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Darakhshan Masroor
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan.

Rafi Akhtar Sultan
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan.

Salman Ahmed
Lecturer, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan.

Muhammad Mohtasheemul Hasan
Associate Professor, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan.

Correspondence
Muhammad Mohtasheemul Hasan
Associate Professor, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan.

Near infrared spectroscopy of *Cicer arietinum* L., seeds

Darakhshan Masroor, Rafi Akhtar Sultan, Salman Ahmed and Muhammad Mohtasheemul Hasan

Abstract
NIRS is an advance, fast, reliable and non-destructive technique extensively used in chemical, food, petrochemical and pharmaceutical industries for rapid and reliable standardization. NIRS of *Cicer arietinum* L. *Desi* and *Kabuli* variety seed flours was performed for qualitative evaluation of secondary metabolites. The major suggested compounds in *Cicer arietinum Desi* and *Kabuli* variety were found as adenine, biochanin-A, biochanin-B, biochannin A 7-O- β -D-glucoside, calycosin, cerebroside, choline, ciceritol, citric acid, diadzein, fructose, garbanzol, genistein, glucose, inositol, isoliquiritigen, liquiritigenin, methyl tetracosanoate, ononin, pyrimidine, saccharose, sissotrin, stigmasterol, trifolirhizin. β- amyrin and β-sitosterol. The presence of these compounds justifies the use of *Cicer arietinum* as nutritional food and tonic.

Keywords: NIRS, *Cicer arietinum*, secondary metabolites

Introduction
The active chemical constituents of medicinal plants are responsible for various desired therapeutic effects. Several pharmaceutical companies have initiated sophisticated plant screening programs to find out promising active therapeutic compound(s) in plants. The infrared (IR) region in spectroscopic techniques is used in the agriculture, chemical, cosmetic, food, medicine, petrochemical, pharmaceutical and textile industries. In 1800, Frederick William Herschel a British astronomer discovered the light in the near infrared (NIR) region, but the first practical application of NIRS as an analytical method was carried out by Karl Norris (an engineer working at the USDA Instrumentation Research Laboratory) on grains and seeds in 1960s. NIRS is now a recognized analytical method for grains and seeds by the American Association of Cereal Chemists and the American Oil Chemist Association. In the near infrared (NIR) region, the spectral signatures appear due to the stretching, bending and rotating vibrations in the chemical compounds [1, 2]. NIRS is a rapid, reliable and non-destructive technique which provides acceptable accuracy with a minimum sample preparation in both qualitative and quantitative analysis to meet the requirements of quality control. This technique is successfully used to determine ash, carbohydrates, fatty acids, inorganic phosphorus, moisture, protein, starch and total dietary fiber [1, 3-5].
Cicer arietinum belongs to family Fabaceae, commonly known as Chickpea in English and *Channa* in Urdu. Chickpea is distributed in Africa, Asia, Caribbean, Central America, Europe, Middle East and South America. *Desi* (black) and *Kabuli* (white) *Channa* are two cultivated varieties based on color and size. The chickpea seeds have carbohydrates, proteins (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine amino acids), fatty acids (arachidic, linoleic, myristic, oleic, palmitic and stearic acids) and vitamins A-E. In secondary metabolites, saponins, phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, vanillic acid) and β-sitosterol are reported [6, 7]. The seeds have also shown antibacterial, anti-carcinogenic, antidiabetic, antifungal, anti-inflammatory, antioxidant, diuretic, hepatoprotective, hypocholesterolemic, hypoglycemic and purgative activities [6-9].

Materials and methods
Plant material, identification and sample preparation
The seeds of *Cicer arietinum* L., were purchased and authenticated by a taxonomist of the Department of Botany, University of Karachi. The voucher specimen number (CAB-06-15/16) and (CAW-07-15/16) were deposited in the Herbarium. The seeds were separately grinded and powdered then passed through 600µm sieve and kept in an amber bottle at room temperature before experiment.

