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Dangar, Radhika R., 2012, “Development of Computer Aided Technique for the Identification of Selected Herbal Drugs”, thesis PhD, Saurashtra University

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**Development of Computer Aided Technique for
the Identification of Selected Herbal Drugs**

Ph.D. Thesis

MAY 2012

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M. PHARM.**



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Development of Computer Aided Technique for the Identification of Selected Herbal Drugs

A Thesis submitted to

Saurashtra University

Rajkot, Gujarat, India

For the award of

Degree of

**DOCTOR OF PHILOSOPHY
IN PHARMACEUTICAL SCIENCES**

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CERTIFICATE

This is to certify that the thesis entitled '**Development of Computer Aided Technique for the Identification of Selected Herbal Drugs**' represents the bonafide work of **MRS. RADHIKA R. DANGAR** and contains the results of her own investigations carried out at the Department of Pharmaceutical Sciences, **Saurashtra University, Rajkot**. The manuscript has been prepared under my guidance and supervision. The work is up to my satisfaction.

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DECLARATION

I hereby declare that the thesis entitled, '**Development of Computer Aided Technique for the Identification of Selected Herbal Drugs**' is genuine record of research work carried out by me under the guidance of **Prof. (Dr.) Navin R. Sheth**, Head, Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India. No part of this thesis has been submitted to any University or Institution for the award of any Degree or Diploma.

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1.1 General Introduction:

Plants are extremely important to the quality of the water we use. A diverse cover of plants aids in maintaining healthy watersheds, streams, and lakes by holding soil in place, controlling stream flows, and filtering sediments from water. The delicate wildflowers that dot the roadsides in Iowa during the spring, summer and fall, protect the soil from erosion caused by heavy rains. Without enough plant cover, wind or water erodes the thin layer of soil that we depend on. Regional climates are impacted by the amount and type of plant cover. Forest and marshes, for example, can cool local climates. Natural disasters, such as drought, have been blamed on the destruction of forests and other critically important plant communities. Plants and plant communities provide the necessary habitat (a place to live) for wildlife and fish populations. The word "ecosystem" means the way in which human, plants and animals all live together supporting each other. Every species serves an important role or purpose in their community¹⁻².

Two hundred and fifty years ago, there were few or no synthetic medicines. The 250,000-300,000 species of higher plants were the main source of drugs for the world's population. Today, 75% of the world's population, the poor 3/4^{ths}, still relies on those plants and other tools of traditional medicine. In the U.S. and Europe the ecological movement has brought about a renewed interest in traditional medicines. Today even among prescription drugs, 25% contain at least one compound derived from higher plants. The percentage might be higher if we include over-the-counter (OTC) drugs. The use of plants in medicines ranges from crude preparations or extracts, to refined extracts and single molecular species; In terms of categories of use this encompasses food supplements, herbal medicines, botanical drugs and prescription medicines. Increased interest in plants as a source of novel pharmacophores recognizes their chemical diversity and versatility, not matched by synthetic chemistry libraries. In spite of the surge of activity in synthetic chemistry over the last 200 years or so, there are so many molecules introduced as drugs, those were derived from plants. Over 100 small molecules derived either directly or indirectly from plants are currently at some point in the clinical trials

process. It is argued that the present use of plant-derived drugs and remedies only scratches the surface of what is a major reservoir of untapped potential, the level of biological and chemical diversity possessed by plants having much to offer in the drive for novel therapeutic agents in the fight against disease. Additionally novel developments in plant biotechnology and molecular biology add further dimensions to the use of plants in the production of therapeutic agents³⁻⁴.

For the most part, the discovery of the drugs can be possible from traditional knowledge that plant parts or extracts can be used to treat one or more diseases in humans. The more interesting of the extracts are then subjected to pharmacological and chemical tests to determine the nature of the active components. Therefore, it should be of interest to ascertain just how important plant drugs are used in the form of crude extracts throughout the world. There is a great deal of interest in support for the search for new and useful drugs from higher plants in countries such as the India, People's Republic of China, Japan, USA, and the Federal Republic of Germany. Virtually every country of the world is active in this search to a limited degree⁴.

Higher plants have been described as chemical factories which are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances whose structures could escape the imagination of synthetic chemist forever. Considering that many of these unique gene sources may be lost forever through extinction and that plants have a great potential for producing new drugs of great benefit to mankind, some action should be taken to reverse the current apathy in the United States with respect to this potential⁵.

Indian subcontinent is gifted with varied agro climates and topography. Therefore, it has one of the richest plant biodiversity of the world and rightly acclaimed as 'Botanical garden of the world'. India accounts for 8 percent of global biodiversity, which exists in 2.4 per cent land area of the world. There are 15,000 species of higher plants, of which about 10 percent are found to have medicinal properties. Out of this only 800 plant species are used in traditional systems of medicines in India. From 1986 to 1996, an all India coordinated research project on ethnobiology was carried out by the

department of environment of the Indian Government. This project concluded that tribal communities alone (who constitute only a small percentage of our population) use over 9,000 species of wild plants, of which the single largest use category medicinal plants number over 7,500 species. Besides this, 3,900 are for edible use, 700 for material and cultural requirements, 300 as gums and dyes, and 100 as incense and perfume⁶.

There is a verse in the Ayurveda classic “Charaka Samhita” that explains how local communities understood and explored nature’s gift of medicinal plants. The western system of medicines too uses complex formulations of plant-based raw material and few of these medicinal plants have generated bulk demand in the organized sector of industry and also export. At present 2,500 plant species are known to be useful and more than 6,000 manufacturers produce about 15,000 Ayurvedic and Unani medicinal preparations from plants. More than 30,000 plant species of medicinal importance are grown all over the world⁷.

The traditional herbal medicines and their preparations have been widely used for thousands of years in India and many oriental countries, such as in China, Korea, Japan, etc. However, one of the characteristics of herbal medicinal preparations is that all the herbal medicines, either presenting as single herb or as collections of herbs in composite formula, is extracted with boiling water during the decoction process. This may be the main reason why quality control of herbal drugs is more difficult than that of western drug. As pointed in “General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines (World Health Organization, 2000)”. Despite its existence and continued use over many centuries, and its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training and research in this area have not been accorded due attention and support. The quantity and quality of the safety and efficacy data on traditional medicines are far from sufficient to meet the criteria needed to support its use world-wide. The reasons for the lack of research data are due to not only to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine”. In general, one or two

markers or pharmacologically active components in herbs and or herbal mixtures were currently employed for evaluating the quality and authenticity of herbal medicines, in the identification of the single herb or herbal medicinal preparations, and in assessing the quantitative herbal composition of an herbal product. This kind of the determination, however, does not give a complete picture of herbal product, because multiple constituents are usually responsible for its therapeutic effects. These multiple constituents may work 'synergistically' and could hardly be separated into active parts. Moreover, the chemical constituents in component herbs in the Herbal products may vary depending on harvest seasons, plant origins, drying processes and other factors⁸.

Thus, it seems to be necessary to determine most of the phytoconstituents of herbal products in order to ensure the reliability and repeatability of pharmacological activity and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance product quality control⁸⁻⁹.

When two or more herbs are used in formulations, they are known as polyherbal formulations. Some time herbs are combined with mineral preparations also. The herbs often exist in crude state and Ayurveda describes method of purification of toxic herbs. The concept of polyherbalism is peculiar to Ayurveda although it is difficult to explain in term of modern parameters. Single plant based formulations may have better acceptance from quality control and standardization aspects, but still not ample amount of evidence has accumulated to prove concept of standardization of herbal drugs based on single constituent. Researchers in the last century identified and isolated Salicin, a glycoside as active principle. From salicin, salicylic acid and finally aspirin was synthesized. Aspirin is known to cause gastric irritation and hypersensitivity. The plant when used alone does not cause gastric irritation, probably due to the presence of tannins. It can be concluded that polyherbal formulations should not be dismissed only on the basis that they do not withstand modern research. Ayurveda and herbal medicine has roots in medicinal herbs and they have been practiced for centuries. The western system of medicines too uses complex formulations of plant-based raw

material and few of these medicinal plants have generated bulk demand in the organized sector of industry and also in the export⁹⁻¹⁰.

The ever increasing demands of herbal drugs led to a spurt of large-scale commercial production with multi crore Rs/\$ investments in many countries including India. This has led to extraordinary demand for the raw materials of herbal drugs, which are mostly collected from the wild. With the ever-increasing demand of medicinal plants the supply line is unfavorably affected, leading to adulteration and substitution for actual drugs. Such adulteration and substitution lead to the poor quality of herbal products. The situation becomes graver due to confusion of botanical identity of a majority of drugs mentioned in the pharmacopoeias of various indigenous systems of medicine¹⁰.

There is a burning need that these drugs are defined in terms of botanicals, Chemicals, physico-chemical and therapeutic principles. Several pharmacopoeias like Ayurvedic Pharmacopoeia of India, Indian Herbal Pharmacopoeia, British Herbal Pharmacopoeia, Japanese Pharmacopoeia, United States Pharmacopoeia, British Herbal Compendium; German Commission E etc. have included monographs to maintain quality of such drugs in their respective nations. But all above Pharmacopoeia are not having description of more than 700 medicinal plants. Lot of work is required to be done to know quality of a single herb¹¹.

Even WHO assembly in a number of resolutions emphasizes the need to ensure quality and prescribes various Quality Control Methods for Herbal Plant materials includes¹²⁻²⁰:

- Sampling Procedure
- Determination of Foreign Matter
- **Visual Examination and Inspection by Microscopy**
- Thin Layer Chromatography
- Determination of Ash

Determination of Extractable Matter

- Determination of Water and Volatile Matter
- Determination of Volatile Oils
- Determination of Bitterness Value
- Determination of Hemolytic Activity

- Determination of Tannins
- Determination of Swelling Index
- Determination of Foaming Index
- Determination of Pesticide Residues
- Determination of Arsenic and Heavy Metals
- Determination of Microorganisms
- Radioactive Contamination

Now characterization of powder vegetable drugs, there are many techniques available like TLC, HPTLC, UV-Visible spectroscopy, HPLC etc. Those techniques are highly costlier and sophisticated, required more attention for maintenance²¹⁻²³.

While microscopy is an important, cheap and handy tool to determine the identity of the used species in order to authenticate genuine powder vegetable drug. However, once the plants have been processed, it is difficult to identify them through macroscopic identification; other means are necessary. A comparative study can be possible in case of closely related species of plants. Only simple microscope is required for this method²⁴.

Authentication of powder vegetable drugs is a critical step in the use of these materials for both research purposes and commercial preparations. Microscopic evaluation and comparison of authenticated and unauthenticated samples of powdered plant material is a cost effective and accurate means of identifying herbal ingredients. Microscopy and computer can be useful tools for the detection of botanical and non-botanical adulterants such as pharmaceutical drugs, microbial contaminants, and inorganic materials. Advancement in microscopic techniques like attachment of digital video eyepiece with microscope and improvements in light, fluorescence, phase contrast, and scanning electron microscopes have improved the accuracy and capabilities of microscopy as a means of botanical authentication. Organoleptic analysis, used in combination with advanced microscopic equipment and attachment with computer, which is computer aided microscopy, provides further accuracy for botanical authentication and characterization of powder vegetable drugs²⁵⁻³⁰.

1.2 Introduction to Microscopy:

Microscopy is the technical branch of using microscopes to view samples and objects those cannot be seen with the naked eye means the objects are not within the resolution range of the normal eye.

1.2.1 The History of Microscope^{31,32}:

- **Circa 1000AD:** The first vision aid was invented (inventor unknown) called a reading stone. It was a glass sphere that magnified when laid on top of reading materials.
- **Circa 1284:** Italian, Salvino D'Armato is credited with inventing the first wearable eye glasses (Visual aid).
- **1590:** Two Dutch eye glass makers, Zaccharias Janssen and son Hans Janssen experimented with multiple lenses placed in a tube. The Janssens observed that viewed objects in front of the tube appeared greatly enlarged, creating both the forerunner of the compound microscope and the telescope.
- **1665:** English physicist, Robert Hooke looked at a sliver of cork through a microscope lens and noticed some "pores" or "cells" in it.
- **1674:** Anton van Leeuwenhoek built a simple microscope with only one lens to examine blood, yeast, insects and many other tiny objects. Leeuwenhoek was the first person to describe bacteria, and he invented new methods for grinding and polishing microscope lenses that allowed for curvatures providing magnifications of up to 270 diameters, the best available lenses at that time.
- **18th century** – Technical innovations improved microscopes, leading to microscopy becoming popular among scientists. Lenses combining two types of glass reduced the "chromatic effect" the disturbing halos resulting from differences in refraction of light.
- **1830** – Joseph Jackson Lister reduces spherical aberration or the "chromatic effect" by showing that several weak lenses used together at certain distances gave good magnification without blurring the image. This was the prototype for the compound microscope.

- **1872** – Ernst Abbe, then research director of the Zeiss Optical Works, wrote a mathematical formula called the "Abbe Sine Condition". His formula provided calculations that allowed for the maximum resolution in microscopes possible.
- **1903** – Richard Zsigmondy developed the ultra-microscope that could study objects below the wavelength of light. He won the Nobel Prize in Chemistry in 1925.
- **1932** – Frits Zernike invented the phase-contrast microscope that allowed for the study of colorless and transparent biological materials for which he won the Nobel Prize in Physics in 1953.
- **1931** – Ernst Ruska co-invented the electron microscope for which he won the Nobel Prize in Physics in 1986. An electron microscope depends on electrons rather than light to view an object, electrons are speeded up in a vacuum until their wavelength is extremely short, only one hundred-thousandth that of white light. Electron microscopes make it possible to view objects as small as the diameter of an atom.
- **1981** – Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope that gives three-dimensional images of objects down to the atomic level. Binnig and Rohrer won the Nobel Prize in Physics in 1986. The powerful scanning tunneling microscope is the strongest microscope to date.

It is not quite certain who invented the microscope. Probably, spectacle makers in Middelburg, The Netherlands, invented both the compound microscope and the refracting telescope, between 1590 and 1610. The names most mentioned are those of Hans and Zacharias Janssen.

The first well known users were van Leeuwenhoek and Hooke. Van Leeuwenhoek used a single lens microscope, Hooke used a compound microscope. The instruments used by Van Leeuwenhoek were far superior to those of Hooke, due to the fact that lens corrections were unknown at the time and the compound microscopes used by Hooke added the lens faults of ocular and objective. So it was Van Leeuwenhoek who made the most discoveries, also due to his sharp eyesight and his unfailing curiosity.

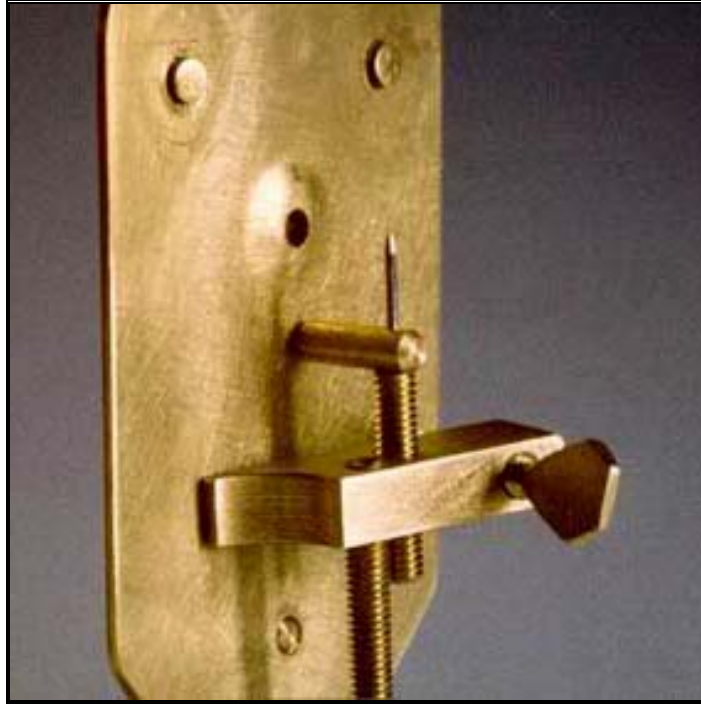


Fig. 1 Replica of microscope by Van Leeuwenhoek

Fig. 1 shows the simplicity of a Van Leeuwenhoek microscope. A subject was placed on the needle and could be positioned with the adjusting screw in the end. The compound microscope however had the future. Although instrument makers like Culpeper, Cuff among others, made beautiful instruments, the real improvement of the microscope came with the invention of the achromatic lens. Achromatic lenses for spectacles were developed by Chester Moore Hall, since 1729. But it was difficult to make small high power achromatic lenses. Jan and Harmanus van Deyl were the first to make these lenses, at the end of the eighteenth century and Harmanus van Deyl started the commercial fabrication of achromatic objectives in 1807. They built on the work of Lister. The lenses with the highest numerical apertures were produced round 1900.



Fig. 2 The First Compound Microscope

A famous producer of top quality microscopes was the firm of Powell and Lealand. Hugh Powell made very high power objectives, an apochromat immersion lens of NA 1,50 in 1896 for instance. Other producers were Ross and Smith and Beck, Nacet, Amici and many other famous names. Carl Zeiss Jena produced its first oil immersion objective in 1880, designed by Ernst Abbe, who was the founder of the optical theory of microscope lenses. Due to better mass fabrication techniques, microscope fabrication was concentrated in Germany after the beginning of the twentieth century. Further developments followed; improvement of the microscope stand and more important even, the development of methods to increase the contrast. The Dutchman Zernike invented the phase contrast microscope in 1934.

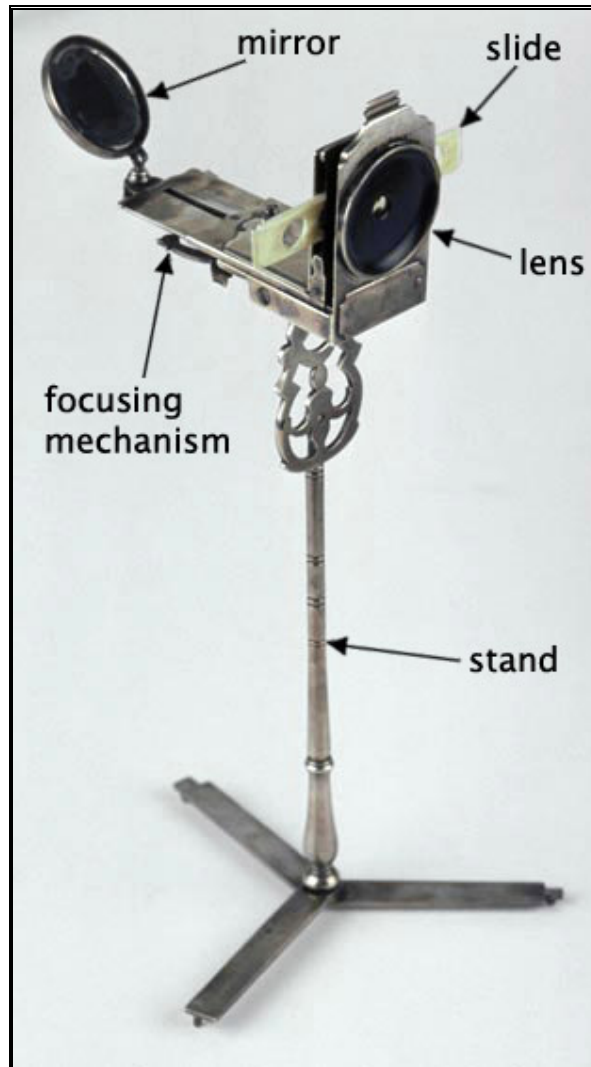


Fig. 3 George Lindsay Single lens Microscope

Other contrast enhancing methods were developed, modulation contrast devised by Hoffman and Gross and differential interference contrast (DIC) all with several variants. A well known DIC system was patented by Nomarsky in 1953. And fluorescence microscopy, in principle already seen by Köhler in 1904, has become a very valuable addition to light microscopy since about 1970.

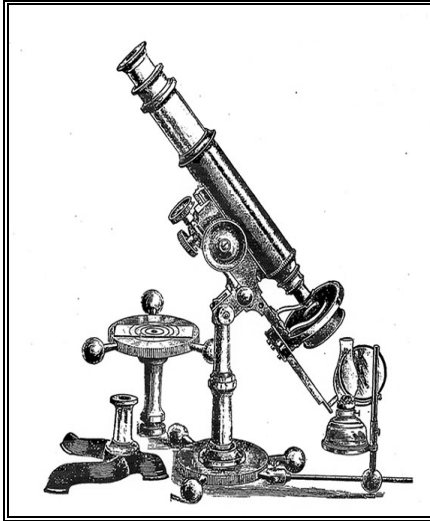


Fig. 4 Griffith Club Microscope

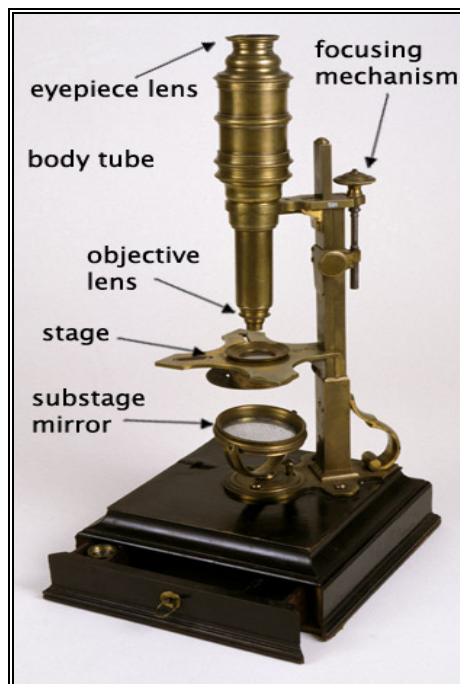


Fig. 5 John Cuff Compound Microscope

1.2.2 Types of Microscope³³:

Microscopes can be classified as following on the different bases. Trinocular microscope is most suitable equipment for the designing digital microscopy. Commercially microscopes classified on the

Based on Ocular Discs:

- (i) Monocular Microscope
- (ii) Binocular Microscope
- (iii) Trinocular Microscope

Based on Commercial Grades:

- (i) Student Microscope
- (ii) Medical Microscope
- (iii) Research Microscope

Based on Medium used for creating Image:

- (i) Optical Microscope
 - Microscope using natural light
 - Microscope using artificial light
- (ii) Electron Microscope
 - Scanning Electron Microscope (SEM)
 - Transmission Electron Microscope (TEM)
- (iii) Scanning Probe Microscope

Based on specific uses:

- (i) Slide Microscope
- (ii) Stereo Microscope
- (iii) Digital Microscope
- (iv) 3D Digital Microscope
- (v) USB Microscope
- (vi) Transmission Electron Microscope
- (vii) Scanning Electron Microscope
- (viii) Scanning Probe Microscope
- (ix) imicroscope

Slide microscope:

- **Magnification:** Uses lenses arranged in series made of optical material that bends light. All magnification takes place via lenses. Uses diascope illumination (that is, light is transmitted through the specimen). Magnification up to about 1000x.
- **Depth of field:** Small
- **Uses:** Pathology, microbiology, forensics, education
- **Advantages:** Color viewing. Require no electricity except for the light source. Can be used on living samples. Simple models are relatively common and inexpensive.
- **Disadvantages:** Usually requires prepared slides and cannot examine objects well in three dimensions.

Stereo microscope, also known as a dissecting microscope³³:

- **Magnification:** As in a slide microscope, but arranged in two optical paths at slightly different angles to allow the user to view objects in three dimensions. Uses episcopic illumination (light is reflected from the specimen's surface). Maximum useful magnification is about 100x.
- **Depth of field:** Large
- **Uses:** Microsurgery, fine repair, dissection, paleontology, quality control, sorting, forensics.
- **Advantages:** Color viewing. Can be used on living samples. Requires little or no preparation of the sample.
- **Disadvantages:** Low magnification.

Digital microscope:

- **Magnification:** Uses both optical lenses and CCD or CMOS sensors; up to at least 1000x magnification.
- **Depth of field:** Like a regular slide microscope.
- **Uses:** All the uses of slide microscopes. Especially useful for applications requiring digital information exchange such as telepathology and continuing medical education.

- **Advantages:** All the advantages of slide microscopes, plus the ability to create "virtual slides" and digital information sharing.
- **Disadvantages:** Requires a power source to operate. Most also require a computer, although some include their own view screens.

3D digital microscope:

- **Magnification:** Works like a regular digital microscope to achieve up to 1000x magnification.
- **Depth of field:** Large, up to 20 times greater than that of a slide microscope.
- **Uses:** All the uses of other digital microscopes, as well as parts machining, materials science, and additional applications.
- **Advantages:** Object can be viewed from almost any angle and its three-dimensional features can be examined.
- **Disadvantages:** As with regular digital microscopes. Can be expensive.

USB microscope:

- **Magnification:** Up to 200x.
- **Depth of field:** Relatively small.
- **Uses:** Document examination, detail work, hobbies, education, entertainment
- **Advantages:** Can be used on almost any object and requires no preparation of the specimen. Inexpensive.
- **Disadvantages:** Not well suited to the same scientific applications as other optical microscopes.

Transmission electron microscope:

- **Magnification:** A series of electrostatic and electromagnetic lenses act on an electron beam to produce up to 50 million times magnification.
- **Depth of field:** Very small.
- **Uses:** Microbiology, pathology, crystallography

- **Advantages:** Very high resolution.
- **Disadvantages:** Requires extensive specimen preparation, including staining and sectioning with an ultramicrotome. Cannot observe the surface of objects. Very specialized equipment that requires a partial vacuum.

Scanning electron microscope³⁴:

- **Magnification:** Electrostatic and electromagnetic lenses, as with a TEM. Magnification ranges from 25x to 250,000x.
- **Depth of field:** Large compared to that of a transmission electron microscope.
- **Uses:** Biology, microbiology, geology, nanotechnology, crystallography
- **Advantages:** Can view objects' three-dimensional surface. Also, an SEM can provide information about the specimen's elemental composition.
- **Disadvantages:** As with TEM, requires specialized equipment and a partial vacuum. Cannot be used on living specimens.

Scanning probe microscope³⁴⁻³⁵:

- **Magnification:** Uses no lenses at all. Instead, the image is formed by making physical contact with a specimen with a probe, which moves across the specimen in a raster pattern. Scanning probe microscopes can detect features as small as a few picometers in width. (A picometer is a trillionth of a meter, or a thousandth of a nanometer.)
- **Depth of field:** Varies according to technique.
- **Uses:** Nanotechnology, biology, microbiology, molecular biology, pathology, food research.
- **Advantages:** Unparalleled resolution. The physical interaction between the probe and specimen can be used to change the specimen, for example by etching it, in a process called nanolithography.
- **Disadvantages:** Image acquisition is slow and the image size is small. Solid-solid and liquid-liquid interfaces provide a challenge.

Imicroscope³⁶:

This is advance type of microscope in which mobile phone can be used as microscope in place of conventional commercial microscope. This application is designed by researcher from the University of California in the smart phone. Smart phones are able to use their heightened senses to perform detail microscopy. This application is specially developed in iphone from apple inc. (USA).

