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Validation of ten *Madhuraskandha* drugs through advanced UV-VIS-NIR spectroscopic technique: A preliminary study

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Abstract
The thematic classification of the drugs is required for a better understanding of the things. Acharya Charaka had implemented *Rasa* as criterion for the classification of the *Asthapana bastidravyas*. He included drugs having same *Rasa* and *Rasa* oriented *Vipaka* or *Prabhava*. Based on the modern pharmaceutical science, validation of the *Skandha* (grouping) can be carried out by using advanced spectroscopic technique. In the present study, ten samples selected from the *Madhuraskandha* were assessed for the total saccharide concentration and also subjected to NIR spectroscopy. The result showed that *Asparagus racemosus* had maximum concentration of saccharide as compared to rest of the samples. NIR spectroscopic study reported to have presence of chemical compounds of starch, cellulose, aldehyde, ketones, methylene group, carboxylate group and amide group etc. These compounds help in the formation of sweet taste. Hence conditionally the grouping of the drugs in *Madhuraskandha* might get validated on the basis of advanced pharmaceutical tools.

Keywords: NIR spectroscopy, total saccharide estimation, *Madhuraskandha* drugs

1. Introduction
Near infra-red (NIR) spectroscopy is a technology that has vast applications in herbal medicine. Near infrared (NIR) is a part of natural sunlight and is generated by several light sources, such as tungsten halogen car driving lights. The color of e.g. an apple in the visible spectrum gives us information on a variety of pigments and chemicals in the fruit, but we cannot 'see' things that do not absorb visible light (e.g. a sugar solution). It happens that water, sugar, acids and a range of other organic substances absorb near infra-red in proportion to their concentration. Spectroscopy is rapid, timely, less expensive, non-destructive, straightforward and sometimes more accurate than conventional analysis. NIR absorption spectra are less useful for identification and more useful for quantitative analysis of compounds containing functional groups made up of hydrogen bonded to carbon, nitrogen, and oxygen [1].
Acharya Charaka had used *Rasa* as a benchmark to classify *Asthapana bastidravya* in the Vimanasthana, known as *Rasaskandha. Madhurarasa* or *Rasa* oriented *Vipaka* or *Madhuraprabhava* drugs are included in the *Madhuraskandha*. In modern chemistry, the tastes are resultant of certain chemical constituents. E.g. Sweet taste may be due to carbohydrates, Sour taste due to acids etc [2]. Foods rich in simple carbohydrates such as sugar are those most commonly associated with sweetness. In biochemistry, carbohydrate is a synonym of saccharide, a group that includes sugars, starch, and cellulose.
The sweet taste is not caused by any single class of chemicals. A list of some of the types of chemicals that cause this taste includes sugars, glycols, alcohols, aldehydes, ketones, amides, esters, amino acids, some small proteins, sulfonic acids, halogenated acids, inorganic salts of beryllium and lead. Especially the most of the organic substances cause sweet taste [3].
In the era of modern pharmaceutical chemistry, the endorsement of the grouping can be done on the basis of functional groups. In the present study, an attempt is made to find out the functional groups incorporated in the drugs of *Madhuraskandha*. The phenol-sulphuric acid derivative method was adopted to estimate the total saccharide content of the selected drugs of *Madhuraskandha* by UV Vis Spectrophotometry as well as powders of test drugs were subjected to NIR spectroscopy. Such spectral information can be useful to identify a selective compound (equivalent dextrose) by establishing the presence or absence of functional group selected drug. Ten drugs had been selected on the basis of its availability, controversial status and activity also revalidated in favor of chemical moiety in the context classical grouping of

drugs.

2. Material and methods

2.1 Collection and identification of drugs

All the ten drugs were procured in the respective seasons according to their part used. The drugs were washed, shade dried and powdered and kept in air tight container. The drugs were authenticated through powder microscopy by following the standard procedures mentioned in Ayurvedic Pharmacopeia of India (API) in the Laboratory of Pharmacognosy, I.P.G.T. &R.A, and Jamnagar [4].

2.2 Preparation of sample

Methanolic extract of samples: About 5g of the test drug powder was macerated with methanol (100ml) in a closed flask for 24 hours where initial shaking frequently during first 6hrs and kept it for 18 hrs. After 24 hours it was filtered. The filtrate was evaporated with the help of water bath and methanolic extract was collected in solid form [5]. The similar procedure has been repeated for the rest of the drugs.

Powder form: The fresh drugs were dried to make it moisture free and then sieved through mesh no.180 to make it in fine texture.

