Analyses of Ethanol, Glucose, Acetaldehyde, Total Proteins and Oil Concentration in *Albizia Lebbeck* benth Seeds Extract

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ABSTRACT

Albizia lebbeck benth (*A. lebbeck*) is an important medicinal plant. The extract of *A. lebbeck* seeds obtained by cold extraction was analysed for ethanol, glucose, acetaldehyde, total protein, and oil content. The *A. lebbeck* seeds were soxhlet extracted using n-hexane to render crude oil. The physiochemical parameters, acid value, saponification value and ester value were determined. Characterization of the oil showed that oil is non-drying oil. The comparatively low acid value and corresponding saponification value implicated that oil has better shelf life and comes in the range of edible oils and can be used in liquid soaps and paints.

Key words: *Albizia lebbeck benth;* extraction; analysis

1. Introduction

Albizzia lebbeck Benth is one of the important medicinal plants**.** It has been reported that the plant possess anti-asthmatic, anti-inflammatory, anti-fertility, anti-allergic and anti-diarrheal properties**.** Ethanolic extract of the plant possess anti-bacterial and methanolic extract possess anti-microbial activities**.** *A. lebbeck* contains, alkaloids, flavonoids, tanins, saponins which have therapeutic value**.**

Zia-Ul-Haq *et al* worked on the composition and antioxidant potential of various parts of the *A. lebbeck* plant. The Compositional study indicated that pods and seeds have carbohydrates while saponin and their oil have linoleic acid as the major fatty acid and α-tocopherol as the major tocopherol component. Seeds have arginine and lysine

and pods have glutamic acid and aspartic acid in the highest concentrations. In vitro antioxidant assays showed that the examined extracts have potent antioxidant potential [1].

Pathak *et al* studied the effect of *A. lebbeck* methanolic extract on the bone erosion. Turnover was studied by analyzing various markers of bone erosion. The anti-arthritic activity of methanol extract of the bark of *A. lebbeck* (Mimosaceae) was evaluated against Freund's complete adjuvant induced-arthritis induce arthritis model in rats. It can be concluded that *A. lebbeck* methanolic extract (AL) possesses strong anti-arthritic property by modulating bone erosion [2].

Saha and Ahmed investigated that the cold extraction of mixture of equal proportions of petroleum ether**,** ethyl acetate and methanol of the bark of *A. lebbeck* was chosen for pharmacological screening [3].

Gupta *et al* worked to evaluate the antifertility activity in the male albino rats from the methanolic pod extract of *A. lebbeck*. A significant decrease in the weights of testis, seminal vesicles, epdidymis and ventral prostate was brought by *A.lebbeck* pod extract. The methanolic extract *of A. lebbeck* pods causes spermatogenic arrest in male albino rats [4].

Jangwan *et al* worked on the methanolic extract of the stem bark of *A. Lebbeck*. The compound which was isolated exhibited potent cytotoxic activity against human aqueous cell carcinoma [HSC-2 and HSC-3] [5].

Rahul *et al* investigated the Phyto-chemical screening and antibacterial activity of *A. lebbeck* leaves was assessed. The phytochemical screening of *A. lebbeck* leaves extract showed the presence of alkaloids, glycoside, tannins, saponins, flavanoids, and carbohydrates. The extracts were sensitive against gram positive and gram negative [6].

Sivakumar *et al* studied the diuretic activity of methanolic extract of *A. lebbeck* in rats by administration a dose of 200 and 400 mg/kg and Frusemide (20mg/kg) was used as a standard. On comparison to control group of rat**,** the extract treated rats showed increase in urine volume and urinary concentration of Na^+**,** K^+ and Cl^- [7].

Jeeva *et al* worked to evaluate the phytochemical constituents and antibacterial activity of the flower extracts of *A. Lebbeck***,** *Cordiasebestena***,** *Thunbergiagrandiflora and Antigonon Leptopus*. The phytochemical antibacterial screening revealed that the methanolic extracts of *A.Lebbeck* and *A.Leptopus* have higher

degree of chemical diversity and antibacterial activity. The methanolic extracts of *A. Lebbeck, A. Leptopus and C. sebestena* show the presence of several bioactive compounds [8].