NIR spectral data

FOSS XDS near infrared Rapid Content Analyzer (FOSS NIR Systems, Inc., USA) was used for NIR spectrum (700 - 2500 nm). XDS RCM Iris was used to center the sample for reproducible sample positioning. NIR reflectance spectra were recorded on FOSS XDS near infrared Rapid Content Analyzer at ambient temperature. Powdered sample (10g) was filled in a disposable glass silicate vial. Prior to the screening, the vial was closed with a plastic lid, then inverted once and tapped twice on a soft surface. The outer surface of the glass vial was clean from any contamination before analysis. The vial then placed onto the sample presentation glass of Foss Rapid Content Analyzer and centered by using the Iris centering device. The Iris was retracted completely after centering and before scanning [10].

Results and Discussion

The NIR spectrum of *C. arietinum Desi* and *Kabuli* variety suggested the presence of adenine, β- amyrin, β-sitosterol, biochanin A 7-O- β -D-glucoside, biochanin A, biochanin B, calycosin, cerebroside, choline, ciceritol, citric acid, diadzein, fructose, garbanzol, genistein, glucose, inositol, isoliquiritigen, liquiritigenin, methyl tetracosanoate, ononin, pyrimidine, saccharose, sissotrin, stigmasterol and trifolirhizin. The results of NIRS are presented in table 1-2 and figure 1-2.

NIR spectroscopy is useful analytical technique to assess the structure of organic compounds containing C -H bonds, O - H, and N - H bonds through the absorption of energy in the NIR region of the spectrum due to relatively weak, broad overtones and combination bands of fundamental vibrations transitions. It has been effectively used for the analysis of natural products and for assessment of product quality [1, 3]. In this study, the spectral data are analyzed between the regions of 1100 – 2400 nm. Each and every peak's wave length of specific spectrum indicates possible functional groups, thus provide fingerprint of a compound in taking powdered plant material. The detail of NIR spectra with specific wavelengths, bond vibrations, possible functional groups and the compounds responsible for the peak present in the seed flour of *C. arietinum* are given in table. The data obtained confirms the presence of chemical compounds reported in the literature.

Conclusion

The results of NIR analysis of the powder confirms the presence of reported chemical compounds. The compounds justify the nutritional importance of *C. arietinum*. This technique can be used as a rapid and authentic method for herbal drug standardization.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this paper.

Table 1: Wave length of spectral peaks obtained from *Cicer arietinum* (*Desi*) seed flour and possible chemical compounds

Band vibrations with peak's wave length (nm)	Possible functional groups	Compounds present in the seed flour that may be responsible for peak
CH second overtone (1205)	CH_2, CH	adenine, biochanin-A, biochanin-B, biochannin a 7-o- β -d-glucoside, calycosin, cerebroside, choline, ciceritol, citric acid, diadzein, fructose, garbanzol, genistein, glucose, inositol, liquiritigenin, ononin, pyrimidine, saccharose, sissotrin, stigmasterol, trifolirhizin, β- amyrin and β-sitosterol.
First overtone of CH combinations; NH and OH first overtone (1460)	CH,$CONH_2$, ROH	
CH first overtone (1765)	CH	
N-H+C-H combination band (2125)	CC, RNH_2	
C-H+C-H combinations band (2285, 2310, 2345)	CH_3,CH_2, CH	

Table 2: Wave length of spectral peaks obtained from *Cicer arietinum* (*Kabuli*) seed flour and possible chemical compounds

Band vibrations with peak's wave length (nm)	Possible functional groups	Compounds present in the seed flour that may be responsible for peak
CH second overtone (1725)	CH_2, CH	adenine, biochanin-A, biochanin-B, biochannin a 7-o- β -d-glucoside, calycosin, cerebroside, choline, ciceritol, citric acid, diadzein, fructose, garbanzol, genistein, glucose, inositol, liquiritigenin, ononin, pyrimidine, saccharose, sissotrin, stigmasterol, trifolirhizin, β- amyrin and β-sitosterol.
OH and NH first overtone (1470)	CH,$CONH_2$, ROH	
CH and SH first overtone (1770)	CH	
C-H+C-H combinations band (2290, 2320)	CH_3,CH_2, CH	
N-H+C-H combination band (2120)	$CONH_2$(R)	adenine