2.3 Sample coding

All the ten samples are coded as follows: S1- *Abutilon indicum* Linn (AI), S2- *Pueraria tuberosa* DC (PT), S3- *Solanum virginianum Linn* (SX), S4- *Ricinus communis* Linn (RC), S5- *Tribulus terrestris* Linn (TR), S6- *Tinospora cordifolia* (Willd.) Miers ex Hook. f. &Thoms. (TC), S7- *Desmodium gangeticum* DC (DG), S8- *Leptadenia reticulata* W &A (LR), S9-*Asparagus racemosus* Willd (AR), and S10- *Boerhavia diffusa* Linn (BD)

The drugs were also grouped into three, Group A contains first five drugs (S1-S5), Group B contains last five drugs (S6-S10) and Group C contains combination of Groups A & B

2.4 Chemical and reagents

Chemicals like phenol, conc. sulphuric acid, and methanol were obtained from Merck specialties Pvt., Ltd, Mumbai.

2.5 Instruments

Double beam UV Vis spectrophotometer 2201(Systronics), Water bath, Dry and cleaned glass wares (test tube, beaker etc.)

Instrument model- Perkin Elmer Lambda 19 UV/VIS/NIR spectrophotometer

Specifications:

- Double beam, double monochromator, ratio recording
- Lamp: Deuterium (UV), Tungsten-Halogen (VIS/NIR), Lead-sulphide cell (PbS) for NIR
- Wavelength range: 200-2500nm
- Scan speed: 240 nm/min

2.6 Determination of total saccharides content

Total saccharides were determined by the phenol-sulfuric method of Chinese Pharmacopoeia [6]. One milligram of the methanolic extract of the drug was dissolved in one milliliter of methanol (1mg of methanolic extract of drug/ml), and it was taken as stock solution. The 120 µl was taken up from the stock solution for further dilution by adding 1 ml of 4% phenol solution and 7ml of sulphuric acid and 880 µl of methanol was added to make volume up to 9 milliliter. Similar procedure has been repeated for rest of samples. One blank solution was also prepared. After a 30-min-incubation at 40 °C, the mixture was left in an ice bath for 5 min and then the absorbance was measured at 400nm by UV spectroscopy. The total saccharides of samples were quantified with the help of anhydrous dextrose linear standard curve. The total saccharide values were presented in microgram (µg) anhydrous dextrose equivalent/ ml extract.

2.7 UV-VIS-NIR spectroscopy

Samples were analyzed using a bench top Perkin Elmer Lambda 19 UV-VIS-NIR spectrophotometer system in range between 750-2500 nm in diffused reflectance mode. The materials powders & extract both were placed in a closed rotating sample lead sulphide cell cup with scan speed 240 nm/min. The contact probe was placed against block surface, and spectral data were collected. At each position the exposure time was twenty five seconds. The spread between the spectra of each material is characteristic of reflectance spectra of powder and dry extract. Particle size of these spectra is represented by the numerical values corresponding to the reflectance of the material at each wavelength (one nanometer interval). The standards selected for wavelength and absorption calibration are traceable to NIST [7]. The NIR spectroscopy study was done NABL accredited SICART at Anand, Gujarat.

2.8 Data analysis

Data were manipulated into two forms: 1) Data were exported as 2D ASCII files at all UV-VIS -NIR wavelength region at 1 nm interval to corresponding reflectance value to build a matrix for ten powder (matrix contain 2250 × 10), i.e., wavelength is one direction and samples in other direction; 2) Data were exported as ASCII files to reduced reflectance value of whole wavelength (a single profile powder sample giving a matrix with 2250 data points). All data operations (preprocessing, etc.) were performed using MATLAB R2008 (Math works) on a computer Intel Pentium 4 processor containing 500 MB RAM and running Microsoft Windows seven.

Data preprocessing:

Raw reflectance is converted to absorbance using the function Absorbance = -log (10)* Reflectance, commonly referred to as log (1/R).Raw data were submitted to chemometric analysis with first order derivative preprocessing for multivariate distribution.

Multivariate analysis:

The PCA were performed using the algorithm Unscrambler Camo student version.

2. Result & Discussion

3.1 Total saccharide estimation by UV spectroscopy

Phenol-Sulphuric acid method is the most easiest and reliable method amongst the quantitative assay for saccharide estimation. It is used mostly in measuring neutral sugar content in oligosaccharides, proteoglycans, glycoproteins and glycolipids. In hot acidic medium, glucose is dehydrated to hydroxyl methyl furfural. This forms a yellow-brown colored product with phenol and has absorption maximum at 400 nm. This is one of the best methods to estimate total Saccharides [8].

