

## STUDY OF SELECTED HERBAL LEAVES FOR THEIR PHARMACEUTICAL IMPORTANCE

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### Abstract:

To extract the phyto-compounds of pharmaceutical importance present in selected herbal leaves. The medicinal plant used for the analysis purpose is Aloe vera. Aloe vera is having many medicinal properties like healing the burns, curing the cold and for face it acts as a best moisturizer and many more. Near infrared reflectance spectroscopy analysis was performed using a Shimadzu (Japan) UV-NIR-3600 Equipped with PMT (photomultiplier tube) for the ultraviolet and visible regions. The Leaf samples were placed in rotating sample cup, and scanned on reflectance mode in the spectral range from 4,000 to 12,500  $\text{cm}^{-1}$  at room temperature ( $\sim 20^\circ\text{C}$ ). In each of the reflectance measurements, 64 scans were run and the resolution used for spectral analysis was 8  $\text{cm}^{-1}$ . Background corrections were made before each sample was scanned. Samples were measured in triplicate, which increased the scanned surface of samples for reducing errors. The spectral absorbance values were recorded as  $\log 1/R$ , where R is the sample reflectance. Phyto-compounds can be found by so many extraction methods and solvents depending on the nature of the compound we are looking for. Every leaf is having a medicinal quality but the concept is that we are not aware of that. How many of us know that babul tree has galactagogue properties? But still in India we think that it drains the ground water and we cut the tree. The main origin of the work started with the basic findings of medicinal properties present in herbal leaves. Here we are using image processing techniques, which makes easier for the identification of phyto-compounds. For this we have chosen some herbal leaves such as tulasi, aloe-vera which are plenty in our surroundings and also have so many health benefits by using. Manual interpretation of herbal leaves which have good sufficient amounts of phyto compounds that are useful in the preparation of Ayurvedic medicines and have good health benefits leads to error. So, it is essential to develop an automatic system for the identification of amount of phyto compounds to avoid the errors. A digital image capturing is proposed to acquire various images of different herbal leaves and they are used to develop a database for the comparison with spectral analysis values obtained using UV visible NIR spectroscopy. The novelty of the work is that comparative analysis using analytical and digital method are determined. In pharmaceutical industries the work can be applied for classifying the leaf based on its medicinal properties.

**Keywords:** Component; Spectral analysis, feature extraction, NIRS, GLCM.

## 1 Introduction

The leaves of the Aloe plant grow from the base in the rosette pattern. Mature plants can grow as tall as 2 and a half inches to 4 feet with the average being around 28 to 36 inches in length. Each plant usually has 12 - 16 leaves that, when mature, may weigh up to three pounds. Each leaf is composed of three layers: An inner clear gel that contains 99% water and rest is made of glucomannans, amino acids, lipids, sterols and Vitamins. The middle layer of latex which is the bitter yellow sap and contains anthraquinones and glycosides. The outer thick layer of 15 - 20 cells called as rind which has protective function and synthesizes carbohydrates and proteins. The plants can be harvested every 6 to 8 weeks by removing 3 to 4 leaves per plant. Aloe vera extract act as an antioxidant due to carotenoids present in it. Lignins present in Aloe vera penetrate deep into the skin and help in introducing other medicinal ingredients to penetrate into the skin. Aloe vera gel contains powerful antioxidants, which belong to a large family of substances known as polyphenols. These polyphenols, along with several other compounds in Aloe vera, can help inhibit the growth of certain bacteria that can cause infections in humans. Aloe vera contains various powerful antioxidant compounds. Some of these compounds can help inhibit the growth of harmful bacteria.

