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Determination of Minerals, Total Phenolic Content, Flavonoids, Antioxidants and Antimicrobial Activities of Ethanolic Extract of Sweet *Lupinus angustifolius* of Palestine

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Authors' contributions

This work was carried out in collaboration among all authors. Author MH designed the study, supervised the work and edited the final version of the manuscript. Author SR managed the experimental analysis of the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors SAO and AQ performed parts of the experiments. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To establish the most suitable extraction method for sweet lupine seeds and to determine minerals, phenolic content, flavonoids, antioxidant activity and antimicrobial activities.
Study Design: Known and standard experimental procedures are employed.
Place and Duration of Study: Department of Chemistry, Bethlehem University- Palestine, from January 2019 to March 2019.
Methodology: Seeds were ground and extracted by Soxhlet extractor using ethanol with different percentages (50%, 60%, 70%, 80% and 95%). Sodium, potassium and ferrous ion content were determined. Resistance to bacteria was performed against *Escherichia coli* and *Staphylococcus aureus*, while antioxidant activity was determined by FRAP method. Two types of flavonoids were measured: Flavonones and dihydroflavonols via the reaction with 2,4-dinitrophenylhydrazine. Phenolics were determined by the Folin-Ciocalteu method.

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Results: 50% ethanol resulted in the highest extract residue (18.6%) while 70% and 60% showed the lowest content (10.0% for both). 80% ethanol extracted sample showed the highest content for sodium (56.51 mg Na/g extract), while 60% and 50% ethanol extracts showed the highest content of potassium (2.25 and 2.33 mg K/g extract, respectively). The maximum concentration of ferrous ion was obtained with 70% ethanol (6.854 mg Fe^{+2}/g extract). 95% ethanolic extract showed the highest antioxidant activity (20.24 mg $FeSO_4$/g extract). Similar results were obtained for total phenolic content and flavonoids: 24.60 mg gallic acid/g extract for phenolics and 116.02 mg rutin/g extract for flavonoids. Extracts showed no bacterial activity against both types of bacteria used.
Conclusion: 95% ethanol extracted samples showed the highest antioxidant activity and the highest flavonoids and phenolic content. Sweet lupine extract did not perform any antimicrobial activity against both Gram positive and Gram negative bacteria.

Keywords: Sweet lupine; Soxhlet extractor; minerals; total phenolics; flavonoids; antimicrobial activity; antioxidant activity.

1. INTRODUCTION

Sweet *Lupinus angustifolius*, also called "narrow-leafed lupine" is a member of the legume family (subfamily Papilionoideae) containing both herbaceous annual and shrubby perennial types with attractive long racemes of flowers [1]. There are twelve lupine species within the *Lupinus* genus, all of which are native to Europe and the Mediterranean regions. Sweet lupine is widely cultivated in Australia, the color of its flower varies from blue, to pink and white in demonstrated forms [2,3]. *Lupinus angustifolius* is one of the four lupines that are widely known and fully domesticated for agriculture purposes (*Lupinus albus, Lupinus angustifolius, Lupinus luteu* and *Lupinus mutablis*).

For several years, lupine flour has been used in pasta, milk, soya substituents and diet products. Lupine seeds are also eaten as snacks in most regions of the world [4]. Lupine seeds can contain toxicologically relevant bitter quinolizidine alkaloids, which cause symptoms of poisoning of humans affecting the nervous, circulatory and digestive systems [5]. Typical symptoms of lupine alkaloid poisoning are dizziness, confusion, tachycardia, nausea and dry mouth, loss of motor coordination and in high doses, cardiac arrest and respiratory paralysis [5]. The levels of quinolizidine alkaloids in lupine seeds vary depending on the botanical and geographical origin of the lupine variety from which they derive. In contrast to bitter lupine, sweet lupine has low level of toxic alkaloid and suitable for human consumption even without debittering [6].

Lupine seeds, like other legumes are sources of vitamin, protein and fibers. Studies reported the pharmacological benefits of lupine alkaloids, with activity on circulatory system, metabolism against obesity and improving bowel health [7].

Due to the low concentration of biologically active materials in plants, it is necessary to use effective methods for extraction of these substances, specially using solvents that are environmentally friendly. Consequently, ethanol was the solvent of choice with different percentages to extract phenolics and flavonoids, which are responsible for the pharmacological properties such as antioxidants and antimicrobials. Therefore, a comprehensive determination of lupine properties is essential, not only because of its potential toxicity to humans, but also for its pharmacological properties.

