Research Article

PHYTOCHEMICAL ANALYSIS OF SEEDS OF CERTAIN MEDICINAL PLANTS

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ABSTRACT

Azardiracta indica (Meliaceae), *Trigonella foenum-graecum* (Fabaceae) and *Pongamia pinnata* (Fabaceae) are some of the ancient plants of great medicinal value. Physicochemical and phytochemical estimation was carried out on the seeds of these three crude drugs revealed the presence of some bioactive constituent, which are then qualitatively and quantitatively analyzed. The bitter components are extracted and isolated by TLC. The R_f values for nimbin, protodioscin and karanjin are 0.57, 0.73 and 0.53 respectively. The corresponding data obtained is comparatively similar with that of standard values. UV absorption spectra's of active components present in crude drugs showed the absorption peaks of nimbin at 217 nm, protodioscin at 366 nm and karanjin at 300 nm.

Keywords: Crude drugs, Nimbin, Protodioscin, Karanjin, Thin layer chromatography, UV Spectroscopy.

INTRODUCTION

The term 'crude' drug generally applies to the products from plant and animal origin found in a raw form. The crude drug is referred in relation to the natural product that has not been advanced in value or improved in condition by any process or treatment beyond that which is essential for its proper packing and prevention from deterioration¹. Neem (*Azadirachta indica*) is a well known in the Indian subcontinent. It is belonging to Meliaceae family is very important medicinal plant which is traditionally used to treat different diseases². All parts of Neem have lot of applications in medical treatment and industrial products³. Neem is bitter in taste and this bitterness is due to an array of complex compounds called "triterpenes"⁴ called Nimbin ($C_{30}H_{36}O_9$) it is representative of ring C seco-tetra-nor-triterpenoids, is bitter principle isolated from seeds⁵. *Trigonella foenum-graecum* commonly known as methi (Hindi) is cultivated all over the world and generally distributed in India, North Africa, and Eastern Mediterranean. It is reported to contain several possible active chemical constituents such as alkaloids, saponins, steroids, tannins, flavonoids, amino acids and trigonillin⁶. Its leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses⁷. The seeds contain protodioscin ($C_{15}H_{84}O_{22}$) compound which is a steroidal saponin found in a number of plant species, and responsible for the bitterness in the fenugreek seed^{8,9}. *Pongamia pinnata* is a medium size glabrous tree, belongs to family Fabaceae (Papilionaceae), popularly known as Karanj or Karanja in Hindi. It is widely distributed in India, Bangladesh, China, Florida, Hawaii, India, Malaysia. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible for the various activities of the plant¹⁰. The seeds have a bitter active compound which is Karanjin ($C_{18}H_{12}O_4$) a furanoflavanoid¹¹.

MATERIALS AND METHOD

Collection of plant seeds

Seeds of *Azadirachta indica*, *Trigonella foenum-graecum* and *Pongamia pinnata* were collected from the surrounding of

Vidisha in different seasons. Identification of seeds were carried out by Dr. Jagrati Tripathi H.O.D of Department of Botany, Unique College, Bhopal M.P., India. The healthy seed samples were used for the qualitative and quantitative analysis.

Solvent Extraction

200 g of *Azadirachta indica* seeds powder was taken and subjected to soxhlet extraction by hexane as a solvent for 8 hours¹². 200 g of *Trigonella foenum graecum* seeds was taken and grinded to obtain Coarse particles was subjected to soxhlet extraction by methanol for 6 hours¹³. 200 g of *Pongamia pinnata* seed powder was taken and subjected to soxhlet extraction by methanol as a solvent for 12 hours¹⁴. After extraction, its extract was filtered and the solvent was evaporated completely by using rotary evaporator. All the crude extracts were filtered using filter paper to obtain particle-free crude extract.

Physicochemical and Phytochemical Screening

The dried seed powder was subjected to Physicochemical and phytochemical tests to assess the qualitative and quantitative chemical composition of different crude bitter drugs by using standard methods. Moisture content acid insoluble ash, total ash, water soluble ash, was determined¹⁵⁻¹⁹. The percentage of Magnesium, Iron, Phosphorous^{17,20-22} and the carbohydrate, protein and Crude fibre were determined^{16,17,21,23-25}. Phytoconstituents²⁶⁻²⁸ were analysed by methods given below:

Test for Alkaloids

Take 0.1 ml extract in a test tube, add one drop of meyer's reagent. It gives a cream precipitate with alkaloids.

Test for flavanoids

Take 1 ml of extract in a test tube, add 5 ml of dil. Ammonia followed by add few drops of concentrated sulphuric acid. A yellow coloration is appears. It is the indication of flavanoids compounds of drug.

Test for Saponin

Take 2 g of the powdered sample and boil with 20 ml of distilled water in a water bath and filter it, 10 ml of filtrate is mix with 5 ml of distilled water and add 3 drops of olive oil and shake vigorously, then observe for the formation of emulsion.

Test for Essential oil/Volatile oil

Crush a small sample of the crude drug between the thumb and forefinger, and examine for the presence of an odour.

Tests for Starch

Take 1 g of dry powder in 50 ml of water boil for one minute and cool, thin and cloudly mucilage is produced, which gives thick and more transparent mucilage. To 10 ml of the mucilage add 0.05 ml of 0.01M Iodine, a dark blue colour is produced, which disappears on heating and reappears on cooling.

