CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2017 Vol.6 (1) January-March, pp.1-8/Abdallah et al.
Research Article

ANTIMICROBIAL, ANTIOXIDANT AND PHYTOCHEMICAL SCREENING OF LUPIN SEEDS (*LUPINUS TERMIS* FORRSK.) FROM SUDAN

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ABSTRACT
Lupin (*Lupinus termis*) seeds are popularly edible and used as snacks in Sudan. The current study aimed to evaluate some biological properties of the Sudanese lupin seeds such as the phytochemical constituents, the antimicrobial and antioxidant activities. The study revealed that the methanolic extract of lupin seeds have no significant antimicrobial activity against all the tested bacteria (6 different Gram-positive and Gram-negative bacteria) and fungi (2 fungal species). The Phytochemical analysis is showed a presence of some bioactive secondary compounds such as alkaloids, cumarins, phytosterols and resin, the study also revealed the presence of some nutritional primary compounds such as proteins, reducing sugars and glycosides. The antioxidant testing exhibited that lupin seeds have significant antioxidant and free radical-scavenging activity. Therefore, lupin seeds could be a good source for natural antioxidant agents for medical and nutraceutical applications.

Keywords: *Antimicrobial, Antibacterial, Antifungal, Antioxidant, Phytochemical, Lupinus Termis*

INTRODUCTION
Human gets most of his food from plants, such vegetarian food are not a source of nutrition only, but they are also a source of many bioactive compounds which promote health, improve immunity and help in curing diseases.
Many edible plants show numerous bioactive properties. Primary products of plants include carbohydrates, proteins and fats, whereas what is known as secondary products (Phytochemical compounds) represents the bioactive compounds. The latter has attracted the attention of the researchers and scholars in the few decades (Abdallah *et al*., 2016).
Lupin is an ancient leguminous plant, it has been cultivated and used as food for human and cattle since around 2000 years ago (Petterson and Fairbrother, 1996). Lupin seeds are used in traditional medicine in Africa and the Middle East as an anti-diabetic agent, and used topically to treat acne, it is used traditionally in the U.S.A. for foot eczema (Quiles *et al.,* 2010). In recent years, Lupin seeds have drawn attention due to their nutritional value, richness in protein, pharmaceutical properties and high alkaloid content (Omer *et al.,* 2016).
There are about 400 species of lupins (Genus: *Lupinus*), among them only a few of them have been extensively studied. Lupins are characterized by the ability to grow on poor agricultural lands (Khan *et al.,* 2015). In Sudan, lupin seeds are a popular snack food, they are boiled and macerated in water several times to get rid of the bitter taste, then salted, spiced and consumed as a snack food.
The wild varieties of Sudanese lupins (*Lupinus termis* Forrsk.) are considered synonymous to *Lupinus albus* L. (Quiles *et al.,* 2010). Unfortunately, lupin seeds are cultivated only for local consumption in Sudan, where there is no interest in lupin production in Sudan, which leads to low seed production and a scarcity of research (El-Nagerbi and Elshafie, 2000). The aim of the study was to evaluate the antimicrobial and antioxidant activity as well as the phytochemical constituents of the Sudanese lupin seeds (*Lupinus termis* Forrsk.).

MATERIALS AND METHODS

Sample Collection and Extraction

The dried seeds of lupin (Figure 1) were purchased from local markets in Khartoum, Sudan. The seeds were purified from peels or unwanted materials and then ground to a fine powder using blender machine (GEEPAS ®, GCG 292, China). 50 grams of the lupin seeds powder was macerated in 500 ml of 80% methanol (v/v) (HPLC grade, Fisher Scientific, UK) in a well tighten glass bottle and left for up to 3 days in an incubator (BINDER GmbH, Germany) at 37 ^{o}C with frequent shaking. Then, the infusion was filtered through Whatman filter papers No.1 (Whatman International Ltd, UK), the filtrate was put again in the incubator at 45^{o}C and allowed to evaporate the solvent for up to 10 days, till getting a semi-solid extract, which was employed in the experiments.

Microorganisms

Six referenced bacterial strains representing Gram-positive and Gram-negative bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10876, *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 35218) and two fungal strains (*Candida albicans* (Clinical isolate) and *Aspergillus niger* ATCC 6275) were used to investigate the possible antimicrobial activity of Lupin seeds. The bacterial and fungal strains were obtained from the Department of Pharmaceutics, Unaizah College of Pharmacy, Qassim University and the Department of Laboratory Sciences, College of Science and Arts, Al-Rass, Qassim University.

