Original Research Article

Dual Effect of Methanolic extract of *Securigera securidaca* as antioxidant and antibacterial activities

Abstract

In the present work, the phytochemical screening, polyphenolic content, antibacterial activity andantioxidant activity of *Securigera securidaca* seeds in methanol were carried out. Phytochemical analysis of seeds showed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids and glycosides. Total phenolic content was estimated by Folin Ciocalteau method and the result showed that methanol extract had the highest phenolic content of 62.28 mg/g. Methanolic extract was screened for antibacterial activity by disc diffusion method and it was found to be potent. The MIC of methanol extract identified by broth dilution method showed a MIC value of 0.25 mg/ml for both *E. coli* and *Kl. Oxytoca*, and also 0.5 mg/ml for both *S. aureus* and *S. epidermis.* The antioxidant effects of the seeds were tested by DPPH scavenging activity as *in vitro* assay. The extract had potent inhibitory activity (IC_{50}) value of 0.057mg/ml. These results suggested that methanol extract of *Securigera securidaca* is an important source for polyphenolic antioxidants and also a potential source for antibacterial activity.

Keywords: *Securigera securidaca*, Antioxidant, Anticorrosion, Polyphenolic content, MIC

1. Introduction

Medicinal plants have been known as pharmacological activities such as green anticorrosion inhibitors [1-6], analgesics, antimicrobial, antioxidant, antispasmodics, and diuretics [7] since ancient times. Therefore, it becomes important starting material of drugs due to the nature of secondary metabolites in plant. Currently, finding new antibacterial and antioxidant agents that have lesser side effects and better efficacy is promising approach in order to use it in some infectious diseases.

Securigera securidaca (S. securidaca) is an annual herb occurring wild in West Asia, Africa and Europe. ~~It is belonging to the Fabaceae family~~, also called *goat pea* and popularly names as *Gandeh Talkheh* [8]. Several experimental studies have shown beneficial effects of *S. securidaca* seeds as natural promise agents for epilepsy in Iranian folk medicine [9], enhancing antidiabetic, chronotropic, treatment of disorders such as hyperlipidemia, hypoglycemic effects, diuretic, hypokalaemic activities [4] and as anti-HIV-1 activity [10]. Moreover, experimental studies showed its role in reducing the level of cholesterol and triglyceride in serum that found on high-fat fed rats [11, 12]. It is reported that the ethanolic and aqueous extract of *S. securidaca* contains various classes of secondary metabolites such as steroids, flavonoids, alkaloids, tannins, cardenolides and penta cyclic triterpenoid type saponins [13,14]. Since these compounds may have the potential to inhibit Gram positive and negative bacteria and act as potent antioxidants, they have the ideal chemical structure for scavenging free radicals [15]. The petroleum ether extract of *S. securidaca* seeds showed antibacterial activities against *Staphylococcus aureus* (*S.aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) while the chloroform extract showed inhibitory effect only for *S. aureus*. The odd thing is, no antimicrobial activity of methanol extract, although there are several important natural compounds in the *S. securidaca* seeds crude [16].

Thus, this study (Fig.1) aims to evaluate the effect of methanolic extract of *S. securidaca* seeds which will be published for the first time as antibacterial activity against *Escherichia coli (E. coli)*, S. aureus (*S. aureus*), *Staphylococcus epidermis (S.epidermis)* and *Klebsiella oxytoca* (*Kl.oxytoca*), also antioxidant effects in order to use it in some infectious diseases.