1.2.3 Types of Microscopy:

There are three well-known branches of microscopy, optical, electron, and scanning probe microscopy. Optical and electron microscopy involve the diffraction, reflection, or refraction of electromagnetic radiation/electron beams interacting with the specimen, and the subsequent collection of this scattered radiation or another signal in order to create an image. This process may be carried out by wide-field irradiation of the sample (for example standard light microscopy and transmission electron microscopy) or by scanning of a fine beam over the sample (for example confocal laser scanning microscopy and scanning electron microscopy). Scanning probe microscopy involves the interaction of a scanning probe with the surface of the object of interest. The development of microscopy revolutionized biology and remains an essential technique in the life and physical sciences.

1.2.3.1 Optical Microscopy³⁷:**Simple Optical Microscopy:**

Optical or light microscopy involves passing visible light transmitted through or reflected from the sample through a single or multiple lenses to allow a magnified view of the sample. The resulting image can be detected directly by the eye, imaged on a photographic plate or captured digitally. The single lens with its attachments, or the system of lenses and imaging equipment, along with the appropriate lighting equipment, sample stage and support, makes up the basic light microscope. The most recent development is the digital microscope, which uses a CCD camera to focus on the exhibit of interest. The image is shown on a computer screen, so eye-pieces are unnecessary.

Bright field optical microscopy: Bright field microscopy is the simplest of all the light microscopy techniques. Sample illumination is via transmitted white light, i.e. illuminated from below and observed from above. Limitations include low contrast of most biological samples and low apparent resolution due to the blur of out of focus material. The simplicity of the technique and the minimal sample preparation required are significant advantages.

Oblique illumination: The use of oblique (from the side) illumination gives the image a 3-dimensional appearance and can highlight otherwise invisible features. A more recent technique based on this method is *Hoffmann's modulation contrast*, a system found on inverted microscopes for use in cell culture. Oblique illumination suffers from the same limitations as bright field microscopy (low contrast of many biological samples; low apparent resolution due to out of focus objects), but may highlight otherwise invisible structures.

Dark field: Dark field microscopy is a technique for improving the contrast of unstained, transparent specimens. Dark field illumination uses a carefully aligned light source to minimize the quantity of directly-transmitted (unscattered) light entering the image plane, collecting only the light scattered by the sample. Dark field can dramatically improve image contrast—especially of transparent objects – while requiring little equipment setup or sample preparation. However, the technique does suffer from low light intensity in final image of many biological samples, and continues to be affected by low apparent resolution.

Rheinberg illumination is a special variant of dark field illumination in which transparent, colored filters are inserted just before the condenser so that light rays at high aperture are differently colored than those at low aperture (i.e. the background to the specimen may be blue while the object appears self-luminous yellow). Other color combinations are possible but their effectiveness is quite variable.

Dispersion staining: Dispersion staining is an optical technique that results in a colored image of a colorless object. This is an optical staining technique and requires no stains or dyes to produce a color effect. There are five different microscope configurations used in the broader technique of dispersion

staining. They include bright field Becke line; oblique, dark field, phase contrast, and objective stop dispersion staining.

Phase contrast: Dispersion staining is an optical technique that results in a colored image of a colorless object. This is an optical staining technique and requires no stains or dyes to produce a color effect. There are five different microscope configurations used in the broader technique of dispersion staining. They include bright field Becke line; oblique, dark field, phase contrast, and objective stop dispersion staining. Disadvantage of phase contrast microscopy is halo formation.

Differential interference contrast: Superior and much more expensive is the use of **interference contrast**. Differences in optical density will show up as differences in relief. A nucleus within a cell will actually show up as a globule in the most often used **differential interference contrast** system according to Georges Nomarski. However, it has to be kept in mind that this is an *optical effect*, and the relief does not necessarily resemble the true shape! Contrast is very good and the condenser aperture can be used fully open, thereby reducing the depth of field and maximizing resolution.

The system consists of a special prism in the condenser that splits light in an ordinary and an extraordinary beam. The spatial difference between the two beams is minimal (less than the maximum resolution of the objective). After passage through the specimen, the beams are reunited by a similar prism in the objective. In a homogeneous specimen, there is no difference between the two beams, and no contrast is being generated. However, near a refractive boundary (say a nucleus within the cytoplasm), the difference between the ordinary and the extraordinary beam will generate a relief in the image. Differential interference contrast requires a polarized light source to function; two polarizing filters have to be fitted in the light path, one below the condenser (the polarizer), and the other above the objective (the analyzer).

Interference reflection microscopy: An additional technique using interference is **interference reflection microscopy** (also known as reflected interference contrast, or RIC). It is used to examine the adhesion of cells to a glass surface, using polarized light of a narrow range of wavelengths to be reflected whenever there is an interface between two substances with different

refractive indices. Whenever a cell is attached to the glass surface, reflected light from the glass and from the attached cell will interfere, while if there is no cell attached to the glass, there will be no interference.

Interference reflection microscopy can be obtained by using the same elements used by DIC, but without the prisms. Also, the light that is being detected is reflected and not transmitted as it is when DIC is employed.

Fluorescence: When certain compounds are illuminated with high energy light, they then emit light of a different, lower frequency. This effect is known as fluorescence. Often specimens show their own characteristic autofluorescence image, based on their chemical makeup.

This method is of critical importance in the modern life sciences, as it can be extremely sensitive, allowing the detection of single molecules. Many different fluorescent dyes can be used to stain different structures or chemical compounds. One particularly powerful method is the combination of antibodies coupled to a fluorophore as in immunostaining. Examples of commonly used fluorophores are fluorescein or rhodamine. The antibodies can be made tailored specifically for a chemical compound. For example, one strategy often in use is the artificial production of proteins, based on the genetic code (DNA). These proteins can then be used to immunize rabbits, which then form antibodies which bind to the protein. The antibodies are then coupled chemically to a fluorophore and then used to trace the proteins in the cells under study.

Highly-efficient fluorescent proteins such as the green fluorescent protein (GFP) have been developed using the molecular biology technique of gene fusion, a process that links the expression of the fluorescent compound to that of the target protein. This combined fluorescent protein is, in general, non-toxic to the organism and rarely interferes with the function of the protein under study. Genetically modified cells or organisms directly express the fluorescently-tagged proteins, which enables the study of the function of the original protein *in vivo*.

Since fluorescence emission differs in wavelength (color) from the excitation light, an ideal fluorescent image shows only the structure of interest that was labeled with the fluorescent dye. This high specificity led to the widespread

use of fluorescence light microscopy in biomedical research. Different fluorescent dyes can be used to stain different biological structures, which can then be detected simultaneously, while still being specific due to the individual color of the dye. To block the excitation light from reaching the observer or the detector, filter sets of high quality are needed. These typically consist of an excitation filter selecting the range of excitation wavelengths, a dichroic mirror, and an emission filter blocking the excitation light. Most fluorescence microscopes are operated in the Epi-illumination mode (illumination and detection from one side of the sample) to further decrease the amount of excitation light entering the detector.

Confocal: Using a scanning point of light instead of full sample illumination confocal microscopy gives slightly higher resolution, and significant improvements in optical sectioning. Confocal microscopy is, therefore, commonly used where 3D structure is important.

Deconvolution: Fluorescence microscopy is extremely powerful due to its ability to show specifically labeled structures within a complex environment and also because of its inherent ability to provide three-dimensional information of biological structures. However, this information is blurred by the fact that, upon illumination, all fluorescently labeled structures emit light no matter whether they are in focus or not. This means that an image of a certain structure is always blurred by the contribution of light from structures that are out of focus. This phenomenon becomes apparent as a loss of contrast especially when using objectives with a high resolving power, typically oil immersion objectives with a high numerical aperture.

However, this phenomenon is not caused by random processes such as light scattering but can be relatively well defined by the optical properties of the image formation in the microscope imaging system. If one considers a small fluorescent light source (essentially a bright spot), light coming from this spot spreads out the further out of focus one is. Under ideal conditions, this produces a sort of "hourglass" shape of this point source in the third (axial) dimension. This shape is called the point spread function (PSF) of the microscope imaging system. Since any fluorescence image is made up of a

large number of such small fluorescent light sources, the image is said to be "convolved by the point spread function".

Knowing this point spread function means that it is possible to reverse this process to a certain extent by computer-based methods commonly known as deconvolution microscopy. There are various algorithms available for 2D or 3D deconvolution. They can be roughly classified in *nonrestorative* and *restorative* methods. While the nonrestorative methods can improve contrast by removing out-of-focus light from focal planes, only the restorative methods can actually reassign light to its proper place of origin. This can be an advantage over other types of 3D microscopy such as confocal microscopy, because light is not thrown away but reused. For 3D deconvolution, one typically provides a series of images derived from different focal planes (called a Z-stack) plus the knowledge of the PSF, which can be derived either experimentally or theoretically from knowing all contributing parameters of the microscope.

(b) Sub-diffraction Optical Microscopy³⁸:

It is well known that there is a spatial limit to which light can focus: approximately half of the wavelength of the light one is using. But this is not a true barrier, because this diffraction limit is only true in the far-field and localization precision can be increased with many photons and careful analysis; and like the sound barrier, the diffraction barrier is breakable. This section explores some approaches to imaging objects smaller than ~250 nm. In 1978, the first theoretical ideas had been developed to break this barrier using a 4Pi microscope as a confocal laser scanning fluorescence microscope where the light is focused ideally from all sides to a common focus that is used to scan the object by 'point-by-point' excitation combined with 'point-by-point' detection.

Vertico SMI – SPDM Phymod Super resolution Microscopy

Localization Microscopy/Spatially Structured Illumination: Around 1995, Christoph Cremer commenced with the development of a light microscopic process, which achieved a substantially improved size resolution of cellular nanostructures stained with a fluorescent marker. This time he employed the

principle of wide field microscopy combined with structured laser illumination (spatially modulated illumination) SMI. In addition, this technology is no longer subjected to the speed limitations of the focusing microscopy so that it becomes possible to undertake 3D analyses of whole cells within short observation times (at the moment around a few seconds).

Also since around 1995, Christoph Cremer developed and realized new fluorescence-based wide-field microscopy approaches that had as their goal the improvement of the effective optical resolution (in terms of the smallest detectable distance between two localized objects) down to a fraction of the conventional resolution (spectral precision distance/position determination microscopy, SPDM).

Combining SPDM and SMI, known as Vertico SMI microscopy Christoph Cremer can currently achieve a resolution of approx. 10 nm in 2D and 40 nm in 3D in wide field images of whole living cells. The Vertico SMI is currently the fastest optical 3D nanoscope for the three-dimensional structural analysis of whole cells world-wide.

The Vertico SMI works with high recording speed and processes a complete 3D stack in 40 seconds (2000 frames: 50 frames/s), the very fast image processing based on specific proprietary algorithms makes the image available after 2min/3min (1-/2-color). The specific wide-field technique captures very large areas up to 5000 μm^2 .

Use of standard dyes like normal GFP: In 2008, Cremer's lab discovered that super resolution microscopy was also possible for many standard fluorescent dyes like GFP, Alexa dyes and fluorescein molecules, provided certain photo-physical conditions are present. Using his specific localization microscopy called SPDM Phymod, it is possible to detect and count two different fluorescent molecule types (this technology is referred to as 2CLM, 2 Color Localization Microscopy).

Near-field scanning: Near-field scanning is also called NSOM. Probably the most conceptual way to break the diffraction barrier is to use a light source and/or a detector that is itself nanometer in scale. Diffraction as we know it is truly a far-field effect: The light from an aperture is the Fourier transform of the aperture in the far-field. But, in the near-field, all of this is not necessarily the

case. Near-field scanning optical microscopy (NSOM) forces light through the tiny tip of a pulled fiber — and the aperture can be on the order of tens of nanometers. When the tip is brought to nanometers away from a molecule, the resolution is limited not by diffraction but by the size of the tip aperture (because only that one molecule will see the light coming out of the tip). An image can be built by a raster scan of the tip over the surface to create an image.

The main down-side to NSOM is the limited number of photons you can force out a tiny tip, and the minuscule collection efficiency (if one is trying to collect fluorescence in the near field).

Local enhancement / ANSOM / optical nano-antennas: Instead of forcing photons down a tiny tip, some techniques create a local bright spot in an otherwise diffraction-limited spot. ANSOM is apertureless NSOM: it uses a tip very close to a fluorophore to enhance the local electric field the fluorophore sees. Basically, the ANSOM tip is like a lightning rod which creates a hot spot of light.

Bowtie nano antennas have been used to greatly and reproducibly enhance the electric field in the nanometer gap between the tips two gold triangles. Again, the point is to enhance a very small region of a diffraction-limited spot, thus improving the mismatch between light and nano scale objects and breaking the diffraction barrier.

Stimulated emission depletion: Stefan Hell at the Max Planck Institute for Biophysical Chemistry (Germany) developed STED microscopy (stimulated emission depletion), which uses two laser pulses. The first pulse is a diffraction limited spot that is tuned to the absorption wavelength, so excites any fluorophores in that region; an immediate second pulse is red-shifted to the emission wavelength and stimulates emission back to the ground state before, thereby depleting the excited state of any fluorophores in this depletion pulse. The trick is that the depletion pulse goes through a phase modulator that makes the pulse illuminate the sample in the shape of a donut, so the outer part of the diffraction limited spot is depleted and the small center can still fluoresce. By saturating the depletion pulse, the center of the donut gets smaller and smaller until they can get resolution of tens of nanometers.

Fitting the point-spread function: Fitting the point spread function (PSF). The methods above (and below) use experimental techniques to circumvent the diffraction barrier, but one can also use crafty analysis to increase the ability to know where a nano scale object is located. The image of a point source on a charge-coupled device camera is called a point-spread function (PSF), which is limited by diffraction to be no less than approximately half the wavelength of the light. But it is possible to simply fit that PSF with a Gaussian to locate the center of the PSF — and thus the location of the fluorophore. The precision by which this technique can locate the center depends on the number of photons collected (as well as the CCD pixel size and other factors). This concept was first used to achieve resolution beyond the diffraction limit with single molecules by Van Oijen et al. in 1998 (Chem. Phys. Lett. V.292, p183). Subsequently at room temperature, groups like the Selvin lab and many others have employed this analysis to localize single fluorophores to a few nanometers. This, of course, requires careful measurements and collecting many photons.

PALM, STORM: What fitting a PSF is to localization, photo-activated localization microscopy (PALM) is to "resolution"—this term is here used loosely to mean measuring the distance between objects, not true optical resolution. Eric Betzig and colleagues developed PALM; Xiaowei Zhuang at Harvard used a similar technique and calls it STORM: stochastic optical reconstruction microscopy. Sam Hess at University of Maine developed the technique simultaneously. The basic premise of both techniques is to fill the imaging area with many dark fluorophores that can be photoactivated into a fluorescing state by a flash of light. Because photoactivation is stochastic, only a few, well-separated molecules "turn on." Then Gaussians are fit to their PSFs to high precision (see section above). After the few bright dots photobleach, another flash of the photoactivating light activates random fluorophores again and the PSFs are fit of these different well-spaced objects. This process is repeated many times, building up an image molecule-by-molecule; and, because the molecules were localized at different times, the "resolution" of the final image can be much higher than that limited by diffraction.

The major problem with these techniques is that to get these beautiful pictures, it takes on the order of hours to collect the data. This is not the technique to study dynamics (fitting the PSF is better for that).

Structured illumination: There is also the wide-field structured-illumination (SI) approach to breaking the diffraction limit of light. SI—or patterned illumination—relies on both specific microscopy protocols and extensive software analysis post-exposure. But, because SI is a wide field technique, it is usually able to capture images at a higher rate than confocal-based schemes like STED (but SI is not actually superfast). The main concept of SI is to illuminate a sample with patterned light and increase the resolution by measuring the fringes in the Moiré pattern (from the interference of the illumination pattern and the sample). "Otherwise-unobservable sample information can be deduced from the fringes and computationally restored."

SI enhances spatial resolution by collecting information from frequency space outside the observable region. This process is done in reciprocal space: The Fourier transform (FT) of an SI image contains superimposed additional information from different areas of reciprocal space; with several frames with the illumination shifted by some phase, it is possible to computationally separate and reconstruct the FT image, which has much more resolution information. The reverse FT returns the reconstructed image to a super resolution image.

But this enhances the resolution only by a factor of 2 (because the SI pattern cannot be focused to anything smaller than half the wavelength of the excitation light). To further increase the resolution, one can introduce *nonlinearities*, which show up as higher-order harmonics in the FT. In reference, Gustafsson uses saturation of the fluorescent sample as the nonlinear effect. A sinusoidal saturating excitation beam produces the distorted fluorescence intensity pattern in the emission. This nonpolynomial nonlinearity yields a series of higher-order harmonics in the FT.

Each higher-order harmonic in the FT allows another set of images that can be used to reconstruct a larger area in reciprocal space, and thus a higher resolution. In this case, Gustafsson achieves less than 50-nm resolving power, more than five times that of the microscope in its normal configuration.

Extensions Optical Microscopy³⁹:

Most modern instruments provide simple solutions for micro-photography and image recording electronically. However such capabilities are not always present and the more experienced microscopist will, in many cases, still prefer a hand drawn image rather than a photograph. This is because a microscopist with knowledge of the subject can accurately convert a three dimensional image into a precise two dimensional drawing. In a photograph or other image capture system however, only one thin plane is ever in good focus.

The creation of careful and accurate micrographs requires a microscopical technique using a monocular eyepiece. It is essential that both eyes are open and that the eye that is not observing down the microscope is instead concentrated on a sheet of paper on the bench besides the microscope. With practice, and without moving the head or eyes, it is possible to accurately record the observed details by tracing round the observed shapes by simultaneously "seeing" the pencil point in the microscopical image. Practicing this technique also establishes good general microscopical technique. It is always less tiring to observe with the microscope focused so that the image is seen at infinity and with both eyes open at all times.

Enhancements Aided Optical Microscopy:

In this technique different kinds of enhancements like digital eyepiece, camera lucida etc can be used in the optical microscopes.

X-ray Optical Microscopy⁴⁰:

As resolution depends on the wavelength of the light, Electron microscopy has been developed since the 1930s that use electron beams instead of light. Because of the much smaller wavelength of the electron beam, resolution is far higher.

Though less common, X-ray microscopy has also been developed since the late 1940s. The resolution of X-ray microscopy lies between that of light microscopy and electron microscopy.

1.2.3.2 Electron microscopy⁴¹:

It is a type of microscopy that uses a beam of electrons to create an image of the specimen. It is capable of much higher magnifications and has a greater

resolving power than a light microscope, allowing it to see much smaller objects in finer detail. They are large, expensive pieces of equipment, generally standing alone in a small, specially designed room and requiring trained personnel to operate them.

History of electron microscopy: By the middle of the 19th century, microscopists had accepted that it was simply not possible to resolve structures of less than half a micrometer with a light microscope because of the Abbe's formula, but the development of the cathode ray tube was literally about to change the way they looked at things; by using electrons instead of light! Hertz (1857-94) suggested that cathode rays were a form of wave motion and Weichert, in 1899, found that these rays could be concentrated into a small spot by the use of an axial magnetic field produced by a long solenoid. But it was not until 1926 that Busch showed theoretically that a short solenoid converges a beam of electrons in the same way that glass can converge the light of the sun, that a direct comparison was made between light and electron beams. Busch should probably therefore be known as the father of electron optics.

In 1931 the German engineers Ernst Ruska and Maximillion Knoll succeeded in magnifying and electron image. This was, in retrospect, the moment of the invention of the electron microscope but the first prototype was actually built by Ruska in 1933 and was capable of resolving to 50 nm. Although it was primitive and not really fit for practical use, Ruska was recognized some 50 years later by the award of a Nobel Prize. The first commercially available electron microscope was built in England by Metropolitan Vickers for Imperial College, London, and was called the EM1, though it never surpassed the resolution of a good optical microscope. The early electron microscopes did not excite the optical microscopes because the electron beam, which had a very high current density, was concentrated into a very small area and was very hot and therefore charred any non-metallic specimens that were examined. When it was found that you could successfully examine biological specimens in the electron microscope after treating them with osmium and cutting very thin slices of the sample, the electron microscope began to appear as a viable proposition. At the University of Toronto, in 1938, Eli

Franklin Burton and students Cecil Hall, James Hillier and Albert Prebus constructed the first electron microscope in the New World. This was an effective, high-resolution instrument, the design of which eventually led to what was to become known as the RCA (Radio Corporation of America) range of very successful microscopes.

Unfortunately, the outbreak of the Second World War in 1939 held back their further development somewhat, but within 20 years of the end of the war routine commercial electron microscopes were capable of 1 nm resolution.

Types of Electron Microscopes:

All electron microscopes use electromagnetic and/or electrostatic lenses to control the path of electrons. Glass lenses, used in light microscopes, have no effect on the electron beam. The basic design of an electromagnetic lens is a solenoid (a coil of wire around the outside of a tube) through which one can pass a current, thereby inducing an electromagnetic field. The electron beam passes through the centre of such solenoids on its way down the column of the electron microscope towards the sample. Electrons are very sensitive to magnetic fields and can therefore be controlled by changing the current through the lenses.

The faster the electrons travel, the shorter their wavelength. The resolving power of a microscope is directly related to the wavelength of the irradiation used to form an image. Reducing wavelength increases resolution. Therefore, the resolution of the microscope is increased if the accelerating voltage of the electron beam is increased. The accelerating voltage of the beam is quoted in kilovolts (kV). It is now possible to purchase a 1,000kV electron microscope, though this is not commonly found.

Although modern electron microscopes can magnify objects up to about two million times, they are still based upon Ruska's prototype and the correlation between wavelength and resolution. The electron microscope is an integral part of many laboratories such as The John Innes Centre. Researchers can use it to examine biological materials (such as microorganisms and cells), a variety of large molecules, medical biopsy samples, metals and crystalline structures, and the characteristics of various surfaces. Nowadays, electron microscopes have many other uses outside research. They can be used as

part of a production line, such as in the fabrication of silicon chips, or within forensics laboratories for looking at samples such as gunshot residues. In the arena of fault diagnosis and quality control, they can be used to look for stress lines in engine parts or simply to check the ratio of air to solids in ice cream!

Transmission Electron Microscope⁴² (TEM)

The original form of electron microscopy, Transmission electron microscopy (TEM) involves a high voltage electron beam emitted by a cathode and formed by magnetic lenses. The electron beam that has been partially transmitted through the very thin (and so semitransparent for electrons) specimen carries information about the structure of the specimen. The spatial variation in this information (the "image") is then magnified by a series of magnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or light sensitive sensor such as a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed in real time on a monitor or computer.

Transmission electron microscopes produce two-dimensional, black and white images. Resolution of the TEM is also limited by spherical and chromatic aberration, but a new generation of aberration correctors has been able to overcome or limit these aberrations. Software correction of spherical aberration has allowed the production of images with sufficient resolution to show carbon atoms in diamond separated by only 0.089 nm and atoms in silicon at 0.078 nm at magnifications of 50 million times. The ability to determine the positions of atoms within materials has made the TEM an indispensable tool for nano-technologies research and development in many fields, including heterogeneous catalysis and the development of semiconductor devices for electronics and photonics. In the life sciences, it is still mainly the specimen preparation which limits the resolution of what we can see in the electron microscope, rather than the microscope itself.

Scanning Electron Microscope⁴³ (SEM)

Unlike the TEM, where the electrons in the primary beam are transmitted through the sample, the Scanning Electron Microscope (SEM) produces images by detecting secondary electrons which are emitted from the surface due to excitation by the primary electron beam. In the SEM, the electron beam

is scanned across the surface of the sample in a raster pattern, with detectors building up an image by mapping the detected signals with beam position.

TEM resolution is about an order of magnitude better than the SEM resolution. Our TEM can easily resolve details of 0.2nm. Our two SEMs at JIC are both relatively recent acquisitions and are high-resolution instruments capable of about 2 nm resolution on biological samples. Because the SEM image relies on electron interactions at the surface rather than transmission it is able to image bulk samples and has a much greater depth of view, and so can produce images that are a good representation of the 3D structure of the sample. SEM images are therefore considered to provide us with 3D, topographical information about the sample surface but will still always be only in black and white.

In the SEM, we use much lower accelerating voltages to prevent beam penetration into the sample since what we require is generation of the secondary electrons from the true surface structure of a sample. Therefore, it is common to use low KV, in the range 1-5kV for biological samples, even though our SEMs are capable of up to 30 kV.

Reflection Electron Microscope⁴⁴ (REM)

This is as in the TEM, an electron beam is incident on a surface, but instead of using the transmission (TEM) or secondary electrons (SEM), the reflected beam of elastically scattered electrons is detected. This technique is typically coupled with Reflection High Energy Electron Diffraction and Reflection high-energy loss spectrum. Another variation is Spin-Polarized Low-Energy Electron Microscopy, which is used for looking at the microstructure of magnetic domains.

Scanning Transmission Electron Microscope⁴⁵ (STEM)

The STEM rasters a focused incident probe across a specimen that has been thinned to facilitate detection of electrons scattered *through* the specimen. The high resolution of the TEM is thus possible in STEM. The focusing action occurs before the electrons hit the specimen in the STEM, but afterward in the TEM. The STEMs use of SEM-like beam rastering simplifies annular dark-field imaging, and other analytical techniques, but also means that image data is acquired in serial rather than in parallel fashion.

Low-voltage Electron Microscope⁴⁶ (LVEM)

This is a combination of SEM, TEM and STEM in one instrument, which operates at relatively low electron accelerating voltage of 5 kV. Low voltage increases image contrast which is especially important for biological specimens. This increase in contrast significantly reduces, or even eliminates the need to stain. Sectioned samples generally need to be thinner than they would be for conventional TEM (20-65 nm). Resolutions of a few nm are possible in TEM, SEM and STEM modes.

Sample Preparation⁴⁷:

Materials to be viewed in an electron microscope generally require processing to produce a suitable sample. This is mainly because the whole of the inside of an electron microscope is under high vacuum in order to enable the electron beam to travel in straight lines. The technique required varies depending on the specimen, the analysis required and the type of microscope:

Cryofixation: freezing a specimen rapidly, typically to liquid nitrogen temperatures or below, that the water forms ice. This preserves the specimen in a snapshot of its solution state with the minimal of artefacts. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy, it is now possible to observe virtually any biological specimen close to its native state.

Fixation: a general term used to describe the process of preserving a sample at a moment in time and to prevent further deterioration so that it appears as close as possible to what it would be like in the living state, although it is now dead. In chemical fixation for electron microscopy, glutaraldehyde is often used to crosslink protein molecules and osmium tetroxide to preserve lipids.

Dehydration - removing water from the samples. The water is generally replaced with organic solvents such as ethanol or acetone as a stepping stone towards total drying for SEM specimens or infiltration with resin and subsequent embedding for TEM specimens.

Embedding - infiltration of the tissue with wax (for light microscopy) or a resin (for electron microscopy) such as araldite or LR White, which can then be polymerized into a hardened block for subsequent sectioning.

Sectioning - the production of thin slices of the specimen. For light microscopy, the sections can be a few micrometers thick but for electron microscopy they must be very thin so that they are semitransparent to electrons, typically around 90nm. These ultra-thin sections for electron microscopy are cut on an ultra microtome with a glass or diamond knife. Glass knives can easily be made in the laboratory and are much cheaper than diamond, but they blunt very quickly and therefore need replacing frequently.

Staining - uses heavy metals such as lead and uranium to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to the electron beam. By staining the samples with heavy metals, we add electron density to it which results in there being more interactions between the electrons in the primary beam and those of the sample, which in turn provides us with contrast in the resultant image. In biology, specimens can be stained "en bloc" before embedding and/or later, directly after sectioning, by brief exposure of the sections to solutions of the heavy metal stains.