In Vitro Antimicrobial Assay

The antimicrobial activity of the methanol extract of lupin seeds was evaluated by disc diffusion method as mentioned in (Abdallah *et al*., 2016) with minor Additions regarding the antifungal test. The tested microorganisms were sub-cultured In Mueller-Hinton agar (Watin-Biolife, KSA) for 18-24 h for bacteria, while the fungal strains were subcultured in Sabouraud Dextrose Broth (Scharlau, Spain) for 24 h (For *Candida albicans*) and 48 hours (For *Aspergillus niger*), the working bacterial samples were prepared and adjusted to be equivalent 0.5 McFarland (Approximately 1-2 × 10^{8} CFU/ml), while the sub-cultured fungi in broth were used directly as working samples. 20 ml of hot autoclaved Mueller-Hinton agar (Watin-Biolife, KSA) for bacteria or Sabouraud Dextrose Agar (Scharlau, Spain) for fungi was poured to a sterile disposable Petri-dish (Size 100x15mm) and left until solidified.

The bacterial or fungal samples were swapped over the surface of the prepared plates using sterile cotton swap. Previously prepared 6 mm filter paper discs (Whatman No.1) were immersed in the reconstituted extracts at 400 and 200 mg/ml, the lupin seeds methanol extract was reconstituted in 10% DMSO (Dimethyl sulfoxide) these discs were put on the agar plates, another antibiotic disc was loaded with the plates; which was gentamicin 10 μg/disc (Oxoid, UK) for bacteria or clotrimazole 10 mg/ml (Canesten, Switzerland) for fungi to serve as positive control, a sterile disc saturated with 10% DMSO was also put on the plate to serve as negative control.

The seeded plates were incubated at 37^{o}C for 18 hours for bacteria and the yeast *Candida albicans* or incubated for up to 48 h for the fungi *Aspergillus niger*, the test was repeated thrice and the mean inhibition zone and standard error of means were calculated.

Phytochemical Analysis

Seeds of lupin were qualitatively screened for some bioactive phytochemical constituents by means of some colorimetric qualitative tests. The crude methanolic extract was screened for the presence of Alkaloids, Anthocyanins, Anthraquinone, Carbohydrates/Reducing sugar, Carboxylic acid, Cardiac glycosides, Coumarins, Emodins, Flavonoid, Leucoanthocyanins, Lipids/Fatty acids, Phenol/Polyphenols, Phlobatannin, Phytosterols, Proteins, Quinones, Resin, Saponins, Steroids, Tannin, Terpenoids and Volatile oil (Sasidharan *et al.*, 2010).

Antioxidant Test

The DPPH radical scavenging activity assay method was modified to determine the antioxidant activity as stated in (Brand-Williams *et al.*, 1995). Briefly, 2 ml of methanolic DPPH solution (30 mg/L) was mixed with 200 μL sample extract (50 mg/mL) obtained from the Sudanese lupin seeds. The sample was incubated in the dark at room temperature for 30 min and then the absorbance of the solution was measured at 516 nm using a UV-visible light spectrophotometer. The percent scavenging of DPPH was calculated using the formula:

Radical Scavenging Activity = $(A_{control} - A_{sample}) / A_{control} \times 100$

Where: $A_{control}$ and A_{sample} are the absorbance values of the control and sample extract at 516 nm, respectively.

RESULTS AND DISCUSSION

The phytochemical investigation of the methanolic seed extract of lupin revealed the presence of some primary compounds which were proteins, reducing sugars, glycosides, cardiac glycosides, while no carboxylic acid, lipids or fatty acids detected (Table 1) which makes it a suitable food to control obesity and diabetes. It was reported that lupin seeds have various nutritional and functional benefits, they are rich in proteins (about 50%) and various fibers and other compounds able to improve and control both of serum cholesterol and serum glucose levels in the blood (Elbandy and Rho, 2014).

There were also some secondary phytochemical compounds, which mostly contains the bioactive and medicinal properties of lupin seeds. As shown in (Table 1), alkaloids, cumarins, phytosterols and resin were present in the methanol extract of lupin seeds while all other tested phytochemicals were negative, which are flavonoids, phenols, polyphenols, phlobatannin, anthocyanins, anthraquinones, emodins, leucoanthocyanins and quinines. On the other hand, lupin seeds were cited in literature containing significant amounts of some bioactive compounds such as alkaloids, polyphenols, carotenoids, tocopherols, phytosterols, as well as antimicrobial, antioxidant, anti-inflammatory, and anti-cancer activity (Khan *et al.*, 2015). This could be related to variations in lupin species or varieties in addition to the impact of environmental conditions on the plant constituents, it could be also attributed to the type of extractions and solvents used.