## 2 Related Works

[1] presented a comprehensive and critical survey on image-based plant segmentation techniques. In this context, “segmentation” refers to the process of classifying an image into plant and non-plant pixels. Good performance in this process is crucial for further analysis of the plant such as plant classification (i.e. identifying the plant as either crop or weed), and effective action based on this analysis, e.g. precision application of herbicides in smart agriculture applications. The survey briefly discusses pre-processing of images, before focusing on segmentation. The segmentation stage involves the segmentation of plant against the background (identifying plant from a background of soil and other residues). Three primary plant extraction algorithms, namely, (i) color index-based segmentation, (ii) threshold-based segmentation, (iii) learning-based segmentation are discussed. Based on its prevalence in the literature, this review focuses in particular on colour Index based approaches. Therefore, a detailed discussion of the segmentation performance of color index-based approaches is presented, based on studies from the literature conducted in the recent past, particularly from 2008 to 2015. Finally, we identify the challenges and some opportunities for future developments in this space [2] presented the evaluation of bioactive compounds of aloe vera using GC/MS. The chemical composition of the n-hexane extract of aloe vera were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of n-hexane extract of aloe vera revealed the existence of twenty six bioactive compounds. The result of this study offers a platform for using aloe vera as herbal drug for cancer studies. [3] presented that phyto chemicals are secondary metabolites which have different health benefits and with respect to plants, they possess color, aroma and flavor. There are different extraction methods of phytochemicals which have been used from the past and which are novel. Those novel techniques are very efficient and they will enable

to extract large yields from small amount of plant material. Further, there are some techniques which can be used for both qualitative and quantitative measurements. Gas chromatography, liquid chromatography, high performance liquid chromatography and high performance thin layer chromatography are some advanced techniques which can be used for quantitative analysis of phytochemicals. The aim of this study is to elaborate different extraction methods and different qualitative and quantitative techniques for screening phytochemicals from plant materials.

[4] presented natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. The focus of this paper is on the analytical methodologies, which include the extraction, isolation and characterization of active ingredients in botanicals and herbal preparations. The common problems and key challenges in the extraction, isolation and characterization of active ingredients in botanicals and herbal preparations are discussed. As extraction is the most important step in the analysis of constituents present in botanicals and herbal preparations, the strengths and weaknesses of different extraction techniques are discussed. The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assays, chromatographic techniques such as HPLC and, TLC as well as non-chromatographic techniques such as immunoassay and Fourier Transform Infra Red (FTIR) are discussed.

[5] There are concerns about using synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) as food additives because of the reported negative effects on human health. Thus, a replacement of these synthetics by antioxidant extractions from various foods has been proposed. More than 8000 different phenolic compounds have been characterized; fruits and vegetables are the prime sources of natural antioxidants. In order to extract, measure, and identify bioactive compounds from a wide variety of fruits and vegetables, researchers use multiple techniques and methods. This review includes a brief description of a wide range of different assays. The antioxidant, antimicrobial, and anticancer properties of phenolic natural products from fruits and vegetables are also discussed.

[6] presented various novel techniques including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and accelerated solvent extraction have been developed for the extraction of nutraceuticals from plants in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield, and enhance the quality of extracts. A critical review was conducted to introduce and compare the conventional Soxhlet extraction and the new alternative methods used for the extraction of nutraceuticals from plants. The practical issues of each extraction method were discussed. Potential uses of those methods for the extraction of nutraceuticals from plant materials was finally summarized.

[7] presented the study to evaluate relative contribution of different polyphenols (total phenolics, flavonoids, flavonols) and their antioxidants activities in aqueous extracts of different parts of some plants; *Argemone mexicana*, *Datura metel*, *Calotropis procera*, *Thevetia peruviana*, and *Cannabis*

sativa. The antioxidants (total phenolics, flavonoids, flavones) were determined by chemical methods. The antioxidant capacities of these extracts were evaluated by FRAP assay. The results demonstrated that phenolic content was maximally present in leaves of *T. peruviana*. This plant exhibited minimum phenolic content in its flower as compared to other plants. The flower of *D. metel* contained maximum phenolic content. The flavonoids were present in highest quantity in leaves of *C. procera* while *T. peruviana* flowers showed maximum flavonoid content. The fruits of *C. sativa* contained maximum quantity of flavonoid as compared to other plants tested. The flower extract of *C. sativa* possessed highest FRAP value followed by *A. mexicana* and fruit of *C. procera*. The values of ratios of different polyphenolic compounds present in plant extracts indicated that flower of *D. metel* contained maximum total flavonoids and minimum phenolics. These results suggested that levelsof total phenolics, flavonoids and their FRAP indices exhibited specificity to different plants and their parts.