Freeze-fracture and **freeze-etch** - a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The fresh tissue or cell suspension is frozen rapidly (cryofixed), then fractured by simply breaking or by using a microtome while maintained at liquid nitrogen temperature. The cold, fractured surface is generally "etched" by increasing the temperature to about -95°C for a few minutes to let some surface ice sublime to reveal microscopic details. For the SEM, the sample is now ready for imaging. For the TEM, it can then be rotary-shadowed with evaporated platinum at low angle (typically about 6°) in a high vacuum evaporator. A second coat of carbon, evaporated perpendicular to the average surface plane is generally performed to improve stability of the replica coating. The specimen is returned to room temperature and pressure, and then the extremely fragile "shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The floating replica is thoroughly washed from residual chemicals, carefully picked up on an EM grid, dried then viewed in the TEM.

Sputter Coating - an ultra-thin coating of electrically-conducting material, deposited by low vacuum coating of the sample. This is done to prevent charging of the specimen, which would occur because of the accumulation of static electric fields due to the electron irradiation required during imaging. It also increases the amount of secondary electrons that can be detected from the surface of the sample in the SEM and therefore increases the signal to noise ratio. Such coatings include gold, gold/palladium, platinum, chromium etc.

Disadvantages of Electron Microscopy⁴⁸:

Electron microscopes are very expensive to buy and maintain. They are dynamic rather than static in their operation: requiring extremely stable high voltage supplies, extremely stable currents to each electromagnetic coil/lens, continuously-pumped high/ultra-high vacuum systems and a cooling water supply circulation through the lenses and pumps. As they are very sensitive to vibration and external magnetic fields, microscopes aimed at achieving high resolutions must be housed in buildings with special services.

A significant amount of training is required in order to operate an electron microscope successfully and electron microscopy is considered a specialized skill.

The samples have to be viewed in a vacuum, as the molecules that make up air would scatter the electrons. This means that the samples need to be specially prepared by sometimes lengthy and difficult techniques to withstand the environment inside an electron microscope. Recent advances have allowed some hydrated samples to be imaged using an environmental scanning electron microscope, but the applications for this type of imaging are still limited.

1.2.3.3 Scanning Probe Microscopy:

Scanning probe microscopy covers several related technologies for imaging and measuring surfaces on a fine scale, down to the level of molecules and groups of atoms. At the other end of the scale, a scan may cover a distance of over 100 micrometers in the x and y directions and 4 micrometers in the z direction. This is an enormous range. It can truly be said that the

development of this technology is a major achievement, for it is having profound effects on many areas of science and engineering⁴⁹.

SPM technologies share the concept of scanning an extremely sharp tip (3-50 nm radius of curvature) across the object surface. The tip is mounted on a flexible cantilever, allowing the tip to follow the surface profile. When the tip moves in proximity to the investigated object, forces of interaction between the tip and the surface influence the movement of the cantilever. These movements are detected by selective sensors. Various interactions can be studied depending on the mechanics of the probe. In contact mode, the tip is usually maintained at a constant force by moving the cantilever up and down as it scans. In non-contact mode or intermittent contact mode the tip is driven up and down by an oscillator. Especially soft materials may be imaged by a magnetically-driven cantilever. In non-contact mode, the bottom-most point of each probe cycle is in the attractive region of the force-distance curve. In intermittent contact mode the bottom-most point is in the repulsive region. Variations in the measured oscillation amplitude and phase in relation to the driver frequency are indicators of the surface-probe interaction⁵⁰.

To image frictional force, the probe is dragged along the surface, resulting in a torque on the cantilever. To image the magnetic field of the surface, a magnetically-susceptible probe is used. In other variations, the electric charge distribution on the surface or the surface capacitance is imaged. For thermal scanning microscopy (TSM) the thermal conductivity of the surface with is probed with a resistive tip that acts as a tiny resistance thermometer. In addition to these modes, many instruments are also designed to plot the phase difference between the measured modes, for example frictional force versus contact profile. This plot is called phase mode. Several types of probes with different tips are used in scanning probe microscopy. Tip selection depends on the mode of operation and on the type of sample⁵¹.

Applications

These techniques have the ability to operate on a scale from microns down to nanometers and can image clusters of individual atoms and molecules. STM relies on the electrical conductivity of the sample, so at least some features on the sample surface must be electrically conductive to some degree. AFM is

used for studies of non-conductors and is the technique more commonly used for studies of macromolecules and biological specimens. AFM has been used for measurements on a wide variety of samples⁵².

Choice of Technique

The analytic mode is chosen based on the surface characteristics of interest and on the hardness or stickiness of the sample. Contact mode is most useful for hard surfaces. However, a tip in contact with a surface is subject to contamination from removable material on the surface. Excessive force in contact mode can also damage the surface or erode the sharpness of the probe tip. Near-contact mode has fewer tendencies to deform a soft surface, but is more sensitive to environmental vibrations and to variations in the film of moisture that coats samples in a normal atmosphere. Moisture or other thin liquid films exert an attractive capillary force on the probe as it is withdrawn from the surface. In non-contact mode this force becomes significant⁵³.

Vibrating mode or intermittent contact modes are particularly suited for imaging soft biological specimen. However, biological samples are successfully imaged in the "harder" contact mode. Unfixed soft specimens are deformed in the z-dimension to a degree dependent on the imposed probe force, although spreading in the x-y plane may not be significant. Biological samples may be hardened to reduce probe-induced deformation by aldehyde fixation or frozen in a cryo-AFM.

Accuracy and Calibration

Instrumental factors; the performance of a scanning probe instrument is limited by a number of factors. One of these is the resolution of the mechanical components used to move the tip and measure its position. The sharpness and stability of the probe tip determine the area of contact and the reproducibility of imaging. Obviously, environmental vibrations must be controlled to a high degree. In addition, most positioners depend on piezoelectric drive, which is subject to problems of non-linearity and to overshoot during rapid movements. The major manufacturers of SPM equipment have made substantial improvements in mechanical and electronic

design. These improvements and advanced electronic calibration routines result in measurements that are more linear and accurate than the early models. Accurately nanofabricated gratings are the basis for two and three-dimensional calibrations. Such calibration gratings and calibration software are commercially available. Probe-Related Image Distortions; at very high magnifications and high-relief sample surfaces, the mode of imaging and the geometry of the probe tip can influence the scanned image. Knowledge of the probe geometry then becomes important for interpretation of the image. To image individual atoms and molecules it is necessary for the tip-surface interaction to depend only on the nearest atom(s) of the tip. This occurs in scanning tunneling microscopy because the tunneling current passes only through the nearest atom of the tip. Tunneling current falls off very steeply with distance from the surface. In atomic force microscopy the tip-surface interaction forces fall off less steeply with distance. Thus an AFM probe responds to the average force of interaction for a number of tip atoms, depending on the sharpness of the tip. An AFM image does not show individual atoms, but rather an averaged surface. For ordered surfaces this will reflect the average unit cell.

Probe Deconvolution (Image Restoration). Imaging very sharp vertical surfaces (surfaces with high relief) is also influenced by the sharpness of the tip. Only a tip with sufficient sharpness can properly image a given z-gradient. Some gradients will be steeper or sharper than any tip can be expected to image without artifact. False images are generated that reflect the self-image of the tip surface, rather than the object surface. Mathematical methods of tip deconvolution can be employed for image restoration. The effectiveness of these methods will depend on the specific characteristics of the sample and the probe tip. A number of scientists have investigated this area⁵⁴.

1.2.3.4 Infrared microscopy:

The term infrared microscope covers two main types of diffraction-limited microscopy. The first provides optical visualization plus IR spectroscopic data collection. The second technique employs focal plane array detection for

infrared chemical imaging, where the image contrast is determined by the response of individual sample regions to particular IR wavelengths selected by the user. The major concern with this type of microscopy is the ability to capture satisfactory photomicrographs using conventional imaging technology. Currently, several film emulsions are available that respond to the infrared spectrum, but electronic detectors are rapidly becoming the imaging device of choice for studies in this wavelength range⁵⁵.

1.2.3.5 Digital holographic microscopy:

It is digital holography applied to microscopy. Digital holographic microscopy identified itself from other microscopic methods by not recording the projected image of the object. Here in this technique light wave front information originating from the object is digitally recorded as a hologram, from which a computer calculates the object image by using a numerical reconstruction algorithm. The image forming lens in traditional microscopy is thus replaced by a computer algorithm⁵⁶.

Closely related other microscopic methods to digital holographic microscopy are interferometric microscopy, optical coherence tomography and diffraction phase microscopy. Common to all methods is the use of a reference wave front to obtain intensity and phase information. The information is recorded on a digital image sensor or by a photo detector from which an image of the object is created by the computer. In traditional microscopy, which does not use a reference wave front, only intensity information is recorded and essential information about the object is lost. This technique has mostly been applied to light microscopy. However, digital holography has also been applied to electron microscopy. Holography was invented by Dennis Gabor to improve the electron microscope. For various reasons holography never made it into the electron microscope. Digital electron holography may finally bring home holography to its birth place and fulfill Gabor's vision⁵⁷.

Principle of Digital Holographic Microscopy:

Digital holography is based on the classic holographic principle, with the difference that the hologram recording is performed by a digital image sensor, e.g. a CCD or CMOS camera. The subsequent reconstruction of the

holographic image that contains the information about the object wave is carried out numerically with a computer. Fig. 6 depicts the schematics of two “off-axis” setups for digital holographic microscopy that are particularly suitable for the modular integration into commercial microscopy systems. The coherent light of a laser is divided into object illumination and reference wave, using single mode optical fibers⁵⁸.

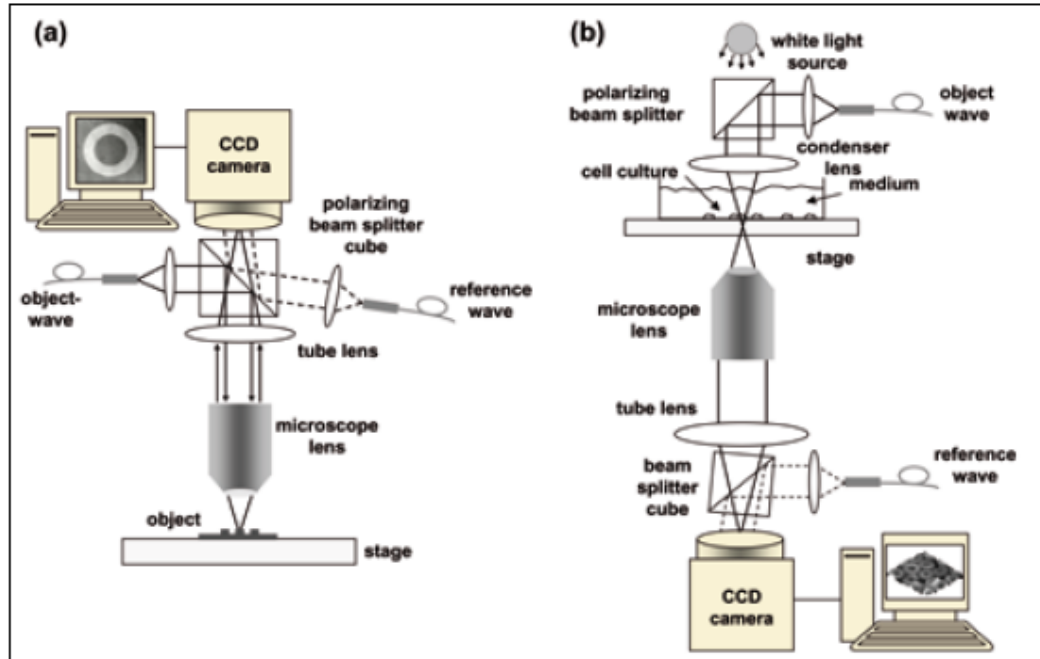


Fig. 6 Schematics for digital holographic microscopy using incident light (left) and inverse transmission arrangements (right)

Here Fig. 6 shows an incident light illumination arrangement for the investigation of reflective samples. The set-up in Fig. 6 is designed to investigate transparent specimen such as living cells. For both setups the coherent laser light for the illumination of the sample is coupled into the optical path of the microscope’s condenser by a beam splitter cube. The reference wave is superimposed with the light that is reflected or transmitted by the object by a second beam splitter with a slight tilt against the object wave front in order to generate “off-axis” holograms which are recorded by a CCD camera. After hologram acquisition, the data is transmitted by an IEEE1394 interface to a PC based image processing system, thus avoiding

cost intensive frame grabber cards with hardware specific software. The modular add-on approach provides the advantage that common commercial microscope lenses with high numerical aperture (e.g. water and oil immersion) can be used in combination with an optimized illumination of the sample. Furthermore, the integration of the additional optical components for digital holography does not restrict the conventional functions of the microscopy systems⁵⁹.

Applications of Digital Holographic Microscopy:

Digital holographic microscopy can be used successfully in a range of application areas. However, due to DHM's capability of non-invasively visualizing and quantifying biological tissue, bio-medical applications have received most attention in the field of pharmacy as well as in the medicine⁶⁰.

1. Label-free cell counting in adherent cell cultures: DHM makes it possible to perform cell counting and to measure cell viability directly in the cell culture chamber today, the most commonly used cell counting methods, haemocytometer or Coulter counter, only work with cells those are in suspension.
2. Label-free viability analysis of adherent cell cultures: Digital holography has been used to study the apoptotic process in different cell types. The refractive index changes taking place during the apoptotic process are easily measured with DHM.
3. Label-free cell cycle analysis: The phase shift induced by cells has been shown to be correlated to the cell dry mass. The cell dry mass can be combined with other parameters obtainable by digital holography, such as cell volume and refractive index, to provide a better understanding of the cell cycle.
4. Label-free morphology analysis of cells: Digital holography has been used in different contexts to study cell morphology using neither staining nor labeling. This can be used to follow processes such as the differentiation process where cell characteristics change. DHM has also been used for automated plant stem cell

monitoring, and made it possible to distinguish between two types of stem cells by measuring morphological parameters.

5. Label free nerve cell studies. Digital holographic microscopy makes it possible to study undisturbed processes in nerve cells as no labeling is required. The swelling and shape changing of nerve cells caused by cellular imbalance was easily studied.
6. Label-free high content analysis. Fluorescent high content analysis/screening has several drawbacks. Label-free alternatives based on phase shift images have therefore been proposed. The capability of DHM to obtain phase shift images rapidly over large areas opens up new possibilities of very rapid quantitative characterization of the cell cycle and the effects of specific pharmacological agents.
7. Red blood cell analysis. Phase shift images, created by diffraction phase microscopy, have been used to study red blood cell dynamics. Diffraction phase microscopy is very similar to digital holographic microscopy and creates phase shift images identical to the phase shift images created by digital holography. It has also been shown that phase shift information discriminates immature red blood cells from mature, facilitating unstained reticulocyte count.
8. Flow cytometry and particle tracking and characterization: Images created by digital holography are calculated from the recorded hologram at any time after the actual recording and at any given focal plane. By combining several images calculated from the same hologram, but at different focal planes, an increased depth of field may be obtained, which is vastly superior to what can be achieved with traditional light microscopy.
9. Tomography studies: Digital holographic microscopy allows for label-free and quantifiable analysis of sub cellular motion deep in living tissue.

1.2.3.6 Digital Pathology (Virtual Microscopy):

Virtual microscopy is a method of posting microscope images on, and transmitting them over, computer networks. This allows independent viewing of images by large numbers of people from different locations. It involves a synthesis of microscopy technologies and digital technologies. The use of virtual microscopes can transform traditional teaching methods by removing the reliance on physical space, equipment, and specimens to a model that is solely dependent upon computer-internet access. This increases the convenience of accessing the slide sets and making the slides available to a broader audience. Digitized slides can have a high resolution and are resistant to being spoiled or busted over time⁶¹.

The Digital Pathology Virtual Microscope Slides allow science students and allied health professionals to access microscope slides through an online database for distance learning and enhanced curriculum delivery. The Digital Pathology Virtual Microscope Slide database can be created with high quality resolution of 83X oil immersion, which is necessary for the proper examination of specimen. The use of virtual microscopes can transform traditional teaching methods by removing the reliance on physical space, equipment, and specimens to a model that is solely dependent upon computer-internet access. This rich database is enhanced with patient clinical presentations, laboratory data, comprehensive slide interpretations, and diagnoses⁶².

1.2.3.7 Amateur Microscopy:

It is the examination and inspection of biological and non-biological specimens for spare time purposes. Collectors of minerals, insects, seashells, and plants may use microscopes as tools to expose features that help them classify their collected items. Other amateurs may be interested in observing the life originate in pond water and of other samples. Microscopes may also prove useful for the water quality assessment for people that keep a home aquarium. Photographic documentation and drawing of the microscopic images are additional tasks that augment the spectrum of tasks of the amateur. There are even competitions for photomicrograph art. Participants of

this pastime either may use commercially prepared microscopic slides or may engage in the task of specimen preparation⁶³.

While microscopy is a central tool in the documentation of biological specimens, it is, in general, insufficient to justify the description of a new species based on microscopic investigations alone. Often genetic and biochemical tests are necessary to confirm the discovery of a new species. A laboratory and access to academic literature is a necessity, which is specialized and, in general, not available to amateurs. There is, however, one huge advantage that amateurs have above professionals: time to explore their surroundings. Often, advanced amateurs team up with professionals to validate their findings and describe new species⁶⁴.

In the late 1800s, amateur microscopy became a popular hobby in the United States and Europe. Several 'professional amateurs' were being paid for their sampling trips and microscopic explorations by philanthropists, to keep them amused on the Sunday afternoon. In 1995, a loose group of amateur microscopists, drawn from several organizations in the UK and USA, founded a site for microscopy based on the knowledge and input of amateur microscopists. This was the first attempt to establish 'amateur' microscopy as a serious subject in the then-emerging new media of the Internet. Today, it remains as a powerful established international resource for all ages, to input their findings and share information. It is a nonprofit-making web presence dedicated to the pursuit of science and understanding of the small-scale world⁶⁵.

1.2.3.8 imicroscopy:

This technique is designed by researcher from the University of California in the smart phone. Smart phones are able to use their heightened senses to perform detail microscopy. This application is specially developed in iphone from apple inc. (USA)⁶⁶

These researchers are not the first group to build a phone microscope, but they thought they can make something cheaper and simpler. Their first attempt took simplicity too far. They started with a drop of water on the camera's lens. The water formed a meniscus, and its curved surface acted like a magnifying lens. It worked fine but water evaporated too fast.



Fig. 7 imicroscope in iphone

Then the team turned to ball lenses, finally ground glass spheres that act as low powered magnifying glasses. The team used a 1-millimeter ball lens that costs approximately Rs. 1971 in their prototype. To build the microscope's lens, Kaiqin Chu, a researcher, inserted a ball lens into a hole in a rubber sheet, then simply taped the sheet over the camera. At 5X magnification, the ball lens no more powerful than a child's magnifying glass. Yet when paired with the phone camera, the microscope could resolve features on the order of 1.5 microns, small enough to identify different types of cells. There are two reasons why such low magnification produces such high-res images. First ball lenses excel at gathering light, which determines resolution. Second, the camera's sensor consists of millions of light-capturing cells. Each cell is only about 1.7 microns across. This is small enough to capture the tiny image through the lens⁶⁷.

1.2.4 Importance of Microscopy:

Authentication of botanical material is a critical step in the use of these materials for both research purposes and commercial preparation. Microscopic evaluation and comparison of authenticated and unauthenticated samples of whole, cut, or powdered plant material is a cost effective and accurate means of identifying herbal ingredients. Microscopy can be a useful tool for the detection of botanical and non-botanical adulterants such as pharmaceutical drugs, microbial contaminants, and inorganic materials. Advances in microscope technology and improvements in light, fluorescence,

phase contrast, and scanning electron microscopes have improved the accuracy and capabilities of microscopy as a means of botanical authentication. Organoleptic analysis, used in combination with advanced microscopy equipment, provides further accuracy for botanical authentication⁶⁸.

1.2.5 Computer Aided Microscopy:

Computer-aided microscopists collect microscope images which they manipulate to produce the "best" or optimized structure(s) and then analyze to produce a statistical description of the important features. It is first of all assumed that the microscope lighting and sample preparation have been optimized so that the crispest possible picture of the specimen is obtained prior to image processing. For example, differences in gray level can be associated with poor sample etching, scratches, dust and so that quantitative definition becomes impossible.

After the image is recorded in analog form, it is digitized. There are many procedures which can be used to manipulate a digitized image. The first step is to improve the image: the image should be corrected for non-uniform illumination and then a smoothing operation can be applied to remove spurious or noisy signals. This is followed by a series of specialized edge sharpening algorithms to ensure exact location of the boundary of interest. The image is then segmented. The purpose of segmentation is to divide the image into well-defined regions (that is, well-defined in terms of the features which are to be later measured). One way of achieving this is to divide the gray scale into specific bands and use gray level thresholds to obtain boundary points. Segmentation requires quantitatively locating boundaries of interest. An exact binary Image of the regions of interest is then obtained. The images are subsequently processed, using binary logic operations, so that essential features can be extracted and the parameters of importance more easily measured.

The operator then determines features of binary representation which are to be measured. A feature is defined as an attribute that describes the image, such as shape and size. Then, the parameters which define the image

attribute are selected; for example, percent area occupied by the object in the field of view, object area, perimeter, diameter, length, shape, and so forth. Finally, the selected parameters are measured and the data statistically evaluated. Computer-aided microscopy can be categorized into the following classes of processing operations: Image Retrieval, Image Enhancement, Segmentation, Binary (Boolean) Operations and Quantitative Analysis⁶⁸.

Now a day computer aided microscopy reveals in terms of microscopical images are directly acquired in the computers and stored in the digital library of the computer. Here images can be refined for the different purposes like comparison. All over observation suggest that this mechanism is very tough to establish. Computers can be act as storage device for the microscope. We can think alternative to organize microscopical data in the computers and that is library created with description of microscopic characters.

2.1. Current Status of Microscopic Technique for Identification of Herbal Drugs:

When will Pharmacognosists (PCs) to talk to personal computers (PCs)?

Pharmacognosy has also seen a lots of ups and downs driven by the growth of pharmaceutical industry, changing consumer habits and desires, and recent consumer movement toward the use of natural materials. There is some resurgence in herbals, which has brought pharmacognosy again to attention. However, the aspect of identification and study of pharmacognostic properties of raw herbs appears to have not got the attention it deserves. The methodology of teaching students the first few chapters of PC in making them identify morphologic and microscopic characteristics of herbals has not changed much. Though chemotaxonomy, genotaxonomy and some amount of molecular taxonomy have evolved, the routine aspects of methodology used to confirm botanical identity of plants and their parts have remained more or less the same²¹.

While the industrial usage of plants and their parts, either as such or after processing, has gone up (with their use in dietary supplements, additives to foods, as ingredients in cosmetics as well as to formulate traditional medicines on industrial scale), the technology for confirming their botanical identity has not seen much changes.

Concurrently, the fear of substitution and adulteration of raw herbs is also on the rise. Availability of qualified and experienced botanists and taxonomists, who have expertise in identifying the 'consignment of plant parts that are coming to an industry, is on the decline as this career is all not very encouraging.

Performing macroscopy and microscopy as a routine test and documenting their results properly to meet GMP requirements, especially when large consignments of plant parts are received by industry, is a difficult, time consuming job, and one does not see good documentation leaving doubts about such quality control tests being done.

Ayurvedic Vaidyas (a qualified Ayurvedic practitioner), who are endowed with qualities to look at, taste and evaluate to confirm the herbs they are using to make the medicines, but it is becoming difficult to see such Vaidyas in large numbers. To add to this, controversial botany is another aspect. The movement of the industry to use pre-powdered plant parts as a means of reducing processing in the factories and value addition to collectors and cultivators of plant supplies demands identity testing and confirmation from differing substrates of the materials. Newer techniques and technologies need to be evaluated by pharmacognosists (PCs) for this purpose.

All of this will have an impact on the safety, quality and efficacy of the finished, product containing a plant or its parts or processed plant materials. Proper identification of the plant used is the first step to build quality and safety.

Some of the pharmacopoeias have introduced mandatory thin layer chromatography (TLC) testing of the plant material under examination, for their TLC profiles, in comparison with the TLC profile of a botanical reference. Substance issued by the pharmacopeia commissions. This has improved the identity testing, but cannot replace the botanical identity testing. It is also true that one meets Pharmacognosy experts who can reel off individual, distinct, and differentiating characteristics that can confirm identities and also differentiate the substituents and adulterants.

Very few books are written on them and even if they are available they suffer from the lack of length (coverage of large number of plants), breadth/depth (coverage of the plant with substituents and adulterants characteristics). An analysis of the area reveals that there are a number of parameters that can be used in the identification process such as starch grains and their structures, lignified parenchyma, cork and their structures, calcium oxalate crystals, stomata and differences and stomata index, the most famous trichomes, vessels and tracheids, stone cells type, varieties of fibers, pollen grains and others. It is the need of the hour that PCs should consider using today's technology to aid in proper identification of plant parts, even in their powdered forms. The use of a computer-based system that can identify, count and statistically analyze the number of chromatoids and those chromosomes that

have been "aberrated" when one performs a "chromosomal aberration test" as per OECD guidelines is well known. If it can do this, it is not impossible to adopt a similar approach to the powder identification of plant-based materials. **It is not easily understood why Indian Pharmacognosists have not looked at adopting technology and come up with databank and a computer based approach for providing technology that can be as easy as "someone taking an IR of a chemical and the PC (personal computer) searching its databases and in less than a minute gives the identity"**

2.2. Plan of work:

The Present Project Deals with the Following Study:

1. Collection of fresh plant materials from field.
2. To authenticate the plant materials by the Government Herbarium Authority.
3. Preparation of different churnas from different parts of plant according to the Ayurvedic formulary of India.
4. To study morphology and microscopy of prepared churnas.
5. Observation of macroscopical as well as microscopical parameters and prepare data bank for the same.
6. Deep Discussion with botanists, taxonomist and software experts to develop identification system for the plant material based on computer by using prepared data bank.
7. Development of computer aided techniques for the identification of herbal drugs based on morphological and microscopical parameters.

2.3. The Promising Aspects of the Project:

Our aim is to develop computer aided identification system for the common Indian herbal drugs based on morphology and microscopy so that common man can explore benefits of common Indian plants without hesitations. Ultimate our aim is

1. To generate databank for the macroscopical and microscopical parameters of common Indian herbs for the quick identification.
2. Study will be helpful to the Government authorities like FDCA, small as well as large scale herbal industries, Academician and Students.
3. This study can inspire common people to use common Indian herbs with authenticity.
4. Study will be useful to the Ayurvedic Practitioners for the identification of genuine herbs during the practice.
5. To generate morphological and microscopical databank to know the identity, purity and quality of the common Indian herbs.
6. To develop computer aided techniques for the quick identification of medicinal plants and plant materials.

There are two major concepts created here for the identification of herbal drugs plant materials by using the computer aided techniques:

3.1 Image Compare:

This is very simple computer program to compare the images of herbal drugs. Image may be morphological image or microscopical image. Minimum parameters are defined to compare the images in the program to minimize complexity in the program. Here any image consists of definite pixels, so no. of pixel is one of the criteria.

