ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF JORDANIAN *SIMMONDSIA CHINENSIS* (LINK) C.K. SCHNEID

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Abstract

Context: Various parts of *Jojoba* plant have been used in ethno-medicine for skin disorders, wound healing and many others.

Objective: The purpose was to examine the effect of the total extracts of *Jojoba* leaves (male & female), testa, seeds and oil on the growth of certain microorganisms in addition to evaluating the antioxidant activity of these extracts.

Materials and methods: Plant parts were subjected for hot extraction by solvents of different polarity (hexane, methanol and ethanol) in addition to cold pressing extraction for seed. Furthermore, the active compounds were isolated by preparative thin layer chromatography, followed by identification for simmondsin and three of its derivatives namely Simmondsin-3'-ferulate, 4, 5-Didemethylsimmondsin and 4-Demethylsimmondsin-2'- ferulate, in addition to phenolic compounds, phytosterols, toccopherols and fatty acids. This was accomplished using chemical reagents along with various methods of chromatography and spectral analysis. The evaluation of antioxidant activity of extracts was done by 2, 2-Diphenyl-1-pircryl hydrazyl (DPPH) free radical scavenging method comparing to ascorbic acid activity. Antibacterial and antifungal activities were done using two methods; the agar well diffusion and disc diffusion methods, against (*Staphylococcus aureus*,

Bacillis subtilis, Escherichia coli, Klebsiella pneumonia and Candida albicans).
Results: The methanolic extract of testa and ethanolic extracts of both male and female leaves all possess antioxidant activity. Crude extracts do not exhibit antimicrobial activity, neither antibacterial nor antifungal activity.
Discussion and conclusion: Plant extracts induced variable degrees of antioxidant activity and proposes its potential as an antioxidant agent for pharmaceutical applications. However, they do not have antimicrobial activity.

Keywords: Simmondsin, Simmondsin ferulate, DPPH, *Staphylococcus aureus, Bacillis subtilis, Escherichia coli, Klebsiella pneumonia, Candida albicans*

Introduction

The most important antimicrobial drugs in clinical usage are naturally derived substances (Heinrich et al., 2008). The fungistatic and fungicidal effects of fatty acids have been well documented (Satish et al., 2007).

Furthermore studies have shown that free fatty acids have antibacterial, antiviral and antifungal activities (Desbois, 2010; Pohl et al., 2011).

Plants have a wide range of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins (C and E), terpenoids (including carotenoids), and other endogenous metabolites (Cai et al., 2004). These natural antioxidants are spread in different plants parts (Al-Mustafa, 2008). Many studies indicate that phytosterols may have anti-inflammatory activities and antioxidant properties (Woyengo et al., 2009).

Simmondsia chinensis (Link) C.K. Schneid is from the family (*Simmondsiaceae*) known as *Jojoba*. In Jordan, it is cultivated in the Jordan University of Science & Technology farms since 1986 and recently in Al-Ghoor area (AI-Zoubi, 1996).

Jojoba oil has a unique chemical structure in the plant kingdom (Elias-Cesnik, 1982). *Jojoba* seeds produce 45% to 75% by weight of an odorless oil or liquid wax. This oil contains certain quantities of sterols, stanols and different toccopherols (Tada et al., 2005; El-Mallah et al., 2009).

Furthermore seed meal, the fraction left after the wax (oil) has been extracted, contains a group of nitrile glycosides known as simmondsin's compounds (Van Boven et al., 2000 a; Laszlo et al., 2006). Also *Jojoba* seeds and meal have been shown to contain considerable amounts of tannins (Wiseman, 1987 a & b).

Materials and methods

The plant materials and oil of *S. chinensis* cultivated in Jordan were collected from the farms of Jordan University of Science and Technology (JUST) / Irbid, during the months of October, November and December (2011), with the co-operation of Agricultural Services Department in JUST. The leaves (male & female), seeds and testa were dried at room temperature (25°C) in the shade for about 15 days and then milled and weighed.