[8] discussed that plants are in need nowadays in human life. There are certain plants which are prone with medicinal qualities by nature. Many medicinal plants have a medicinal quality from root to leaf. Leaves play major role in our ecosystem. Identifying the leaf from look- alike is becoming a major task in our day to day life. Since there is a mistake in human vision for medicinal leaf with lookalike leaf computer vision is required. In these days identifying leaf is not possible. Therefore computer technique is must in identifying them. Image processing plays a vital role in leaf identification. A database is created with 127 herbal leaves. For creating a database 11 texture parameters are taken into account. The parameters are Sum of Variance, Inverse Difference Moment, Aspect ratio, Correlation, Sum Entropy, Mean, and Sum Average. Gray level co-occurrence matrix (GLCM) is used for determining the parameters like entropy, homogeneity, contrast and energy. A test image is taken and compared with the database; the dissimilarity is calculated with the extracted parameters. The one with least dissimilarity is identified as the leaf and the output isdisplayed.

[14] Using linear interpolation the classification was carried out. The texture analysis using a wavelet transformation technique has brought a clear output. Normal Differential Vegetation index approach was used.

[9] presented that plants are recognized in the pharmaceutical industry due to their broad spectrum of structural diversity and their wide range of pharmacological activities. The biological active compounds that are present in plants referred as phytochemicals. These phytochemicals derived from different parts of plants such as leaves, barks, seed, seed coat, flowers, roots and pulps and thereby used as sources of direct medicinal agents. Phytochemistry describes the large number of secondary metabolic compounds present in the plants. The plants are the reservoirs of naturally occurring chemical compounds and of structurally diverse bioactive molecules. The extraction of bioactive compounds from the plants and their quantitative and qualitative estimation is important for exploration of new biomolecules to be used by pharmaceutical and agrochemical industry directly or can be used as a lead molecule to synthesize more potent molecules. This review mostly highlighted on the analytical methodologies, which includes the extraction methods and the analysis of bioactive compounds present in the plant extracts through the various techniques involving the applications of chromatographic techniques such as HPLC (High

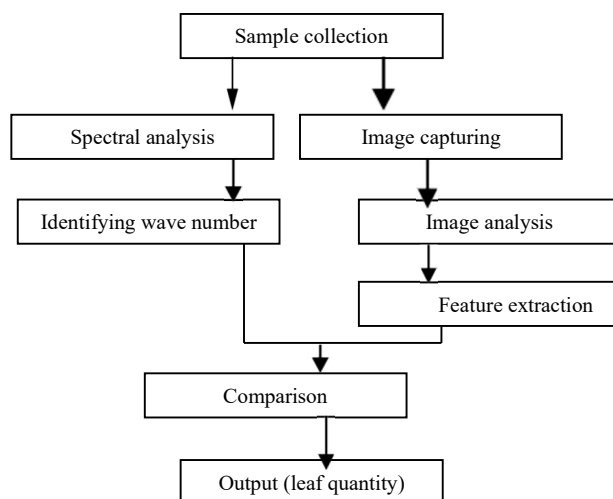
Performance Liquid Chromatography), TLC (Thin Layer Chromatography), HPTLC (High Performance Thin Layer Chromatography), OPLC (Optimum Performance Laminar Chromatography), GC (Gas Chromatography), PC (Paper Chromatography), CC (Column Chromatography) and its detection through Fourier Transform Infra-Red spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS).

[10] described the feature extraction methods for crop and fruit diseases based on computer image processing in detail. Crop and fruit diseases are most important agricultural products. In order to obtain more value added products, a proper quality control is essentially required. There are various applications claimed to extract the accurate information from the colored image database. The main purpose of this paper is to provide an interface for digitally illiterate users, especially farmers to efficiently and effectively retrieve information through internet. In addition, to enable the farmers to identify the disease in their crop, its causes and symptoms using image processing without classical approach and identify the disease.