Several other such studies of other plants have also been done. The proximate composition of seeds of *A. saman, Millettia griffonianus and Tamarindus indica* and chemical analysis of the oils was done. By AAS composition of the metals in the seeds and oils were find out and by the use of GC the fatty acid composition was evaluated [9]. The Chemical, physicochemical and functional properties of fibrous materials from freeze dried (FDPSP) and oven dried (ODPSP) *Parkiaspeciosa* pod (PSP) was evaluated [10]. The physicochemical parameters of oil extracted from the seeds of *Ceiva pentandra Linn* were determined and compared with those recommended by the international codex standards for edible oils and the nutritional and anti-nutritional content of *A. Lebbeck* like the crude protein, ether extract, crude fibre, ash, mineral and antinutrients like phytate, cyanide, oxalate, saponin, and tannins but the presence of tannins was not detected. They studied the physical and chemical properties of fats and oils extracted from the white and red seeds of *sesame* seeds and compared with those reported by the other workers. The *sesame* seeds oil content was obtained in high amount which strongly indicates its prospects for commercial extraction [11-13]. Akpan *et al* extracted and characterized crude and refined *castor* oil using n-hexane as a solvent. The crude and refined oil was characterized by (parameters) specific gravity, refractive index, acid value, saponification value and iodine value [14].

In the present study an attempt is made to determine the concentration of ethanol, glucose, acetaldehyde and total proteins in locally grown *A. lebbeck* seeds extract.

2. Experimental

2.1. Materials and Methods

All experimental work was performed at Analytical Chemistry lab, Abdul Wali Khan University Mardan, Pakistan. First of all the distillation of all commercially available solvents were carried out. The distilled solvents were used for extraction and in different tests carried out for analysis of the extract.

2.2. Collection of A. lebbeck seeds

The *A. lebbeck* seeds were collected from the plants grown in Nizampur, District Nowshera, KP, Pakistan.

2.3. Preparation of sample

First of all the pods were separated from the seeds and then the seeds were dried kept in shaded room for a period of days at ambient temperature and ventilated condition (air shaded drying in our room). The seeds were chopped by blender to obtain dry powdered material.

2.4. Cold Extraction

50g of sample material was taken and soaked in 200ml of ethanol solvent for three weeks. The mixture was shaken twice a day. 1st layer was removed and 100 ml was added to the soaked sample material. After a week the 2nd layer was removed and a third layer (100 ml) was introduced to the sample flask at room temperature, the layers were then mixed and filtered. In Rotary evaporator, the Ethanol filtrate was evaporated at reduced pressure to get dark yellow extract. After this the extract was placed at room temperature for two days, by doing this ethanol content evaporated and we got concentrated ethanolic extract which was collected in a vial and stored for analysis.

2.5. Concentrating the A. lebbeck seeds cold extract with rotary evaporator

The *A. Lebbeck* seeds cold extract was taken in a cleaned round bottom flask. Temperature of rotary evaporator was set at 78 ^{o}C according to the boiling point of ethanol used for extraction. The extract was then treated in rotary evaporator. When all the solvent was removed, the concentrated extract was then taken out from Rb flask. The concentrated extract was stored for futher analysis.

2.6. Physicochemical studies of A. lebbeck seeds cold extract

Different analytical tests were done for determination of ethanol, glucose, acetaldehyde, total protiens and oil. Ethanol concentration was determined by using saccharides removal kit followed by ethanol quantification kit. Glucose concentration in sample was determined by DNS method. Acetaldehyde kit was used for the quantification of acetaldehyde in the sample. For protein determination standard curve was generated using Bradford assay.

In the ethanolic extract of *A. lebbeck* seeds the protein concentration was determined by using strip. The strip indicates presence of proteins by changing color. The strip was dipped in the sample when the sensitive strip changed the color, it was compared with scale to check protein amount.

2.7. Hot extraction

In hot extraction method 50 gm of *A. Lebbeck* seeds was taken inside filter paper and 450 ml n-hexane was taken in the round bottom flask. After collecting the first extract 450 ml fresh n-hexane was added. The two extracts were then combined and concentrated in the rotary evaporator.

2.7. Physicochemical studies of A. lebbeck seeds hot extract

The hot extract obtained with soxhlet apparatus was oily in nature. Different physicochemical tests were done for determination of acid value, saponification value and ester value. And percentage oil yield was calculated.

3. Results and Discussion

3.1 Cold extraction

Two methods were used for the oil extraction from *A. lebbeck* seeds cold extraction and hot extraction method. The cold ethanolic extract of *A. lebbeck seeds* obtained after rotary treatment was subjected for further analysis and different tests were applied on this extract. The results of those tests are given in Table 3.

Table 3.1 Compositional analysis of ethanolic crude extract of *A. lebbeck* seeds after rotary evaporation

Sample	Ethanol (g/L)	Glucose (g/L)	Acetaldehyde (g/L)	Total Proteins (mg/ml)
A. Lebbeck seeds	50.941	0.695	0.039	2.513

The compositional analysis of ethanolic extract of *A. lebbeck* seeds revealed the presence of ethanol, glucose and acetaldehyde and proteins as indicated in Table 3.1. The ethanol and proteins are present in large amount while glucose and acetaldehyde are present in lesser amount. But comparative to acetaldehyde glucose has higher amount.