Image library implements a system by which 2 image files can be compared, using a variety of comparison methods. In general, those methods operate on the images on a pixel-by-pixel basis and reporting statistics or data based on color value comparisons.

Image::Compare makes heavy use of the Imager module, although it's not necessary to know anything about it in order to make use of the compare functions. However, Imager must be installed in order to use this module, and file import types will be limited to those supported by your installed Imager library.

In general, to do a comparison, you need to provide 3 pieces of information: the first image to compare, the second image to compare, and a comparison method. Some comparison methods also require extra arguments -- in some cases a Boolean value, some a number and some require a hash reference with structured data. See the documentation below for information on how to use each comparison method.

Image::Compare provides 3 different ways to invoke its comparison functionality. you can construct an Image::Compare object and call set_* methods on it to give it the information, then call compare () on that object, or you can construct the Image::Compare with all of the appropriate data right off the bat, or you can simply call compare () with all of the information. In this third case, you can call compare () as a class method, or you can simply invoke the method directly from

the `Image::Compare` namespace. If you'd like, you can also pass the word `compare` to the module when you use it and the method will be imported to your local namespace.

3.1.1 Methods for Comparison:

3.1.1.1 Exact Method: The exact method simply returns true if every single pixel of one image is exactly the same as every corresponding pixel in the other image, or false otherwise. It takes no arguments.

```
$cmp->set_method(  
  method => &Image::Compare::EXACT,  
);
```

3.1.1.2 Threshold Method: The THRESHOLD method returns true if no pixel difference between the two images exceeds a certain threshold, and false if even one does. Note that differences are measured in a sum of squares fashion (vector distance), so the maximum difference is $255 * \sqrt{3}$, or roughly 441.7. Its argument is the difference threshold. (Note: EXACT is the same as THRESHOLD with an argument of 0.)

```
$cmp->set_method(  
  method => &Image::Compare::THRESHOLD,  
  args   => 50,  
);
```

3.1.1.3 Threshold_Count: The THRESHOLD_COUNT method works similarly to the THRESHOLD method, but instead of immediately returning a false value as soon as it finds a pixel pair whose difference exceeds the threshold, it simply counts the number of pixels pairs that exceed that threshold in the image pair. It returns that count.

```
$cmp->set_method(  
  method => &Image::Compare::THRESHOLD_COUNT,  
  args   => 50,  
);
```

3.1.1.4 Average_Threshold: The `AVG_THRESHOLD` method returns true if the average difference over all pixel pairings between the two images is under a given threshold value. Two different average types are available: `MEDIAN` and `MEAN`. Its argument is a hash reference, contains keys "type", indicating the average type, and "value", indicating the threshold value.

```
$cmp->set_method(  
  method => &Image::Compare::AVG_THRESHOLD,  
  args   => {  
    type  => &Image::Compare::MEAN,  
    value => 35,  
  },  
);
```

3.1.1.5 Image: The `Image` method returns an `Imager` object of the same dimensions as your input images, with each pixel colored to represent the pixel color difference between the corresponding pixels in the input. Its only argument is a boolean. If the argument is omitted or false, then the output image will be grayscale, with black meaning no change and white meaning maximum change. If the argument is a true value, then the output will be in color, ramping from pure red at 0 change to pure green at 50% of maximum change, and then to pure blue at maximum change.

```
$cmp->set_method(  
  method => &Image::Compare::IMG,  
  args   => 1, # Output in color  
);
```

3.2 Herbal Drugs Identification System:

This is web based computer program prepared by using JavaScript as a programming language. This is database type web based computer program where characteristics of herbal drugs are stored permanently in the database of the program. Database is created in MySQL so we can manage huge database. As the world's most popular open source database, MySQL is used by a wide range of organizations to manage their data.

MySQL is the most popular Open Source Relational SQL database management system. MySQL is one of the best RDBMS being used for developing web based software applications.

3.2.1 JavaScript:⁶⁹⁻⁷²

JavaScript is a prototype-based scripting language that is dynamic weakly typed and has first-class functions. It is a multi-paradigm language. It also supports object oriented, imperative, and functional, programming styles.

JavaScript was formalized in the ECMAScript language standard and is primarily used in the form of client-side JavaScript, implemented as part of a Web browser in order to provide enhanced user interfaces and dynamic websites. This enables programmatic access to computational objects within a host environment. JavaScript's use in applications outside Web pages for example in PDF documents, site-specific browsers, and desktop widgets is also significant. Newer and faster JavaScript VMs and frameworks built upon them (notably Node.js) have also increased the popularity of JavaScript for server-side web applications. JavaScript uses syntax influenced by that of C. JavaScript copies many names and naming conventions from Java, but the two languages are otherwise unrelated and have very different semantics. The key design principles within JavaScript are taken from the self and Scheme programming languages. JavaScript is the most popular scripting language on the internet, and works in all major browsers, such as Internet Explorer, Firefox, Chrome, Opera, and Safari. Java and JavaScript are not same. Java and JavaScript are two completely different languages in both concept and design! Java (developed by Sun

Microsystems) is a powerful and much more complex programming language in the same category as C and C++.

Several interesting facts about JavaScript are as follow:

- JavaScript was designed to add interactivity to HTML pages
- JavaScript is a scripting language
- A scripting language is a lightweight programming language
- JavaScript is usually embedded directly into HTML pages
- JavaScript is an interpreted language (means that scripts execute without preliminary compilation)
- Everyone can use JavaScript without purchasing a license
- **JavaScript gives HTML designers a programming tool** - HTML authors are normally not programmers, but JavaScript is a scripting language with a very simple syntax! Almost anyone can put small "snippets" of code into their HTML pages
- **JavaScript can react to events** - A JavaScript can be set to execute when something happens, like when a page has finished loading or when a user clicks on an HTML element
- **JavaScript can read and write HTML elements** - A JavaScript can read and change the content of an HTML element
- **JavaScript can be used to validate data** - A JavaScript can be used to validate form data before it is submitted to a server. This saves the server from extra processing
- **JavaScript can be used to detect the visitor's browser** - A JavaScript can be used to detect the visitor's browser, and - depending on the browser - load another page specifically designed for that browser
- **JavaScript can be used to create cookies** - A JavaScript can be used to store and retrieve information on the visitor's computer

JavaScript = ECMAScript

JavaScript is an implementation of the ECMAScript language standard. ECMA-262 is the official JavaScript standard. JavaScript was invented by

Brendan Eich at Netscape (with Navigator 2.0), and has appeared in all browsers since 1996. The official standardization was adopted by the ECMA organization (an industry standardization association) in 1997. The ECMA standard (called ECMAScript-262) was approved as an international ISO (ISO/IEC 16262) standard in 1998. The development is still in progress. This is the script being used by the different programmer for the development of web based program.

3.2.2 Database:⁷³⁻⁷⁴

A database is a separate application that stores a collection of data. Each database has one or more distinct APIs for creating, accessing, managing, searching, and replicating the data it holds. Other kinds of data stores can be used, such as files on the file system or large hash tables in memory but data fetching and writing would not be so fast and easy with those types of systems. So now days we use relational database management systems (RDBMS) to store and manager huge volume of data. This is called relational database because all the data is stored into different tables and relations are established using primary keys or other keys known as foreign keys. A Relational Database Management System (RDBMS) is software that:

- Enables you to implement a database with tables, columns, and indexes.
- Guarantees the Referential Integrity between rows of various tables.
- Updates the indexes automatically.
- Interprets an SQL query and combines information from various tables.

RDBMS Terminology:

Before we proceed to explain MySQL database system, let's revise few definitions related to database.

- Database: A database is a collection of tables, with related data.
- Table: A table is a matrix with data. A table in a database looks like a simple spreadsheet.
- Column: One column (data element) contains data of one and the same kind, for example the column postcode.

- Row: A row (= tuple, entry or record) is a group of related data, for example the data of one subscription.
- Redundancy: Storing data twice, redundantly to make the system faster.
- Primary Key: A primary key is unique. A key value can not occur twice in one table. With a key you can find at most one row.
- Foreign Key: A foreign key is the linking pin between two tables.
- Compound Key: A compound key (composite key) is a key that consists of multiple columns, because one column is not sufficiently unique.
- Index: An index in a database resembles an index at the back of a book.
- Referential Integrity: Referential Integrity makes sure that a foreign key value always points to an existing row.

MySQL is a fast, easy-to-use RDBMS used being used for many small and big businesses. MySQL is developed, marketed, and supported by MySQL AB, which is a Swedish company. MySQL is becoming so popular because of many good reasons.

- MySQL is released under an open-source license. So you have nothing to pay to use it.
- MySQL is a very powerful program in its own right. It handles a large subset of the functionality of the most expensive and powerful database packages.
- MySQL uses a standard form of the well-known SQL data language.
- MySQL works on many operating systems and with many languages including PHP, PERL, C, C++, JAVA etc.
- MySQL works very quickly and works well even with large data sets.
- MySQL is very friendly to PHP, the most appreciated language for web development.
- MySQL supports large databases, up to 50 million rows or more in a table. The default file size limit for a table is 4GB, but you can increase

this (if your operating system can handle it) to a theoretical limit of 8 million terabytes (TB).

- MySQL is customizable. The open source GPL license allows programmers to modify the program.

3.2.3 Apache Tomcat:⁷⁵⁻⁷⁶ Apache Tomcat (or Jakarta Tomcat or simply Tomcat) is an open source web server and servlet container developed by the Apache Software Foundation (ASF). Tomcat implements the Java Servlet and the JavaServer Pages (JSP) specifications from Oracle Corporation, and provides a "pure Java" HTTP web server environment for Java code to run.

Tomcat should not be confused with the Apache web server, which is a C implementation of an HTTP web server; these two web servers are not bundled together, although they are frequently used together as part of a server application stack. Apache Tomcat includes tools for configuration and management, but can also be configured by editing XML configuration files.

Tomcat is a Java Servlet container and web server from the Jakarta project of the Apache software foundation. A web server dishes out web pages in response to requests from a user sitting at a web browser. But web servers are not limited to serving up static HTML pages; they can also run programs in response to user requests and return the dynamic results to the user's browser. Tomcat is very good at this because it provides both Java servlet and JavaServerPages (JSP) technologies (in addition to traditional static pages and external CGI programming). The result is that Tomcat is good choice for use as a web server for many applications; also if we want a free servlet and JSP engine. It can be used standalone or used behind traditional web servers such as Apache httpd, with the traditional server serving static pages and Tomcat serving dynamic servlet and JSP requests.

These are some of the key tomcat directories, all relative to \$TOMCAT_HOME (Tomcat home dir sometimes also called \$CATALINA_HOME):

/bin - Startup, shutdown, and other scripts. The *.sh files (for Unix systems) are functional duplicates of the *.bat files (for Windows systems).

/conf - Configuration files and related DTDs. The most important file in here is server.xml. It is the main configuration file for the container.

/logs - Log files are here by default.

/webapps - This is where your webapps go.

All of the information in the configuration files is read at startup, meaning that any change to the files necessitates a restart of the container.

3.2.4 Wamp Server:⁷⁷ Wamp server is a Windows' web development environment. It allows you to create web applications with Windows, Apache, MySQL, PHP/Perl/Python. WAMP is a form of mini-server that can run on almost any Windows Operating System. WAMP includes Apache 2, PHP 5 (SMTP ports are disabled), and MySQL (phpMyAdmin and SQLitemanager are installed to manage your databases) preinstalled. An icon on the taskbar tray displays the status of WAMP, if; a) WAMP is running but no services are opened (the icon will appear red), b) WAMP is running and one service is opened (the icon will appear yellow) or c) WAMP is running with all services opened (the icon will appear white). Apache and MySQL are considered to be services (they can be disabled by left-clicking on the taskbar icon, guiding us cursor over the service we wish to disable and selecting "Stop Service"). The files/web pages that are hosted on WAMP server can be accessed by typing <http://localhost> or <http://127.0.0.1/> in the address bar of web browser. WAMP must be running in order to access either of the above addresses.

3.3 Basic Parameters of Software:

Following parameters are considered for the preparation of computerized Databank of the characters of herbal drugs and development of Software that is **Herbal Drug Identification System**. In this system drop down menu is created in the computer based program by using java language. Following columns are used to create database in the sql server. Java is the programming language used to create the computer based program. This database can be explored in the internet browser. To create local host wamp server and Tomcat platforms are used.

3.3.1 Type of Specimen: Powder

3.3.1.1 Morphological Parameters:

1. **State:** State of a substance is solid, semisolid or liquid. Here we are concentrating on powder vegetable drugs, so in case of microscopical characters only solid state is given.
 - a. Solid
2. **Texture:** Generally texture of the powder is fine, if churna is passed through 80 # sieve. When any fibrous drug is present like liquorice powder, then state is coarse. Intermediate state is semicoarse.
 - a. Fine
 - b. Semi coarse
 - c. Coarse
3. **Colour:** Color is very important morphological character for the identification of herbal drug. Generally green color indicates leaf and reddish color indicate bark while creamish buff colour indicates presence of root in the powder drugs.
 - a. All Basic Colors
 - b. Others and allow input manually
4. **Odour:** It is very important organoleptic character for identification of herbal drug. It is also called as fragrance/aroma/smell/typical odour. Volatile oil containing drugs are easily identified by its aroma.
 - a. Aromatic

- b. Nauseating
- c. Characteristic
- d. Other and allow input manually

5. Taste: It is another organoleptic character that can be sensed by our tongue. Kalmegh, chirata, quassia, cinchona, etc. are very bitter in taste. Ginger, garlic, capsicum, etc. are pungent in taste. Drugs like stevia and liquorice are sweet in taste. Drugs containing tannins are astringent in taste.

- a. Bitter
- b. Pungent
- c. Sweet
- d. Acrid
- e. Astringent
- f. Other and allow input manually

3.3.1.2 Microscopical Parameters:

1. Trichomes: Trichomes are very important microscopical character for the identification of leaf drugs as well as stem, root, seed and fruits also. For example Kantkari is identified by stellate trichome. *Digitalis purpurea* is identified by multicellular trichomes, glandular trichomes and also collapsed cell trichomes. In case of seed drug in nux vomica lignified multicellular trichomes are present, which is unique characteristic of Nux vomica seed.

- a. None
- b. Simple Covering Unicellular
- c. Simple Covering Multicellular
- d. Glandular Trichomes with Unicellular head and unicellular stalk
- e. Glandular Trichomes with Unicellular head and multicellular stalk
- f. Glandular Trichomes with multicellular head and unicellular stalk
- g. Glandular Trichomes with multicellular head and multicellular stalk

- h. Clavate
- i. Club Shaped
- j. Collapsed Cell
- k. Other and allow input manually
- l. Trichomes Present (Search)

2. Stomata: Stomata present in the outermost layer (epidermis) of any part of plant but more found in leaf drug. It is absent in xerophytic plants like pink Karen (sunken stomata), like Bhoyringni (In upper epidermis it is absent). Generally four types of stomata are found in nature.

- a. None
- b. Paracytic (Rubiaceous)
- c. Dicytic (Caryophyllous)
- d. Anisocytic
- e. Anisocytic
- f. Sunken
- g. Other and allow input manually
- h. Stomata Present (Search)

3. Types of epidermal cells: Epidermal cells can be observed in powdered drug in surface view, but not in transverse view. Epidermal cell wall is different in different drugs like in senna anticlinal cell wall while in digitalis wavy wall is present.

- a. Straight wall
- b. Wavy wall
- c. Beaded wall
- d. Anticlinal cell wall
- e. Other and allow input manually

4. Types of non-living cell-content: Non living cells contents are the form of calcium oxalate or calcium carbonate (cystolith). They are also called as ergastic substances of the plant cell. They are typical

character for characterization of entire as well as powder drugs. They can be microscopically observed by mounting unstain slide. Unstain slide can be prepared by clearing the powder in test tube with chloralhydrate and then take few fragments in middle of the slide and add mounting reagent i.e. lactophenol and put cover-slip. For example, in quassia wood, prisms of calcium oxalate are only distinguishing character and in shatavari root raphides are present.

- a. None
- b. Crystals of Calcium Oxalate
- c. Microsphenoidal Crystals of Calcium Oxalate
- d. Rosette of Calcium Oxalate
- e. Micro Rosette of Calcium Oxalate
- f. Raphides of Calcium Oxalate
- g. Cystolith of Calcium Carbonate
- h. Aleurone grains
- i. Fixed oil globules
- j. Pollen grains
- k. Other and input manually
- l. Non-living cell Present (Search)

5. Types of Starch grains: Starch grains are important character for the underground parts of the plant like roots and rhizomes, but they are also present in aerial parts of the plant. They can be observed microscopically by preparing slide with dilute iodine solution (N/10). If starch grains are present, they will stain blue. In powder drug if single starch grains are present then it is called as simple starch grains and if bunch of 2-10 are present they are called as compound starch grains. For example in ginger rhizome powder abundant simple starch grain with accentric hilum is present

- a. None
- b. Simple
- c. Compound
- d. Accentric Hilum

- e. Concentric Hilum
- f. Others and input manually
- g. Starch Grain Present (Search)

6. Quantity of starch grains: In general underground parts of the plants store abundant starch grains while aerial parts have less starch grains.

- a. None
- b. Abundent
- c. Minute / Less

7. Stone cells: Stone cells are typical lignified character of the plant. They give strength to the plant i.e. coriander fruit and cinnamon bark.

- a. Present
- b. Absent

8. Parenchyma: They are very soft tissues. If chlorophyll is present in parenchyma then it is called as chlorenchyma e.g. senna leaf. If free air spaces are present in it then it is called as arenchyma e.g. Vaj rhizome and clove flower bud.

- a. Chlorenchyma
- b. Arenchyma
- c. Simple parenchyma
- d. Others and input manually

9. Collenchyma: They are parenchymatous cells and its intercellular spaces are filled with suberin. They are generally present in leaf and stem of plants. e.g Mentha stem

- a. Input manually

10. Sclerenchyma: They are highly lignified cells either in the form of sclerides or stone cells. They are generally found in the wood and bark drugs.

- a. Sclerides
- b. Stone cells

11. Special characters: Special characters generally not found in the drug, but sometimes they are present in the plants; which put significant identity of that plant.

- a. None
- b. Others and input manually
- c. Special Characters Present (Search)

12. Lignified characters: Lignified characters can be visualized in stained slide only. Stained slide is prepared by clearing the powder in chloral hydrate and then add few drops of phluoroglucinol solution and concentrated hydrochloric acid, allow to stand for one minute; then discard the solution, and mount it by mounting in lactophenol.

- a. None
- b. Phloem fibres
- c. Xylem fibres
- d. Xylem vessels
- e. Tracheid
- f. Others and input manually
- g. Lignified character Present (Search)

13. Non-lignified characters: Many characters non-lignified in nature in few exceptional drugs. For example, in ginger xylem vessels and fibres are non-lignified.

- a. None
- b. Others and input manually
- c. Non-lignified Fibres Present (Search)

14. Types of xylem vessels: Xylem vessels have different types of thickening and it is very important microscopical character for the powdered drugs.

- a. Reticulate
- b. Annular
- c. Spiral
- d. Pitted

e. Border pitted

15. Colouring Matter: In many drugs coloring matter is present and is easily identified by microscope like reddish coloring matter in capsicum fruit powder.

- a. Yellowish
- b. Brownish
- c. Reddish
- d. Others and input manually

3.3.2. Type of Specimen: Plant

3.3.2.1 Morphological Parameters:

1. Leaf: Many leaf drugs are used in ayurvedic formulations. For example, Senna, vasaka, mentha, tulsi, digitalis, brahmi, shankhpushpi etc. Leaf drug can be identified by following morphological parameters.

a. Type: If leaf is not divided into different segments from midrib, then it is called simple leaf e.g. Datura.

- i. Simple
- ii. Compound
- iii. None

b. Texture: Touch to feel for e.g. pili Karen is glabrous (smooth), bhoy ringni is spiny, digitalis is hairy in texture.

- i. Glabrous
- ii. Pubescent
- iii. Spiny
- iv. Leathery
- v. None

c. Colour: Generally leaves are green in colour but exceptions are also present.

- i. All Basic Colors
- ii. Others and allow input manually

d. **Shape:** Shape is distinguishing characters for identification of leaf. For e.g. Brahmi is kidney shaped (reniform) and grasses are acicular shaped.

- i. Acicular
- ii. Linear
- iii. Lanceolate
- iv. Orbicular
- v. Elliptical
- vi. Ovate
- vii. Obovate
- viii. Oblong
- ix. Reniform
- x. Cordate
- xi. Lyrate
- xii. Oblique
- xiii. Sagitate
- xiv. None

e. **Arrangement:** It is called as Phyllotaxy. It is mode of arrangement of leaves on the stem.

- i. Opposite
- ii. Alternate/spiral
- iii. Opposite & decussate
- iv. Whorled
- v. None

f. **Apex:** Apex of the leaf is typical morphological character for each leaf drugs for e.g. vinca is having mucronate apex and kachnar is having emarginate apex.

- i. Acute
- ii. Acuminate/Caudate
- iii. Cuspidate
- iv. Obtuse
- v. Mucronate

- vi. Emarginate
 - vii. None
 - g. **Base:** Base of the leaf may be equal or unequal. For e.g. senna have equal base and datura is having unequal base.
 - i. Equal
 - ii. Unequal
 - iii. None
 - h. **Venation:** Arrangement of veins in leaf is called as venation. If veins are running parallel to midrib then it is a monocot leaf, while if reticulate venation then it is called as dicot leaf.
 - i. Reticulate
 - j. Parallel
 - k. None
 - I. **Margin:** If margin is smooth it is called as entire margin e.g. vinca leaf.
 - i. Entire
 - ii. Crenate
 - iii. Serrate
 - iv. Dentate
 - v. Incised
 - vi. Ciliate
2. **Stem:** Many stem drugs are used in powder form in ayurvedic formulations like mentha, ephedra, shankhpushpi, etc.
- a. **Shape**
 - i. Cylindrical
 - ii. Sub-cylindrical
 - iii. Quadrangular
 - iv. Tortuous
 - v. Others and input manually
 - b. **Colour:** Color is very important morphological character for the identification of herbal drug. Generally green color indicates leaf and reddish color indicate bark while creamish buff colour indicates presence of root in the powder drugs.

- i. All Basic Colors
- ii. Others and allow input manually

c. Texture

- i. Smooth
- ii. Hairy
- iii. Spiny
- iv. Others and allow input manually

3. Bark: Many bark drugs are used in herbal drug industries like cinchona, cinnamon, kurchi, cascara, arjuna, ashoka, quillaia, wild cherry bark, etc.

a. **Shape:** During drying of bark, it acquires a characteristic shape and it is a diagnostic character for identification of bark drug. For e.g., kurchi is recurved bark and arjuna is flat bark.

- i. Flat
- ii. Curved
- iii. Recurved
- iv. Chanelled
- v. Quill
- vi. Double quill
- vii. Compound quill
- viii. None

b. **Colour:** Most of the bark is reddish brown in color but there are many exceptions like kurchi is creamish in color.

- i. All Basic Colors
- ii. Others and allow input manually

c. Outer surface: Characters present on outer surface of bark is identical morphological parameter.

- i. Smooth
- ii. Lenticels
- iii. Cracks and Fissures
- iv. Corky warts
- v. Furrows and Wrinkles
- vi. Exfoliated cork

- vii. Rhytidoma
 - viii. Epiphytes
 - ix. None
- d. **Inner surface:** When parallel longitudinal ridges are formed during drying, it is called striations. When parallel transverse wrinkles are formed during drying, it is called corrugations.
- i. Striations
 - ii. Corrugation
 - iii. None
- e. **Fracture surface:** When we break the bark, the exposed surface is called as fractured surface.
- i. Short
 - ii. Granular
 - iii. Splintery
 - iv. Fibrous
 - v. Laminated
4. **Wood:** Many wood drugs are used in different formulations like sandalwood, quassia wood, etc.
- a. **Type**
 - i. Sapwood
 - ii. Heartwood
 - iii. None
 - b. **Colour**
 - i. All Basic Colors
 - ii. Others and allow input manually
5. **Flower:** Many flower drugs are used in ayurveda like saffron, clove, palash, etc. They are morphologically identified by following parameters
- a. **Type of inflorescence:** Inflorescence is the arrangement of flowers on floral axis or the branch system of the floral region bearing a group of flowers.

- i. Recemose: Lower flowers are older while upper flowers are younger.
 - ii. Cymose: development of flower at the apex.
 - iii. Others and allow input manually
 - iv. None
- b. Type of flowers based on floral whorls:** Floral whorls are calyx, corolla, androecium and gynoecium
- i. Complete
 - ii. Incomplete
 - iii. None
- c. Type of flowers based on symmetry**
- i. Actinomorphic flower/regular
 - ii. Zygomorphic/Irregular
 - iii. None
- d. Type of flowers based on reproductive organs**
- i. Unisexual
 - ii. Bisexual (Hermaphrodite)
 - iii. None
- e. Type of flowers based on position of thalamus**
- i. Hypogynous/ Superior
 - ii. Perigynous
 - iii. Epigynous
 - iv. None
- f. Type of flowers based on no. of floral parts**
- i. Dimerous
 - ii. Trimerous
 - iii. Tetramerous
 - iv. Pentamerous
 - v. Polymerous
- g. Colour of sepals (calyx)**
- i. All Basic Colors
 - ii. Others and allow input manually
- h. Colour of petals (corolla)**

- i. All Basic Colors
- ii. Others and allow input manually
- i. Type of sepals**
 - i. Polysepalous : free sepals
 - ii. Gamosepalous: united sepals
 - iii. None
- j. Type of petals**
 - i. Polypetalous: free petals
 - ii. Gamopetalous: united petals

3.2.2.2 Microscopical Parameters:

- 1. Trichomes:** Trichomes are very important microscopical character for the identification of leaf drugs as well as stem, root, seed and fruits also. For example Kantkari is identified by stellate trichome. *Digitalis purpurea* is identified by multicellular trichomes, glandular trichomes and also collapsed cell trichomes. In case of seed drug in nux vomica lignified multicellular trichomes are present, which is unique characteristic of Nux vomica seed.
 - a. None
 - b. Simple Covering Unicellular
 - c. Simple Covering Multicellular
 - d. Glandular Trichomes with Unicellular head and unicellular stack
 - e. Glandular Trichomes with Unicellular head and multicellular stack
 - f. Glandular Trichomes with multicellular head and unicellular stack
 - g. Glandular Trichomes with multicellular head and multicellular stack
 - h. Clavate
 - i. Club Shaped
 - j. Collapsed Cell
 - k. Other and allow input manually
 - l. Tirchomes Present (Search)

2. Stomata: Stomata present in the outermost layer (epidermis) of any part of plant but more found in leaf drug. It is absent in xerophytic plants like pink Karen (sunken stomata), like Bhoiringni (In upper epidermis it is absent). Generally four types of stomata are found in nature.

- a. None
- b. Paracytic (Rubiaceous)
- c. Dicytic (Caryophyllous)
- d. Anisocytic
- e. Anisocytic
- f. Actinocytic
- g. Other and allow input manually
- h. Stomata Present (Search)

3. Types of non-living cell-content: Non living cells contents are the form of calcium oxalate or calcium carbonate (cystolith). They are also called as ergastic substances of the plant cell. They are typical character for characterization of entire as well as powder drugs. They can be microscopically observed by mounting unstain slide. Unstain slide can be prepared by clearing the powder in test tube with chloralhydrate and then take few fragments in middle of the slide and add mounting reagent i.e. lactophenol and put cover-slip. For example, in quassia wood, prisms of calcium oxalate are only distinguishing character and in shatavari root raphides are present.

