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Research Article

NEAR INFRA RED SPECTROSCOPIC ANALYSIS OF *MACROTYLOMA UNIFLORUM* (LAM.) VERDC., *PHASEOLUS LUNATUS* LINN. AND *PHASEOLUS VULGARIS* LINN.

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ABSTRACT

The present study is designed to determine the types of secondary metabolites in three selected seed powders by fast, reliable, and non-destructive Near Infra Red spectroscopic analytical technique. NIRS analysis of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., seed flours were done for qualitative evaluation of secondary metabolites. The fine powder of crude drug was directly applied in FOSS XDS near infrared Rapid Content Analyzer. The properly filled powdered sample in a disposable glass silicate vial and closed with plastic lid was centrally placed onto the sample presentation glass of Foss Rapid Content Analyzer. The scanning of sample at ambient temperature gives NIR spectra within 30 seconds. The major proposed compounds in *M. uniflorum* were 3,4-dihydroxybenzoic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, kaempferol, linolenic acid, niacin, quercetin, sinapinic acid, syringic acid, and vanillic acid. Whereas, coumaric acid, fructose, galactose, L-serine, raffinose, stachyose, sucrose, threonine, and tyrosine were detected in *P. lunatus* and the NIR suggested the presence of caffeic acid, ferulic acid, sinapinic acid, phytic acid, leucine, and valine in *P. vulgaris*. NIRS as an advance innovative technology extensively used in chemical, food, petrochemical, and pharmaceutical industries for rapid and reliable standardization. The present study revealed a good correlation to conclude that flour samples of all the three legume seeds could potentially be added to food system to achieve nutritional and functional benefits.

Key words: NIRS, *Macrotyloma uniflorum, Phaseolus lunatus, Phaseolus vulgaris,* Secondary metabolites.

INTRODUCTION

The active chemical constituents of medicinal plants which are found in low concentrations are responsible for various desired therapeutic effects. Several pharmaceutical companies have initiated sophisticated plant screening programs, in order to find out promising active therapeutic compound(s). Therefore, the use of gas chromatography (GC), liquid chromatography (LC), high performance liquid chromatography (HPLC), mass spectrometry (MS), and Nuclear Magnetic Resonance (NMR) were focused for the elucidation of isolated compounds[1]. The increasing population of the world requires quick, reliable, and accurate techniques for the determination of food quality. The non-destructive analytical methods have come to stay with promising results and its application runs through all fields of science. NIRS as a non-destructive technology is now specially used to determine chemical and physical properties of food and related products, in order to monitor their quality[2]. *Macrotyloma uniflorum* is distributed in Pakistan, India, and Sri Lanka; *Phaseolus lunatus* is a native of tropical America, now widely cultivated throughout the tropics of the world including Pakistan and *Phaseolus vulgaris* is widely cultivated in tropical and temperate regions[3]. The seeds of *M . uniflorum* are ovoid and gray to brown with pale fawn in color, sometimes with faint mottles or with small scattered black spots or with both. The reported length and width (mm) is 6 - 8 x 3 - 4[4]. The seeds of *P. lunatus* are of kidney or boat shape and white to green, grey and yellow to brown, red, purple, and black with 19.02 - 31.7 x 12.7 - 18.9 mm in size[5]. Whereas, the seeds of *P. vulgaris* are of kidney or boat shape and dark red in color. The reported size of the seeds is 11 - 15 x 5 - 8 mm[6]. The seeds of *M. uniflorum*, *P. lunatus,* and *Phaseolus vulgaris* are reported to possess caffeic, coumaric acid, delphinidin, ferulic acid, leucine, linoleic acid, lysine, malvidin, oleic acid, petunidin, phytic acid, sinapinic acid, and valine[7-13]. The present study is designed to determine the types of secondary metabolites in *M. uniflorum*, *P. lunatus* and *P. vulgaris* seed flours by using NIRS analysis.

MATERIALS AND METHODS

Plant material, identification, and sample preparation

The seeds of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., were purchased and authenticated by a taxonomist of the Department of Botany, University of Karachi. The voucher specimen number of *Macrotyloma uniflorum* (Lam.) Verdc., (G.H.No.86483), *Phaseolus lunatus* Linn., (G.H.No.86451), and *Phaseolus vulgaris* Linn., (G.H.No.86536) were deposited in the Herbarium of University of Karachi. The seeds were separately grinded and powdered then passed through 600µm sieve and kept in an amber bottle at room temperature before commencing the experiment.

