

Saponins test

Half gram of each extract was dissolved in 100 ml of potato-glucose solution using 250 ml Erlenmeyer flasks and 2 g of agar were added. Control was prepared by using potato-glucose-agar mixture and water instead of saponins solution. All contents were autoclaved, and

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Table (1): Names, synonyms, vernacular names, collecting places and traditional uses of the studied plants.

***Pergularia tomentosa* L.**	
Family	Asclepiadaceae
Synonyms	*Daemia cordata* (Frossk), *Deamia tomentosa* L.
Vernacular name	Ghalqa
Collecting place	Wadi Allaqi
Traditional uses	Depilatory, poultice, laxative, anthelmintic and abortive.
Reference:	Ibn El Betar (1809)
***Spergularia marina* (L.) Bessler**	
Family	Caryophyllaceae
Synonyms	*Arenaria ruba* L. var. *marina* L., *Arenaria marina* (l.) Roth
Vernacular name	Gileglaag
Collecting place	Wadi Abaddi
Traditional uses	Lithontripic, diuretic, relax muscle walls of urinary tubules, treatment of kidney stones, chronic cystitis and catarrh of the bladder.
Reference	Copra *et al.* (1956)
***Alhagi maurorum* Medic**	
Family	Leguminosae
Synonyms	*Alhagi mannifera* Desv. *Hedysarum alhagi* L.
Vernacular name	Aqool
Collecting place	Aswan
Traditional uses	Anti-asthmatic, aphrodisiac, anti-pyretic, appetizer, anti-rheumatic, diuretic, and laxative
Reference	Atta and Mouneir (2004)
***Lippia nodiflora* (L.) Michx**	
Family	Verbenaceae
Synonym	*Phylla nodiflora* (L.) Greene
Vernacular name	Hasheesh libbeia
Collecting place	SouthValley University campus (Aswan)
Traditional uses	Sedative, spasmolytic, expectorant, anti-septic and treating respiratory infections.
Reference:	Pascual *et al* (2001)
***Tribulus terrestris* L.**	
Family	Zygophyllaceae
Synonym	*Tribulus lanuginosus* L
Vernacular name	Khrshoom
Collecting place	Wadi Allaqi
Traditional uses	Aphrodisiac, aperient, tonic, diuretic, refrigerant, carminative, and treatment of anemia, spermatorrhoea, asthma, and burning sensation.
Reference	El-Antaki (1923)
***Zygophyllum album* L.**	
Family	Zygophyllaceae
Synonym	*Zygophyllum proliferum* Forssk.
Vernacular name	Bahsanaan, Orfass
Collecting place	Wadi El-Gimal (Red Sea)
Traditional uses	For rheumatism, gout, cough, asthma, hypertension, flatulent colic and as diuretic.
Reference:	El-Antaki (1923)

then the content of each flask was poured into Petri dishes (30 ml in each dish). Five millimeter diameter discs of *Trichderma viride* (72 hrs age grown in control conditions) were inoculated in each Petri dish. Dishes were incubated at 30°C and the diameter of fungus growth were measured after 48 hrs.

Preparation of standard curve

Standard curve was prepared by dissolving 10, 20, 30, 40, 50 and 60 mg of alfa-alfa biologically active saponins in 25 ml of distilled water. Five ml of each solution were completed to 100 ml by adding 95 ml of potato-glucose solution and 2 gm of agar. All prepared solution were sterilized and tested with *Trichoderma viride* as described before.

The growth inhibition of *T. viride* was calculated from the following equation:

$$\text{Growth inhibition} = 100 - (Ds \times 100) / Dc$$

Where **Ds** is the diameter of *T. viride* growth on the sample medium and **Dc** is the diameter of *T. viride* growth on the control medium.