Test for Terpenoids (Salkowski test)

Take five ml of extract, mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) is added to form a layer. A reddish brown colouration on the inner face is formed. It indicates the presence of terpenoids.

Test for Tannins

Take 2 ml of extract in a test tube. Add few drops of 0.1 % ferric chloride and observe for brownish green or a blue-black coloration.

Test for Steroids

To 2 ml of extract add 2 ml chloroform and 2 ml conc. H_2SO_4 . Shake well, chloroform 1 layer appear red and acid layer show greenish yellow florescence.

Separation and Identification of Components

Isolation of bitter component was carried out by thin layer chromatography. Different solvents were used for the separation of bitter components from seeds. TLC^{10,29,30} and UV methods^{11,30,31} were used for the isolation and identification of Nimbin, Protodioscin, Karanjin from seed extracts. The identification of Nimbin, Protodioscin and Karanjin content was done by U.V Spectrophotometer. For stock solution, pipette out 2 ml of filtered *Azadirachta indica* seed extract and dilute to 25 ml, by methanol. From stock solution pipette out 1 ml and dilute to 25 ml. Measure the absorbance of the standard solution and extract at 217 nm against methanol as a blank. Similarly the stock solutions are prepared for the *Trigonella foenum-graecum* and *Pongamia pinnata* seeds. But, the diluted 10 times for *Trigonella foenum-graecum* seed extract and 4 times for *Pongamia pinnata* seeds extract. The absorbance of the *Trigonella foenum-graecum* and *Pongamia pinnata* standard solutions are measured at 366 nm and 300 nm against methanol as a blank.

Nimbin³² Protodioscin⁹ Karanjin¹⁰**Table 1: Yield of crude extracts**

S. No.	Name of Plant	Weight of Powder taken	Solvent used	Yield	% Yield
1	<i>Azadirachta indica</i>	200 g	Hexane	65 g	32.5 %
2	<i>Trigonella foenum-graecum</i>	200 g	Methanol	58 g	29 %
3	<i>Pongamia pinnata</i>	200 g	Methanol	68 g	34 %

Table 2: Physicochemical Evaluation of crude drug of medicinal plants

S. No.	Test	<i>A. indica</i>	<i>T. foenum graecum</i>	<i>P. pinnata</i>
1	Colour	Pale yellow	Dark brown	Dark reddish brown
2	Odour	Bitter	Bitter	Bitter
3	Taste	Garlic like	sweet smell	Dissagreeble smell
4	Total ash	7.8 %	6.7 %	2.8 %
5	Acid insoluble ash	1.87 %	1.48 %	0.1 %
6	Moisture Content	6.8 %	9.2 %	19.1%
7	Water soluble ash	21.4 %	34.67 %	19.8 %
8	Carbohydrates	5.21%	44.5 %	10.79 %
9	Protien	55.12 %	27.95 %	17.325 %
10	Crude fibre	34.2 %	4.5 %	4.95 %
11	Iron	970 ppm	11.5 mg/100 g	0.1 %
12	Phosphorus	0.37 %	78.5 mg/100 g	0.6 %
13	Magnesium	0.31 %	200 mg/100 g	0.61 %

Table 3: Phytochemical Evaluation of the crude drug of medicinal plants

S. No.	Tests	<i>A. indica</i>	<i>T. foenum graecum</i>	<i>P. pinnata</i>
1.	Alkaloid	+	+	+
2	Flavanoids	+	+	+
3	Saponin	+	+	+
4	Essential Oils	+	+	+
5	Starch	+	+	+
6	Terpenoids	+	+	+
7	Tannin	+	+	+
8	Steroid	+	+	+

RESULT AND DISCUSSION

In the present study the different plant seeds were analyzed. For the determination of the various elements, powder of the seeds and extract were qualitatively and quantitatively analyzed. The findings of the physicochemical and phytochemicals test results were depicted in the respective tables. The Characterization of the isolated Nimbin, Protodioscin and Karanjin was done by TLC and UV Spectroscopy. The R_f values were obtained from TLC of bitter components matched with the standards and the results found to be within the prescribed range. The R_f values of Nimbin (1:10), Protodioscin (1:20) and Karanjin (1:30) are

found to be 0.57, 0.73 and 0.53 respectively. The UV absorption maxima of isolated compounds, nimbin, protodioscin and karanjin were recorded using methanol as a solvent. A U.V spectrum of the isolated compounds shows peak at almost same wavelength and intensity with standards. Nimbin peak obtained at 217 nm, Protodioscin at 366 nm and karanjin at 300 nm. Nimbin⁵, Protodioscin^{8,9}, and Karanjin¹¹ are responsible for bitter taste in seeds. The seeds of *Azardiracta indica*, *Trigonella foenum-graecum* and *Pongamia pinnata* showing various pharmacological activity^{3,7,10}. The graphs of U.V absorption spectrum has been shown below:

Figure 1: U.V peak of nimbin in *Azardiracta indica* seed extract at 217 Figure 2: U.V peak of Protodioscin in *Trigonella foenum graecum* seed extract at 366 nm Figure 3: U.V peak of Karanj in *Pongamia pinnata* seed extract at 300 nm

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