The phenol sulphuric acid method is carried out by preparing a set of solutions with known dextrose concentration and mixing them with phenol-sulphuric acid reagent. A standard curve can be made and concentration of carbohydrates in the samples can be derived from the standard curve.

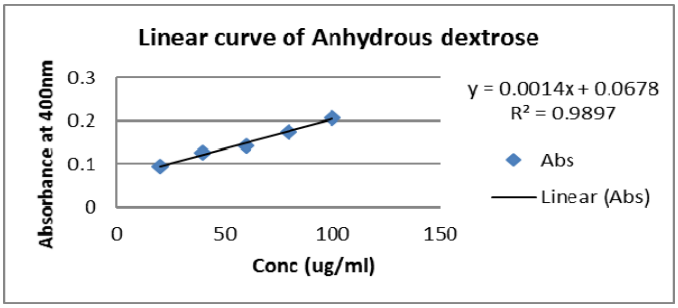


Fig 1: Linear graph of standard anhydrous dextrose

The linear graph for different concentration of anhydrous dextrose is presented in Fig 1. It shows the relation between the absorbance (y-axis) and the concentration of different carbohydrates in µg/ml (x-axis) for the phenol-sulphuric method. Using the proposed method, the linear curve was found to be in the range of 20-100 µg/ml. The correlation coefficient (R^2) of linear regression is 0.9897 which indicates high linearity between concentration and absorbance. The total saccharide content in the ten samples of *Madhuraskandha* was calculated using regression equation obtained from the linear graph of the standard drug. (Table 1)

Table 1: Total saccharide concentration in ten drugs of *Madhuraskandha*

Sample	Drugs	Absorbance at 400nm	Total saccharides concentration %w/w
S1	*Abutilon indicum* Linn. Sweet	0.145	23.24
S2	*Pueraria tuberosa* DC.	0.136	13.95
S3	*Solanum virginianum Linn*	0.210	90.63
S4	*Ricinus communis* Linn	0.156	30.62
S5	*Tribulus terrestris* Linn	0.166	43.99
S6	*Tinospora cordifolia* (Willd.) Miers ex Hook. f. &Thoms	0.161	41.38
S7	*Desmodium gangeticum* DC.	0.202	63.41
S8	*Leptadenia reticulata* W. & A.	0.181	81.91
S9	*Asparagus racemosus*Willd.	0.128	95.32
S10	*Boerhavia diffusa* Linn	0.123	23.27

Carbohydrate, synonym of saccharide (sugar), a group that includes sugars, starch, and cellulose. While the scientific nomenclature of carbohydrates is complex (nothing but a polyhydroxy aldehyde and ketone) [9]. Hence the taste of the carbohydrates can be considered as sweet. The preliminary qualitative analysis of all the samples showed the presence of carbohydrate [4]. Total saccharides estimation by phenol sulphuric acid method through UV Vis Spectrophotometry showed that maximum content is present in the S9, S3, S7, S8 followed by S6 and S4. It may be one of the similar chemical constituents found in the ten drugs of *Madhuraskandha*.

2.2 UV-VIS-NIR spectroscopy

Spectral derivatization used in order to select the spectral region of ten sample of *Madhuraskandha*. The ten sample of *Madhuraskandha* showed that all the samples have difference in the relative intensity of absorbance in the context of various functional groups like protein, cellulose protein, carbohydrate, starch, methyl group etc. (Table 2)