2. MATERIALS AND EXPERIMENTAL DETAILS

2.1 Raw Materials and Equipment

Sweet lupine seeds were obtained from the local market, while reagents/chemicals were purchased from Sigma-aldrich. Deionized water was used in all preparations, and commercial ethanol was used for extraction. An Analytik Jena Specord 40 UV-VIS spectrophotometer was used for the determination of the antioxidant activity, phenolic content and flavonoids. A model FP 640 flame photometer was used for the measurements of sodium and potassium content. Bacteria strains were provided from Holy Family Hospital in Bethlehem-Palestine.

2.2 Extraction of Seeds

Lupine seeds were ground and extracted by Soxhlet extractor using different percentages of

ethanol (50%, 60%, 70%, 80% and 95%) for three hours. The solvent was evaporated under vacuum and the residue was stored in refrigerator away from direct light.

2.3 Stock Solution

Residue was dissolved in 50% ethanol (200 mg/100 mL) and this served as stock solution for the determination of sodium, potassium, ferrous ion, antioxidant activity, total phenolic content and flavonoids.

2.4 Determination of Sodium and Potassium

Sodium and potassium were determined by flame photometry against reference standards for both elements. From the calibration curves, the concentration of the extracted samples was determined.

2.5 Determination of Ferrous Ion (Fe^{+2})

Fe^{+2} in sample extract was determined by a titrimetric method: redox titration of Fe^{+2} with potassium dichromate using sodium diphenylamine sulfonate, a pH independent redox indicator. Endpoint was detected as the color turned to violet.

2.6 Determination of Antimicrobial Activity

Antibacterial activity was studied on sweet lupine against *S. aureus* (Gram positive) and *E. coli* (Gram negative) bacteria. An "Agar Well" method was used to test the resistance of extract to bacteria [8]. In this method, three wells were created in the Agar plates of the Muller-Hinton broth [9]: the first of which was for negative control (H_2O), the second was for positive control (Amoxicillin), and the third one was for sample (the extract). High concentrations of extracts (1.2 g/100 mL) were used for the determination of antibacterial activity. Petri dishes were incubated at 37ºC for 24-48 hours.

2.7 Antioxidant Activity

The antioxidant activity was determined by the ferric reducing antioxidant power (FRAP) [10] method that relies on reduction by antioxidants of the complex ferric ion-TPTZ (2,4,6-tri (2-pyridyl)-1,3,5-triazine). The binding of Fe^{+2} to the ligand makes a complex that gives the blue color intensity. The absorbance was measured to test the concentration of iron reduced, which is correlated with the concentration of antioxidant.

2.7.1 Analysis

For sample extract: 800 µL of sample (Stock solution) was mixed with 1000 µL FRAP, and for standard: 80 µL of standard $FeSO_4$ (0.1–2.0 mM) was mixed with 1000 µL H_2O and 1000 µL FRAP. Solutions were incubated at 37ºC for 15 minutes and the absorbance of the colored product was measured at λ=593 nm against 50% ethanol as blank.

2.8 Total Phenolics Content

The total concentration of phenolic compounds was determined using Folin-Ciocalteu method [11,12].

2.8.1 Analysis

For sample extract, 1.20 mL of 7.5% Na_2CO_3 was mixed with 100 µL sample and 1.8 mL diluted Folin- Ciocalteu reagent (1:1). Standard preparation was done as the follows, 1.20 mL Na_2CO_3 was mixed with 40 µL standard Gallic acid (90-900 ppm) and diluted Folin- Ciocalteu reagent (1:1). The mixtures were incubated for one hour at 30ºC where the sample was turned to greenish-blue, and absorbance was measured at λ=765 nm.

2.9 Flavonoids

The colorimetric identification and quantification of the two types of flavonoids (flavonones and dihydroflavonols) was based on their reaction with 2,4-dinitrophenylhydrazine (DNP) in the presence of KOH in methanol [13,14].

2.9.1 Analysis

For sample extract and standard (rutin, 5 – 100 ppm), 200 µL of stock solution was mixed with 400 µL 2,4-dinitrophenylhydrazine and placed in a water bath at 50ºC for 60 minutes. After cooling to room temperature, 800 µL of a 10% KOH/methanol solution was added to the mixture, where after 350 µL of the total mixture was diluted to 5.0 mL with 100% methanol. Absorbance was measured at λ=486 nm using a UV-VIS spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Extraction

Lupine seeds were extracted with different percentages of ethanol. Results are summarized in Table 1. As shown, the highest percentage of extract was obtained when 50% ethanol was used (18.6%). On the other hand, the lowest percentage was obtained when 60% and 70% ethanol were used (10.0% for both).