The concentrations of saponins were calculated using the standard curve and the percentage of saponins content were calculated using the following equation:

$$\text{Saponins \%} = (C \times 100) / W$$

Where, **W** is the weight of the sample (mgs), and **C** is the concentration of saponins content

RESULTS

The results of the present study showed that the growth of *Trichoderma viride* fungus is affected by the saponins contents of the plant. The growth diameter, the inhibition percentage of *T. viride* growth and the saponins contents of the different plant species are provided in table (2).

Table (2): Average diameter of *Trichoderma viride* growth, inhibition percentage, and saponins concentrations of the plants extracts.

Plant species	Weight of extract (gm)	Diameter of *T. viride* Growth (cm)	Trichoderma growth inhibition %	Saponins conc. mg/100 ml
Pergularia tomentosa L.	0.5	4.1	24.03	3.8
Spergularia marina (L.) Bessler	0.5	3.21	40.67	6.06
Alhagi maurorum Medic	0.5	4.92	8.85	1.88
Lippia nodiflora (L.) Michx	0.5	5.2	3.67	1.14
Tribulus terrestris L.	0.5	4.7	12.96	3.31
Zygophyllum album L.	0.5	4.75	12.33	2.31

The data revealed that the minimum growth diameter of *Trichodema viride* was recorded in the media which contain the extract of *Spergularia marina* (3.21 cm). This data means that the inhibitory substance (saponins) is present in a high concentration (6.06 mg/100 ml) in this plant extract. Meanwhile, the maximum value of growth diameter of *T. viride* were recorded in media that contain the extracts of *Lippia nodiflora* and *Alhagi maurorum* (5.2 cm and 4.92 cm, respectively). These maxima growth diameters of *T. viride* were resulted from the low concentrations of saponins in the two plants (1.14 and 1.88 mg/100 ml, respectively).

Adequate amounts of saponins (2.31-3.8 mg/100 ml) were determined in the extracts of *Pergularia tomentosa*, *Tribulus terrestris* and *Zygophyllum album*. Saponins causes an inhibition for the growth of *T. viride* and the growth diameters of the fungus were relatively low in the media that contain these extracts (Fig. 1).

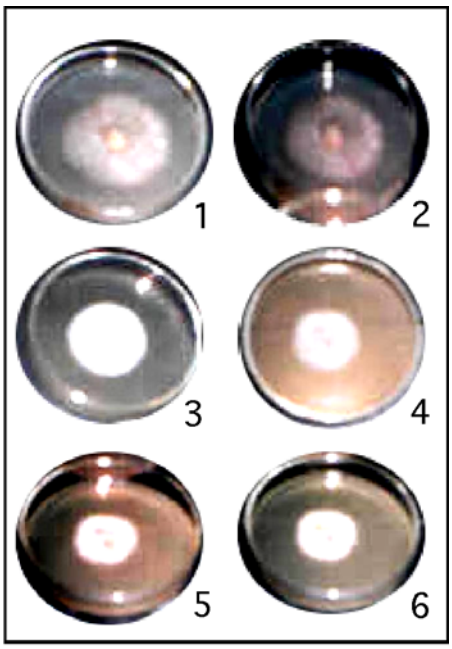

Figure (1): Effects of tested plant extracts on *Trichoderma viride* growth: (1) *Lippia nodiflora*, (2) *Alhagi maurorum*, (3) *Zygophyllum album*, (4) *Tribulus terrestris*, (5) *Pergularia tomentosa*, and (6) *Spergularia marina*.

Discussion

Many works have been done on the isolation, identification, and structure elucidation of new saponins by using different chromatographic techniques (e.g. Oleszek and Bialy 2006), but no more works were done on biodetermination of saponins.

This study explains a method to determine the plant saponins contents quantitatively. Saponins content was determined by using *Trichoderma viride* fungus. The growth of this fungus has a high sensitivity to saponins compounds (Zimmer *et al.,* 1967). The potential activity of saponins in the investigated samples was indicated by the level of the inhibition of *T. viride* growth.