[11-12] The nano methodology uses a powdered herbal leaf mixed with chemicals like ethanol and methanol. This method has improved the functionality, stability and cytotoxicity.

[13,15] Uses a spectral analysis for identifying the contaminated wheat grain and soluble content in apple respectively. Image analysis and spectral analysis were compared for determining the soluble in apple. Texture parameters were analyzed

### 3 Proposed Framework



**Fig: 1: Block diagram**

Samples collected in which the amount of phytochemicals is obtained using UV visible NIR spectroscopy. The collected samples are tulasi (rama tulasi, Krishna tulasi, vana tulasi, amrita tulasi) and alo vera. The acquired leaf samples are analysed under UV visible NIR spectroscopy to obtain the

amount of phytochemicals present in the solid leaf samples.

UV-Vis-NIR spectrophotometer is a high sensitivity, high resolution, low stray light instrument for transmission and absorption measurements. NIR spectra consists of overtones and combination bands of the fundamental absorptions in the mid infra-red region. The number of photons absorbed is dependent on the type of the chemical bonds present in the sample. There is no direct way to measure the number of photons absorbed as they disappear one-by-one.

### 3.1 NIR of Aloe vera

The raw spectral data of selected samples exhibited the general spectral features (Fig: 2 a&b). It is obviously shown that peaks and valleys were presented in the spectra, which indicated the different chemical component characteristics of aloe vera samples each potentially contributing to the NIR spectrum. The raw NIR absorption spectrum collected from the leaf surface of the aloe vera (Fig: 2 a) shows broad overtone and combination bands. The second derivative obtained from the leaf sample (Fig: 2 b) has been displayed to demonstrate the improved spectral characteristics