- a. None
- b. Crystals of Calcium Oxalate
- c. Microsphenoidal Crystals of Calcium Oxalate
- d. Rosette of Calcium Oxalate
- e. Micro Rosette of Calcium Oxalate
- f. Raphides of Calcium Oxalate
- g. Systolith of Calcium Carbonate
- h. Other and input manually
- i. Non-living cell Present (Search)

- 4. Types of Starch grains:** Starch grains are important character for the underground parts of the plant like roots and rhizomes, but they are also present in aerial parts of the plant. They can be observed microscopically by preparing slide with dilute iodine solution (N/10). If starch grains are present, they will stain blue. In powder drug if single starch grains are present then it is called as simple starch grains and if bunch of 2-10 are present they are called as compound starch grains. For example in ginger rhizome powder abundant simple starch grain with eccentric hilum is present
- None
 - Accentric Hilum
 - Concentric Hilum
 - Others and input manually
 - Starch Grain Present (Search)
- 5. Quantity of starch grains:** In general underground parts of the plants store abundant starch grains while aerial parts have less starch grains.
- None
 - Abundent
 - Minute / Less
- 6. Stone cells:** Stone cells are typical lignified character of the plant. They give strength to the plant i.e. coriander fruit and cinnamon bark.
- Present
 - Absent
- 7. Parenchyma:** They are very soft tissues. If chlorophyll is present in parenchyma then it is called as chlorenchyma e.g. senna leaf. If free air spaces are present in it then it is called as arenchyma e.g. Vaj rhizome and clove flower bud.
- Present
 - Absent
 - Other and allow input manually
 - none

- 8. Collenchyma:** They are parenchymatous cells and its intercellular spaces are filled with suberin. They are generally present in leaf and stem of plants. e.g Mentha stem
- Present
 - Absent
 - Other and allow input manually
 - none
- 9. Sclerenchyma:** They are highly lignified cells either in the form of sclerides or stone cells. They are generally found in the wood and bark drugs.
- Present
 - Absent
 - Other and allow input manually
 - none
- 10.Special characters:** Special characters generally not found in the drug, but sometimes they are present in the plants; which put significant identity of that plant.
- None
 - Others and input manually
 - Special Characters Present (Search)
- 11.Lignified fibres:** Lignified characters can be visualized in stained slide only. Stained slide is prepared by clearing the powder in chloral hydrate and then add few drops of phluoroglucinol solution and concentrated hydrochloric acid, allow to stand for one minute; then discard the solution, and mount it by mounting in lactophenol.
- None
 - Phloem fibres
 - Xylem fibres
 - Others and input manually
 - Lignified Fibres Present (Search)

12. Non-lignified fibres: Many characters non-lignified in nature in few exceptional drugs. For example, in ginger xylem vessels and fibres are non-lignified.

- a. None
- b. Others and input manually
- c. Non-lignified Fibres Present (Search)

4.1 Image Compare:

This is very simple computer program to compare the images of herbal drugs. Image may be morphological image or microscopical image. Minimum parameters are defined to compare the images in the program to minimize complexity in the program. Here an image consists of definite pixels, so no. of pixel is one of the criteria.

Image library implements a system by which 2 image files can be compared, using a variety of comparison methods. In general, those methods operate on the images on a pixel-by-pixel basis and reporting statistics or data based on color value comparisons.

In general, to do a comparison, we need to provide 2 pieces of information: the first image to compare, and the second image to compare.

The exact method simply returns true if every single pixel of one image is exactly the same as every corresponding pixel in the other image, or false otherwise. It takes no arguments.

Here first of all start the program “image compare”

Window of image compare will be opened as shown in the Fig. 8 then click on the left big box on the window. New browse window opens that gives facility to select image file from the location. After selecting image file click on the right side box again browse windows opens, here select another image file which is to be compared with image of left side box. Then click on compare image button, exact comparison will be carried out on the basis of pixels presents in both the images. After finishing comparison between two similar images program will give message “Match Successful”. If images are not similar then Program will display the message “Image do not match”. (Fig. 8 to 13)

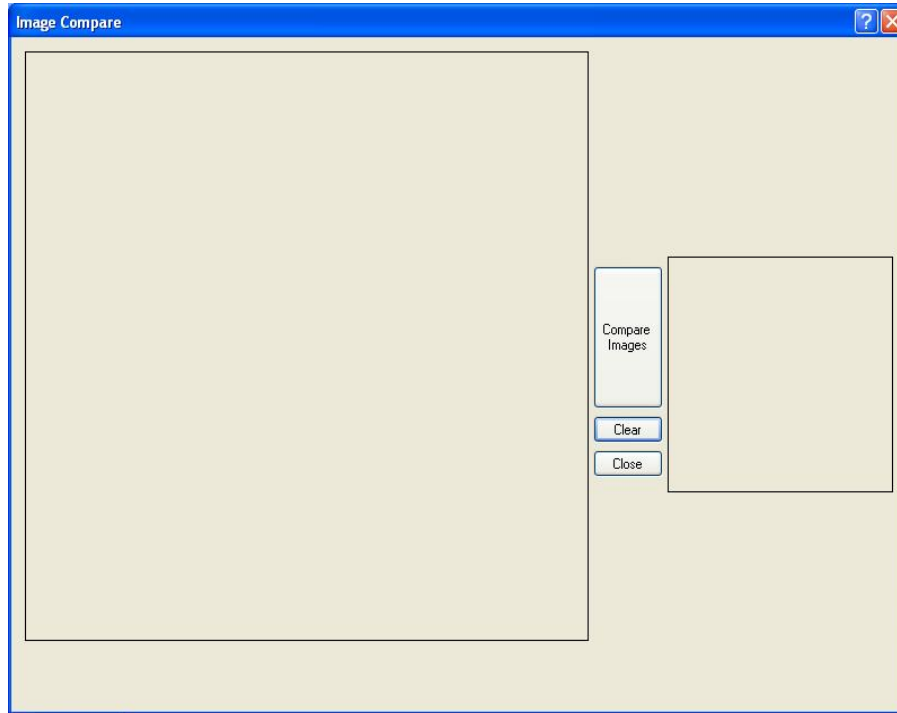


Fig. 8 Preview of Image Compare Program

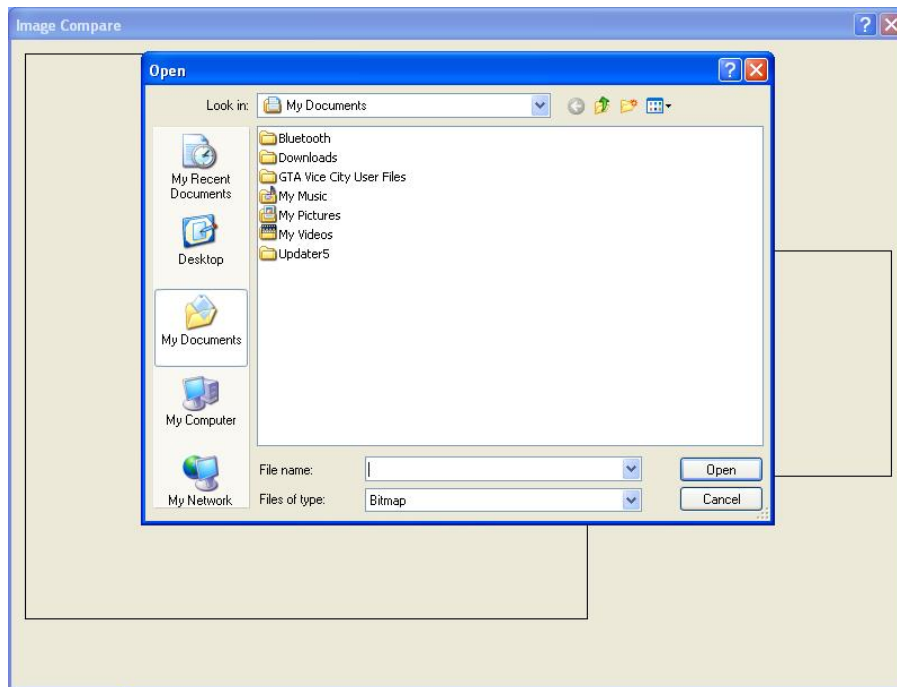


Fig. 9 Preview of Image Compare Program with Browse Window

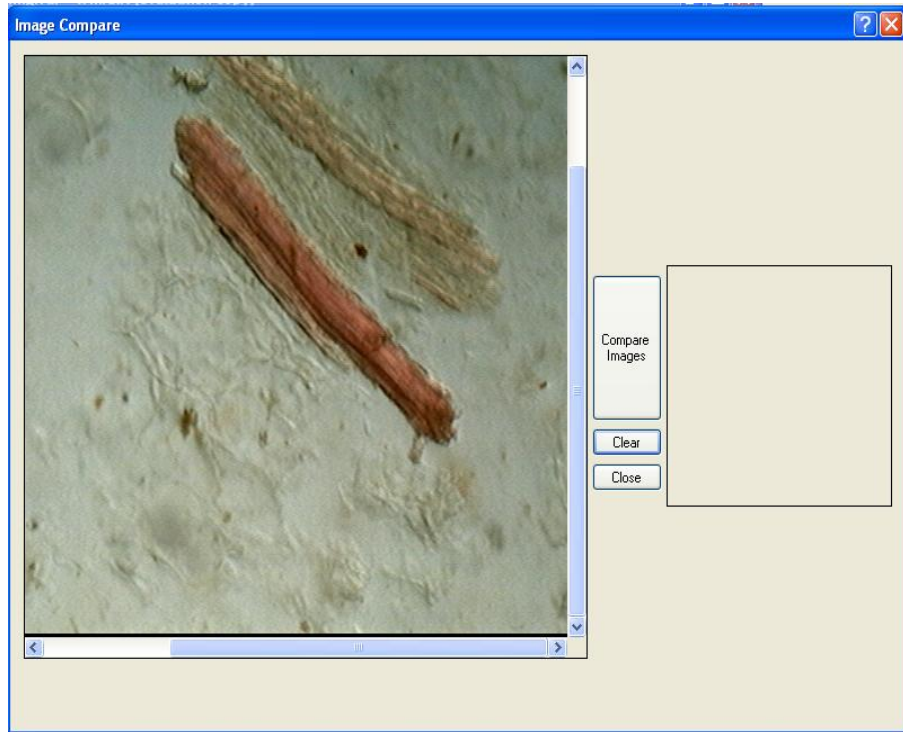


Fig.10 Preview of Image Compare Program with Selected Image

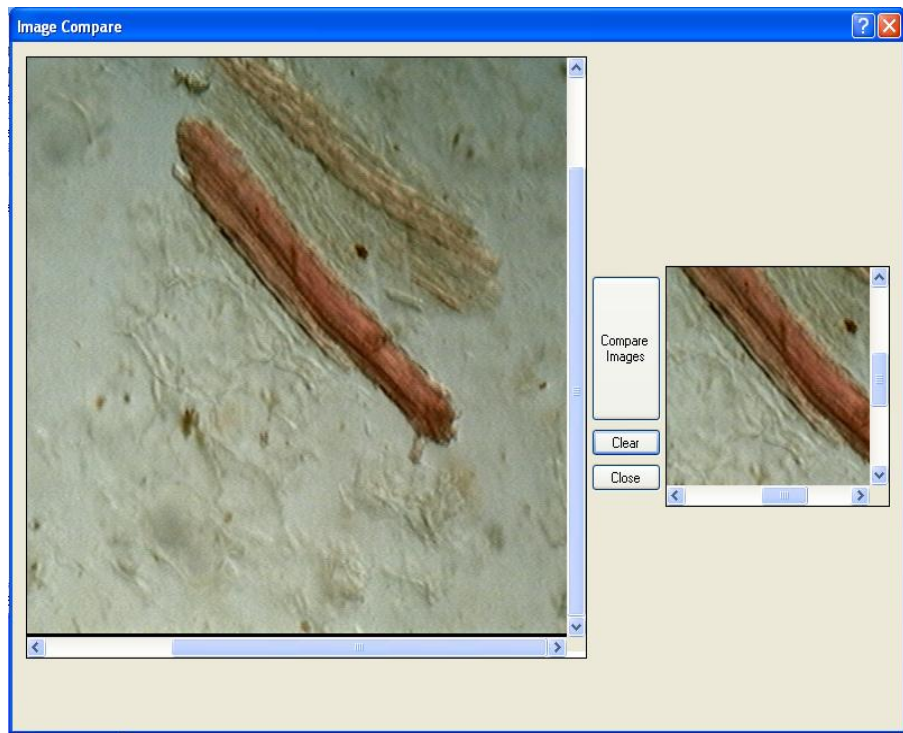


Fig.11 Preview of Image Compare Program with comparing Images

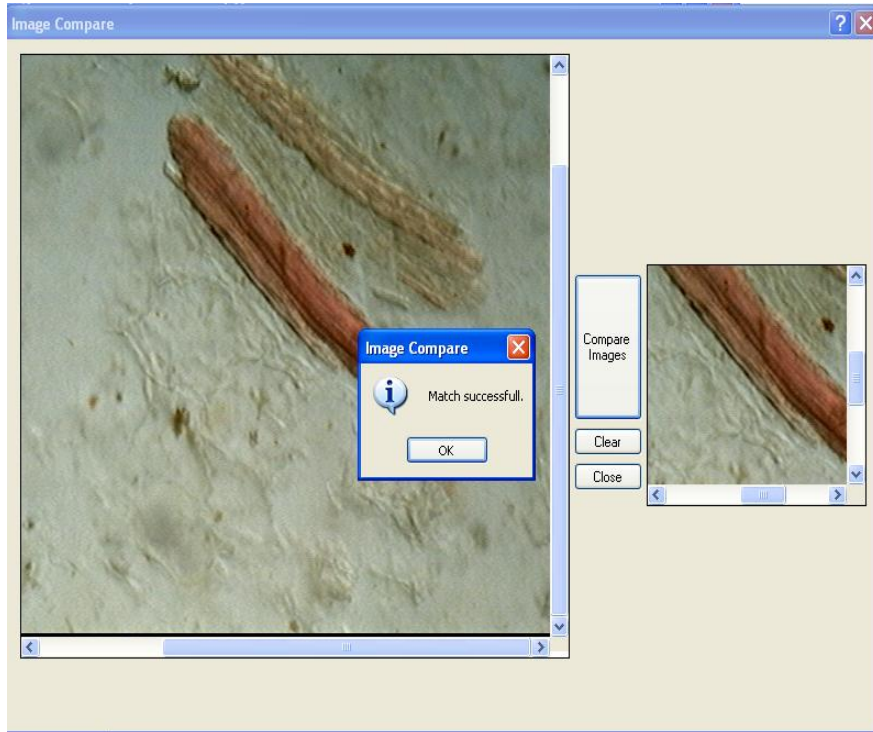


Fig.12 Preview of Image Compare Program with Similar Image

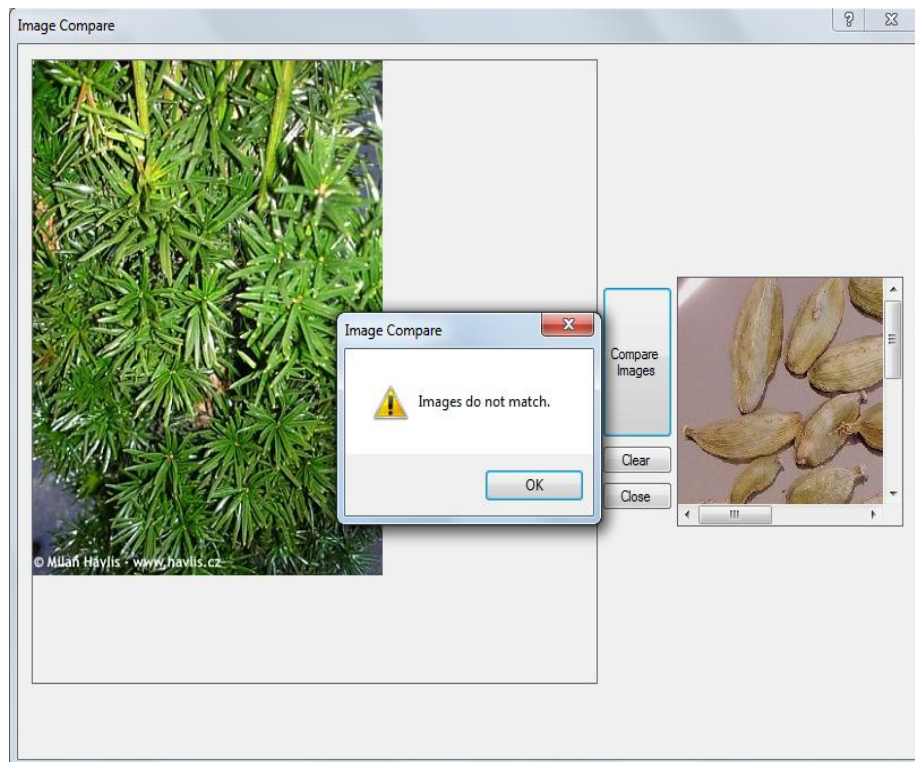


Fig.13 Preview of Image Compare Program with not similar Images

4.2 Herbal Drugs Identification System:

Here efforts made to develop handy tools for the researcher and common man to identify common unknown medicinal plants in the minutes. This is web based computer program prepared by using JavaScript as a programming language. This is database type web based computer program where characteristics of herbal drugs are stored permanently in the database of the program. Database is created in mySQL so we can manage huge database.

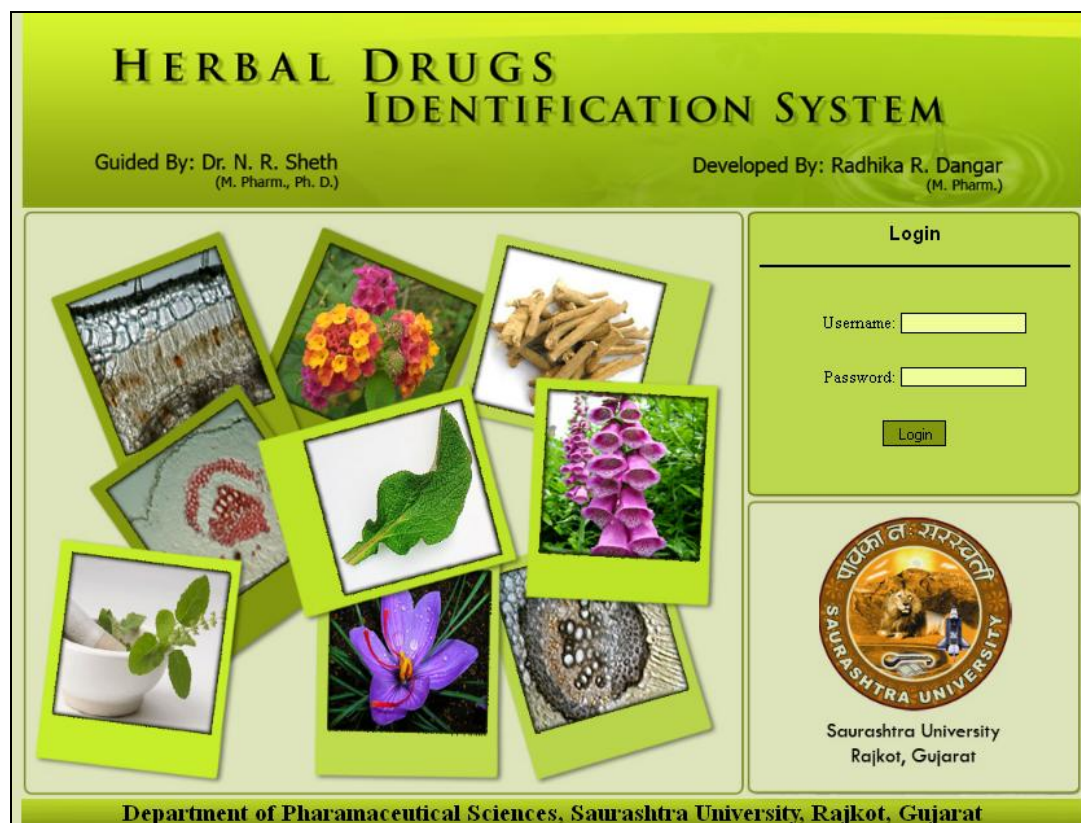
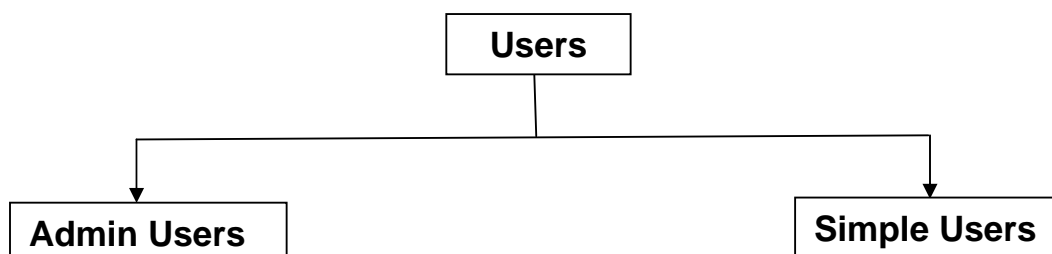


Fig.14 Home Page of Herbal Drugs Identification System

Here computer program having some features; on the home page login window is displayed, because login is very important to explore the features of computer program. Users are two types



4.2.1 Admin Users: Admin users are program administrators, they can manage total program. Default user name and password for the administrative privileges in the software are as follows:

User Name: admin

Password: admin123

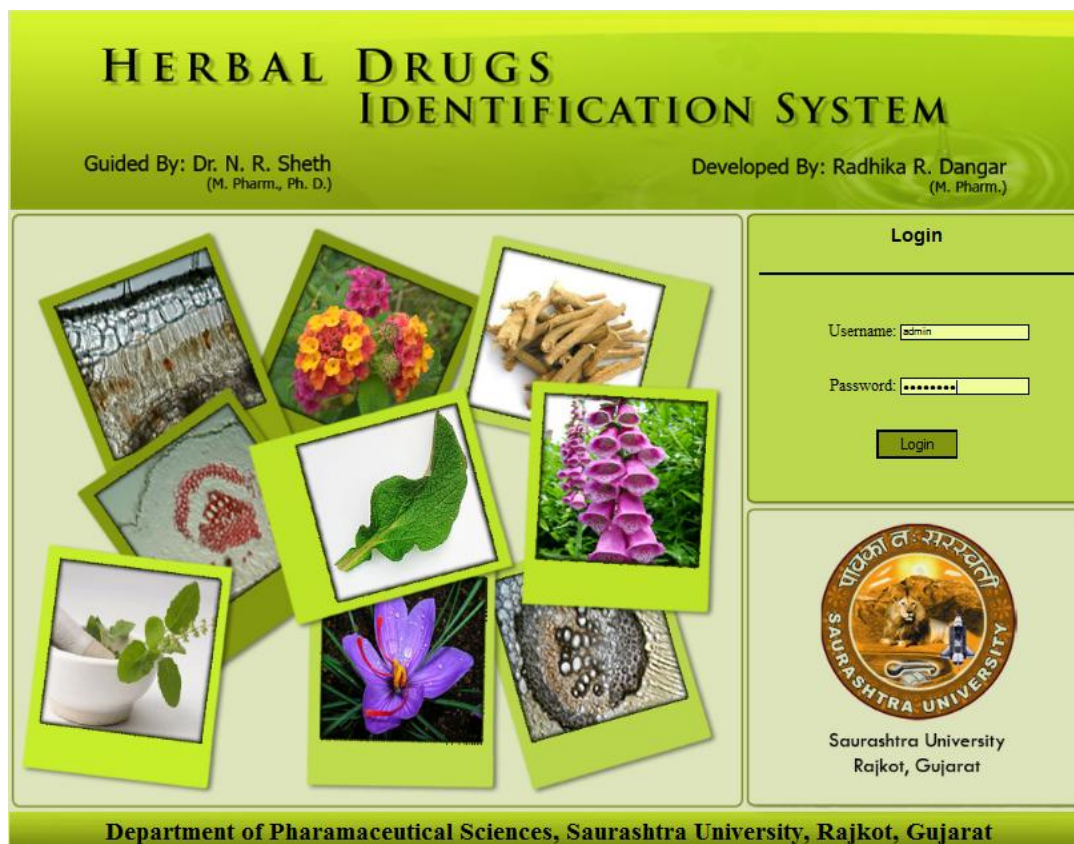


Fig. 15 Home Page of Herbal Drugs Identification System Showing Admin Login Area

By entering above user name and password, user will login to main software window, where following six buttons are present.

1. Add User
2. View User Details
3. View Drug Details
4. Add New Drug Details
5. Manage Database
6. Sing out

**HERBAL DRUGS
IDENTIFICATION SYSTEM**

Guided By: Dr. N. R. Sheth
(M. Pharm., Ph. D.)

Developed By: Radhika R. Dangar
(M. Pharm.)

[Add User](#) |
 [View User Details](#) |
 [View Drugs Details](#) |
 [Add New Drug Details](#) |
 [Manage Database](#) |
 Welcome, admin |
 [SIGN OUT](#)

Page: Add New Herbal Drug Details

Latin Name:

English Name:

Common Name: (optional)

Drug Family Name:

Type of Specimen:

Department of Pharamaceutical Sciences, Saurashtra University, Rajkot, Gujarat

Fig.16 Window of Herbal Drugs Identification System Showing Admin Area

4.2.1.1 Add User: Here we can add different types of users; either user having administrative privilege or having only viewing privilege. Master user of the program can create new user. When user is going to create in the software, some basic details are required like First name, Last name, email, User name and Password, after adding these details admin user can decide user access rights in the program. By adding all required details new user can be added.

4.2.1.2 View User Details: How many users are present in the program, which can be viewed by clicking view user details button. Here in the window user details will be explore in the columns, so we have easy to view all the user details. In this window master user can edit or delete the particular user.