Most of Saponins isolated from *Spergularia marina* and *Zygophyllum album* belong to triterpenoidal saponins (Elgamal *et al.*, 1995; De Tommasi *et al.*, 1998). While saponins isolated from *Pergularia tomentosa* belong to cardiac glycosides (Hamed *et al*., 2006). In addition, most saponins isolated from *Tribulus terrestris* were steroidal saponins (Cai *et al.*, 2001; Xu *et al.*, 2000). Both triterpenoidal and steroidal glycosides contain in their structures many hydrophobic groups. These groups have a high capacity to inhibit the growth of *Trichoderma viride* (Lalitha and Venkatraman, 1991). Meanwhile, the chemical constituents of *Alhagi maurorum* were characterized by the presence of tanins, flavonoids, unsaturated sterols and trace saponins (Atta and Mouneir, 2004). Also, the phenolic compounds (flavonoids and phenylpropanol) and essential oils are the major constituents of *Lippia nodiflora* **(**Pascual *et al.*, 2001). The tanins and flavonoids compounds contain in their aglycone more polar than triterpenoidal and steroidal saponins, and hence they showed a low capacity to inhibit the growth of fungi.

The action mechanism of saponins may lie in causing damaging to the membrane resulted in leakage of cellular materials, ultimately leading to cell death (Mashvildaze *et al.*, 2000). This activity has been documented in a number of saponins and the damaging effects have been showed against a variety of fungi including *Candida albicans*, *Saccharomyces cerevisiae* and *Acremonium* spp. (Lalitha and Venkatraman, 1991; Polacheck *et al.*, 1991).

In conclusion, the study provides an idea about saponins contents of the investigated plants (Fig. 2) which give them a pharmacological importance. Moreover, it indicated the antifungal activity of saponins.

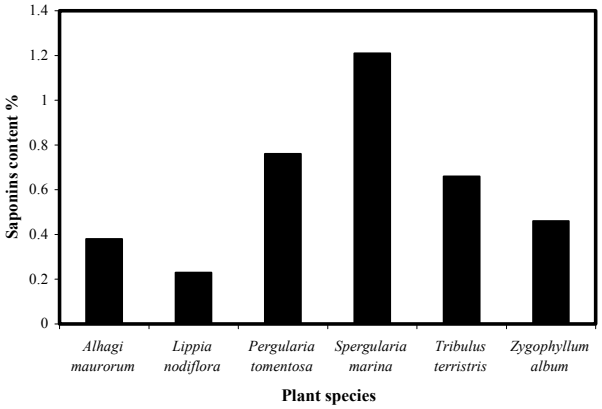

Figure (2): Percentages of the saponins contents of the studied plants.

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التقدير الحيوى لصابونينات النبات باستخدام فطر الترايكوديرما فيريدى

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الملخص العربـــى

يهدف هذا البحث إلى تقدير كمية الصابونينات في مجموعة من النباتات الطبية المحلية وذلك بطريقة حيوية. أعتمدت هذه الطريقة على حساسية نمو فطر الترايكوديرما فيريدى (*Trichoderma viride*) تجاه الصابونينات. وقد تم تجميع النباتات من مناطق مختلفة فى جنوب مصر.

وقد تم عمل مستخلصات من هذه النباتات، وتم زراعة فطر الترايكوديرما فيريدى على مستخلصات هذه النباتات ومتابعة نمو الفطر وقياس نصف قطر النمو. و قد أظهرت النتائج أن أكبر كمية للصابونينات تم تقديرها في نبات سبيرجيولاريا مارينا (*Spergularia marina*) و بلغت 6.06 ملجم/100 مل. وأن أقل كمية للصابونينات (1.88، 1.14 ملجم/100 مل) قدرت في كل من نبات ليبيا نوديفلورا ونبات الهاجي موراىروم (*Lippia nodiflora* and *Alhagi maurorum*) على التوالي. كما اتضح من الدراسة مدى فاعلية الصابونينات على تثبيط نمو فطر الترايكوديرما فيريدى.