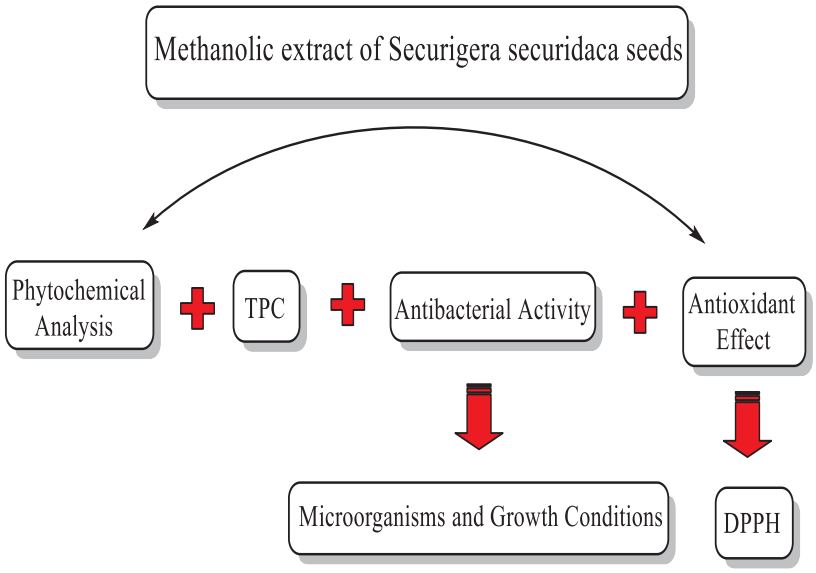

Figure 1: The overall work on methanolic extract of *S. securidaca* seeds

2. Material and Methods

Collection and preparation of the plant materials

S. securidaca seeds brought from local herbal shop (Karak- Jordan) in the summer of 2017. The seeds were grounded to be fine powder using a coffee blender and stored in the special container until use.

Preparation of methanolic extract

Twenty-five grams of seeds powder were soaked in 250 ml of 96% methanol and it was put in the shaker device at 150 rpm, in dark place for four days at room temperature and stored in a refrigerator for three days. The extract was then filtered using a Buchner funnel under vacuum. The filtrate was centrifuged at 3000 rpm for 15 minutes, and then extract concentrated in the rotary evaporator under vacuum at 50 °C. The crude was left in open vials in the fume hood for four days at room temperature and stored thereafter at 4 °C in a glass container until further use [17, 18].

Extraction Yield:

The yield of crude methanol extract was calculated (%, W_1/W_2) as:

Yield= W1/W2 x 100%

Where$W_1$is the weight of dried and ground plant material after evaporation of methanol and W_2 is the weight of powdered plant.

Qualitative phytochemical analysis

Phytochemical screening of primary and secondary metabolic compounds such as alkaloids, tannins, steroids, terpenoids, saponin glycosides, flavonoids, volatile oils, starch, phenols and proteins were conducted on seed extract according to standard phytochemical methods [19, 20].

Determination of total phenolic content (TPC) in the methanolic crude

The Folin-Ciocalteau assay method [21] was used to determine the total soluble phenolic content in plant extract in terms of Gallic acid. In this section: plant extract (0.2 ml, three replicates); 1 ml of Folin-Ciocalteau reagent was introduced into test tubes; The mixtures were neutralized with 0.8 ml of 7.5 % of Na_2CO_3 and the final concentration of the plant extract in the solution was 500 µg/ml. The tubes were mixed and shaken well to allow for reaction and left for 30 minutes at room temperature for color development and the absorbance was measured at 760 nm using a spectrophotometer. TPC was calculated according to the standard

calibration curve of Gallic acid (GA) solutions at different concentration (0 to 25µg/ml). TPC was expressed as Gallic acid equivalents (GAE) in milligrams per gram plant extract [22].

Evaluation of Antibacterial activity

Microorganism and growth conditions

The extraction was tested for antibacterial activity *in vitro* using both Gram negative and Gram positive bacteria (four microorganism including *E. coli*, *S. aureus*, *S. epidermis* and *Kl. oxytoca*). These stock cultures of bacteria were obtained from research Lab., Department of Biology, Mutah University. Antimicrobial activity evaluations were performed using the agar disc diffusion method [23, 24]. TPZ (10 µg) was employed as positive control, whereas a negative control contains pure methanol or distilled water. The plates were incubated at 37ºC for 18 - 24h. The antimicrobial activity of the extract was determined by measuring the diameter of inhibition zone (mm) against each bacterium. The tests were performed in triplicate and reported as mean ± standard deviation (SD).

Minimum Inhibitory Concentration (MIC)

Broth dilution method [25] was used to determine MIC. The extract dissolved in 10% DMSO in methanol was first diluted to the highest concentration (200 mg/ml) to be tested and then six-fold serial dilution was made in the concentration range of 0.0625 - 200 mg/ml. The extract solutions were added to a nutrient broth in separate test tubes inoculated with the respective standardized suspension of a strain adjusted to a concentration of 1x 10^8 colony/ml. Each tube contains various extract at concentration of 0 (control), 0.0625, 0.125, 0.250, 0.500, 1 and 2 mg/ml in broth medium. These tubes were incubated at 37 °C overnight and observed for visible growth (turbidity). MIC can be determined by examining broth tubes compared to control tube, containing only broth and inoculums without extract. Tubes that remain clear indicate no active growth and show the lowest concentration of extract, it was considered as MIC.