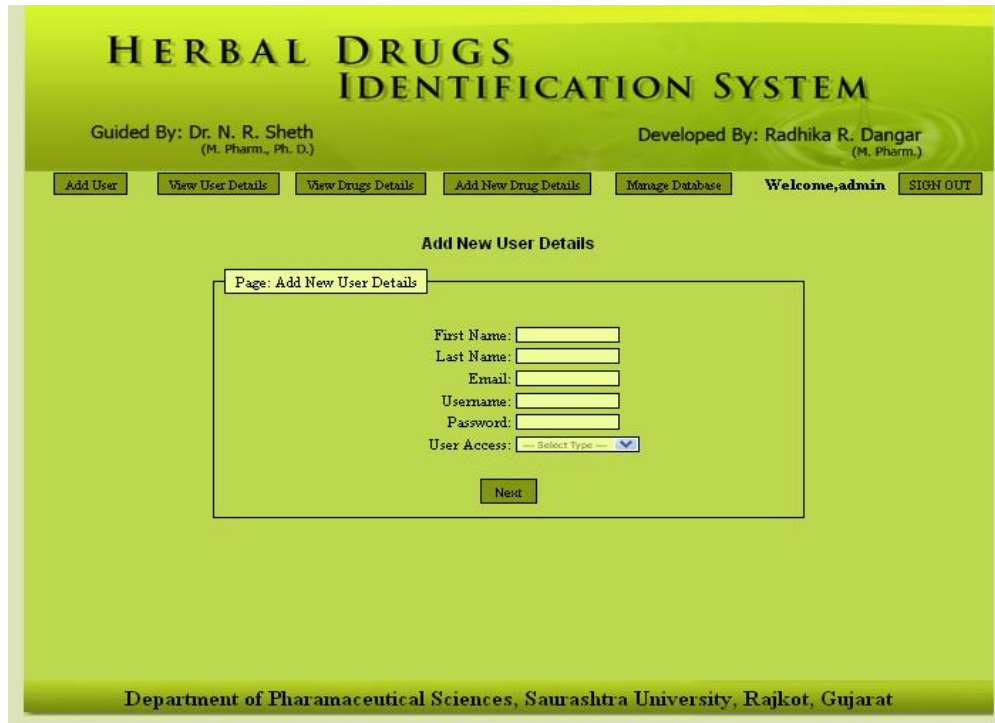


Fig. 17 Page of Herbal Drugs Identification System Showing Add User Window

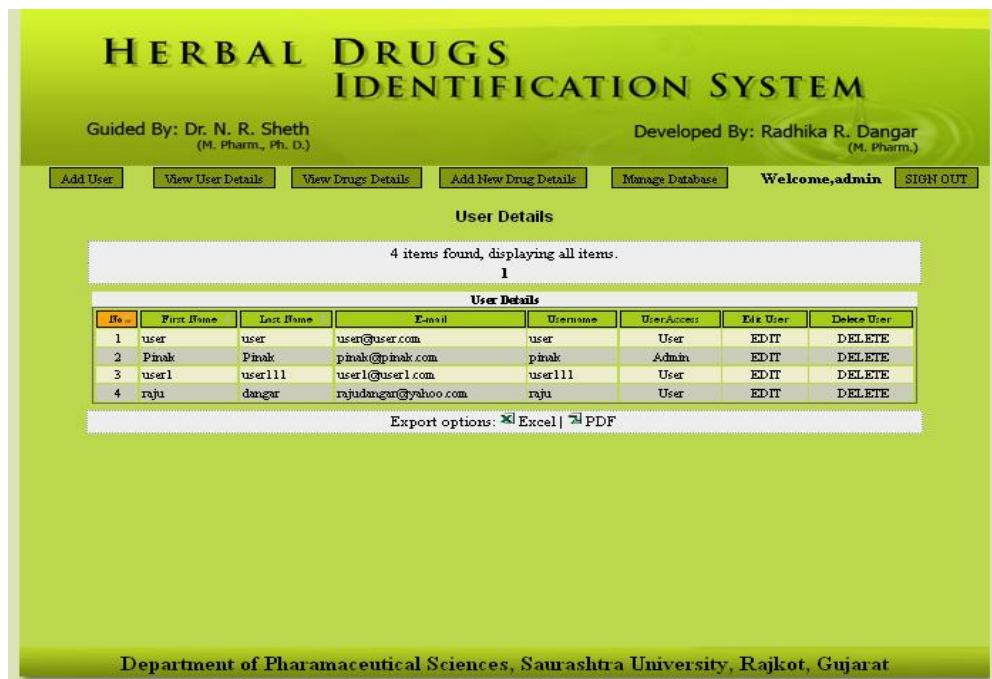


Fig.18 Page of Herbal Drugs Identification System Showing User Details

4.2.1.3 View Drug Details: This window shows drugs details entered in the software. Drug details will be displayed according to the alphabetical order. Here in this window by clicking heading of the columns we can rearrange the drugs presents in the database, this way columns' headings use as filters, so we can easily have the idea of added drugs in the program. These dug details either can be export in the excel sheet or in the form of portable document format (PDF). This facility provides hard as well as soft backup of database. This backup can be used to check drugs present in the database and also used to restore database in case of system crash. In this window any drugs can be edited or deleted directly if necessary. Window will also display no. of drugs added in the herbal drugs identification system. We can move directly on the desired page no. by clicking on the displayed numbers.

The screenshot shows the 'HERBAL DRUGS IDENTIFICATION SYSTEM' interface. At the top, it is guided by Dr. N. R. Sheth (M. Pharm., Ph. D.) and developed by Radhika R. Dangar (M. Pharm.). The interface includes navigation buttons: 'Add User', 'View User Details', 'View Drugs Details', 'Add New Drug Details', 'Manage Database', 'Welcome, admin', and 'SIGN OUT'. The main section is titled 'Drug Details' and displays '132 items found, displaying 1 to 10.' with pagination links: '[First/Prev] 1, 2, 3, 4, 5, 6, 7, 8 [Next/Last]'. Below this is a table with columns: No., Latin Name, English Name, Common Name, Family Name, Specimen Type, View Drug, Edit Drug, and Delete Drug. The table lists 10 items, including *Abies spectabilis*, *Abies precatorius*, *Acacia catechu*, *Acacia leucophloea*, *Acacia nilotica*, *Aconitum napellus*, *Ashwagandha*, *Ashwagandha*, *Aegle marmelos*, and *Ailanthus excelsa*. At the bottom, there are 'Export options: Excel | PDF' and the footer text: 'Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat'.

No.	Latin Name	English Name	Common Name	Family Name	Specimen Type	View Drug	Edit Drug	Delete Drug
1	<i>Abies spectabilis</i>	Indian silver fir, West himalayan high level fir, East himalayan fir leaf powder	Bang, chilrao, talispatra, dodimma, morunda	Pinaceae	Powder	VIEW	EDIT	DELETE
2	<i>Abies precatorius</i> Linn.	Jequirity, Crab's eye, Indian liquorice	Rati, Chanothee leaf powder, Ghanghi	Fabaceae	Powder	VIEW	EDIT	DELETE
3	<i>Acacia catechu</i>	Black catechu, Cutch bark powder	Khair, kathe	Mimosaceae	Powder	VIEW	EDIT	DELETE
4	<i>Acacia leucophloea</i>	Arimeda bark powder	Hannobaval, pilobaval, safed kiker	Mimosaceae	Powder	VIEW	EDIT	DELETE
5	<i>Acacia nilotica</i> Syn. <i>A. arabica</i> var. <i>indica</i>	Babula tree, Indian gum arabic tree bark powder	Baval, Kalobaval, Babula	Mimosaceae	Powder	VIEW	EDIT	DELETE
6	<i>Aconitum napellus</i> Linn.	Aconite root powder	Ativish, Vachag, Monkshood	Ranunculaceae	Powder	VIEW	EDIT	DELETE
7	<i>Ashwagandha</i> Nees	Vasaka leaf powder	Adusi	Acanthaceae	Powder	VIEW	EDIT	DELETE
8	<i>Ashwagandha</i> seylanica	Arhusi leaf powder	Vasaka, Vasa, Arhusi, arusa	Acanthaceae	Powder	VIEW	EDIT	DELETE
9	<i>Aegle marmelos</i>	Bael bark powder	Biltra, Bael, Bili, Bilspthal, Bengal Quince	Rutaceae	Powder	VIEW	EDIT	DELETE
10	<i>Ailanthus excelsa</i>	Tree of heaven	Moto Arhuso, Mahanim, Aruh	Samarubaceae	Powder	VIEW	EDIT	DELETE

Fig. 19 Page of Herbal Drugs Identification System Showing Drug Details

4.2.1.4 Add New Drug Details: New drug details can be added by clicking the button "Add New Drug Details". Basic drug details added first on the same window, after adding these details chews type of specimen whether it is

powder or vegetative part of the plant. Then click 'next' button, new window will be displayed and where we have to add all morphological and microscopical details of the given specimen. If plant specimen is there then next page (page. 2) will appear where we have to select (check the appropriate boxes) which vegetative part of the plant is going to add in the software, that is to be checked, after that page No. 3 will be appears regarding the morphology and microscopy. In case of powder specimen; directly morphological and microscopical parameters will be added, because powder does not represent any vegetative part of the plant. After adding basic detail, morphological and microscopical details click next button present at bottom of the page, added details will be displayed. This can help to check entered details before accommodated in the database. After checking all the details the submit button will be clicked, it add all displayed details in the database. 'Drug details have been added successfully' message will be displayed on the window, which indicates confirmation of drug entry in the database. Once any drug is added in the software and further same drug details is going to add in the software; software will intimate regarding presence of same drug in the database. Basic details of the drugs are act as filters while assessing database of the software, so person can easily search any drugs in the database. Lastly 'click here add new drug details' button will be displayed that facilitate to add new drug in the same sequence.

HERBAL DRUGS IDENTIFICATION SYSTEM

Guided By: Dr. N. R. Sheth (M. Pharm., Ph. D.) Developed By: Radhika R. Dangar (M. Pharm.)

Welcome, admin

Page: Add New Herbal Drug Details

Latin Name:
 English Name:
 Common Name: (optional)
 Drug Family Name:
 Type of Specimen:

Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat

Fig. 20 Window of Software showing Add New Drug Detail

HERBAL DRUGS IDENTIFICATION SYSTEM

Guided By: Dr. N. R. Sheth (M. Pharm., Ph. D.) Developed By: Radhika R. Dangar (M. Pharm.)

Welcome, admin

Page: Add New Herbal Drug Details

Latin Name:
 English Name:
 Common Name: (optional)
 Drug Family Name:
 Type of Specimen:

Fig. 21 Window of Software showing Basic Drug Detail of Plant

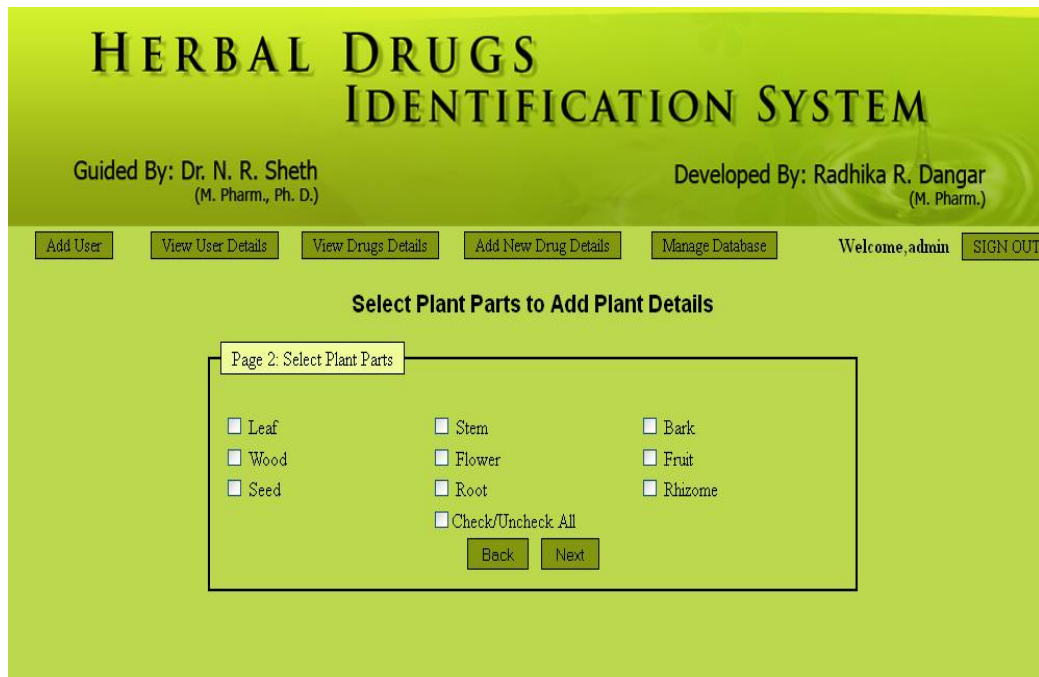


Fig. 22 Window of Software showing Plant Parts with Check Boxes

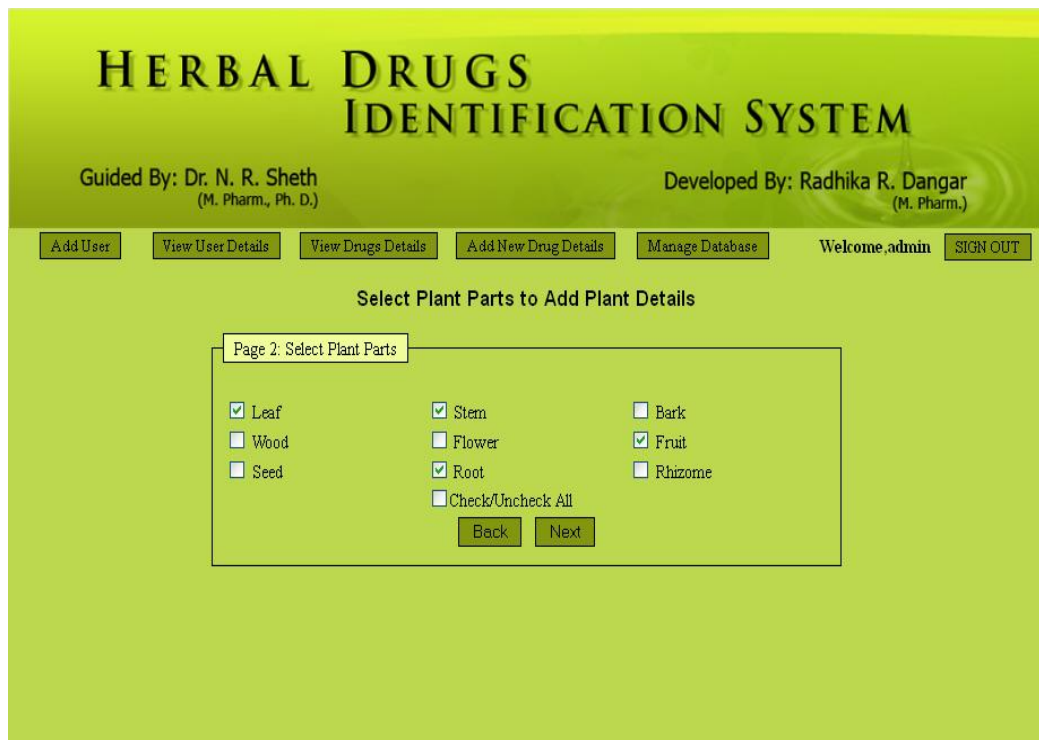


Fig. 23 Window of Software showing Plant Parts with Checked Boxes

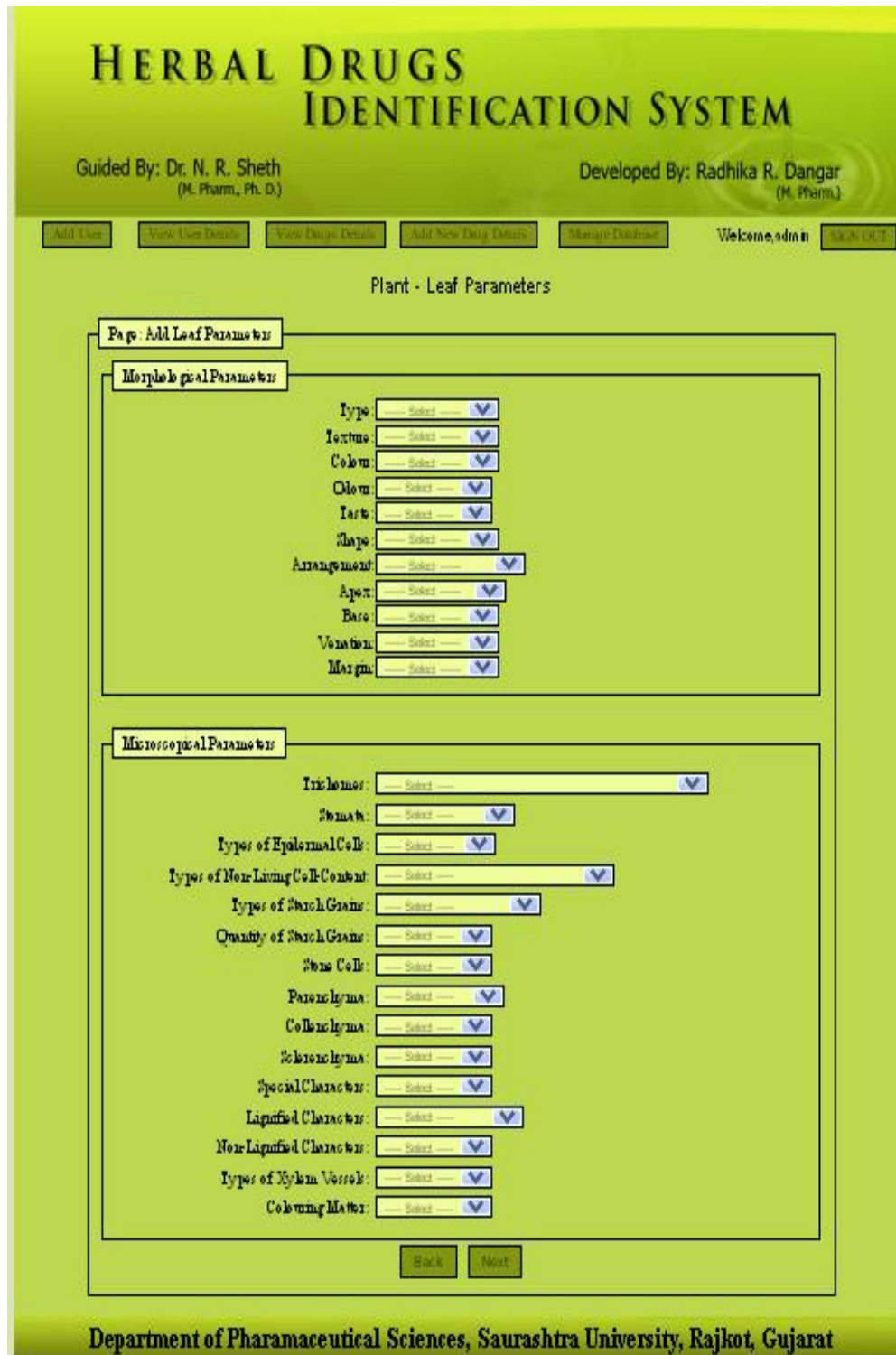


Fig. 24 Window of Software showing options for Leaf Details

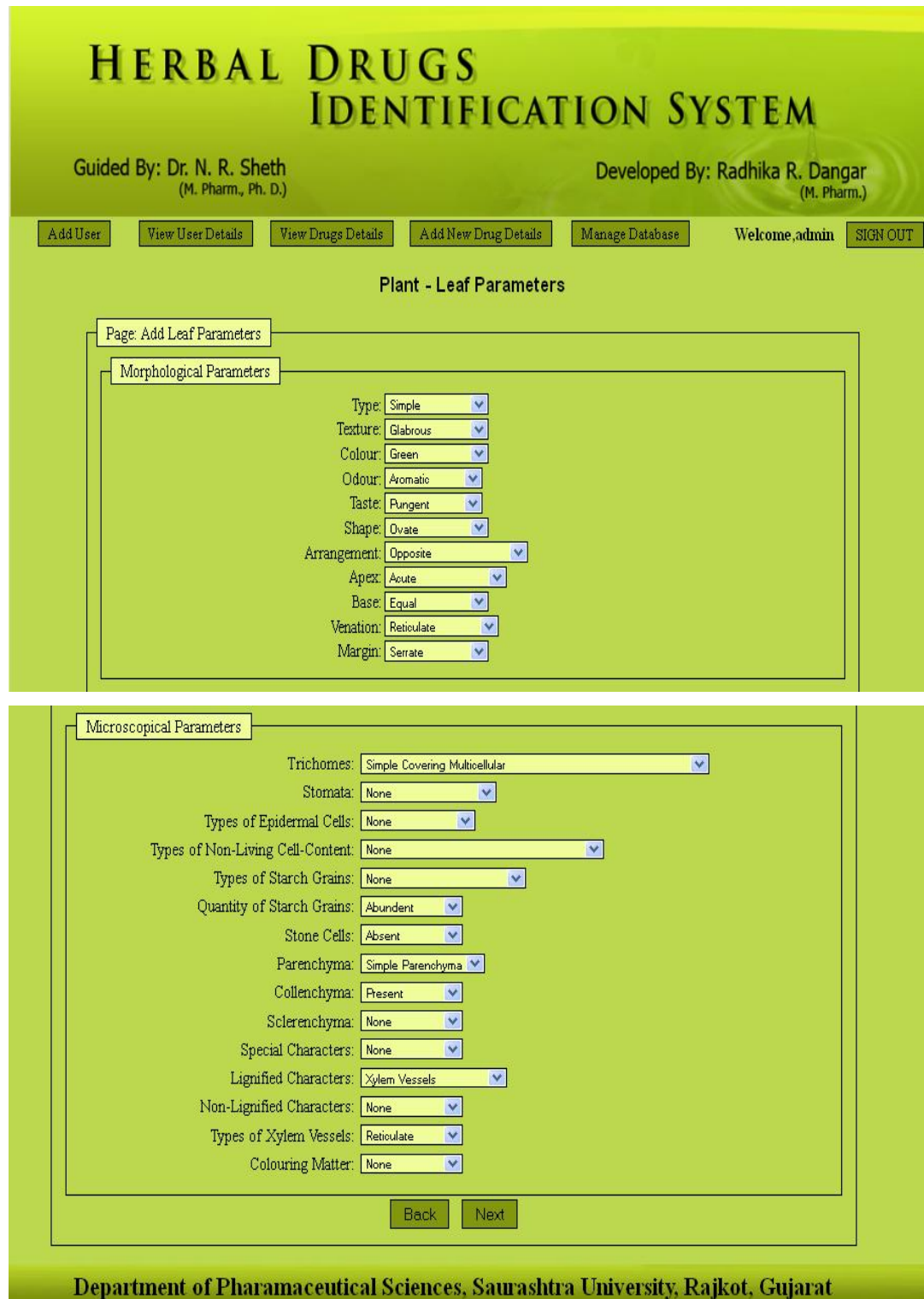


Fig. 25 Window of Software showing selected Leaf Parameters

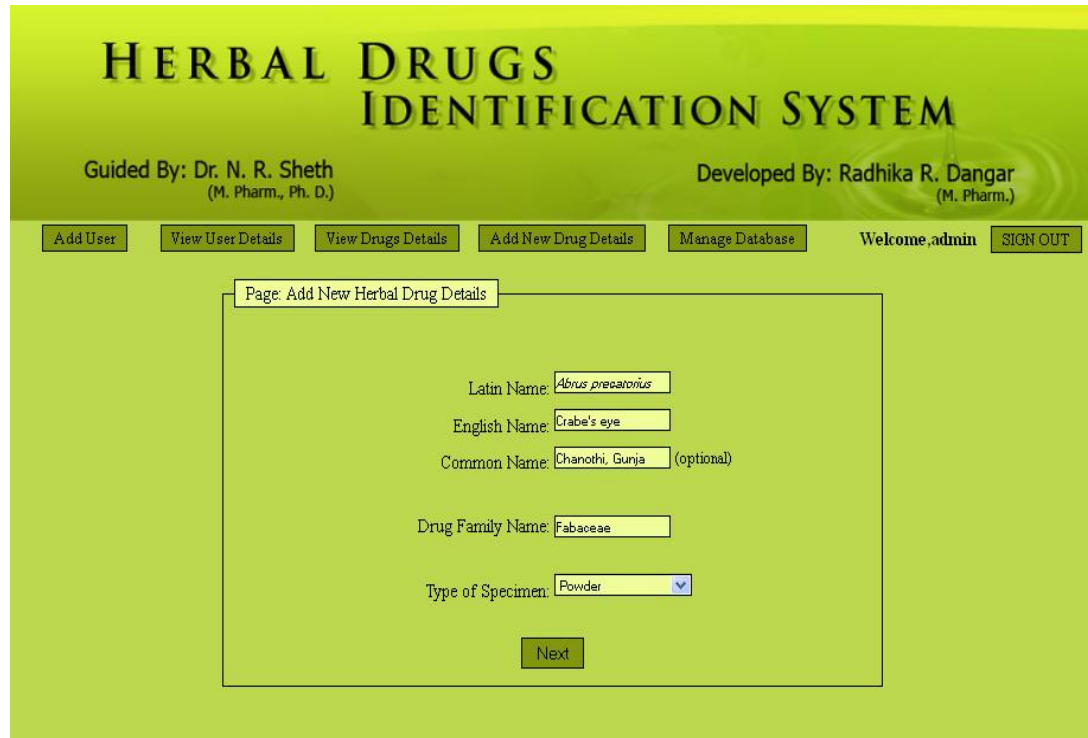
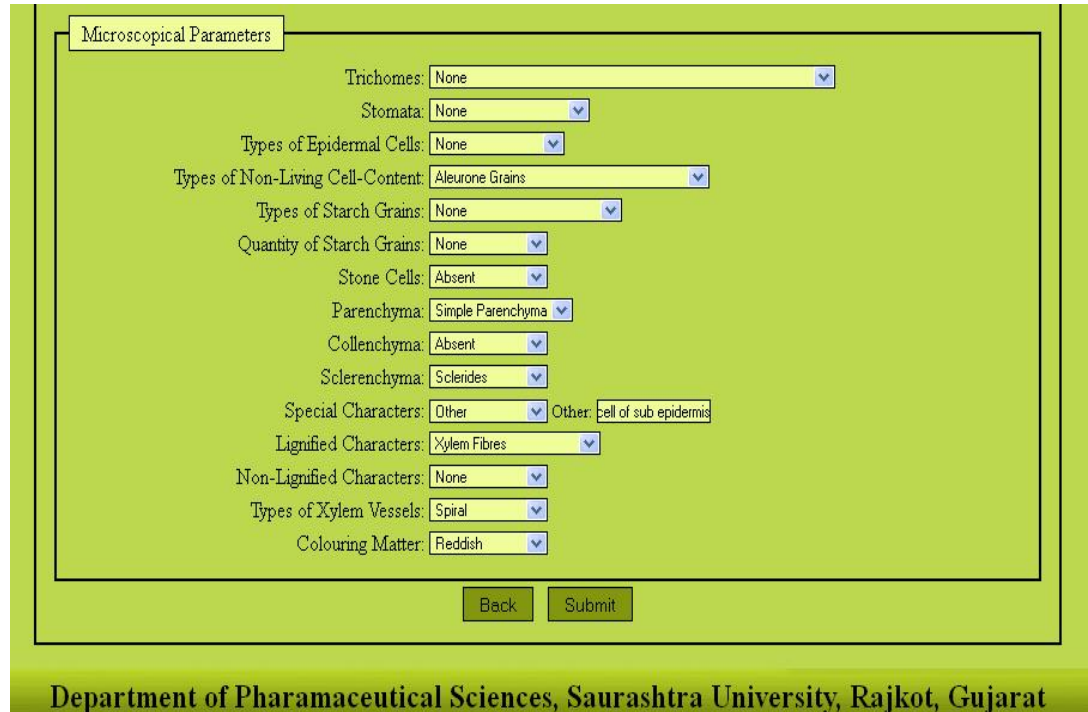


Fig. 26 Window of Software showing Basic Drug Details of Powder



Department of Pharamaceutical Sciences, Saurashtra University, Rajkot, Gujarat
 Fig. 27 Window of Software showing Morphological Parameters of Powder

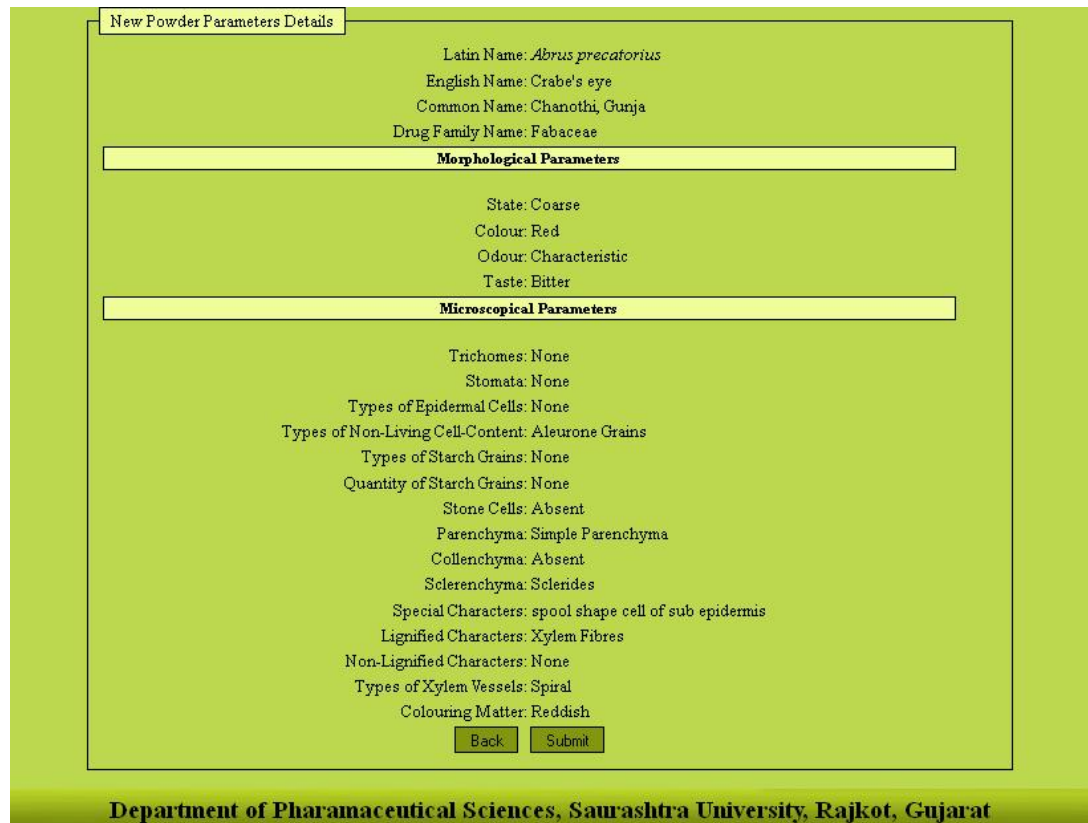


Fig. 28 Window of Software showing Powder Drug Details



Fig. 29 Window of Software showing Confirmation Message of Drug Added

4.2.1.5 Manage Database: Database management is very much important when program or software runs on the basis of preserved data. These types of software can analyze problems on the base of data sets present in the program. Preservation and store of data sets is the prime requirement in this software. Rich data sets can effectively analyze the given task. Interpretations quality and results are definitely affected by the type of data present in the database of the program. Manage database button provides facility of handling database. Here we can take backup of database either in the hard form or in the soft form. Hard form backup is prepared and can be stored for long time, while soft backup of database is the facility of storing database in other location of the computer or on the other storing media.