NIR Spectral data

FOSS XDS near infrared Rapid Content Analyzer (FOSS NIR Systems, Inc., USA) was used for NIR spectrum, and the range is 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 of 19 ml. Prior to screening, the vial was closed with a plastic lid. Then it was 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[14].

RESULTS

The suggested compounds in *M. uniflorum* were 3,4-dihydroxybenzoic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, kaempferol, linolenic acid, niacin, quercetin, sinapinic acid, syringic acid, and vanillic acid. Whereas, coumaric acid, fructose, galactose, L-serine, raffinose, stachyose, sucrose, threonine, and tyrosine were detected in *P. lunatus*. The NIR spectrum of *P. vulgaris* suggested the presence of caffeic acid, ferulic acid, phytic acid, sinapinic acid, leucine and valine. Ferulic acid, leucine, and phytic acid, and sinapinic acid were commonly estimated in *M. uniflorum, P. lunatus* and *P. vulgaris*. The spectra of *M. uniflorum* and *P. lunatus* showed the presence of three same compounds as coumaric acid, linoleic acid and oleic acid. Caffeic acid, delphinidin, malvidin, petunidin, and valine were common in the *M. uniflorum* and *P. vulgaris*. The results of NIRS analysis are presented in table-1(a-c) and figure -1(a-c).

Table-1(a): Wave length of spectral peaks obtained from *Macrotyloma uniflorum* 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	3,4-dihydroxybenzoic acid, caffeic acid, chlorogenic acid, cyanidin, daidzein, delphinidin, ferulic acid, gallic acid, genistein, kaempferol, linoleic acid, linolenic acid, malvidin, myricetin, niacin, oleic acid, *p*-coumaric acid, petunidin, *p*-hydroxybenzoic acid, phytic acid, protocatechuic acid, quercetin , sinapinic acid, syringic acid, vanillic acid, β-carotene.
OH and NH first overtone(1455)	CH,$CONH_2$, ROH	
CH and SH first overtone(1760)	CH, SH	
C-H+C-H combinations band(2290, 2320)	CH_3,CH_2, CH	
First overtone of CH combinations; NH and OH first overtone (1470)	CONHR, ROH	3,4-dihydroxybenzoic acid, caffeic acid, chlorogenic acid, cyanidin, daidzein, delphinidin, ferulic acid, gallic acid, genistein, kaempferol, malvidin, myricetin, *p*-coumaric acid, petunidin, *p*-hydroxybenzoic acid, protocatechuic acid, quercetin , sinapinic acid, syringic acid, vanillic acid.
NH and N-H+C-H combination band (2080)	$CONH_2$(R), ROH	
C-O+O-H and O-H combination band, first overtone (1930)	$CONH_2$, H_2O, POH, RCO_2R'	3,4-dihydroxybenzoic acid, caffeic acid, chlorogenic acid. ferulic acid, gallic acid, kaempferol, linolenic acid, niacin, oleic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, phytic acid, protocatechuic acid, quercetin , sinapinic acid, syringic acid, vanillic acid.
N-H+C-H combination band (2120)	$CONH_2$(R), RNH_2	Niacin

Table-1(b): Wave length of spectral peaks obtained from *Phaseolus lunatus* 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(1200)	CH_2, CH	Alanine, arachidonic acid, arginine, aspartic acid, behenic acid, coumaric acid, cysteine, ferulic acid, fructose, galactose, glutamic acid, glycine, histidine, isoleucine, leucine, lignoceric acid, linoleic acid, L-serine, lysine, methionine, myristic acid, oleic acid, palmitic acid, palmitoleic acid, phenylalanine, phytic acid, proline, raffinose, sinapinic acid, stachyose, stearic acid, sucrose, threonine, tryptophan, tyrosine, valine, verbascose.
CH and SH first overtone(1750)	CH_2, CH, SH	
C-O+O-H and O-H combination band, first overtone (1930)	$CONH_2$, H_2O, POH, RCO_2R'	
C-H+C-H combinations band (2290, 2320, 2350)	CH_3,CH_2, CH	
First overtone of CH combinations; NH and OH first overtone (1470)	CONHR,ROH	Coumaric acid, fructose, galactose, L-serine, raffinose, stachyose, sucrose, threonine, tyrosine, verbascose.
N-H+C-H combination band(2100)	$CONH_2$(R)	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, L-serine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine.