Evaluation of Antioxidant Activity of the Extracts

DPPH radical scavenging activity

The free radical scavenging activity of crude extract was estimated based on the previous reported procedure using the stable 2, 2-diphenyl-1-picrylhydrazyl radical, known as DPPH [26]. Briefly, different concentrations of the extracts were mixed with 5 ml of 0.004% methanol solution of DPPH (plant concentration in the solution varies from 0 to 2000µg/mL). The mixture was shaken vigorously and left to stand for 30 min with incubation at 37 °C. After that, the absorbance of the resulting mixture (DPPH with extract) was read against methanol at 517

nm using a spectrophotometer. All determinations were done at least in triplicate. The radical scavenging activity (capability to scavenge the DPPH radical) was calculated as a percentage of DPPH discoloration using the following equation:

Percentage of DPPH discoloration = $[(A_c - A_s)/A_c]$ x 100 (%)

Where A_c is the absorbance of the control reaction (DPPH solution without the tested extract), and $A_s$is the sample is the absorbance of the presence of all of the extract samples and reagents. IC_{50} (crude concentration providing 50% inhibition) was determined by a graph plotting the percentage inhibition against crude concentration. Trolox equivalent per gram dry weight can be calculated by creating a standard curve of Trolox standards (concentration 0 to 1.5 µg/ml) versus their absorbance. This curve was used to be a standard for the construction of the calibration curve, and the percentage of DPPH discoloration was expressed as mg Trolox equivalents per gram of plant extract [27].

Statistical analyses

All Experimental data were recorded in triplicate and the results were expressed as a mean ± standard deviation (SD). The IC_{50} value was calculated by Microsoft Excel 2010.

3. Results

Phytochemical screening

Qualitative phytochemical analysis of the phytochemical constituents presents in methanolic extract of S. securidaca seeds showed the presence of alkaloids, flavonoids and saponins in high content seeds extract; terpenoids, steroids and glycosides in mild content of the extract (Table 1).

Table 1. Phytochemical analysis for the methanolic extract of S. securidaca seeds

Phytochemical constituents	Methanolic extract
Alkaloids	+++
Flavonoids	+++
Terpenoids	++
Steroids	++
Glycosides	++
Saponins	+++

Where: (++) means mild content, and (+++) high content

Total phenolic content

~~TPC in the crude was estimated by Folin Ciocalteu's method and the Gallic acid (GA) as standard compound. A standard calibration curve for the TPC was constructed by GA. After that, the TPC value of methanolic extract of S. securidaca seed was calculated using the~~ standard curve equation of Gallic acid equivalent (GAE) in mg/g plant extract.

$Y = 0.0469X + 0.2703$, $R^2 = 0.9601$ Eq. (1)

Where Y is the absorbance at 760 nm and X is the amount of total phenolics in the plant extract. The TPC of the plant crude was 62.28 mg GAE/g of plant extract.

Antimicrobial effects

Table 2and Figure 2show the antibacterial activity of methanolic extract of the planta gainst four bacterial strains including *E. coli*, *S. aureus*, *S. epidermis* and *Kl. Oxytoca.* The extraction has an inhibitory effect on the growth of bacterial strains in disc diffusion method and in agar well diffusion method with an inhibition zone from 4 mm to 13 mm diameters at different extract concentration.

Table 2. Antibacterial activity of methanolic extract of S. securidaca seeds

Bacterial strains	Zone of inhibition (mm) / Mean ± SD		
	2 mg/disc	1 mg/disc	0.5 mg/disc
E. coli	09 ± 1.1	07 ± 1.0	05 ± 1.6
S. aureus	13 ± 2.1	11 ± 1.1	09 ± 1.0
S. epidermis	13 ± 1.3	11 ± 1.0	08 ± 1.6
Kl. oxytoca	08 ± 1.2	05 ± 1.3	04 ± 1.5