Table 3.2 Protein concentration in different layers of *A. lebbeck* seeds cold extract measured by stripping method.

Nature of sample	Proteins (mg/dl)
First layer	~30
Second layer	~30
Third layer	~10
Layer after rotary treatment	~30

3.2. Protein determination by stripping method

The protein concentration was determined in the above samples by stripping method. The results shown in Table 3.2, indicate that the *A. lebbeck* seeds have enough amounts of proteins in all the layers. The third layer shows comparatively less amount of protein because the proteins are already extracted in the first two layers. The protein content for *A. lebbeck* seeds obtained in this study is slightly lower than the range mentioned in literature.

3.3. Oil obtained by Soxhlet extraction

The n-hexane extract of *A. lebbeck* seeds obtained from hot extraction method was analysed by applying different physiochemical tests and the percentage oil of the extract was calculated. The results are given in Table 3.3.

Table 3.3 Percentage Oil yield extracted from *A. Lebbeck* seeds using n-hexane as solvent in soxhlet extract system

Amount/color	Results
Percentage of oil	14%
color of extracted oil	Dark Yellow

After complete evaporation of the solvent the concentrated oil was left and then percentage oil yield was calculated. The percentage oil yield with respect to dry mass of 25g of *A. lebbeck* seeds was found experimentally to be 7% and calculating for

50g of *A. lebbeck* seeds the percentage oil yield is 14%. The dark yellow oil of *A. lebbeck* seeds had pleasant smell.

Different physicochemical tests were applied for determining various parameters of the obtained oil from *A. lebbeck* seeds. The results of the physiochemical tests applied on the crude oil are given below in the table 3.4.

Table 3.4. Content and physiochemical parameters of the crude oil of *A. lebbeck* seeds obtained by soxhlet extraction.

Physiochemical Parameters	**Value (mgKOH/g)**
Saponification value S.V.	78.54
Acid value A.V.	1.122
Ester value	77.418

The content and the Physiochemical characteristics of the crude oil extracted from the seeds of *A. lebbeck* have been presented in Table 3.4, all these Physiochemical parameters were determined using the official methods and recommended practices of the American oil Chemist's Society (AOCS). These physiochemical parameters are used for the characterization of the oil or fat to evaluate its storage, edibility and other important application.

Acid value is the number of potassium hydroxide in mg required to neutralize the free fatty acids in 1g of fat or oil. Acid value gives an indication of the quality, condition and edibility of the oil. Edible oils must have low acid value. High acid value indicates presence of more free fatty acids which in turn indicates that the oil is old, unrefined or probably heated. Free fatty acids are responsible for aroma of the oil. The acid value of the crude oil from *A. lebbeck* seeds was determined to be 1.122 mg KOH/g which is higher than *Ceiva pentandra Linn* seed oil (0.56) and lower than palm oil (16.4) which indicates that the

oil is edible and is advantageous in paint and liquid soap making.

The saponification number is the measure of total free and combined acids present in the oil. In combination with acid value, saponification value are also helpful in determining quantity of glyceroids and mean weight of the acids in a given oil sample. Saponification value has inverse relation with long chain fatty acids and molecular weight. Saponification value are also significant in soap making. The saponification value of the crude oil of *A. lebbeck* seeds was determined to be 78.54 mg KOH/g which is lower than Jatropa seed oil (122.49 mg KOH/g) but closer to beeswax (88-100).It indicates that oil can be used in soap formation.

The ester value of crude oil from *A. lebbeck* seeds was determined to be 77.418 mgKOH/g. The ester value helps to measure the intactness of the ester bond between the glycerol molecule and the fatty acids present in the oil. Oils having higher ester value are more intact and are less prone to oxidation.

Conclusions

The successive extraction of *A. lebbeck* seeds was done with ethanol and n-hexane**.** The ethanolic extract was evaluated for proximate analysis**.** The results of the proximate analysis revealed the presence of ethanol, glucose, acetaldehyde and protein quantity**.** The study showed that seeds have high quantity of ethanol and proteins and low quantity of glucose and acetaldehyde**.**

Physio-chemical characterization of the oil obtained from n-hexane extract was done**.** On the basis of results obtained from characterization the oil was found to be edible and can be used in foods, paints, liquid soap making and pharmaceutical industries due to its low acid value and saponification value. The oil yield capacity can be improved through different conditions and concentrations of the solvent used. Further advanced spectroscopic studies are required for the structural elucidation and identification and characterization of the bioactive compounds present in seeds of *A. lebbeck*.

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