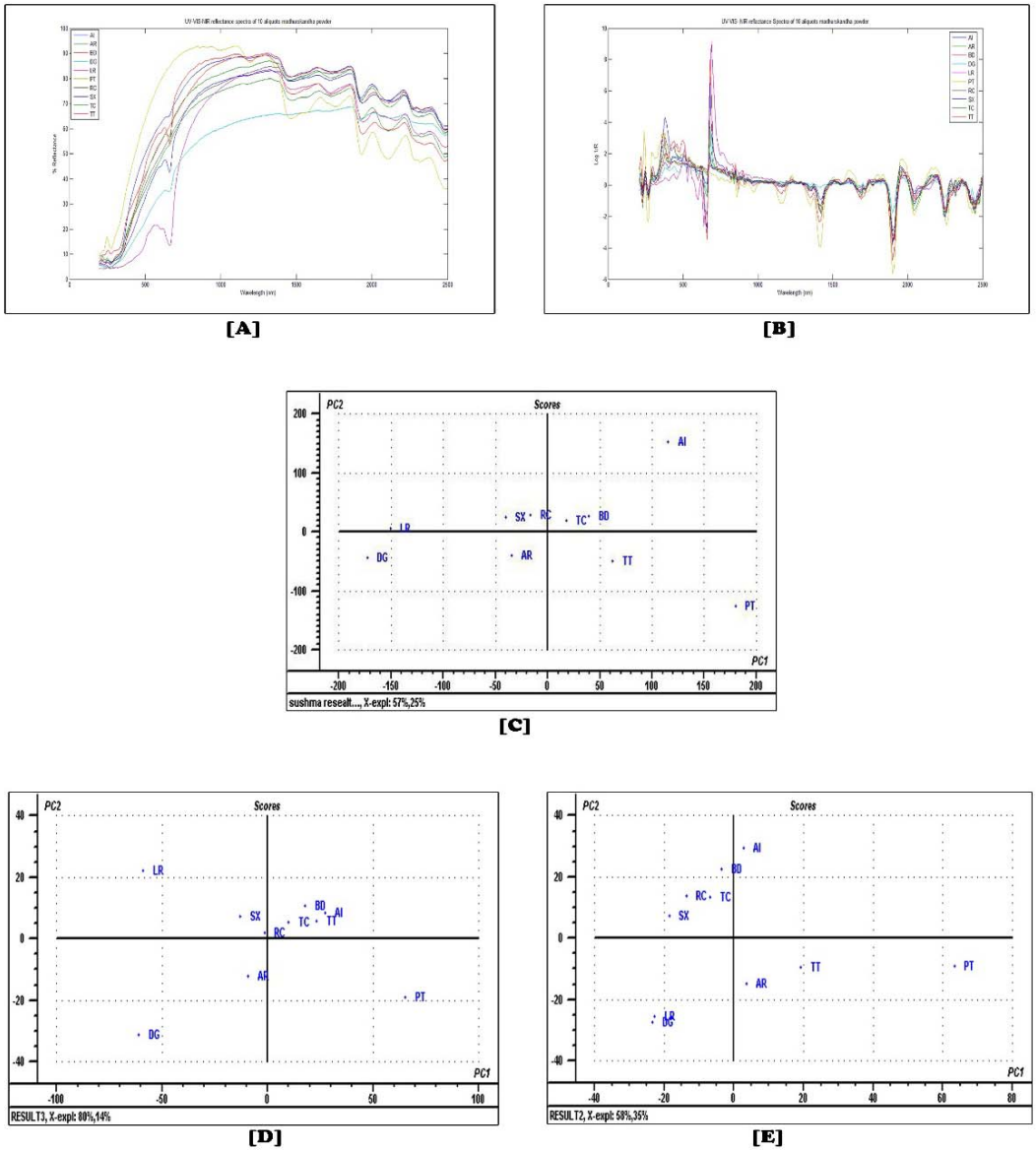
Table 2: dissimilar pattern of spectra observed in ten samples of *Madhuraskandha*

Type of electromagnetic radiation	Wavelength	Bond vibration	Structure
UV	350	-ONO	Nitrile
VIS	400	Chromophore	Conjugation
	450	Chromophore	Conjugation
	500	Chromophore	Conjugation
NIR	900	Chromophore	Conjugation
	1150	C-H second overtone	Aromatic
	1600	C−−H stretch first overtone	==CH_2
	1700	C−−H stretch first overtone	-CH_3
	1800	O−−H combination	-H_2O
	1950	C==O stretch second overtone	−−CO_2R
	2300	C−−H bend second overtone	Protein
	2350	CH_2 bend second overtone	Cellulose Protein
	2450	C−−H combination Sym C−−N−−C stretch first overtone	-CH_2 Protein
	2488	C−−H stretch/C−−C stretch combination	Cellulose
	2500	C−−H stretch/C−−C and C−−O−−C stretch	Starch

The ten samples of *Madhuraskandha* revealed the presence of starch, water, cellulose, aldehyde, ketones, methylene group, carboxylate group, and amide group having similar relative intensity of absorbance at specified wavelength. (Table 3)

Table 3: similar pattern of spectra observed in ten samples of *Madhuraskandha*

Type of electromagnetic radiation	Wavelength	Bond vibration	Structure
UV	250	-S-S-(disulfide)	Dimethyl disulfide
	300	-ONO,CHO	Nitrile & aldehyde
VIS	650	Chromophore	Conjugation
	675	Chromophore	Conjugation
	700	Chromophore	Conjugation
	760	Chromophore	Conjugation
NIR	1160	C==O stretch fourth overtone	-C==O
	1200	C−−H second overtone	-CH2
	1240	C−−H second overtone	-CH
	1450	O−−H stretch first overtone C==O stretch third overtone	-Starch -H2O Cellulose
	1500	N−−H stretch first overtone	-NH
	1580	N−−H stretch first overtone	CONH
	1900	C==O stretch second overtone	−−CO2H
	2040	N−−H/Amide IIb or N−−H/Amide IIIb or combination	CONH -CONH2
	2200	C−−H stretch/C==O stretch combination	−−CHO