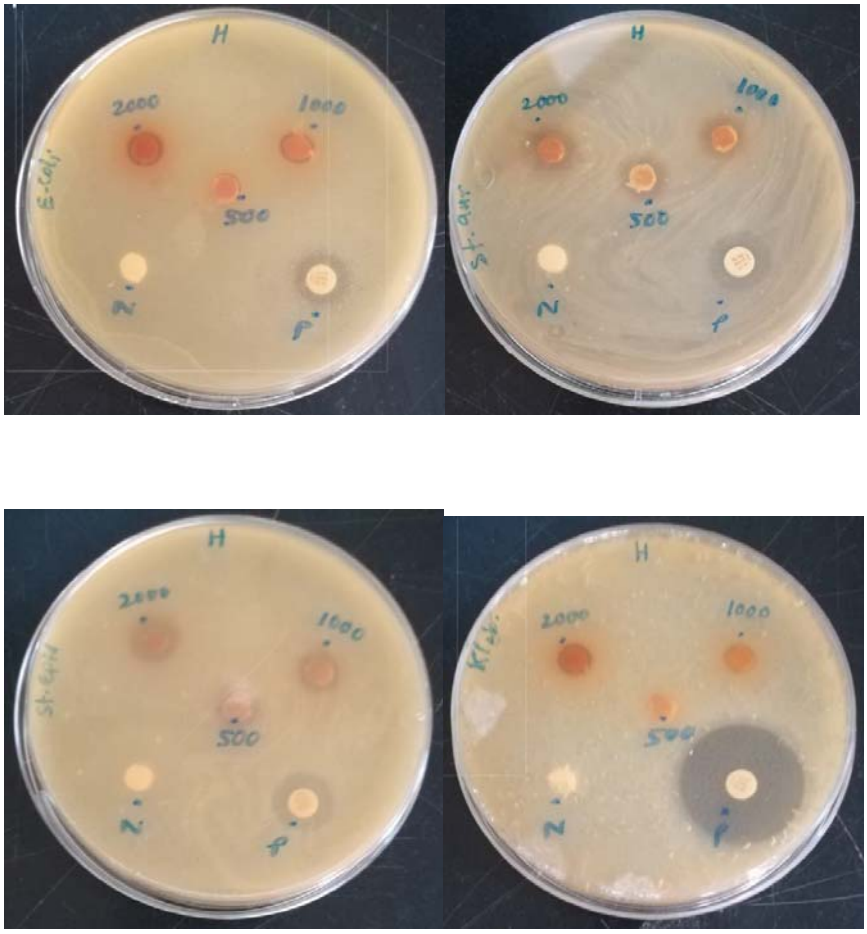

Figure 2. Antimicrobial activity of methanol extract of seeds of S. securidaca against four bacterial strains showing zone of inhibition, concentration:2000, 1000 and 500 µg/ml, P= positive control (TPZ), N= negative control (water)

Table 3 summarizes the MIC results of plant extract on the different bacterial strains. The MIC values showed that negative Gram bacteria (*E. coli* and *Kl.oxytoca*) were inhibited at 0.25 mg/ml and the positive Gram bacteria (*S. aureus* and *S. epidermis*) were inhibited at 0.50 mg/ml. (MIC tests pictures in Supplementary data).

Table 3. MIC of S. securidaca seeds Methanolic extract (mg/ml)

Microorganism	**MIC mg/ml**
E. coli	0.25
S. aureus	0.50
S. epidermis	0.50
Kl. oxytoca	0.25

Antioxidant Activity

The DPPH radical scavenging activity of S. securidaca seeds methanol extract is shown in Figure 3. This result has been evaluated using the DPPH radical method by reference standard (Trolox). The concentration spreadof 1-100 µg/ml. The IC_{50} value (half maximal inhibitory concentration) was 0.057mg/ml for S. securidaca.