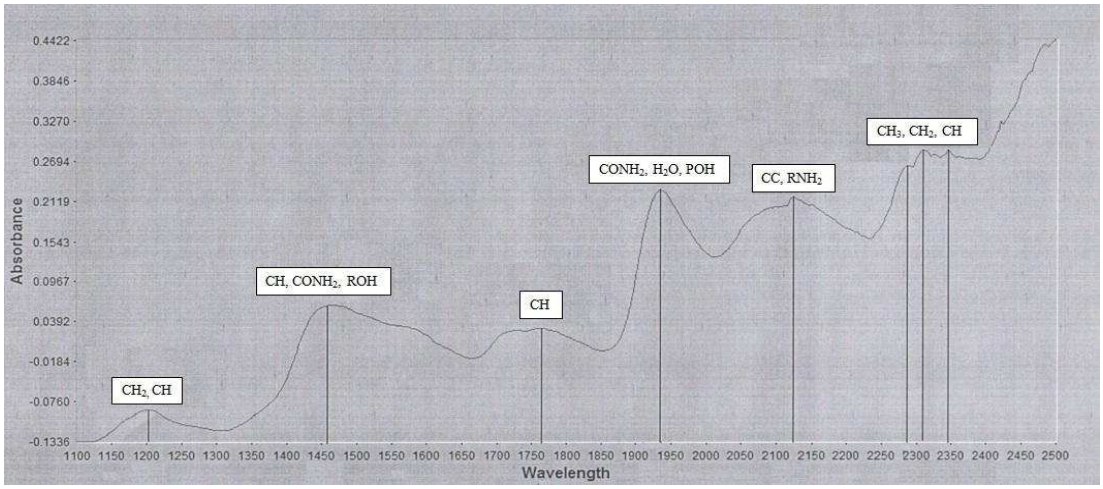

Fig 1: NIR spectrum of *Cicer arietinum* (*Desi*) seed flour

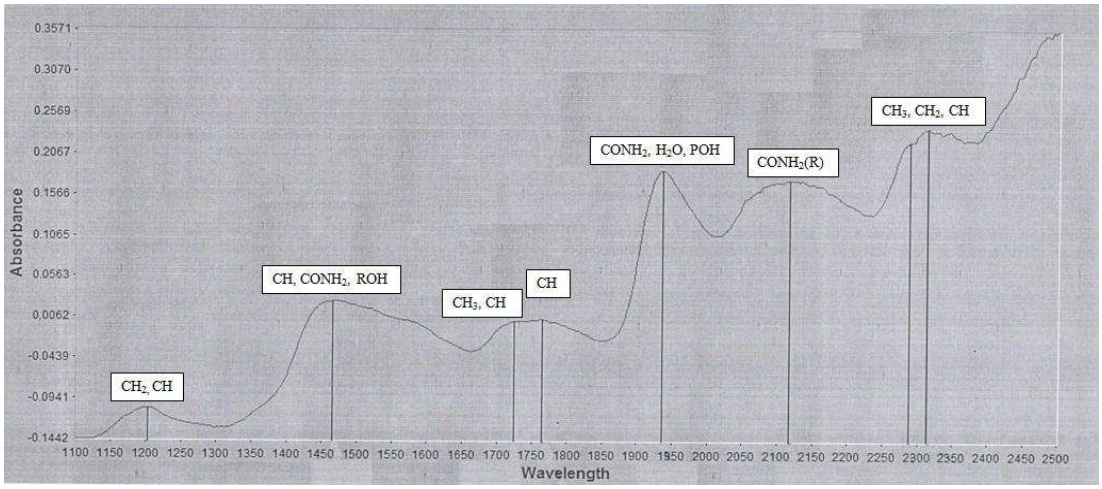

Fig 2: NIR spectrum of *Cicer arietinum* (*Kabuli*) seed flour.

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