Table-1(c): Wave length of spectral peaks obtained from *Phaseolus vulgaris* 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(1200)	CH_2, CH	Caffeic acid, ferulic acid, leucine, lysine, malvidin 3,5-diglucoside, malvidin 3-glucoside/oenin, myrtillin / delphinidin 3-glucoside, petunidin 3,5-O-diglucoside, petunidin 3-glucoside, phytic acid, sinapinic acid, valine.
First overtone of CH combinations; NH and OH first overtone(1460)	CH,$CONH_2$, ROH	
CH first overtone(1770)	CH	
N-H+C-H combination band(2150)	CC, RNH_2	
C-H+C-H combinations band(2280, 2330, 2380)	CH_3,CH_2, CH	
C-O+O-H and O-H combination band, first overtone(1930)	$CONH_2$, H_2O, POH, RCO_2R'	Caffeic acid, ferulic acid, sinapinic acid, malvidin 3,5-diglucoside, phytic acid, lysine, leucine, valine.
N-H+C-H combination band(2100)	$CONH_2$(R)	Leucine, lysine, valine.

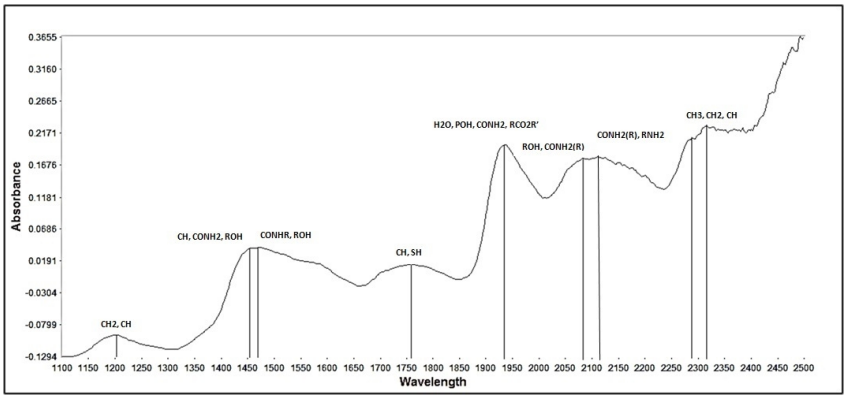

Figure-1(a): NIR spectrum of *Macrotyloma uniflorum* seed flour

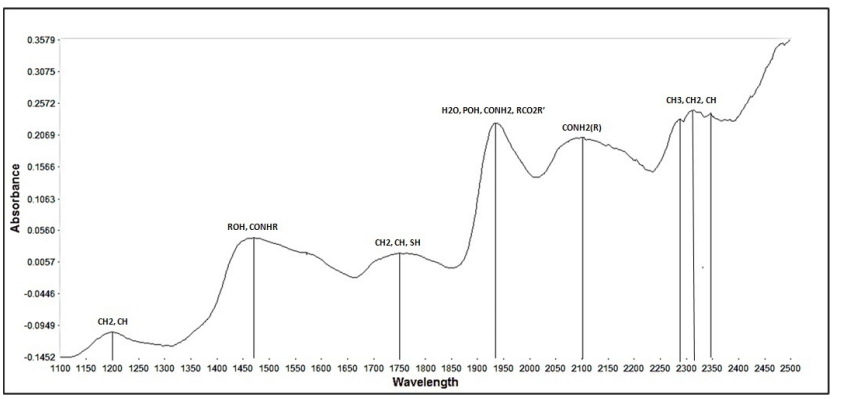

Figure-1(b): NIR spectrum of *Phaseolus lunatus* seed flour

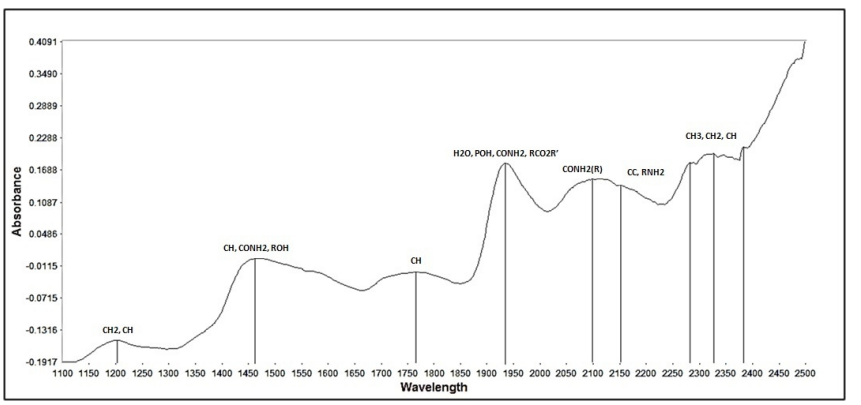

Figure-1(c): NIR spectrum of *Phaseolus vulgaris* seed flour

DISCUSSION

The electromagnetic spectrum between the visible and the microwave wavelengths from 700 - 2500 nm region. NIR spectroscopy is useful to assess the structure of organic compounds containing C - 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, assessment of product quality and for controlling production. NIR spectroscopy has been widely applied in both qualitative and quantitative analysis with a minimum sample preparation. This technique is quick, reliable, accurate and of low cost[1,2]. It has been successfully used to determine ash, carbohydrates, fatty acids, inorganic phosphorus, moisture, protein, starch, and total dietary fiber in crops such as chickpeas, peas, soybean, etc. NIR spectroscopy is also helpful in developing calibration models to determine the levels of moisture, protein, starch, fat, and seed weight in ground and intact common beans seeds. The NIR spectrum can give a global signature of the composition as a fingerprint[15].

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 *M. uniflorum*, *P. lunatus* and *P.vulgaris* are given in table-1 (a-c). The data obtained confirms the presence of chemical compounds reported in the literature.

CONCLUSION

The current study has qualitatively indicated the presence of chemical compounds and their major contribution in the chemistry of the sample. The data obtained confirms the presence of chemical compounds reported in literature. NIR is a rapid (no sample preparation is required and take < 30 seconds analysis time), reliable, and non destructive technique and, therefore, it can be used as a rapid and authentic method for herbal drug standardization.

