

Chapter 2

Pharmacognostical Studies

Abstract The chapter deals with tools and techniques employed in pharmacognosy. Pharmacognostic evaluation helps to screen the commercial varieties, substitutes, adulterants and any other quality control of the drugs. It is a simple and reliable tool, helps to obtain information about biochemical and physical properties of crude drug. Methods such as macroscopic and microscopic analysis, maceration, histochemical colour reaction, photomicrography, organoleptic character of plant powder and extracts, fluorescence analysis of plant powder with different chemical reagents, determination of pH of plant powder, water solubility index (WSI) and water absorption index (WAI) and acid value are discussed.

Introduction

Pharmacognosy is the study of medicinal material derived from natural source. It is the study of the physical, chemical, biochemical and biological properties of drug found in nature as well as the search of new drug from natural origin. Pharmacognostic evaluation helps to screen the commercial varieties, substitutes, adulterants and any other quality control of the drugs. It is a simple and reliable tool, by which the complete information of the crude drugs can be obtained (WHO 1998).

Aim

To find out the macroscopic, microscopic, histochemical and physicochemical characteristic features of the plant sample.

Principle

When the sample is treated with particular chemical agent, it forms specific colour or it predicts specific substances or cells through which it aids for the quality control of drug.

Materials

1. Test tubes, Whatman No. 1 filter paper, measuring cylinder, funnel, water bath, embryo cup with lid, slides and cover slip.
2. Formalin (mix formalin, acetic acid and 70 % ethanol in ratio of 1:1:12).
3. Safranin (mix 1 g of safranin in 10 mL of 95 % ethanol).
4. Fast Green (take ethanol and methyl salicylate in the ratio of 1:1 and then mix with 14 mg of Fast Green).
5. Clearing solution (mix methyl salicylate, absolute alcohol and xylene in the ratio of 2:1:1).
6. Jeffrey's maceration solution (1:1 of 10 % nitric acid and 10 % chromic acid).

Protocol

2.1 Macroscopic Analysis

The morphological characters (Trease and Evans 1983; Wallis 1985) of the plant sample being observed are as follows:

1. Shape;
2. Surface;
3. Colour;
4. Size.

2.2 Microscopic Analysis

1. Initially, pile up the sections of plant parts in formalin for fixation.
2. Then stain the sections with safranin for 5 min and then wash it with water.
3. Treat the sections with 30, 70, 90 and 100 % ethanol for 5, 1, 1 and 1 min, respectively, for dehydration.
4. After that, stain the sections with Fast Green for 15 s.
5. Wash the sections with absolute alcohol for 1 min and with clearing solution for 2 min.
6. Then treat the sections with xylene for less than 10 s.
7. Finally, mount the sections on the slide using D.P.X. liquid mountant (Pandey 2005).

2.3 Maceration

1. Macerate the plant samples with Jeffrey's maceration solution.
2. Decant remaining acid and then wash the bleached powder fragments with water repeatedly.
3. Add a few drops of ammonium hydroxide for neutralization.
4. Stain the macerated plant samples with safranin and mount using glycerine (Pandey 2005).

2.4 Histochemical Colour Reaction

The histochemical colour reactions of plant samples are performed separately in order to identify major cell components by chemical reagents. The following table represents the procedure of histochemical reaction (Khandelwel et al. 1996).

S. No.	Reagents used	Test	Colour formation	Histochemical zone
1	T.S. of plant parts + iodine solution	Starch	Blue	Spongy parenchyma
2	T.S. of plant parts + iodine solution + H_2SO_4	Cellulose	Bright yellow	Chlorenchyma
3	T.S. of plant parts + safranin	Lignin	Red	Vascular zone
4	T.S. of plant parts + methylene blue	Mucilage	Deep violet	Spongy parenchyma
5	T.S. of plant parts + amido black	Protein	Green	Cambium

2.5 Photomicrography

Photographs of microscopic section of different magnifications are taken with Olympus BX51 light microscopic unit. Descriptive terms of the anatomical features are as given in the standard anatomy book (Esau 1965).

2.6 Organoleptic Character of Plant Powder and Extracts

The organoleptic parameters (Trease and Evans [1983](#)) of plant powder and the extracts are as follows:

1. Colour;
2. Texture;
3. Odour.

2.7 Fluorescence Analysis of Plant Powder with Different Chemical Reagents

1. Take a pinch of plant powder and treat it with different chemicals separately.
2. Use the chemicals such as sodium nitroprusside, lead acetate solution, potassium hydroxide, 1 N NaOH, 1.5 N HCl, conc. H_2SO_4 , HNO_3 , 50 % H_2SO_4 and 0 % HNO_3 .
3. Then allow the mixture to stand at room temperature for 5 min.
4. Then filter it using Whatman No. 1 filter paper.
5. After this process, observe the colour changing behaviour of the plant powders under daylight and UV light (Kokoshi et al. [1958](#)).

2.8 Determination of PH of Plant Powder

1. Take 1 g of plant powder in the conical flask.
2. Add 10 mL of distilled water to the conical flask and blend it.
3. Then allow it to stand for 5 min at room temperature.
4. Measure the pH of sample using pH meter (Indian Pharmacopoeia [2010](#)).

2.9 Water Solubility Index (WSI) and Water Absorption Index (WAI)

1. Take 2.5 g of plant powder in a 50-mL centrifuge tube and add 30 mL of distilled water to it at 30 °C and stir intermittently for 30 min.
2. Then centrifuge for 10 min at $5100 \times g$.
3. Pour the supernatant carefully into a Petri dish and then allow both supernatant and pellet to dry overnight (Gomez [1984](#)).

Calculation

WSI = Amount of solid in the dried supernatant/weight of plant powder

WAI = Weight of dry solid/weight of plant powder

2.10 Acid Value

1. Take 1 g plant sample and then dissolve it in 50 mL of equal volume of ethanol (95 %) and petroleum ether.
2. Then filter the sample using Whatman No. 1 filter paper.
3. Then add few drops of phenolphthalein and then titrate it with 0.1 M potassium hydroxide until it remained faintly pink after shaking for 30 min.

Calculation

Acid value is calculated by the formula:

$$\text{Acid Value} = 5.61 \, n/W,$$

where n = number of mL of 0.1 M potassium hydroxide required and W = weight in grams of substance (Morkhade et al. 2006; Ohwoavworhwa and Adelakun 2005).

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<http://www.springer.com/978-3-319-26810-1>

Pharmacological Assays of Plant-Based Natural
Products

Thangaraj, P.

2016, XV, 188 p., Hardcover

ISBN: 978-3-319-26810-1