Extraction methods

Different parts of *S. chinensis*, namely testa, male and female leaves went through extraction by a soxhlet apparatus. Different solvents were added separately (ethanol and methanol) in different patches and refluxed for 12 hours, with optimum control of temperature and pH. The seeds were soaked in *n*-hexane (95%) followed by extraction using soxhlet apparatus for 12 hours. The contents were filtered and dried. The extract was evaporated under reduced pressure using rotatory evaporator. The liquid wax extracts were kept in amber glass containers to avoid any photo-oxidation. The residual parts were soaked in ethanol and methanol separately, followed by extraction using soxhlet apparatus for 12 hours (Holser, 1999; Zaher et al., 2004).

Another extraction method used was a cold pressing technique by hydraulic presser machine designed specifically for this purpose in the *Jojoba's* cold-pressing unit (AI-Zoubi, 1996). This method was used for the extraction of liquid wax and other related compounds, after complete removal of testa from the seeds. The seeds weighed two kilograms and the volume of oil produced was measured to be one litter. Quantities used in extraction methods are shown in Table (1). All different extracts were dried, cooled and subjected to identification process.

Screening for the presences of the active constituents

The analysis was performed on 20 cm x 20 cm (0.25 mm thick) Thin Layer Chromatography (TLC) plates with aluminum support, silica gel matrix, and fluorescent indicator λ_{max} 254 nm and λ_{max} 365 nm (Merck, Germany). The best developing solvent systems used were: ethyl acetate/ethanol (70:30, v/v), chloroform/methanol (80:20, v/v) (Van Boven et al., 2000 b), and benzene (100, v/v) (Spencer & List, 1988).

Identification reagents for nitrile glycosides were conducted by: 1-naphthol spray: The spray was prepared by adding 10.5 ml of a 15% ethanolic solution of 1-naphthol to a mixture of 40.5 ml of ethanol, 4 ml of water, and 6.5 ml of sulfuric acid. After spraying with the reagent, the TLC plates are heated in an oven at 100°C for 5 min (Van Boven et al., 2001).

Other reagents used include sulfuric acid (10%) spray and sodium picrate solution (Müller-Schwarze, 2009).

Identification reagents for phenolic compounds and tannins were: 3-5 % w/v solution of ferric chloride in ethanol (95%) and sulfuric acid (10%) spray (Van Boven et al., 2000 b).

Furthermore, the identification reagents for fixed oil (fatty acids), phytosterols and toccopherols were: Iodine vapor (Tada et al., 2005) and a mixture of concentrated sulfuric acid in ethanol (50:50, v/v) spray. The TLC plates were heated at 100°C in the oven for 15 min (Van Boven et al., 2000 c).

Antioxidant activity (DPPH free radical scavenging activity)

They Antioxidant activities were assessed on the basis of the radical scavenging effect of the stable 2, 2-Diphenyl-1-pircryl hydrazyl (DPPH) free radical activity by modified method of reported procedure by (Yadav et al., 2012). This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non-radical form DPPH-H.

The diluted working solutions of the test extracts were prepared in methanol. The stock solution of extracts (1000 µg/ml) was prepared by dissolving weighed amount of the crude extract in 99.9% methanol. The dilution samples of the extracts were prepared from the stock solution using suitable diluted solutions (1, 5, 10, 20, 40, 50, 80, 100, 200, 300, 400, 500, 800 and 1000 µg/ml) and were applied according to the dilution calculation equation.

In contrast, the diluted working solutions for the oil extracted from the seeds in the two methods mentioned previously were: methanol, chloroform, zylene, dichloromethane, petroleum ether and toluene. The Ascorbic acid was used as a standard. DPPH (0.002%) was prepared in methanol. One ml of this solution was mixed with one ml of sample solution of each extract concentration and the same procedure was done on standard solution (ascorbic acid). These solution mixtures were kept in dark for thirty minutes at room temperature (25°C) and the optical density (absorbance) was measured at λ_{max} 517 nm. The optical density (absorbance) was recorded and % inhibition was calculated using the formula given as the following:

Percent (%) inhibition of DPPH activity = [(Absorbance of blank – Absorbance of test sample) / Absorbance of blank] x 100

Antimicrobial activity

All bacterial and fungal strains were obtained from the microbiology laboratory of Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Jordan.