The results of the antimicrobial activities are demonstrated in Table 2. As indicated form these results there was no significant antibacterial activity (Figure 1) or antifungal activity (Figure 2) compared with the antimicrobial drug (Gentamicin for bacteria or clotrimazole for fungi). This can be explained in light of the phytochemical results. It was reported that the major secondary phytochemical compounds that have antimicrobial potential are phenolic compounds, flavonoids, saponins and alkaloids (Abdallah, 2016). Most of these compounds did not detect on the methanol extract of lupin seeds, except alkaloids. Alkaloids are enormous diverse group of natural phytochemical products, there are more than 18,000 types of alkaloids known up to date (Cushnie *et al.*, 2014). Accordingly, more future chemical studies should necessarily isolate and study the nature of the alkaloids present in lupin seeds. Moreover, the negative antibacterial results of the current study are in agreement with previous studies; Shahhat *et al.*, (2014) published that there was no antibacterial activity for *Lupinus termis* oil extract against all the tested bacterial strains. Lampart-Szczapa *et al.*, (2003) found that lupin seeds of three species (*Lupinus albus*, *Lupinus luteus* and *Lupinus angustifolius*), exhibited no antibacterial activity of the cotyledons of the seeds while the testa (The coat) of the same seeds showed antibacterial effects, their study suggests that alkaloids of the cotyledons have no influence as antibacterial and the detected antibacterial activity are related to development of some phenolic compounds in the testa.

The results of the antioxidant testing showed that the lupin seeds have remarkable antioxidant and free radical-scavenging activity compared to the pure ascorbic acid (Table 3). This result is compatible with the finding of (Tsaliki *et al.*, 1999), they cited that the methanol extract of the flour of lupin seeds (*lupines albus*) showed a marked antioxidant activity, higher than that of soybean. It is also in agreement with (Aniess *et al.*, 2015), they are mentioned that the flour of lupine seeds exhibited higher antioxidant activity with Diphenyl-P-Picryl Hydrazyl (DPPH) and 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) than the flour of wheat seeds.

Antioxidant phytochemicals have many health benefits in preventing various chronic diseases such as cardiovascular diseases, inflammations, cancers, diabetes and neurodegenerative diseases (Zhang *et al.*, 2015). The natural antioxidants derived from plants are preferred over the synthetic chemical antioxidants, due to the multiple mechanisms of reaction, non-toxic nature and their bioactive phytochemicals (Padmanabhan and Jangle, 2012). Accordingly, lupin seeds could be a good source for natural antioxidants for in food processing industries.

Figure 1: The Seeds of Lupin (*Lupinus tremis* Forrsk.)

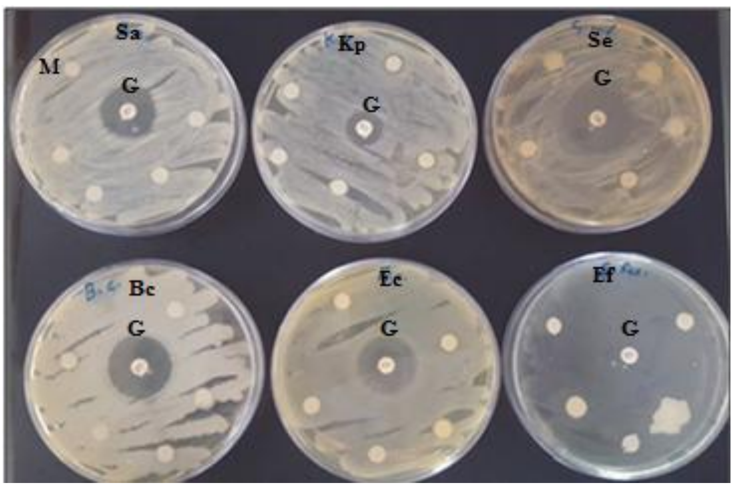

Figure 2: The Absence of Antibacterial Effects of the Methanol Extracts (Terminal Discs) of Lupin Seeds Compared to Gentamicin (G)