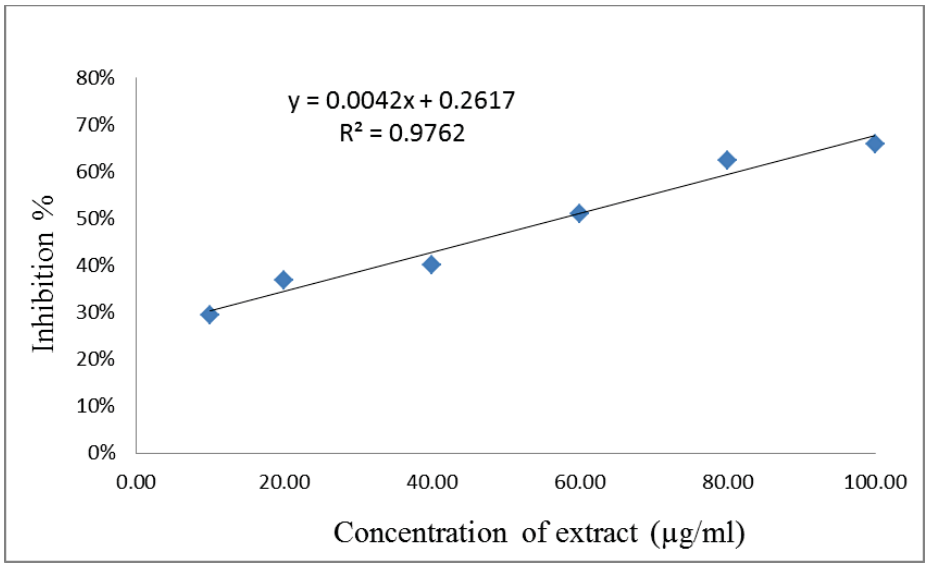

Figure 3: Scavenging effect of S. securidaca seeds extract on DPPH radical

4.Discussion

Recently, drug development and phyto-medicine are the hot topics in the world in order to find new and develop the known potential antioxidant and antibacterial. Plants are becoming more valuable in these topics because they are rich in several classes of secondary metabolites like alkaloids, polyphenols, terpenoids, steroids, glycosides and other natural products. These constituents showed an important value of herbal medicine in advances clinical research in infection diseases and improve health care [29]. The Folin-Ciocalteu's method has been used to find total phenol concentration presents in the extract. This method has been applied for finding polyphenols and other interfering compounds because of their antimicrobial and antioxidant activities. These compounds allow the extract to act as antioxidants due to their redox properties and chemical structures [30]. The bacterial activity of alkaloids and their derivatives such as highly aromatic planar quaternary is referred to their ability to intercalate with DNA [31, 32]. The methanolic extract of *S. securidaca* seeds uptake several natural phytochemical constituents with a percentage yield of 15.4 % (Table 4). The presence of these compounds is thought to be responsible for antimicrobial and antioxidant activity.

Table 4. Antioxidant and total phenolic content and percentage yield

Phytochemical tests	**The seeds parts of *S. securidaca* methanolic extract**
Antioxidant activity (IC_{50})	56.74 ± 1.2 µg/ml plant extract
Total phenolic contents	62.28 ± 1.7 mg/ml plant extract
Percentage yield %	15.4% plant extract

The phytochemical constituents of *S. securidaca* seeds extract have several secondary metabolites products such as flavonoids and cardiac glycosides. Furthermore, S. securidaca extract has some flavonoids that act as potent cytotoxicity against HT-29 (colon carcinoma), T47D (breast ductal carcinoma) and Caco-2 (colorectal adenocarcinoma) which known as Human cancer cell line [33].Meanwhile, the extract had potent antioxidant activity against DPPH and all the free radical investigated (IC_{50}= 56.74 ± 1.2 µg/ml plant extract).Previous report [16] showed that Petroleum ether and chloroform fraction of *S. securidaca* seeds have antimicrobial effects on the growth of *S. aureus* and *P. aeruginosa* (etheric extract) and only *S. aureus* has inhibited by chloroform extract. Moreover, methanolic extract has no microbial effect. This is the first report on methanol extract of *S. securidaca* seeds as antioxidant and antibacterial effects. Methanolic extract showed potent antibacterial activities against *S. aureus* and *S. epidermis* as Gram positive bacteria, also *E. coli* and *Kl. Oxytoca* as Gram negative bacteria with MICs 0.5, 0.5, 0.25 and 0.25 mg /ml, respectively. The extract work as a potent antimicrobial for both type go bacteria, but the inhibition zone diameter of extract against Gram positive is ranging from 8-13 mm, while it is 4-9 mm for Gram negative bacteria and it depends on the concentration of the plant.

5. Conclusion

Based on the findings of this paper, it can be concluded that S. securidaca seeds extract is a potent source of antioxidant and antimicrobial agents against Gram positive and negative bacterial strains. In addition, it could be used as preservative food and non-food systems and natural antioxidant. Further studies on other microbial with isolated secondary metabolites are needed to elucidate the active ingredient that shows a broad spectrum of pharmacological activity.

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