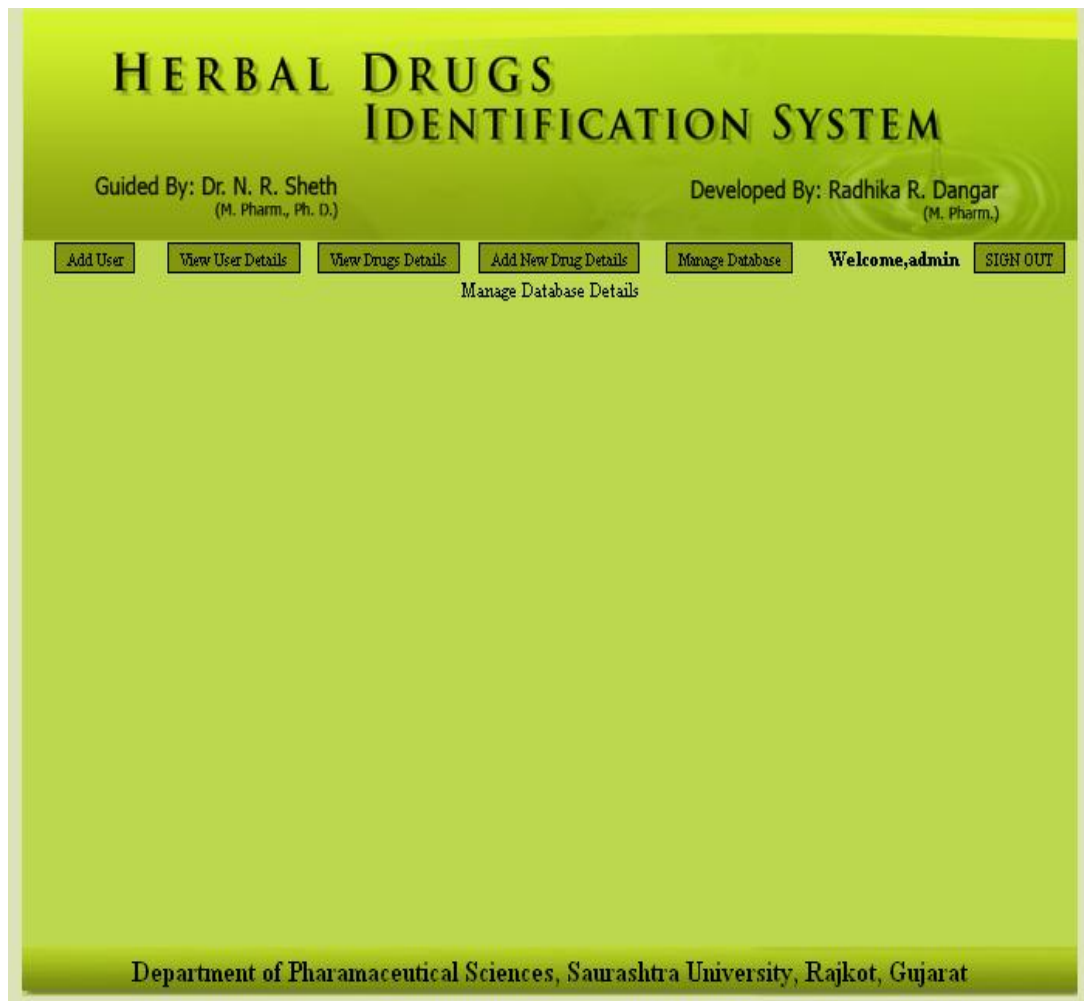


Fig. 30 Window of Software showing selected Leaf Parameters

4.2.2 Simple Users: Simple users are having only rights to explore the software, but they are not having right to modify anything in the program. Here In the software General user name and password are followings. Here user can check the identity of the plants and plant parts on the basis of morphological and microscopical characters.

User Name: user

Password: user123

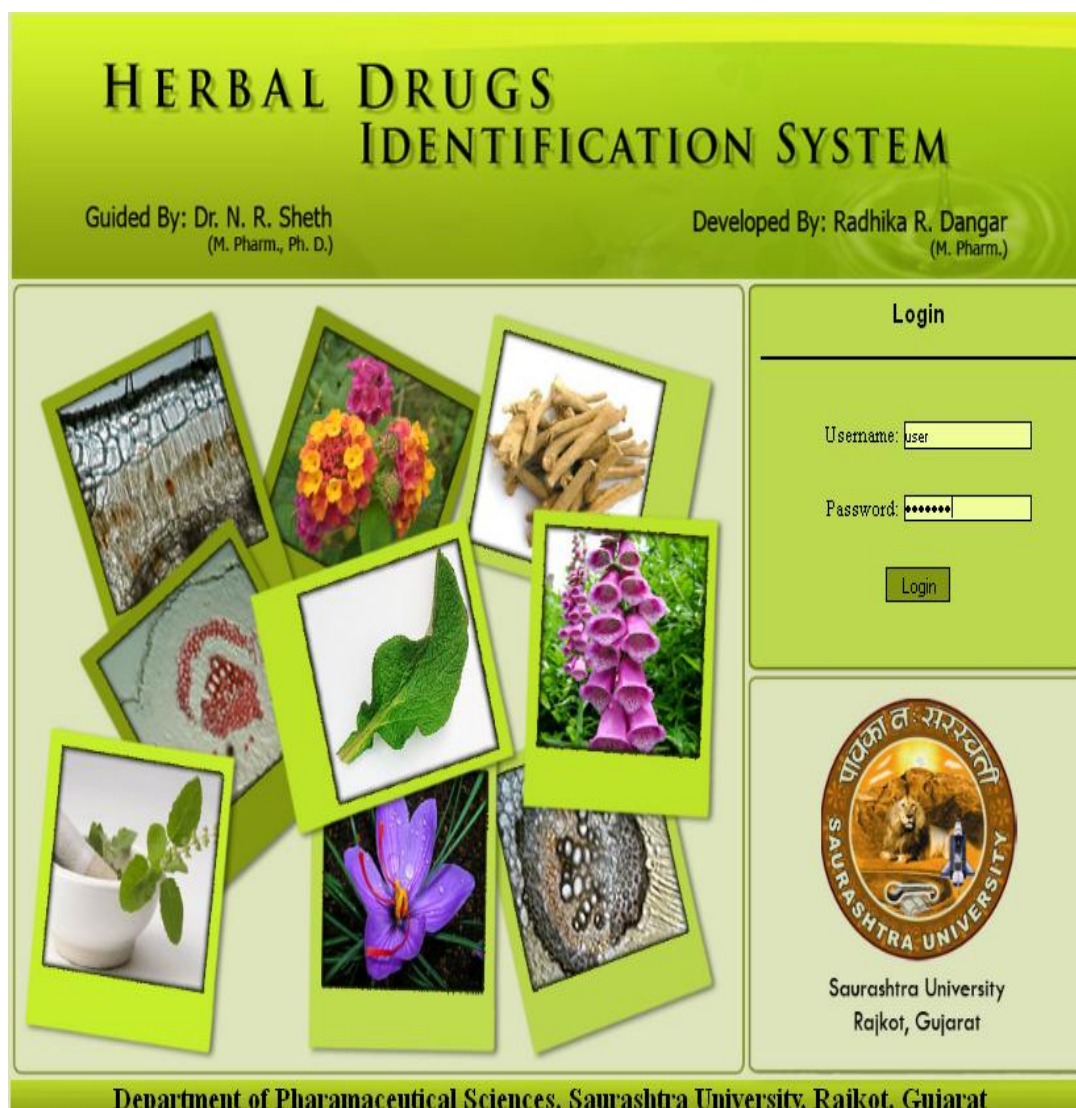


Fig. 31 Window of Software showing User's login Area

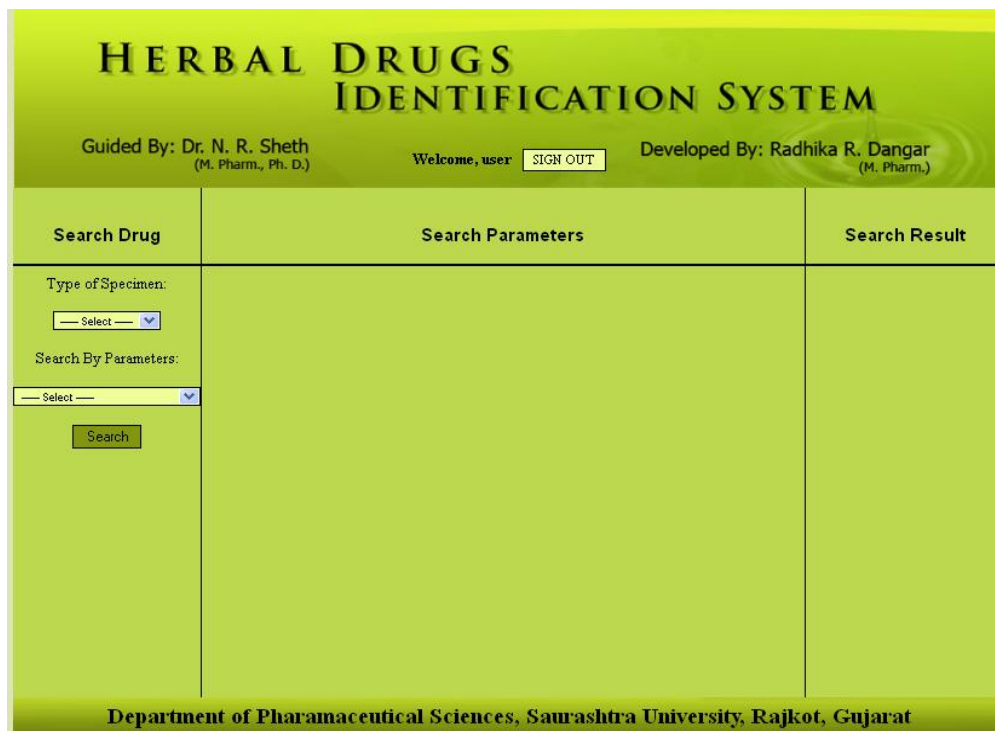


Fig. 32 Window of Software showing User Interface

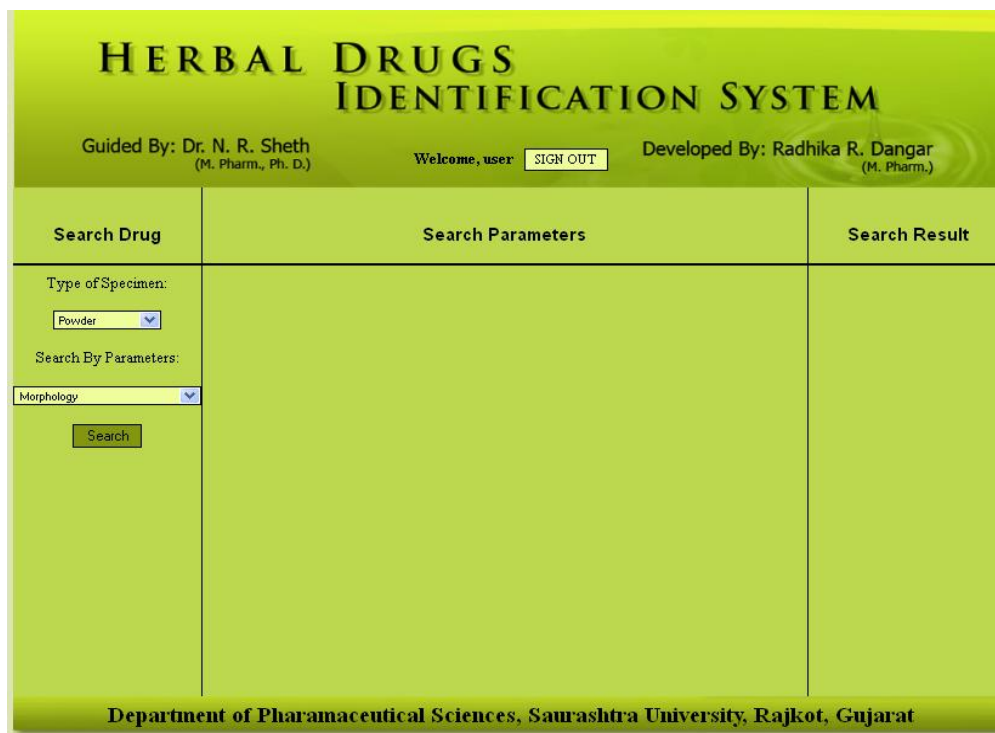


Fig. 33 Window of Software showing User Interface with Selected Parameters

After login to user interface, window will display three columns like search drug, search parameters and search result. First column search have two types of main filters, one is type of specimen and another is search by parameters. Search drug column is interpretation column, search parameters column is observation column and search result column is inference column.

4.2.2.1 Search Drugs by Type of Specimen: Drugs can be broadly identified on the basis of 'type of specimen'. This filter separate database in to two categories, one is powder drug and another is plant parts. Drop down menu provides two options. If we select type of specimen plant, then more one more option will be shown below the 'select parts of plant' button. Below this button all vegetative parts of the plants will be displayed, here we can check the box before the vegetative parts; user can also check multiple boxes. This option can make easy identification of any vegetative parts of the plants; technically database can be scrutinized by applying this filter on the basis of primary features. Check or uncheck all box provides facility to check or uncheck all boxes at a time.

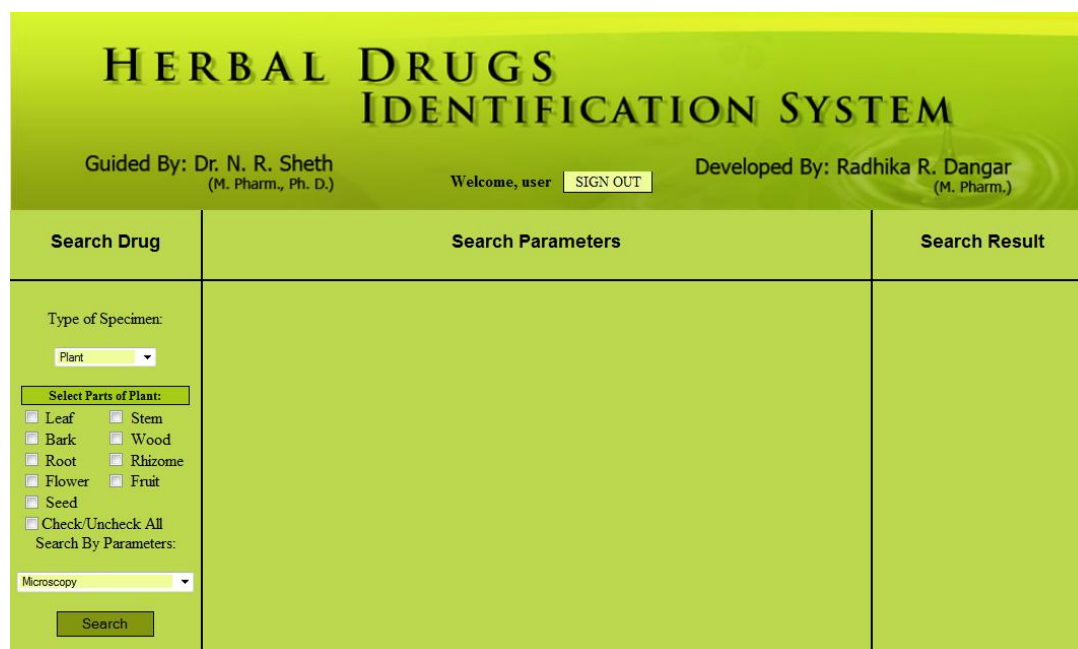


Fig. 34 Window of Software showing Plant and its Parameters

Fig.35 Window of Software showing selected Plant Parameters

4.2.2.2 Search Drugs by Plant Parameters: These parameters are very important for the searching the drugs. These are main parameters of the software like 'Morphology', 'Microscopy' and 'Morphology & Microscopy Both'. According to the selection of given parameters in the first column, another column which is 'search parameters' changes its view. Middle column provide facility of drop down menu where user can select option from the drop down menu. All drop down menu act as a filter for the database. When user select any option from the drop down menu according to that right side column 'search result' shows appropriate results which directly shown from scrutinized database. When user selects more than one option from the middle column according to that displayed results changes in the right column.

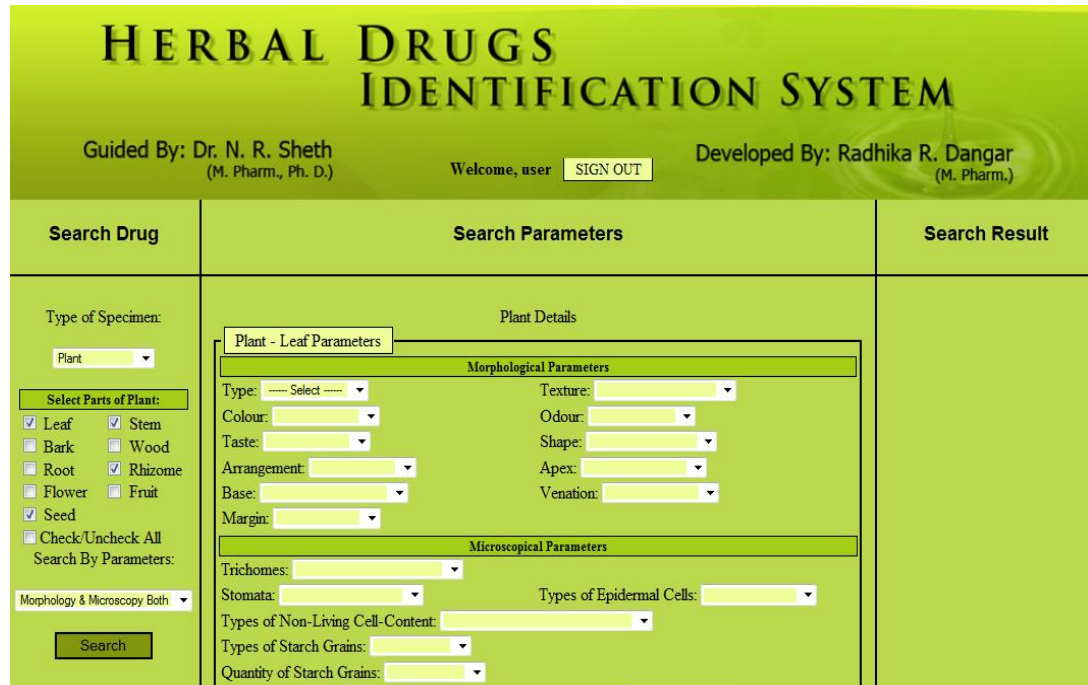


Fig. 36 Window of Software showing Drop down Menu in the middle Column

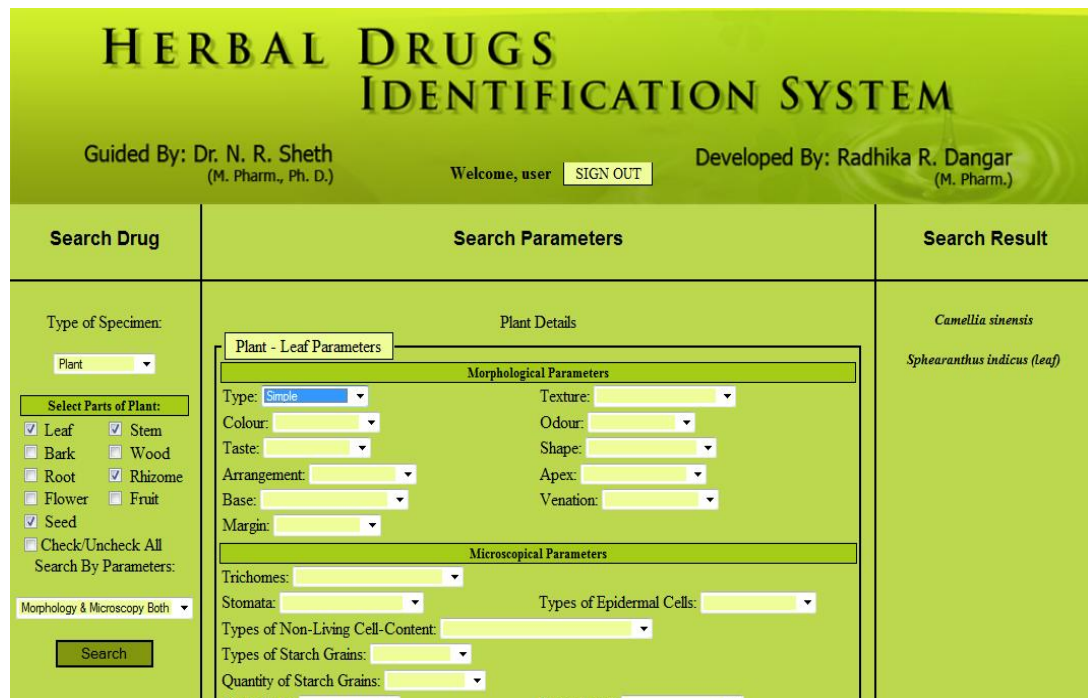


Fig. 37 Window of Software showing Drop down menu with Selected Option and Results

4.3 Drugs Details Added in the Software Library:

No.	Latin Name	English Name	Common Name	Family Name	Specimen Type
1	<i>Abies spectabilis</i>	Indian silver fir, West himalayan high level fir, East himalayan fir leaf powder	Bang, chilrao, talispatra, dodimma, morunda	Pinaceae	Powder
2	<i>Abrus precatorius</i>	Crabe's eye	Chanothi, Gunja	Fabaceae	Powder
3	<i>Abrus precatorius</i> Linn.	Jequirity, Crab's eye, Indian liquorice	Rati, Chanothee leaf powder, Ghungehi	Fabaceae	Powder
4	<i>Acacia catechu</i>	Black catechu, Cutch bark powder	Khair, kathe	Mimosaceae	Powder
5	<i>Acacia leucophloea</i>	Arimeda bark powder	Harmobaval, pilobaval, safed kikar	Mimosaceae	Powder
6	<i>Acacia nilotica</i> Syn. <i>A. arabica</i> var. <i>indica</i>	Babula tree, Indian gum arabic tree bark powder	Baval, Kalobaval, Babula	Mimosaceae	Powder
7	<i>Aconitum napellus</i> Linn	Aconite root powder	Ativish, Vachnag, Monkshood	Ranunculaceae	Powder
8	<i>Adhatoda vasica</i> Nees	Vasaka leaf powder	Adusi	Acanthaceae	Powder
9	<i>Adhatoda zeylanica</i>	Ardusi leaf powder	Vasaka, Vasa, Ardusi, arusa	Acanthaceae	Powder
10	<i>Aegle marmelos</i>	Bael bark powder	Bilva, Bael, Bili, Bilvaphal, Bengal Quince	Rutaceae	Powder
11	<i>Ailanthus excelsa</i>	Tree of heaven	Moto Arduso, Mahanim, Aralu	Simarubaceae	Powder
12	<i>Albizia lebbeck</i>	Siris tree bark powder	Siris, shirish, Lebbeck tree	Mimosaceae	Powder
13	<i>Alstonia scholaris</i>	Devil's tree bark powder	Saptaparni, satawana, sathi	Apocynaceae	Powder
14	<i>Amlakyadi churna</i>	Amlakyadi churna	Amlakyadi churna	-	Powder
15	<i>Ammi visnaga</i>	Khella, Visnaga fruit	Visnaga	Umbelliferae	Plant
16	<i>Andrographis paniculata</i> Nees.	Kalmegh herb powder	Kalmegh, kariyatu	Acanthaceae	Powder
17	<i>Anethum sowa</i> Kurz.	Indian dill fruit powder	Sowa, dill	Umbelliferae	Powder

