Original Research Article

Determination of Minerals, Total Phenolic Content, Flavonoids, Antioxidant and Antimicrobial Activities of sweet *Lupinus Angustifolius* Ethanolic Extract in Palestine

ABSTRACT

Aims: To find out the best extraction method of sweet lupine seeds and to determine minerals, phenolic content, flavonoids, antioxidant and antimicrobial activities.
Study design: Known and standard experimental procedures are employed.
Place and Duration of Study: Department of Chemistry, Bethlehem University, from January 2019 to March 2019.
Methodology: Seeds were grinded and extracted by Soxhlet extractor using ethanol with different percentages (50%, 60%, 70%, 80% and 95%). Sodium, potassium and ferrous ion content were determined. As for pharmacological properties, 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 in this work: Flavonones and dihydroflavonols via the reaction with 2,4-dinitrophenylhydrazine. Phenolics were determined by the Folin-Ciocalteu method.
Results: 50% ethanol produced the highest content of extract (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 percentage of potassium (2.25 and 2.33mg K/g extract, respectively). The maximum concentration of ferrous was obtained with 70% ethanol (6.854mg Fe^{+2}/g extract). 95% ethanolic extract showed the highest antioxidant activity (20.24mg $FeSO_4$/g extract). The same result was 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 best 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 Papilioniodeae) 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 (*Lupinus albus, Lupinus angustifolius, Lupinus luteu L.* 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 in the world [4]. Lupine seeds can contain toxicologically relevant bitter quinolizidine alkaloids which cause symptoms of poisoning in humans affecting the nervous, circulatory and digestive systems. Typical symptoms of lupine alkaloid poisoning are dizziness, confusion, tachycardia, nausea, dry mouth, loss of motoric 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 poisoning alkaloid and suitable for human consumption even without debittering [6].

Lupine seeds, like other legumes are good 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 polyphenols and flavonoids which are responsible for the pharmacological properties such as antioxidants and antimicrobial. Therefore, a complete determination of lupine properties is essential, not only because of its toxicity but also for its pharmacological properties.

2. MATERIAL AND EXPERIMENTAL DETAILS

2.1. Raw Materials and Equipment

Sweet lupine seeds were obtained from the local market, while all other fine chemicals were purchased from Sigma Aldrich Company. Deionized water was used in all preparations, and commercial alcohol was used for extraction. Analytik Jena Specord 40 UV-VIS spectrophotometer was used for the determination of the antioxidant capacity, phenolic content and flavonoids. 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.

2.2. Extraction of Seeds

Lupine seeds were grinded and extracted by Soxhlet extractor using different percentages of ethanol (50%, 60%, 70%, 80% and 95%) for three hours. Solvent was then evaporated under vacuum and the residue was stored away from direct light.

2.3. Preparation of Samples (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 iron, 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 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. "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 is 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 or capacity 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 amount of iron reduced which is correlated with the amount 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 37 ºC 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 amount 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 following: 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 30 ºC where color 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 sample (Stock solution) was mixed with 400 µL 2,4-dinitrophenylhydrazine and placed in water bath at 50 ºC for 60 minutes. After cooling to room temperature, 800 µL of 10% KOH/methanol was added to the mixture, then 350 µL of the total mixture was diluted to 5.0 mL with methanol. Absorbance was measured at λ=486 nm using appropriate UV-VIS spectrophotometer.

3. RESULTS AND DISCUSSION

3.1. Extraction

Lupine seeds were extracted with different percentages of ethanol and results are summarized in table 1. As shown, the highest percentage of extract was obtained when 50% of ethanol was used (18.6%). On the other hand, the lowest percentage obtained was when 60% and 70% of 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. As table 2 shows, the highest amount of sodium was obtained when 80% of ethanol was used while the lowest amount was 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 bitter *Lupinus albus* seeds [15], the highest amount of sodium we obtained was with 50% ethanol suggesting that sodium is present in inorganic complexes in bitter seeds. The highest amount of potassium in sweet lupine obtained was when 50% and 60% ethanol were used. This result is in agreement with results of bitter lupine seeds where 60% ethanol produced the highest amount [15].

3.3. Determination of Ferrous Ions

Table 2 shows as the percentage of ethanol decreases, the ferrous content increases until it reaches 70% ethanol, where the maximum content is observed. However, below 70% alcohol, the ferrous content decreases.

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

3.4. Antimicrobial activity

Sweet lupine extract showed no inhibition against neither *E. coli* nor *S. aureus*. Our negative results are in agreement with previous studies in terms of *E. coli* [16]. The extract of *Lupinus Angustifolius* was weakly active on *E. coli*.

3.5. Determination of Antioxidant Activity and Total Phenolics Content

As illustrated in table 3, the highest activity of antioxidants was obtained when using 95% ethanol. The same result was obtained for phenolics that play a significant antioxidant role as phytochemical in sweet lupine seeds. This result was expected since antioxidants such as phenolics are organic compounds that tend to dissolve in ethanol more than water [17]. Ethanol was used in accordance with the literature data, to ensure optimum extraction of phenols, since the efficiency of ethanol extraction from plant material is greater and environmentally friendly when using ethanol-water system than methanol-water [18, 19]. Compared to bitter lupine, it was found that bitter seeds have a higher antioxidant activity

since it contains a higher content of phenols [15]. Since ethanolic extract contains other compounds in addition to phenols, Folin-Ciocalteu method was used due to its low sensitivity.

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

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 showed significant amount of flavonoids (115.02 mg Rutin/g extract). It is worth mentioning that the concentration of these bioactive chemicals depends on many factors including climate and soil conditions [20].

Table 4. Results obtained for different flavonoids content

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 above mentioned results, antioxidants existing in sweet lupine are organic compounds and are more likely to dissolve in ethanol than in water. Moreover, polyphenols 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 percent of extract content, yet it may have inorganic compounds or compounds with no biological effect to bacteria or oxidation reactions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

Not applicable

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