Antibacterial activity screening

Antibacterial activity screening was performed on different samples from ethanolic and methanolic extracts of male, female leaves, testa and seeds extracts in addition to the oil obtained from the seeds by two different extraction methods. In addition, three chemical standards were used in the screening and they were ferrulic acid, gallic acid and tannic acid.

Antibacterial screening was generally performed by two methods; the first method is the agar well diffusion method, where the medium was inoculated with the test microorganism. Once the agar solidified, it was punched with a sterile six millimeters diameter wells and filled with 200 µl of the samples (Perez et al., 1990).

The second method is the disc diffusion method (Greenwood et al., 1997; Cavalieri et al., 2005). This test is based on diffusion and consists of impregnating the discs with the compound to be tested on the agar and incubating to examine the degree of sensitivity by measuring the diameters of the zone of growth inhibition. The bacteria (1.5×10^8 colony forming units (CFU) / ml) were swabbed uniformly across culture plate, and then the disc that is impregnated with the compound to be tested is placed on the surface of the agar. The compound diffused from the filter paper into the surrounding agar and produced a diminishing gradient of concentrations.

The bacterial strains used were two Gram-positive (*Staphylococcus aureus*, *Bacillis subtilis*), and two Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*). The screening of the extracts' antibacterial effect was carried out by determining the zone of inhibition using sterilized paper disc (6 mm in diameter, Whatman No. 1). The obtained microorganism strains were inoculated in Petri dishes at 37°C for 24 h and were referred as seeded agar.

Two types of culture media were used in this study, which were nutrient agar and sabouraud dextrose media. In order to investigate the desired antimicrobial concentrations, the sterile discs were impregnated with extracts solutions in different concentrations and placed on inoculated agar. The different concentrations used are illustrated in Table (3). Amoxicillin with clavulonic acid (pen-strep) and Trimithoprim (pen-strep) susceptibility discs impregnated disc were used as positive controls. After incubation overnight at 37°C, inhibition zones were measured and recorded as mean diameter (mm). Antibacterial activity was also expressed as inhibition percentage of Amoxicillin with clavulonic acid and Trimithoprim.

Antifungal activity screening

Antifungal activity screening was performed on the same samples that were subjected to antibacterial activity screening. It was performed by agar well diffusion and disc diffusion assays against *Candida albicans*. In the same way used in the antibacterial activity screening (Perez et al., 1990; Greenwood et al., 1997; Cavalieri et al., 2005), the sterile discs were impregnated with extracts solutions in concentrations as illustrated in Table (3). Then the discs were placed on the inoculated media in the Petri dishes and incubated at temperature 37°C. Nystatin susceptibility discs (pen-strep) impregnated disc were used as positive controls. Inhibition zones were measured and recorded as mean diameter (mm). Antifungal activity was also expressed as inhibition percentage of Nystatin.

Results

Antioxidant activity

Jojoba extracts were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. DPPH absorption inhibition (%) of *Jojoba* extracts and ascorbic acid (vitamin C) in addition to the calculated (IC_{50}) values are shown in Figure (1), Figure (2) and Table (2).

The results indicated that male leaves (ethanolic extract), female leaves (ethanolic extract) and testa (methanolic extract) all possess antioxidant activity. But the antioxidant activity of ascorbic acid is higher than the crude extract. Furthermore, the relation between vitamin C, *Jojoba* extracts' concentrations and DPPH absorption inhibition (%) is shown in Figure (3).

Moreover, the relation between *Jojoba* extracts types and DPPH absorption inhibition (%) is illustrated in Figure (4).

Antimicrobial activity

All samples showed no activity against *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumonia*, and *C. albicans* as a result there was no need to conduct the minimum inhibitory concentration (MIC) measurement.