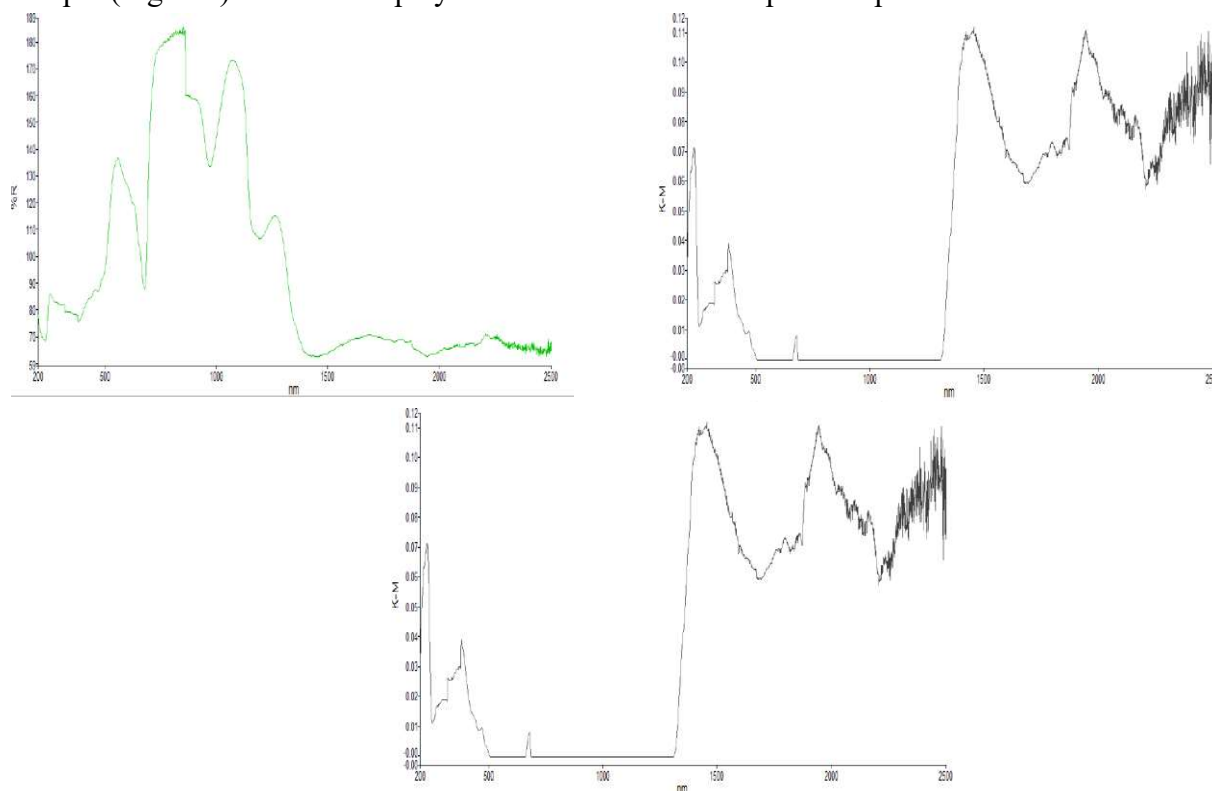


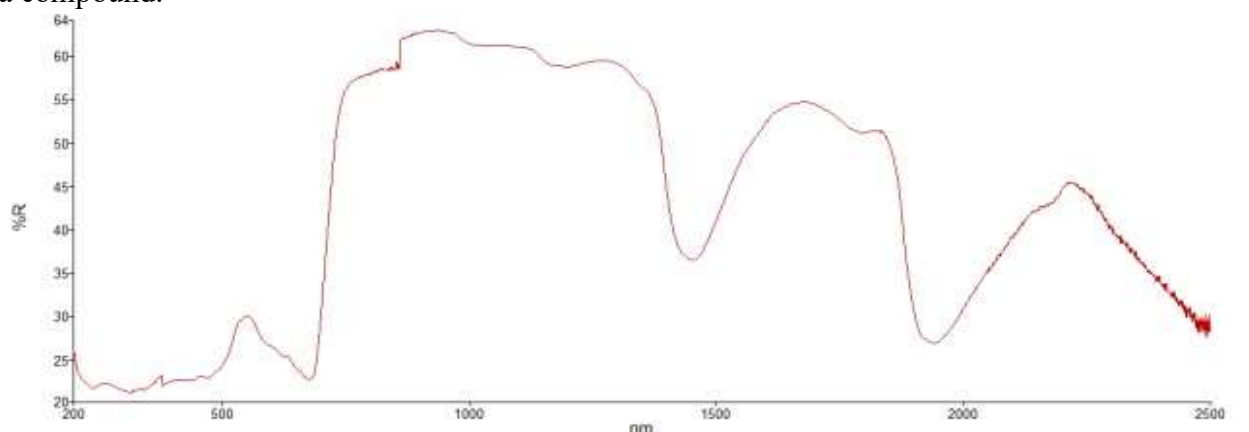
Fig: 2 a. Typical NIR Spectra of Aloe vera; b. Second Derivative

After comparison to the origin of near-infrared absorption bands, we found that these wavelengths related to critical functional groups as carbon atoms and hydrogen (C-H), hydrogen atoms and oxygen (O-H).

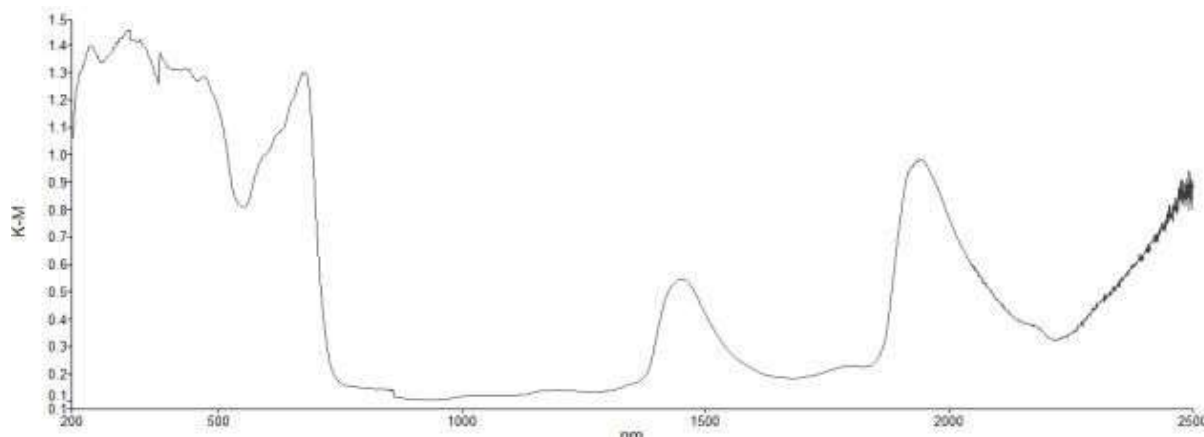
**Table 1: Spectral analysis of Aloevera**