Table 1. Percentages of residue obtained from sweet lupine seeds

Solvent	Result
95% EtOH	12.2%
80% EtOH	10.9%
70% EtOH	10.0%
60% EtOH	10.0%
50% EtOH	18.6%

3.2 Determination of Sodium and Potassium

Results of sodium and potassium are illustrated in Table 2. The highest concentration of sodium was obtained when 80% of ethanol was used while the lowest concentration was obtained with 50% ethanol. This can be attributed to the fact that sodium is present in sweet lupine as organic salts that tends to dissolve in ethanol more than in water. In a previous study on *Lupinus albus* seeds [15], the highest concentration of sodium was obtained with 50% ethanol suggesting that sodium is present as inorganic complexes in the seeds. The highest concentration of potassium in sweet lupine was obtained when 50% and 60% ethanol were used. This result is in agreement with results reported by Hanania et al. (2018) where bitter lupine seeds were extracted with 60% ethanol, which resulted in highest potassium concentrations [15].

3.3 Determination of Ferrous Ions

Table 2 also shows that as the percentage of ethanol decreases, the ferrous content increases until the 70% ethanol extraction, where the maximum content of ferrous was extracted. However, below 70% ethanol, the ferrous content decreases.

3.4 Antimicrobial Activity

Sweet lupine extract showed no inhibition against neither *E. coli* nor *S. aureus* bacteria. The negative results reported here are in agreement with previous studies in terms of *E. coli*, but it does not agree with the results of the study on *S. aureus*, where significant activity was observed [16,17]. The extract of *Lupinus angustifolius* was weakly active on *E. coli.*

Table 2. Sodium, potassium and ferrous content of extracts (mg/g)

Ethanol %	Sodium	Potassium	Ferrous
95%	10.29	0.15	3.726
80%	56.51	1.00	4.340
70%	17.59	0.6	6.854
60%	10.51	2.25	2.424
50%	9.20	2.33	1.839

3.5 Determination of Antioxidant Activity and Total Phenolics Content

As illustrated in Table 3, the highest activity of antioxidants was obtained when 95% ethanol was used. Similar results were obtained for phenolics which is an important antioxidant as phytochemical in sweet lupine seeds. This result was expected since antioxidants such as phenolics are organic compounds that tend to dissolve in ethanol rather than water [18]. Our results showed higher content of phenolics and similar antioxidant activity to those reported in literature [19,20].

Ethanol was used in accordance with the literature data, to ensure optimum extraction of phenols, because the extraction efficiency of plant material using ethanolic-water is greater and environmentally friendly than methanolic-water extraction [21,22]. Compared to bitter lupine, it was found that bitter seeds have a higher antioxidant activity since it contains a higher content of phenols [15].

3.6 Determination of Flavonoids Content

Flavonones and dihydroflavones are the two types of flavonoids that were determined in sweet lupine. As illustrated in Table 4, 95% ethanolic extract resulted in the highest concentrations of flavonoids i.e. 115.02 mg rutin/g extract. It is worth mentioning that the concentration of these bioactive chemicals depends on many factors including climate, precipitation and soil conditions [23].

Table 3. Antioxidant activity and total Phenolics for sweet lupine extracts

Ethanol %	mg $FeSO_4$/g extract	mg Gallic acid/g extract
95%	20.24	24.60
80%	19.22	20.98
70%	12.03	18.35
60%	9.15	11.92
50%	7.23	12.28

Table 4. Rutin (flavonoids) concentrations obtained from for different percentage ethanol extraction

Ethanol %	mg Rutin/g extract
95%	115.02
80%	11.77
70%	35.19
60%	22.56
50%	39.83

4. CONCLUSION

Based on the results, antioxidants present in sweet lupine are organic compounds and are more likely to dissolve in ethanol than in water. Sodium ion was shown to be present in high percentages, especially in 80% ethanolic extract. Potassium, on the other hand, showed high concentration when extracted with 60% ethanol. It was found that sweet lupine has higher ferrous ion concentration than bitter lupine. Moreover, phenolics and flavonoids have many biological properties in plant especially as antioxidants, while antibacterial agents are absent from sweet lupine seeds. Although 50% ethanol was the highest percentage of extracted content (residue), yet it may have inorganic compounds or compounds with no biological effect to bacteria or oxidation reactions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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