Discussion

The antioxidant results of Jordanian *Jojoba* were in agreement with *Jojoba* data conducted in other parts of the world (Kampf et al., 1986; Mallet et al., 1994; Reda et al., 2009; Elnimiri & Nimir, 2011; Ibrahim et al., 2011). This can be explained by the fact that phenolic compounds, phytosterols and alpha-toccopherpols were constituent of *S. chinensis* , and these compounds have an antioxidant activity as previously declared by (Evans, 2009) and (Ismail et al., 2012). *Jojoba* also has a group of nitrile glycosides known as simmondsin's. These pharmacologically active substances may have a role in

the antioxidant activity. As the antioxidant activity of glycosides was reported by (Abdel-Wahhab et al., 2007) and (Mehta et al., 2009).

Previous studies have shown that free fatty acids have antibacterial, antiviral and antifungal activities (Desbois, 2010; Pohl et al., 2011). The same activities were found in the tannins (Bhat et al., 2005), phenolic compounds (Bruneton, 1999) and phytosterols (Singh et al., 2012). Although *Jojoba* contains phenolic compounds, phytosterols, saturated and unsaturated fatty acids, no antimicrobial activities were observed in this study.

On the other hand, it was reported that *Jojoba* leaves' extract has antiherpitic activity as explained in (Yarmolinsky et al., 2010) and *Jojoba* oil has intense inhibitory action on the growth of *Mycobacterium tuberculosus* (Daugherty et al., 2012). Moreover, it was reported that *Jojoba* essential oil has fungicidal activity against plant fungal diseases (Dayan et al., 2009).

However, the finding from this study regarding Jordanian *Jojoba* oil antimicrobial activity agrees with reported data of previous investigations (Elias-Cesnik, 1982; Elnimiri & Nimir, 2011).

Conclusion

Crude extracts from different parts of *S. chinensis* exhibit antioxidant activity, this activity may be related to their phenolic compounds, phytosterols, toccopherols and fatty acids contents, because all the previous phyto-compounds were reported as antioxidant. Furthermore, the ethanolic extracts of male & female leaves as well as the methanolic extract of testa were more potent than other parts of the plant according to the IC_{50} values. Moreover, this will open new horizons for a wide range of pharmaceutical applications. To name a few, topical pharmaceutical formulations and therapeutic applications for example, antioxidant-based formulations. These formulations are used for prevention and treatment of many illnesses such as atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer by dealing with the oxidative stress, which leads to cell degeneration.

Crude extracts from *S. chinensis* do not exhibit antimicrobial activity, neither antibacterial nor antifungal activity against *(S. aureus*, *B.subtilis*, *E. coli, K. pneumonia* and *C. albicans*). This study regarding Jordanian *Jojoba* oil antimicrobial activity comes in agreement with the negative results obtained in other literature sources.

This is a pioneer results regarding *Jojoba* which is cultivated in Jordan under different environmental conditions.

Recommendations

Further antimicrobial activity investigation against other microorganisms is recommended.

Detailed further investigation regarding the differences in the chemical constituents and biological activity between the male and female *Jojoba* leaves, since this study found that there were differences in the chemical composition between the two genders of leaves and they possessed higher antioxidant activities than other parts of the plant.

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Tables and Figures

Table 1. Quantities used in extraction methods of testa, leaves (male & female) and seeds of *Jojoba* plant.

Parts used	Weight	Ethanol (95 %)	Ethanolic dry extract	Weight	Methanol (99.9%)	Methanolic dry extract
Testa	60 g	300 ml	6.5 g	40 g	300 ml	4.4 g
Male leaves	60 g	300 ml	14.8 g	40 g	300 ml	9.7 g
Female leaves	60 g	300 ml	15.6 g	40 g	300 ml	10.2 g
Seeds	60 g	300 ml	6.3 g	40 g	300 ml	4.9 g

Table 2. The IC_{50} values of DPPH absorption inhibition (%) of *Jojoba* extracts.