REFERENCES

1. Cozzolino D. Near infrared spectroscopy in natural products analysis. Planta Med 2009;75:746-756.
2. Teye E, Huang, Afoakwa N. Review on the potential use of near infrared spectroscopy (NIRS) for the measurement of chemical residues in food. Am J Food Sci Technol 2013;1:1-8.
3. Nasir E, Ali SI. *Flora of Pakistan*. Vol. 100 Papilionaceae. 1977. 1-389.
4. Chauhan JS, Tomar YK, Badoni A, Singh NI, Ali S. Morphology and influence of various plant growth substances on germination and early seedling growth in *Macrotyloma uniflorum* (Lam.). J Am Sci 2009;5:43-50.
5. Aniszewski T, Karttunen A-L, Hyvarinen H. Structure of *Phaseolus lunatus* testa at its central point. Acta Biolo Cracov Bot 2006;48:69-76.
6. Giurcă D. Morphological and phenological differences between the two species of the *Phaseolus* genus (*Phaseolus vulgaris* and *Phaseolus coccineus*). Cercet Agron Mold 2009;42:39-45.
7. Kawsar, SMA, Huq E, Nahar N, Ozeki Y. Identification and Quantification of Phenolic cids in *Macrotyloma uniflorum* by Reversed Phase-HPLC. Am J Plant Physiol 2008;3:165-172.
8. Sreerama YN, Sasikala VB, Pratape VM. Nutritional implications and flour functionality of popped/expanded horse gram. Food Chem 2008;108:891-899.
9. Morris JB, Wang ML, Grusak MA, Tonnis B. Fatty acid, flavonol, and mineral composition variability among seven *Macrotyloma uniflorum* (Lam.) Verdc. accessions. Agriculture 2013;3:157-169.
10. Sreerama YN, Sashikala VB, Pratape VM. Variability in the distribution of phenolic compounds in milled fractions of chickpea and horse gram: evaluation of their antioxidant properties. J Agr Food Chem 2010;58:8322-8330.
11. Ezeagu IE, Ibegbu, MD. Biochemical composition and nutritional potential of ukpa: a variety of tropical lima beans (*Phaseolus lunatus*) from Nigeria-a short report. Pol J Food Nutr Sci 2010;60:231-235.
12. Luthria DL, Pastor-Corrales MA. Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. J Food Comp Anal 2006;19:205-211.
13. Guzmán-Maldonado SH, Acosta-Gallegos J, Paredes-López O. Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L). J Sci Food Agr 2000;80(13):1874-1881.
14. Foss NIR Systems. *Installation and user guide ,Foss Rapid Content Analyzer TM*, in *Part Number 720-750-6540; Version 1.0*. 1998, Foss NIR Systems, Inc.: U.S.A.
15. Plans M, Simó J, Casañas F, Sabaté J, Rodriguez-Saona, L. Characterization of common beans (*Phaseolus vulgaris* L.) by infrared spectroscopy: comparison of MIR, FT-NIR and dispersive NIR using portable and benchtop instruments. Food Res Int 2013;54(2):1643-1651.

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