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Physiochemical and Photochemical Transmission of Plant Justicia Adhatoda Meandering

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Abstract

Ayurveda–Ancient Science of Life is believed to be prevalent for last 5000 years in India. It is one of the most noted systems of medicine in the world (Kokate et al., 2007). Atharveda (around 1200 BC), Charak Samhita and Sushrut Samhita (100-500 BC) are the main classics that give detailed descriptions of over 700 herbs. Researches on pharmacognosy, chemistry, pharmacology and clinical therapeutics have been carried out on Ayurvedic medicinal plants and many of the major pharmaceutical corporations have renewed their strategies in favour of natural products drug discovery.

Keywords: Carbohydrate, Glycosides, Tannins, Lipids, Alkaloids.

Introduction

Herbal medicines are the oldest remedies known to mankind. Man's dependence on plants for health care is as old as the existence of mankind on this planet. India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. There are very few medicinal herbs of commercial importance, which are not found in this country. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects.

Standardization

Standardization is the process of delivering a product with a specified minimum level of one or more phytoconstituents, where we can make sure about the quality of the product; broadly it covers the qualitative and quantitave part of analysis (Harborne, 1998). Standardization is adjusting the herbal drug preparation to a defined content of the active constituent. It refers to the process of delivering a product with a specified minimum level of one or more plant constituents.

Need of Standardization

Depending upon whether the active principle of the plant is known or not, different concepts ('normalization' vs. 'standardization') have to be applied in order to establish relevant criteria for uniformity (Rudolf, 1998). Reproducible efficacy and safety of phytopharmaceuticals is based on reproducible quality. compound in a plant mixture**.**

Attributes of Standardization

Three attributes are desirable for standardization:

- **Authenticity:** It relates to proving that the material is true and corresponds to the right identity. It involves many parameters like gross morphology, microscopy, chemical analysis etc.
- **Purity:** It pertains to evaluate that there are no adulterants present in the plant material. It can be evaluated by pharmacognostic studies.
- **Assay:** This part of standardization is chemical and biological profiling by which chemical and biological effects can be assessed and curative values are established.

Chemical Constituents

Chemical constituents of Rauwolfia serpentina are **indole** alkaloids.It contains at least 30 alkaloids, which total some 0.7 – 2.4 %. Other substances present include phytosterols, fatty acids, unsaturated alcohols and sugars.These are esters derived from methyl reserpate and trimethoxy benzoic acid in the case of reserpine and trymethoxy cinnamic acid in the case of rescinnamine.

Medicinal And Traditional Uses

Ajmaline is a class Ia anti arrhythmic agent. ,Rauwolfia serpentina alkaloids are also used as a tranquilizer and Anti hypertensives., Reserpine is used to treat high blood pressure and mental disorders including schizophrenia. It has been used as an antihypertensive and an antipsychotic as well as a research tool, but its adverse effects limit its clinical use, papaverine is a direct-acting smooth muscle relaxant used in the treatment of impotence and as a vasodilator.

Materials and Method

Foreign matter analysis

A 100 g of the plant material was spread in a thin layer and the foreign matter was sorted into groups by visual inspection and using a hand lens. The remainder of the sample was sifted through a no. 250 sieve; dust was regarded as mineral admixture. The sorted foreign matter was weighed. The content of each group was calculated in grams per 100 g of air dried sample. The observations were recorded in Table 4.2.

Extractive Values

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.

Successive extractive value

The powdered material of the drug (50 g) was packed in a Soxhlet apparatus and was subjected to successive extraction with different solvents like Petroleum ether, Ethyl acetate and methanol. Then it was filtered rapidly taking precaution against the loss of solvent. 25 ml of filtrate was evaporated to dryness in a tarred bottom china dish. It was then dried at 105 °C and weighed. The percentage of solvent soluble extractive with reference to air dried drug was calculated. The observations were recorded in Table.

Loss on Drying (LOD)

2.0 gm of powder was accurately weighed in a petridish and kept in a hot-air oven maintained at 105^0C for four hours. After cooling in a desiccator, the loss in weight was recorded.

This procedure was repeated till constant weight was obtained.

The ash remaining following ignition of medicinal plant materials is determined by 3 different methods which measure total ash, acid insoluble ash and water soluble ash.

The **total ash** is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash” which is derived from the plant tissue itself and “non-physiological ash” which is the residue of the extraneous matter (sand and soil) adhering to the plant surface.

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

A. Total ash

Accurately weighed 2g of ground dried material was taken in a previously ignited and tared crucible (usually of platinum and silica). The material was spreaded in an even layer and ignited by gradually increasing the heat to 500-

600^0C until it was white, indicating the absence of carbon. Then it was cool in desiccators and weighed. The content of total ash was calculated in mg per g of air dried material.