***Jojoba* Extracts**	**IC_{50} (µg/ml)**
Vitamin C	1.34
Testa (methanolic extract)	39.95
Seeds (methanolic extract)	122.66
Female leaves (ethanolic extract)	32.54
Male leaves (ethanolic extract)	1.56
Seeds (ethanolic extract)	154.30
Oil (dissolved in zylene)	963.06
Oil (dissolved in chloroform)	164.84
Oil (dissolved in methanol)	111.44
Oil (dissolved in toluene)	793.60
Oil (dissolved in dichloromethane)	148.66
Oil (dissolved in petroleum ether)	751.13

Table 3. Jojoba extracts' quantities that used in the antimicrobial activity study.

***Jojoba* extract used**	**Concentration (mg / ml sterile distilled water)**			**Type of extract**
	Experiment 1	Experiment 2	Experiment 3	
Testa	97	100	198	Methanol
Male leaves	49	58	159	Ethanol
Female leaves	52	64	160	Ethanol
Seeds ethanolic extract	62	149		Ethanol
Supernatant from seeds ethanolic extract	25	49		Ethanol
Seeds oil	10	498		Hexane

Seeds oil	10	510	Cold extraction
Ferrulic acid	10		Chemical standard
Gallic acid	10		Chemical standard
Tannic acid	10		Chemical standard

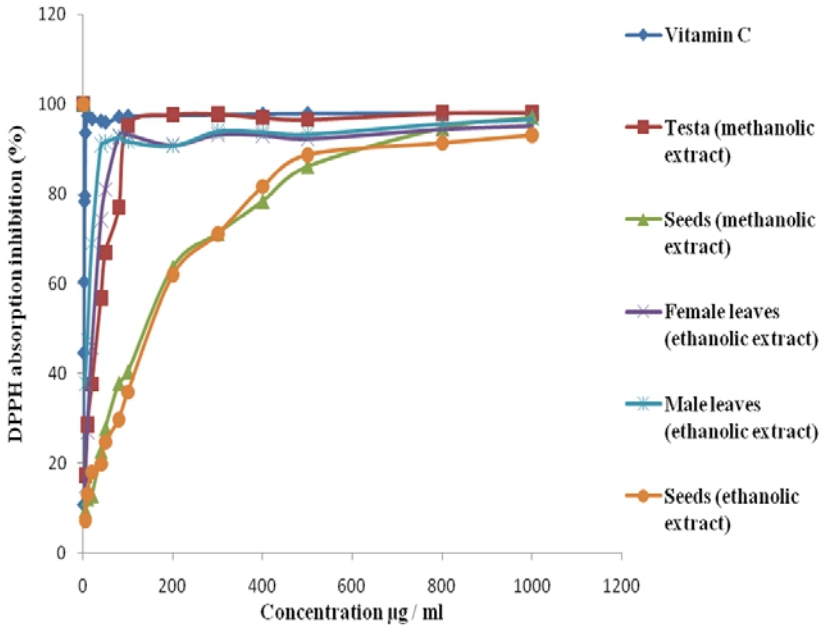


Figure 1. DPPH absorption inhibition (%) of different parts of *Jojoba* extracts (part one)

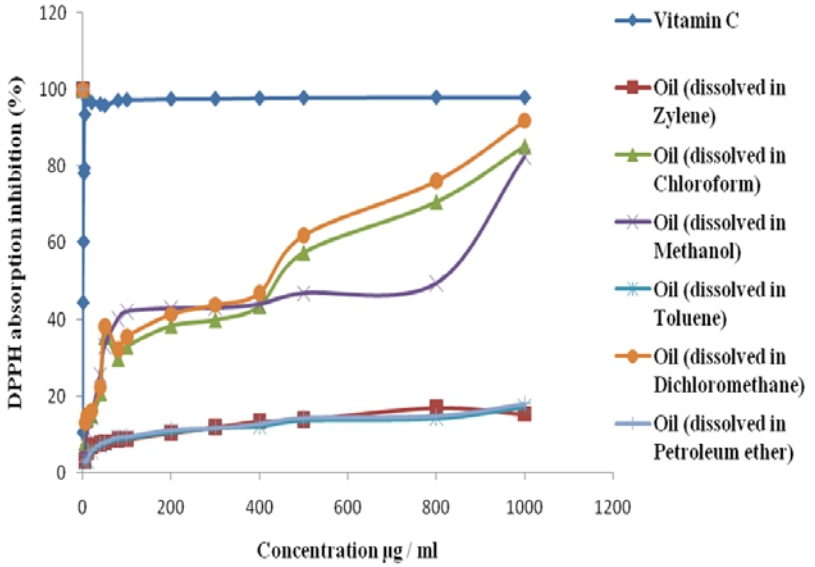


Figure 2. DPPH absorption inhibition (%) of different parts of *Jojoba* extracts (part two)