[A]: Spectral comparison of ten samples of *Madhuraskandha*, **[B]:** First derivative spectra of ten samples of *Madhuraskandha*, **[C]:** score plot of ten samples of *Madhuraskandha* drugs, **[D]:** Score plot of similar pattern between ten samples of *Madhuraskandha* drugs**, [E]:** Score plot of dissimilar pattern between ten samples of *Madhuraskandha* drugs

Fig 2: UV-VIS-NIR spectroscopic analysis of *Madhuraskandha* drugs

The first step to developing an analytical method using NIR is to measure a spectrum of the sample using an NIR spectrophotometer. It is helpful to note that the near-infrared spectrum obtained by using a spectrophotometer is the result of the convolution of the measuring instrument function with the unique optical and chemical characteristics of the sample measured [10]. First derivatization of the spectra is a convenient way to conduct situ spectra region selection because it increases the selectivity of interacting bond [11] and eliminates any baseline offsets and slope changes due to irrelevant physical effects on the ten samples.

Principal component analysis (PCA) is the most widely used multivariate analysis technique for transforming the original measurement variables into new variables called principal components (PC) [12]. Each PC is a linear combination of the original measurement variables. Often, only two or three principal components are necessary to explain all of the information present in the data. By plotting the data in a coordinate system defined by the two or three largest principal components, it is possible to identify key relationships in the data, that is, find similarities and differences among objects in a data set. In Herbal drug studies, PCA is commonly-used multivariate tool for classification and discrimination [12]. It is an unsupervised clustering technique for reducing the dimensionality of a data set, without losing important information.

PCA attempted with view of frequencies or different wavelength was selected attributes/ variables. In the present study, whole data was explained by 9 PCs (principal components) but major information found in 2 PC. In the present study PCA was explained 95% with two principal components. The score plot shows the similarity and dissimilarity among the all samples based on the wavelength. The loading shows the variables responsible to discriminate the samples.

PCA attempted with component load and score. Separated components are selected attribute for the all the samples in different wave length. The PCA plots for all the samples when different wave length is considered as a variable. The score plot showed that *A.indicum, B.diffusa* and *T.cordifolia* were considered to be similar because they fell in the same quadrant i.e. Right side in upper quadrant of score plot, while *T.terrestris* and *P.tuberosa* were found to be similar because they fell in the same quadrant i.e. Right side in lower quadrant of score plot. They were differentiated from *S. virginianum L.reticulata, R.communis* and *D.gangeticum*, *A.racemosus* because they fell in the upper and lower side of the left quadrant of the score plot. The load plot exhibited that frequency or wavelength is the variable responsible for discrimination among all the samples. 82% of the data can be explained in two principal components, (Fig 2[C]) 100% data was explained through 9 principal components.

Ten drugs selected from the *Madhuraskandha* can be differentiated into three groups i.e. *Atibala, Vidari, Jivanti* belongs to *Madhurarasa*; *Guduchi, Shatavari, Gokshura* and *Shalaparni* referred to have *Madhura vipaka* and *Kantakari, Eranda, Punarnava* samples act based on the *Madhuraprabhava* (the drugs act as *Madhurarasa* or *Madhuravipaka*, but do not possess either of them) [14].

NIR spectroscopy showed that the samples of *Madhuraskandha* contain starch, water, cellulose, aldehyde, ketones, methylene group, carboxylate group and amide group. However, they have difference in the relative intensity of absorbance which sorted them dissimilar. As all the samples contains the compounds responsible for sweet or *Madhura rasa*, it might be criteria for grouping all the drugs into *Madhuraskandha*.

4. Conclusion

The pattern adopted by Acharya Charaka for the classification of the *Asthapana bastidravyas* was based on the *Rasa*. The drugs included in the *Skandhas* were either of the same *Rasa* or possess *Rasa* oriented *Vipaka* or *Prabhava.* The percentage of saccharides was observed to be maximum in *A.racemosus* as compared to other samples. NIR spectroscopy showed the presence of chemical compounds like starch, water, cellulose, aldehyde, ketones, methylene group, carboxylate group and amide group. The tools like NIR spectroscopy, UV-Vis spectrophotometry of modern pharmaceutical chemistry may be useful to endorse the grouping of the drugs in the *Skandhas*.

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