B. Acid- insoluble ash

To the crucible containing total ash, 25ml of hydrochloric acid was added. Crucible was covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper (Whatman No. 41) and was washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccators for 30 minutes, and was weighed. The content of acid-insoluble ash was calculated in mg per gm of air dried material.

C. Water- soluble ash

To the crucible containing the total ash, 25ml of distilled water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible or on an ash less filter-paper. It was washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^0 C. The weight of this residue in mg was subtracted from the weight of total ash. The content of water soluble ash was calculated in mg per gm of dried material.

D. Sulphated ash value

Take about 2g, accurately weighed crude drug powder in a tared platinum or silica crucible previously ignited and weighed. The drug is treated with dil. Sulphuric acid before ignition. In this all oxides and carbonates are converted to sulphates and ignition is carried out at a higher temp. (600^0c). after ignition then it was cool in a dessicator and weighed. The content of sulphated ash was calculated in mg per g of air dried material.

Phytochemical Screening

Methanolic extract was subjected to preliminary phytochemical investigation for detection of Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Proteins and Amino acids, Saponins, Phytosterols, Acidic compounds, Resins and Reducing sugars.

Extraction, Fractionation, Isolation of Chemical Constituents and Standardization

Extract preparation: he coarsely powdered aerial roots of Rauwolfia serpentina were extracted with methanol. The method of preparation is described below.

Methanolic extract

Dry Plant of Rauwolfia serpentina was ground to coarse powder. The coarsely powdered aerial parts (800mg) were packed in a percolator, soaked in a methanol (15litres) and kept for one week. The extract was drained, filtered and concentrated under reduced pressure using rotary film evaporator. The extraction process was repeated three times more under similar conditions. The combined extract was finally dried in vacuum desiccators and weighed.

Fractionation of methanolic extract

The dried methanolic extract was dissolved in (750ml) distilled water. The solution thus formed was defatted with n-Hexane (5 litres). The aqueous extract was dissolved in 10% acetic acid to adjust the pH 3.5 and extracted with chloroform three times (3×2liter) to yield alkaloid fraction(A1).The acidic aqueous filtrate thus obtained was basified to pH 9-10 by adding ammonium hydroxide and extracted with chloroform (3×2liter) to afford crude alkaloid gummy fraction(A2).

Compounds were isolated from the Ethyl acetate extract by using column chromatography and fractions were monitored on TLC.

Slurry Formation

Dried Ethyl acetate soluble extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent .The adsorbed extract was then charged into the column.

Elution of the column

The column was first eluted with petroleum ether. Then column was eluted with the solvent by gradually increasing the percentage of ethyl acetate in petroleum ether.

Each fraction of 250 ml was collected and concentrated on rotavapour. A total of 222 fractions were collected and TLC of all 222 fractions were done using different developing solvents. The fractions were pooled on the basis of the TLC pattern shown by them.

Developing solvents used for the TLC in various proportions

1. chloroform: methanol
2. Pet ether: acetone: diethyl amine

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

Preparation of Dragondorff's reagent:

A. Bismuth sub nitrate (0.85g) + water (20ml) + add 2 drops of conc. Nitric acid and heat till mixture become homogenous

B. Potassium iodide 8g dissolved in 20ml of water.

Mix 5ml of A & 5ml of B + 20ml acetic acid.

Fractions 38-59 eluted in 15% ethyl acetate in petroleum ether, which gives pure compound in TLC plate. Dry at rotavapour and subjected to further process and its melting point was found to be 195 - 204◦C.

Fractions 84 - 116 eluted in 20% ethyl acetate in petroleum ether, which on concentration and filtration yielded a solid mass, which was re-crystallized in methanol to give a crystalline compound. The melting point of the compound was found to be 147˚C. The compound was identified as **Ajmaline**, on the basis of TLC pattern, melting point and spectroscopic data in comparison with reported data of **Ajmaline** in the literature.

Chromatography of Basified Extract

Isolation of Markers

Compounds were isolated from the Basified extract by using column chromatography and fractions were monitored on TLC.

Slurry Formation

Dried basified extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent .

Elution of the column

The column was first eluted with petroleum ether. Then column was eluted with the solvent by gradually increasing the percentage of ethyl acetate in petroleum ether.

Each fraction of 250 ml was collected and concentrated on rotavapour. A total of 222 fractions were collected and TLC of all 222 fractions was done using different developing solvents. The fractions were pooled on the basis of the TLC pattern shown by them.

Developing solvents used for the TLC in various proportions

1. chloroform: methanol
2. Pet ether: acetone: diethyl amine

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

Preparation of Dragondorff's reagent:

(A) Bismuth sub nitrate (0.85g) + water (20ml) + add 2 drops of conc. Nitric acid and heat till mixture become homogenous

(B) Potassium iodide 8g dissolved in 20ml of water.

Mix 5ml of A & 5ml of B + 20ml acetic acid.