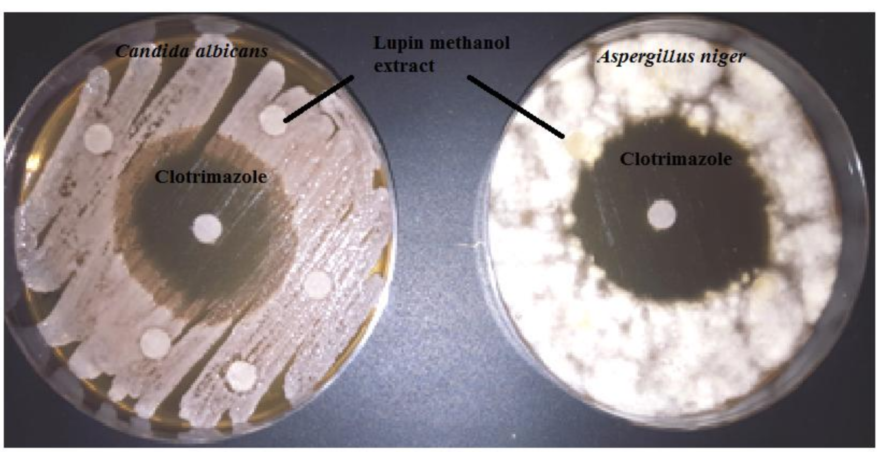

Figure 3: The Absence of Antifungal Effects of the Methanol Extracts (Terminal Discs) of Lupin Seeds Compared to Clotrimazole (In the Center)

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Table 1: Screening of some Chemical Compounds from the Methanol Seed Extract of *Lupinus termis*

Chemical Constituents	Methanol Crude Extract
Primary compounds	
Carbohydrates/Reducing sugar	+
Carboxylic acid	-
Cardiac glycosides	+
Glycosides	+
Lipids/Fatty acids	-
Proteins	+
Secondary compounds	
Alkaloids	+
Anthocyanins	-
Anthraquinone	-
Coumarins	+
Emodins	-
Flavonoid	-
Leucoanthocyanins	-
Phenol/Polyphenols	-
Phlobatannin	-
Phytosterols	+
Quinones	-
Resin	+
Saponins	-
Steroids	-
Tannin	-
Terpenoids	-
Volatile oil	-

Table 2: The Antimicrobial Activity of the Methanol Seed Extract of *Lupinus termis*

Tested Compound	Mean Zone of Growth Inhibition in mm (Omitting the Disc Diameter 6mm)							
	Gram-Positive Bacteria				Gram-Negative Bacteria		Fungi	
	Sa	Se	Ef	Bc	Ec	Kp	An	Ca
Methanol extract (400 mg/ml)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.3±0.2	6.0±0.0	6.0±0.0
Methanol extract (200 mg/ml)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.1±0.2	6.0±0.0	6.0±0.0
Gentamicin (10 µg/disc)	22.6±1.5	28.6±0.5	7.6±2.0	25.3±1.1	23.3±1.5	11.3±1.5	N/A	N/A
Clotrimazole (10 mg/ml)	N/A	N/A	N/A	N/A	N/A	N/A	35.0±1.0	32.0±2.6
10% DMSO	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

Key: Disc diameter=6.0 mm, 6.0±0.0= No activity (the disc diameter), Zone of inhibition is the mean of three replicates ±standard deviation, N/A=Not applicable, Sa=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 12228, Ef=*Enterococcus faecalis* ATCC 29212, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 35218, Kp=*Klebsiella pneumoniae* ATCC 700603, An=*Aspergillus niger* ATCC 6275 and Ca=*Candida albicans* (Clinical isolate). DMSO= Dimethyl sulfoxide.

Table 3: DPPH Scavenging Activity of Methanolic Seeds Extract of Lupin Compared to Ascorbic Acid

Compound	Concentration	% Inhibition
Methanolic seed extract of lupin	50 mg/ml	78.73684
Ascorbic acid	100 µg	68.73684

Conclusion

Lupin seeds are a typical functional food, rich in nutritional and health benefits. Although seeds did not show antimicrobial activity, it was found that lupin seeds have a potential antioxidant activity, which requires more future studies in order to isolate these antioxidant compounds. Alkaloids are interested phytochemical compound detected in the seeds extract.

Though, it is recommended to study, since the majorities of alkaloids have antimicrobial properties while that of lupin seeds have not. In conclusion, the study introduces lupin seeds as a promising source of natural antioxidant compounds.

Funding

None

Conflict of Interest

All authors have no conflict of interest to report.

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