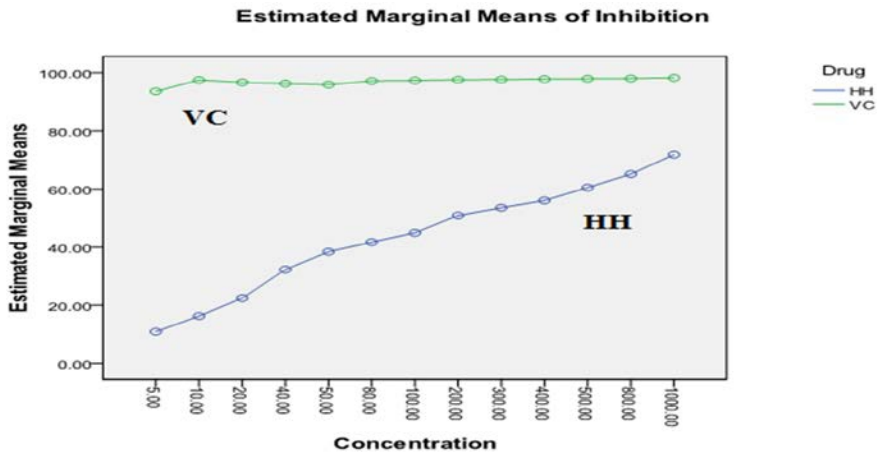
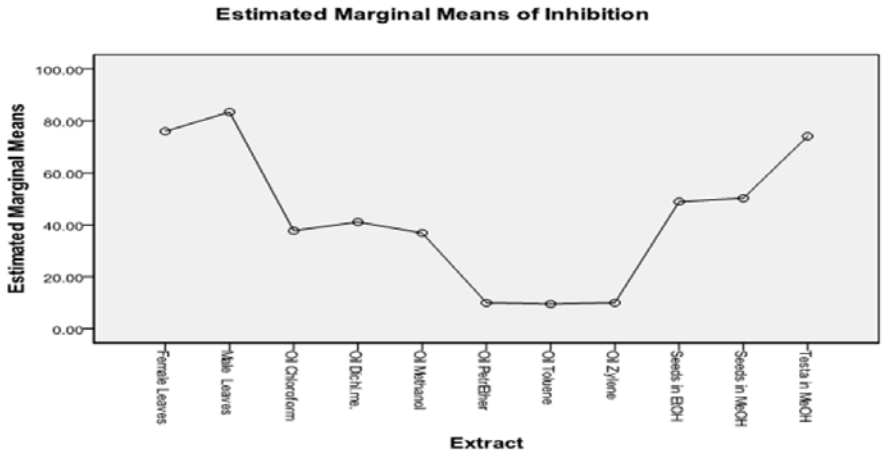


Figure 3. The relation between Vitamin C (VC), *Jojoba* extracts' concentrations (HH) and DPPH absorption inhibition (%)



Female leaves=Female leaves (ethanolic extract), Male leaves=Male leaves (ethanolic extract), Oil Chloroform=Oil (dissolved in chloroform), Oil Dichl. me. =Oil (dissolved in dichloromethane), Oil Methanol=Oil (dissolved in methanol), Oil PetrEther=Oil (dissolved in petroleum ether), Oil Toluene=Oil (dissolved in toluene), Oil Zylene=Oil (dissolved in zylene), Seeds in EtOH=Seeds (ethanolic extract), Seeds in MeOH=Seeds (methanolic extract), Testa in MeOH=Testa (methanolic extract)

Figure 4. The relation between *Jojoba* extracts' types and DPPH absorption inhibition (%)

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