Fractions 35-40 eluted in 50% ethyl acetate in petroleum ether, which gives pure compound in TLC plate. Dry at rotavapour and subjected to further process. The melting point of the compound was found to be 147˚C. The compound was identified as **β sitosterol**, on the basis of TLC pattern, melting point and spectroscopic data in comparison with reported data of **β sitosterol** the literature

Fractions 41- 115 eluted in 100% ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound.

Fractions 141- 162 eluted in 10% methanol in ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Fractions 163- 198 eluted in 20% methanol in ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Chromatography of Basified and centrifuged Extract

Isolation of Markers

Compounds were isolated from the Basified and centrifuged extract by using column chromatography and fractions were monitored on TLC.

Slurry Formation

Dried basified and centrifuged extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent .

Elution of the column

The column was first eluted with petroleum ether. Then column was eluted with the solvent by gradually increasing the percentage of ethyl acetate in petroleum ether.

Each fraction of 250 ml was collected and concentrated on rotavapour. A total of 330 fractions were collected and TLC of all 330 fractions was done using different developing solvents. The fractions were pooled on the basis of the TLC pattern shown by them.

Developing solvents used for the TLC in various proportions

1. Chloroform: methanol
2. Pet ether: acetone: diethyl amine

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

Preparation of Dragondorff's reagent:

(C) Bismuth sub nitrate (0.85g) + water (20ml) + add 2 drops of conc. Nitric acid and heat till mixture become homogenous

(D) Potassium iodide 8g dissolved in 20ml of water.

Mix 5ml of A & 5ml of B + 20ml acetic acid.

Fractions 43- 60 eluted in 40% ethyl acetate in petroleum ether , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Fractions 191- 200 eluted in 70% ethyl acetate in petroleum ether , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Fractions 201- 225 eluted in 80% ethyl acetate in petroleum ether , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Fractions 226- 245 eluted in 100% ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Chromatography of Acid soluble Extract

Isolation of Markers

Compounds were isolated from the Acid soluble extract by using column chromatography and fractions were monitored on TLC.

Slurry Formation

Dried Acid soluble extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent .The adsorbed extract was then charged into the column.

Elution of the column

The column was first eluted with chloroform. Then column was eluted with the solvent by gradually increasing the percentage of methanol.

Developing solvents used for the TLC in various proportions

1. chloroform: methanol
2. Pet ether: acetone: diethyl amine

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

Preparation of Dragondorff's reagent:

(E) Bismuth sub nitrate (0.85g) + water (20ml) + add 2 drops of conc. Nitric acid and heat till mixture become homogenous

(F) Potassium iodide 8g dissolved in 20ml of water.

Mix 5ml of A & 5ml of B + 20ml acetic acid.

Fractions 35-40 eluted in 50% ethyl acetate in petroleum ether, which gives pure compound in TLC plate. Dry at rotavapour and subjected to further process. The melting point of the compound was found to be 147˚C. The compound was identified as **β sitosterol**, on the basis of TLC pattern, melting point and spectroscopic data in comparison with reported data of **β sitosterol** the literature

Fractions 41- 115 eluted in 100% ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Fractions 116- 140 eluted in 5% methanol in ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Fractions 141- 162 eluted in 10% methanol in ethyl acetate , which on concentration and filtration yielded a solid mass,

which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Chromatography of Basified and centrifuged Extract

Isolation of Markers

Compounds were isolated from the Basified and centrifuged extract by using column chromatography and fractions were monitored on TLC.

Slurry Formation

Dried basified and centrifuged extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent .The adsorbed extract was then charged into the column.

Weight of silica gel – G used (column)	100 gm
Diameter of column used	4 cm
Length of column used	95 cm

Fractions 246- 266 eluted in 5% methanol in ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Fractions 267- 299 eluted in 10% methanol in ethyl acetate , which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Slurry Formation

Dried Acid soluble extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent .The adsorbed extract was then charged into the column.

Elution of the column

The column was first eluted with chloroform. Then column was eluted with the solvent by gradually increasing the percentage of methanol.

Developing solvents used for the TLC in various proportions

1. Chloroform: methanol
2. Pet ether: acetone: diethyl amine

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with **Dragondorff'S reagent**

Fractions 14- 18 eluted in 5% methanol in ethyl acetate, which on concentration and filtration yielded a solid mass, which was recrystallized in methanol to give a crystalline compound. Then the compound was found to be a mixture of compounds on the basis of HPLC studies.

Results And Discussion

In the present study, Isolation of chemical constituents and standardization of plant extracts of Rauwolfia serpentina was done, Rauwolfia serpentina is the well-known medicinal plant used from the ancient era to till date for their medicinal values. Standardization was done on the basis of marker compounds isolated from the plant extracts. The plant was collected and analyzed.

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