18	<i>Anthocephalus chinensis</i>	Wild cinchona bark powder	Kadamb, kadam, vrttapuspa	Rubiaceae	Powder
19	<i>Areca catechu</i> Linn	Areca nut powder	Sopari, arecanut	Palmae	Powder
20	<i>Argyreia speciosa</i>	Samudrasosha leaf powder	Samandarkapat, samandarsokh	Convolvulaceae	Powder
21	<i>Atropa belladonna</i> Linn	Belladonna leaf powder	Deadly nightshade, Indian belladonna, Angurshefa	Solanaceae	Powder
22	<i>Azadirachta indica</i>	Margosa tree	Limda, Nim, Limbdo	Meliaceae	Powder
23	<i>Bacopa monnieri</i>	Brahmi leaf powder	Thyme leaved gratiola, Neer brahmi	Scrophulariaceae	Powder
24	<i>Bauhinia purpurea</i>	Koilara bark powder	Raktakanchan, Vanaraja	Caesalpinaceae	Powder
25	<i>Bauhinia variegata</i>	Mountain Ebony bark powder	Kachnar, champakati, kachanar	Caesalpinaceae	Powder
26	<i>Bombax ceiba</i> Linn.	Silk cotton tree bark powder	Shimlo, semal, salmali, Semar	Bombacaceae	Powder
27	<i>Butea monosperma</i>	Palash bark powder, Flame of the forest	Kesudo, Khakharo, palaspapda	Fabaceae	Powder
28	<i>Calotropis gigantea</i>	Shallow wart	Akado	Asclepiadaceae	Powder
29	<i>Calotropis procera</i>	Madar tree, Arka	Akado	Asclepiadaceae	Powder
30	<i>Camellia sinensis</i>	Tea leaves	Tea leaf powder	Theaceae	Plant
31	<i>Cannabis sativa</i> Linn.	Bhang leaf powder	Bhang, Charas, Ganja	Cannabinaceae	Powder
32	<i>Capsicum minimum</i> Roxb	Capsicum fruit powder	Capsicum, Red Chilli powder	Solanaceae	Powder
33	<i>Cardiospermum halicacabum</i> Linn.	Balloon vine, Winter cherry leaf powder	Karocho, Kanphata, Indravalli	Sapindaceae	Powder
34	<i>Carissa carandas</i> Linn	Karaunda bark powder	Karamada, karaonda	Apocynaceae	Powder
35	<i>Carum carvi</i> Linn	Caraway fruit powder	Shahjira, caraway	Umbelliferae	Powder
36	<i>Cassia angustifolia</i>	Senna leaf powder	Senna ki patti, Mindhiaval	Leguminosae	Powder
37	<i>Cassia angustifolia</i>	Senna	Mindhi Aval, sonamukhi	Leguminosae	Plant
38	<i>Cassia occidentalis</i> Linn	Kasondi leaf powder	Kasuvayee	Caesalpinaceae	Powder

39	<i>Cassia sophera</i> Linn.	Kasondi leaf powder	Kasundri, kuwadice, Banar	Caesalpinaceae	Powder
40	<i>Cassia tora</i>	Chakramadu leaf powder	Kawario, Chakvada	Caesalpinaceae	Powder
41	<i>Catharanthus roseus</i>	Madagascar periwinkle, Vinca leaf powder	Vinca, Barmasi, Sadabahar	Apocynaceae	Powder
42	<i>Centella asiatica</i>	Indian pennywort	Mandukparni, Motibrahmi, Brahmi leaf powder	Umbelliferae	Powder
43	<i>Cephaelis ipecacuanha</i>	Ipecac root powder	Ipecacuanha root	Rubiaceae	Powder
44	<i>Cinchona calisaya</i>	Cinchona bark powder	Jesuit's bark, peruvian bark	Rubiaceae	Powder
45	<i>Cinnamomum camphora</i>	Camphor tree	Karpur, Camphor tree	Lauraceae	Powder
46	<i>Cinnamomum cassia blume</i>	Cassia Bark powder	Taj, Dalchini powder	Lauraceae	Powder
47	<i>Cinnamomum sulphuratum</i> Nees	Jungli dalchini leaf powder	Jungli dalchini	Lauraceae	Powder
48	<i>Cinnamomum tamala</i>	Indian cassia leaf powder	Tejpat, tamalpatra	Lauraceae	Powder
49	<i>Cinnamomum zeylanicum</i> Blume	Ceylon cinnamon, Cinnamon bark powder	Dalchini	Lauraceae	Powder
50	<i>Claviceps purpurea</i> on <i>Secale cereale</i>	Ergot powder	Ergot of Rye	Ascomycetes	Powder
51	<i>Clitoria ternatea</i>	Winged leaved clitoria	Aparajita, Gokani	Papilionaceae	Powder
52	<i>Coffea arabica</i> Linn	Coffee seed powder	coffee	Rubiaceae	Powder
53	<i>Colchicum autumnale</i> L.	Colchicum seed powder	Indian colchicum, Meadow saffron corm, Meadow saffron corm	Liliaceae	Powder
54	<i>Coldenia procumbens</i> Linn	Trailing coldenia leaf powder	Tripankhi, tripakshi, Basario-Ookhraad	Boraginaceae	Powder
55	<i>Convolvulus pluricaulis</i>	Shankhpuspi leaf powder	Shankhpuspi, shankhavalli	Convolvulaceae	Powder
56	<i>Cordia dichotoma</i>	Lasora bark powder	Bargund, Bahuvaraka, Sita	Boraginaceae	Powder

57	<i>Coriandrum sativum</i> Linn.	Coriander fruit powder	Dhana, coriander, Dhaniya fruits	Umbelliferae	Powder
58	<i>Costus pictus</i> D.	Insulin plant leaf powder	Pakarmula	Zingiberaceae	Powder
59	<i>Crateva magna</i>	Three leaved caper bark powder	Varuna, Varano, Vayvarno, Baruna	Capparaceae	Powder
60	<i>Crocus sativus</i>	Saffron	Kesar, Saffron	Iridaceae	Plant
61	<i>Curcuma longa</i> Linn.	Turmeric rhizome powder	Haldi, Indian saffron, curcuma	Zingiberaceae	Powder
62	<i>Cymbopogan citratus</i>	Cymbopogan leaf powder	Gandharina, Bhustrina	Poaceae	Powder
63	<i>Dalbergia sissoo</i> Roxb.	Sissoo tree bark powder	Sisam, Seesam	Fabaceae	Powder
64	<i>Datura innoxia</i> Linn.	white Datura leaf powder	Safed daturo, Dhaturu	Solanaceae	Powder
65	<i>Datura metel</i> Linn.	White thron apple leaf powder	Dhatura, Dhaturu	Solanaceae	Powder
66	<i>Datura stramonium</i>	Devil's apple, stramonium leaf powder	Dhatura, Kanaka, Shivpriya, Unmatta	Solanaceae	Powder
67	<i>Digitalis lanata</i>	Grecian foxglove leaf powder	Hritpatri	Scrophulariaceae	Powder
68	<i>Digitalis purpurea</i> Linn.	Foxglove	Digitalis	Scrophulariaceae	Powder
69	<i>Eclipta alba</i> Hassk	Bhringraj leaf powder	Bhringraj, Kesari, Kesaraja	Asteraceae	Powder
70	<i>Eledi churna</i>	Eledi churna	Eledi churna	-	Powder
71	<i>Elettaria cardamomum</i>	Cardamom seed powder	Elaichi, Cardamom	Zingiberaceae	Powder
72	<i>Elettaria cardamomum</i> var. <i>minuscula</i>	Cardamom seed	Elaichi, Cardamom	Zingiberaceae	Plant
73	<i>Emblica officinalis</i>	Amla fruit pericap powder	Amla, Anola, Ambala, Amlaki	Euphorbiaceae	Powder
74	<i>Ephedra gerardiana</i>	Ephedra stem powder	Ephedra, Ma-huang, Somlata	Gnetaceae	Powder
75	<i>Erythrina variegata</i> Linn.	Coral tree bark powder	Panderavo, Pangara, Paribhadra	Fabaceae	Powder
76	<i>Erythroxylum coca</i>	Coca leaf powder	Coca	Erythroxylaceae	Powder
77	<i>Eucalyptus globulus</i>	Eucalyptus leaf powder	Nilgiri	Myrtaceae	Powder
78	<i>Evolvulus alsinoides</i> Linn	Vishnukarnta leaf powder	Nila puspa, Parijata, vishnukranti	Convolvulaceae	Powder

79	<i>Ficus benghalensis</i> Linn.	Banyan tree bark powder	Vad, vadalo, vata	Moraceae	Powder
80	<i>Ficus racemosa</i> Linn	Cluster Fig, country fig bark powder	Umbro, Umerdo, Gular	Moraceae	Powder
81	<i>Ficus reliogiosa</i> Linn	Pipal tree bark powder	Pipalo, Piparo, Asvattha	Moraceae	Powder
82	<i>Foeniculum vulgare</i>	Fennel fruit powder	Vadiyali, varyali, sauf	Umbelliferae	Powder
83	<i>Foeniculum vulgare</i>	Fennel fruit powder	Vadiyali, varyali, sauf	Umbelliferae	Plant
84	<i>Gentiana lutea</i> Linn.	Gentian root powder	Bitter root, karu, kutki	Gentianaceae	Powder
85	<i>Ginkgo biloba</i> Linn	Ginkgo leaf powder	Bai-guo-ye	Ginkgoaceae	Powder
86	<i>Glycyrrhiza glabra</i> Linn.	Liquorice root powder	Jethimadh	Leguminosae	Powder
87	<i>Gmelina arborea</i> Roxb.	Candahar, cashmere root bark powder	Sawan, shewan, Gambhari, madhuparnika	Verbenaceae	Powder
88	<i>Gmelina arborea</i> stem bark	Candahar, cashmere stem bark powder	Gambhari, sawan, shewan	Verbenaceae	Powder
89	<i>Gymnema sylvestre</i>	Gymnema leaf powder	Medhasingi, Gudamara, Dhuleti	Asclepiadaceae	Powder
90	<i>Holarrhena antidysentrica</i> Wall	Kurchi bark powder	Kurchi, Kadachhal, Kutaja	Apocynaceae	Powder
91	<i>Hyoscyamus niger</i> Linn.	Henbane, Black henbane leaf powder	Khorasaniajimo	Solanaceae	Powder
92	<i>Indigofera tinctoria</i> Linn	Nil leaf powder	Gali, Gari, Rangpatri, Nilla	Fabaceae	Powder
93	<i>Jasminum auriculatum</i>	Juhi leaf powder	Juthika, Jui, Juhi	Oleaceae	Powder
94	<i>Jasminum grndiflorum</i> Linn	Jasmine leaf powder	Chameli	Oleaceae	Powder
95	<i>Kalanchoe pinnata</i>	Jakhmehayat, pranabija	Asthibhaksha leaf powder	Crassulaceae	Powder
96	<i>Lannea cormandelica</i>	Jhingan bark powder	Kaimil, Jiyal, Jeol, Mavedi	Anacardiaceae	Powder
97	<i>Lawsonia inermis</i> Linn	Henna leaf powder, samphire	Mahendi, Madayantika	Lythraceae	Powder
98	<i>Lepatadenia teticulata</i>	Jivanti leaf powder	Jivanti, dodi, Nanidodi, Khirkhodi	Asclepiadaceae	Powder

99	<i>Linum usitatisimum L.</i>	Linseed powder	Linseed, Flax seed, Alsi	Linaceae	Powder
100	<i>Lobelia nicotianifolia Linn.</i>	Lobelialeaf powder	Nala, Nali, Narsala	Lobeliaceae	Powder
101	<i>Madhuca longifolia</i>	The Indian butter tree bark powder, Mahawash tree	Mahudo, Mahuwa, Gudapuspa	Sapotaceae	Powder
102	<i>Matricaria chamomilla Linn</i>	Chamomile flower powder	Chamomile	Compositae	Powder
103	<i>Melia azedarach Linn</i>	Bead tree, Persian lilac bark powder	Bakain, mahanimb	Meliaceae	Powder
104	<i>Melis azedarach Linn</i>	Mahanimb, persian liliac leaf powder	Bakain, mahanimb	Meliaceae	Powder
105	<i>Melissa officinalis</i>	Lemon Balm leaf powder	Biliotan, Badaranja boya	Lamiaceae	Powder
106	<i>Mentha piperita Linn</i>	Mentha leaf powder	Peppermint	Labiatae	Powder
107	<i>Mentha spicata Linn.</i>	Pudina leaf powder, Spearmint	Pudina	Labiatae	Powder
108	<i>Mimosups elengi Linn</i>	Bakul, Bullet wood tree powder	Bakul, Borsali, Bakula, madhugandha, sthirpuspa	Sapotaceae	Powder
109	<i>Morinda pubescens Smith</i>	Ach bark powder	Aal, ach, surang	Rubiaceae	Powder
110	<i>Moringa concanensis</i>	Indian horseradish, wild drumstick leaf powder	Karuwa, Shigru, krisnabijawa sajana, Muwa,	Moringaceae	Powder
111	<i>Moringa oleifera</i>	Drumstick, Horse radish tree	Saragavo leaf powder, Sigru	Moringaceae	Powder
112	<i>Murraya koenigii</i>	Curry leaf tree	Kadhi, Limbdo, Gora nimb, Mitha nimb	Rutaceae	Powder
113	<i>Myristica fragrans</i>	Nutmeg	Jayphal, Nutmeg, Jatiphala	Myristicaceae	Powder
114	<i>Myxopyrum smilacifolium Blume.</i>	Hemamalati leaf powder	Ghohora-lata, chathuravalli	Oleaceae	Powder
115	<i>Naveyasa churna</i>	Naveyasa churna	Naveyasa churna	-	Powder

116	<i>Nerium indicum</i> Mill.	Indian oleander, Sweet scented oleander leaf powder	Kanera, lal karen	Apocynaceae	Powder
117	<i>Nyctanthus arbortritis</i> Linn	Night jasmine, Parijata	Parijata leaf powder	Oleaceae	Powder
118	<i>Ocimum americanum</i>	Common basil	Kala tulsi	Labiatae	Powder
119	<i>Ocimum basilicum</i> L.	Sweet basil leaf powder	Mavo, Damaro, Barbari	Lamiaceae	Powder
120	<i>Ocimum gratissimum</i> Linn.	Large basil, shrubby basil leaf powder	Ram tulsi, van tulsi	Labiatae	Powder
121	<i>Ocimum kirimandscharicum</i> Linn.	Karpur tulsi, camphor basil	Karpur tulsi	Labiatae	Powder
122	<i>Ocimum sanctum</i> Linn	Tulasi leaf powder	Krishnatulasi, tulsi	Labiatae	Powder
123	<i>Oroxylum indicum</i>	Sonapatha bark powder	Aralu, Tentoo, Shyonak	Bignoniaceae	Powder
124	<i>Pantago ovata</i> Forsk	Isapghula seed powder	Isapgul, Isapghool	Plantaginaceae	Powder
125	<i>Pelargonium graveolens</i> Linn.	Geranium leaf powder	Geranium	Geraniaceae	Powder
126	<i>Picrasma quassioides</i> Benth	Quassia wood powder	Jamaica quassia	Simarubaceae	Powder
127	<i>Pimpinella anisum</i> Linn.	Anise fruit, aniseed	Anise fruit powder	Umbelliferae	Powder
128	<i>Piper betle</i> Linn	Betel leaf powder	Pan, Nagurvel, Tambuli	Piperaceae	Powder
129	<i>Piper longum</i> Linn	Piper root, pippalimula powder	ganhoda, gantoda, pippalika	Piperaceae	Plant
130	<i>Piper longum</i> Linn (fruit)	Long pepper, pipali fruit powder	Lindi peeper, pipar	Piperaceae	Powder
131	<i>Piper nigrum</i> Linn.	Black pepper fruit powder	Mari, black pepper	Piperaceae	Powder
132	<i>Pluchea lanceolata</i>	Rasna leaf powder	Rayasan, Rasna	Asteraceae	Powder
133	<i>Podophyllum emodi</i>	Indian podophyllum root powder	Podophyllum	Berberidaceae	Powder
134	<i>Pogostemon patchoulli</i>	Patchouli leaf powder	Pacholi, Peholi	Lamiaceae	Powder
135	<i>Polyalthia longifolia</i>	Cemetery tree, asopalav bark powder	Asoka, debdaru	Annonaceae	Powder

136	<i>Pongamia pinnata</i>	Indian Beech bark powder	Karanj, Kanaji, Naktamala	Fabaceae	Powder
137	<i>Prosopis spicigera</i> Linn.	Mesquite	Khijado, Shami, Sami, Jhand	Fabaceae	Plant
138	<i>Prosopis spicigera</i> Linn. (stem)	Mesquite	Sami, shami, khijado	Fabaceae	Plant
139	<i>Pterocarpus marsupium</i>	Indian kino tree bark powder	Biyo, bijasal, Bija	Fabaceae	Powder
140	<i>Punica granatum</i> L.	Pomegranate leaf powder	Dadima, Dadama	Punicaceae	Powder
141	<i>Putranjiva roxburghii</i>	pitanjiya bark powder	Putranjiva, Garbhakara	Euphobiaceae	Powder
142	<i>Quillaia saponaria</i> Molina	Quillaia bark powder	Soap bark	Rosaceae	Powder
143	<i>Rauwolfia serpentina</i>	Rauwolfia root powder	Serpagandha, choota chand	Apocynaceae	Powder
144	<i>Rhamnus purshiana</i>	Cascara bark powder	American frangula, cascara bark	Rhamnaceae	Powder
145	<i>Rheum palmatum</i> Linn.	Rhubarb rhizome powder	Revanchini	Polygonaceae	Powder
146	<i>Rosemarinus officinalis</i>	Rosemary leaf powder	Rosemary, Rusmari	Labiatae	Powder
147	<i>Ruta graveolens</i> Linn.	Gargen rue leaf powder	Satapa, sadab, satari	Rutaceae	Powder
148	<i>Salvia officinalis</i> Linn.	Sage, Garden sage	salvia, sefakuss leaf powder	Labiatae	Powder
149	<i>Santalum album</i> Linn	Sandal wood powder	Chandan, Sweta chandan, liquid gold	Santalaceae	Powder
150	<i>Saraca indica</i> Linn	Ashok bark	Ashoka stem bark powder	Leguminosae	Powder
151	<i>Sesbania bispinosa</i>	Jayanti Bheda leaf powder	Ikad, Jayanti	Fabaceae	Powder
152	<i>Sesbania grandiflora</i>	Swamp pea bark powder	Bak, Agathiyo, agasti	Fabaceae	Powder
153	<i>Sitopaladi churna</i>	Sitopaladi churna	Sitopaladi churna	-	Powder
154	<i>Sphaeranthus indicus</i> Linn	East Indian globe thistle	Gorakhmundi, Munditika	Compositae	Plant
155	<i>Sphearanthus indicus</i> (leaf)	East Indian globe thistle	Gorakhmundi, Munditika	Compositae	Plant
156	<i>Stevia rebaudiana</i> Linn.	Stevia (natural sweetner)	Stevia leaf powder	Asteraceae	Powder
157	<i>Strophanthus gratus</i>	Strophanthus seed powder	Strophanthus	Apocynaceae	Powder

158	<i>Strophanthus kombe</i>	Strophanthus seed powder	Strophanthus	Apocynaceae	Powder
159	<i>Strychnous nuxvomica Linn</i>	Nuxvomica seed powder	Nuxvomica, Zer kachuro, Poison nut tree	Loganiaceae	Powder
160	<i>Swadista virechan churna</i>	Swadista virechan churna	Swadista virechan churna	-	Powder
161	<i>Symplocoa racemosa Roxb.</i>	Symplocos bark powder	Lodha, lodhra, Rodhra	Symplocaceae	Powder
162	<i>Syzygium aromaticum</i>	Caryophylli, Clove flower bud powder	Clove, Lavang, Laving	Myrtaceae	Powder
163	<i>Syzygium cumini</i>	Black plum, Java plum bark powder	Jambu, jambuda, Raja jambu	Myrtaceae	Powder
164	<i>Taxus wallichiana</i>	Taxus leaf powder, Birmi	Birmi, Postil, Thuno	Taxaceae	Powder
165	<i>Tecomella undulata</i>	Rohida bark powder	Rohida, Ragatrohida, Rohira	Bignoniaceae	Powder
166	<i>Terminalia alata or T. tomentosa</i>	Amari, Asan bark powder	Sadar, asan, sain	Combretaceae	Powder
167	<i>Terminalia arjuna</i>	Arjuna bark powder	Sadad, kakubha	Combretaceae	Powder
168	<i>Terminalia belerica</i>	Baheda pericarp of fruit powder	Bibhitaka, Baheda	Combretaceae	Powder
169	<i>Terminalia chebula</i>	Chebolic Myrobalan pericarp of fruit powder	Harde, Haritaki, harda	Combretaceae	Powder
170	<i>Thea sinensis</i>	Tea leaf	Tea	Theaceae	Plant
171	<i>Thespesia populnea</i>	Portia tree bark powder	Paraspippalo	Malvaceae	Powder
172	<i>Trikatu churna</i>	Trikatu churna	Trikatu churna	-	Powder
173	<i>Triphala Churna</i>	Triphala churna	Triphala churna	-	Powder
174	<i>Tylophora indica</i>	Pitmari	Antamul, antamul leaf powder	Asclepiadaceae	Powder
175	<i>Urginea indica</i>	Squill Bulb powder	Squill	Liliaceae	Powder
176	<i>Valeriana wallichii</i>	Indian Valerian rhizome and root powder	Tagar	Valerianaceae	Powder
177	<i>Vitex negundo Linn.</i>	Five leaved chaste tree	Nagod, Nirgundi	Verbenaceae	Powder
178	<i>Wedelia chinensis</i>	Pirabhingraj leaf powder	Swarnabhangra, kesaraja	Asteraceae	Powder

179	<i>Wrightia arborea</i>	Dudhi, dharanli bark powder	Dudhlo, kala indrajau, strikutaja	Apocynaceae	Powder
180	<i>Wrightia tinctoria</i>	Indigo leaf powder	Indigo plant, Indrajav, kalikari	Apocynaceae	Powder
181	<i>Zanthoxylum armatum</i>	Toothche tree	Tejabala, Tejbal, tejovathi, Tejaswinee	Rutaceae	Powder
182	<i>Zingiber officinalis</i>	Ginger rhizome powder	Ginger, Sunth powder	Zingiberaceae	Powder
183	<i>Zizyphus jujuba</i>	Jujube bark powder	Bor, Desi ber, Ber	Rhamnaceae	Powder

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6.1 Abstract of Poster Presented in International Symposium on Herbal and Traditional Medicine held at Saurashtra University, Rajkot



16th National Convention of Society of Pharmacognosy
and International symposium on Herbal and traditional Medicine
"FRONTIERS IN DRUG DISCOVERY FROM HERBAL & TRADITIONAL MEDICINE"

9 to 11 March 2012



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FORMULATION OF SOLID ORAL DOSAGE FORM OF ETHANOLIC EXTRACT OF BOERHAAVIA DIFFUSA L. ROOTS

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Introduction: Ethanolic extract of *B. diffusa* roots is reported to possess various biological activities but so far there is no report on its preformulation and formulation studies. **Objectives:** Tablet formulation of standardized ethanolic extract of *B. diffusa* roots and its evaluation for various pharmaceutical parameters as per Indian Pharmacopoeia (IP). **Materials and Methods:** *B. diffusa* roots were extracted with 70% ethanol by maceration at room temperature and lyophilized after removal of ethanol. The extract was standardized with respect to total flavonoids content (quercetin equivalents) and marker constituent, boeravinone B content by HPLC method. Compatibility study of different ratio of standardized extract and excipients blends were done under isothermal condition at 40°C±2°C/75%±5% RH for 30 days. Preformulation studies of extract were done for hygroscopicity (Callahans method), solubility, particle size by zeta sizer, flow property (angle of repose, tapped and bulk density, carr's index, hausner ratio). The tablet formulation was optimized by various proportions of excipients and was evaluated for its pharmaceutical parameters viz. weight variation, hardness, friability, disintegration time and dissolution. **Results and Discussion:** The content of boeravinone B in the ethanolic extract was found to be within permissible IP limit (NLT 0.005% w/w). Excipients PVP, Aerosil, MCC, Mg stearate, and cross carmellose sodium showed no incompatibility with the studied extract. Preformulation parameters of extract-excipients blend and formulation parameters of tablets were within permitted limits as per IP 2010. **Conclusion and Future Plan:** The ethanolic extract of *B. diffusa* was successfully formulated as tablets and all parameters were within permissible limits. To the best of our knowledge this is the first report on tablet formulation of *B. diffusa*. Accelerated stability study of tablets (as per ICH guidelines) is currently under progress.

Keywords: Boerhaavia diffusa, Formulation, Standardization, Evaluation

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ASSESSMENT OF ADAPTOGENIC POTENTIAL OF METHANOLIC EXTRACT OF RASAYANA DRUG – JIVANTI

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Leptadenia reticulata (Family; Asclepiadaceae) is identified as Jivanti of Ayurveda. *Dregea volubilis* (Family; Asclepiadaceae) and *Pentatropis microphylla* (Family; Asclepiadaceae) are utilized as substitute of original plant. All the three plants selected are described under the category of Rasayana in Ayurveda texts, as the agents which protect the body from nonspecific stress conditions. In modern therapy such type of substances are called as adaptogens. In order to access the adaptogenic potential of the selected plants a well accepted model of swim endurance test was used. Various biochemical parameters viz. serum SGPT, serum Glucose, Serum Triglycerides, Serum Cholesterol and Serum Blood Urea Nitrogen were selected as evaluation parameters to access the protective effect of methanolic extract on stress induced alteration in such parameters. The results of the studies revealed the alteration in the selected biochemical parameters was inhibited in the groups of the animals treated with methanolic extract of the selected plants in dose dependant manner.

Keywords: Leptadenia reticulata, Dregea volubilis, Pentatropis microphylla

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DEVELOPMENT OF COMPUTER AIDED TECHNIQUE FOR THE IDENTIFICATION OF SELECTED HERBAL DRUGS - R. R. Dangar, R. D. Dangar, N.R. Sheth

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Objective: Therapeutic potential of the plants are accepted by the pharmaceutical manufacturers of the world. Safety is also one of the important aspects for the popularity of plant based medicines. Identification of plants is mostly based on morphology and microscopy. Morphology and microscopy are scientific tools even though they are not popular, because they require skill persons. Morphology and microscopy are the tedious parameters for the end users. So our aim is to develop identification technique for the plants and plant parts by using computers. **Materials and Methods:** Macroscopical and microscopical studies were carried out for plants and powdered drug. Information revealed during study is stored in the computer based program. Computer based program is developed by using morphological and microscopical characters of the plants. This web based program assist to store morphological as well as microscopical information of the plants and plant parts. **Result:** We have developed web based program to handle morphological and microscopical information of the plants and plant based drugs like churna. This is the first attempt in the field of Pharmacognosy to develop computer based program for the identification of the plants. MySQL is used for the handling wide database. Web based software is designed in the JavaScript. We have combined pharmacognostical approach with computers. This technique converts tedious process to convenient process. It is very easy for the common man, who doesn't know plants more scientifically. **Conclusion:** We have developed web based software for the identification of plants and plant parts. This is first effort in the Pharmacognosy to use computers in identification of plants across world.

Keywords: Software, Database, Identification, Morphology, Microscopy

Organized by:
Department of Pharmaceutical Sciences, Saurashtra University, Rajkot

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ABSTRACT

6.2. Certificate of Poster Presentation Presented in International Symposium on Herbal and Traditional Medicine held at Saurashtra University, Rajkot