Wavelength (nm)	Assignments
850-950	Third Overtone of C-H Stretching
950-1,100	Second Overtone of N-H and O-H Stretching
1400-1500	First Overtone of N-H and O-H Stretching
1650-1800	First Overtone of C-H Stretching
2200-2450	Combination of C-H Stretching

Different substances, based on their electron configuration, absorb different wavelengths of light. Absorption in the NIR region results from molecular vibrations (mainly stretching and bending) within a compound.



The NIR spectral data of selected samples are shown in Fig: 3 a & b. Many chemical constituents are present in the leaf tissue of Tulsi including water, chlorophyll, flavonoids, and protein, each potentially contributing to the NIR spectrum. Water is the major cell wall constituent, affecting the hydrogen bonding between cell wall components and the strength of the associations between hydrogen-bonded polymers.



**Fig: 3 a. Typical NIR Spectra of Tulsi; b. Second Derivative**

**Table 2: Spectral analysis of Tulsi**

Wavelength (nm)	Assignments
850-950	Third Overtone of C-H Stretching
950-1,100	Second Overtone of N-H and O-H Stretching
1400-1500	First Overtone of N-H and O-H Stretching
1650-1800	First Overtone of C-H Stretching
2200-2450	Combination of C-H

By using Gray level co-occurrence matrix, features of the acquired leaf samples are extracted. And the extracted features are energy, correlation, sum variance, invariance, sum average, sum variance, sum entropy, entropy, difference variance, information measure of correlation 1 & 2. The obtained values are tabulated below.

**Table 3 Features of leaves**

	Rama tulasi	Krishna tulasi	Vana tulasi	Amrita tulasi	Aloe vera
<b>Energy</b>	0.4001	0.4167	0.6478	0.4848	0.4747
<b>Correlation</b>	505.4164	611.6563	369.2458	444.9779	448.3570
<b>Sum variance</b>	20.1539	25.5467	27.5777	22.9286	22.4710



<b>Invariance</b>	0.8816	0.8629	0.8863	0.8649	0.8456
<b>Sum average</b>	8.8502	9.8993	10.3780	9.3135	9.2545
<b>Sum variance</b>	56.5365	75.3581	89.5289	68.4164	66.4911
<b>Sum entropy</b>	1.4397	1.4765	1.0737	1.3617	1.3757
<b>Entropy</b>	1.6921	1.6645	1.2203	1.5313	1.5715
<b>Difference variance</b>	0.0243	0.0238	0.0085	0.0182	0.0199
<b>Information measure of correlation 1 &amp; 2</b>	-0.0612	-0.0689	-0.1929	-0.1061	-0.0949

#### 4 Conclusion

In this paper, it is proposed to automate the identification of various phyto compounds which are present in the herbal leaves. These samples of Tulasi and Aloe vera have been acquired for UV visible NIR spectral analysis. It is so to get the amount of absorbance and reflectance values. The images of five herbal leaves like Rama Tulasi, Krishna Tulasi, Amrita Tulasi, Vana Tulasi, and Aloe vera have been captured to feed as input and extracted the features. The extraction is limited to 5 leaves and classification using SVM classifier and NIR spectral analysis was determined. In future many leaves and their families will be taken into account to determine the accuracy. It is observed that in accordance with the wavelength varieties of tulsi leaves fall under the energy level of 0.4 to 0.5 and Aloe vera falls under the energy level 0.6. More leaves are to be sampled in future to calculate the energy component and bring in to conclusion.

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