PHYTOCHEMICAL EXAMINATION ON LEAVES OF DIFFERENT PLANTS

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Abstract : A wide range of medicinal value is provided by the natural products which are obtained from different plants. The study of traditional medicinal plants and their therapeutic properties plays a very important role in chemistry of natural products. Due to the increasing therapeutic use of natural products interest in this area is also increasing. The focus of this work is on methodologies that include extraction, separation and identification of natural products. The work was carried out on extraction of Piper betle and Justicia gendarussa leaves by three different solvents and then the identification tests of the chemical constituents were done. Different techniques such as chromatography i.e. Column chromatography and Thin Layer Chromatography (TLC) as well as spectroscopic techniques were used for identification of compounds.

Key words : Column Chromatography, Thin layer chromatography, UV-Vis

I. Introduction

Herbal plants cover a broad range of plant taxa and closely related species. There is a wide growth in international market for medicinal plants, which are used equally for herbal drug and for pharmaceutical products. As the natural medicines are reported to be much safer than synthetic drugs, they have gained popularity in recent years, leading to an incredible growth of pharmaceutical usage. India is a rich source of medicinal plant. A variety of range of medicinal plants are grown here by adopting different methods.

Herbal plants are used to cure most diseases in humans and animals. The medicinal value of the plant could be attributed to the phytochemicals such as primary and secondary metabolites. These phyto-constituents are being used as drugs in many pharmaceutical companies. Justicia gendarussa is a rare medicinal plant, which grows in Asian countries like India, Malaysia, Indonesia and Srilanka. Leaf, stem and roots of the plant are frequently used as traditional herbal medicine against some common diseases such as rheumatism, fever, cough, jaundice, thrush, arthritis, cephalgia, hemiplegia, facial paralysis, otalgia, hemicrania, bronchitis, liver and kidney disorders. Its leaves are widely used in India since ancient age for medication.

a. PIPER BETLE LEAVES :

The leaves of Piper betle plant are normally called “Paan” in India. It is one such plant which possess high medicinal importance. Leaves of Piper betle are stimulant, antiseptic and sialogogue which promotes secretion of saliva. It is an active local stimulant used in the treatment of respiratory inflammation of mucus membrane. It is also used as a local application or gargle, also as an inhalant in diphtheria. The fresh leaves and the fresh juice and the oil of betle vine have aromatic, carminative and astringent properties. The warm leaves form a valuable application to the chest in cases of bronchial difficulty, and are applied to the mammae to check the secretion of milk. Due to its properties, the plant Piper betle was undertaken and studied.

Fig. 1: Piper betle leaves

Medicinal Uses (Traditional):

The liquid of boiling betle leaf and decoction of ginger with a little amount of rock salt are given for remedial using in hacking cough, whooping cough and asthma. Salt packed with betle leaf is baked and made into powder. It is taken for coughing. Slightly heated betle leaf smeared with coconut oil is applied on the fontanelle in an infant for coryza and also applied in layers over chest, especially of a child for the treatment of cough, pulmonary affections and bronchitis. The decoction of the betle leaves is used as eye drops in ophthalmic and other painful eye diseases and night blindness. The fresh leaves applied externally around the eyes are also useful in eye diseases. Betle petiole dipped in castor oil is used as a suppository for constipating infants.

In fusion of betle leaf juice and honey are given to children for therapeutic uses in fever, flatulence and digestive disorders. The leaves are chewed to reduce bad breath, to remove foul odour from mouth and to improve the voice. It is widely used in India, Southeast Asia and other subcontinents for its stimulant and psychoactive effects. It is combined with areca nut, slaked lime paste and other mouth freshners and then chewed to freshen the breath which is traditionally known as *"Paan"*.

b. JUSTICIA GENDARUSSA :

Justicia gendarussa is a rare medicinal plant, which grows in Asian countries like India, Malaysia, Indonesia and Srilanka. Leaf, stem and roots of the plant are frequently used as traditional herbal medicine against some common diseases such as rheumatism, fever, cough, jaundice, thrush, arthritis, cephalgia, hemiplegia, facial paralysis, otalgia, hemicrania, bronchitis, liver and kidney disorders. Its leaves are widely used in India since ancient age for medication. The leaves are commonly known as "*Ardusi*" or "*Ardusso*" in India. The major active components of the justicia gendarrusa leaves are the flavonoids, sitosterols, alkaloids and reducing sugars.

Fig. 2: Justicia gendarussa leaves

Medicinal Uses (Traditional) :

Herbal medicine plays a major role in the development of modern civilization. In Ayurveda, the plant is useful for the treatment of inflammation, bronchitis, vaginal discharges, eye infections and fever. The decoction of the leaves and tender shoots are diaphoretic and they are given in chronic rheumatism. Oil prepared from the leaves is useful in eczema, and the mixture of leaves is given internally for hemiplegia, cephalalgia and facial paralysis. Leaves, stem and roots of the plant are frequently used as traditional herbal medicine against some common diseases such as rheumatism, fever, cough, jaundice, arthritis, cephalgia, hemiplegia, facial paralysis, hemicrania, bronchitis, liver and kidney disorders. Justicia gendarussa is an interesting example of plant having traditional medicinal value for many years and have been proved by many research works.

II.Research Methodology : The initial analysis is done by conducting phytochemical tests on the different solvent extracts of the plant for the detection of the chemical constituents present in it. To study the Ethyl acetate extract in detail by using column chromatography and TLC followed by elucidation of structure of the chemical compounds present on the basis of the physical properties viz. melting points and other spectroscopic techniques.

III.Materials and method:

- **Plant Material :**

Piper betle leaves were purchased from local market of Vadodara.

Justicia Gendarussa leaves were collected from local farm of Vadodara , Gujarat , India.

They were identified and authenticated by Parul Institute of Agriculture, Vadodara, India.

- **Preparation of plant material :**

The solvents used for extraction were **Ethyl acetate, Methanol and Water.**

Fresh leaves of Piper betle and Justicia gendarrusa were collected and dried at room temperature for 7 days. 15g of powdered material was packed in soxhlet apparatus and extraction was carried out by 250ml of different solvents along with few tiny pieces of porcelain and temperature was maintained at 60°C for 24 hours.

IV.Procedure:

Tests conducted on the solvent extracts:

- **SHINODA TEST**: Yellow coloration is observed when few drops of NaOH was added to 1 ml extract. On further addition of few drops of dilute HCl it turns colourless. This indicates presence of **FLAVANOIDS.**

- **WAGNERS TEST**: A fraction of extract was treated with 5 drops of Wagner's Reagent (1.2 g of iodine and 2g of KI in 100ml of water) and observed for the formation of brown precipitate or coloration. This indicates the presence of **ALKALOIDS.**

- **PHENOL TEST** : A fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black precipitates or coloration. The change in colour indicates the presence of **PHENOL.**

- **MOLISCH TEST** : Few drops of Molisch reagent (alpha napthol + ethanol) was added to extract. This was followed by the addition of concentrated sulphuric acid. The mixture was allowed to stand for a few minutes. Formation of red or dull violet colour at interphase of two layers indicates the presence of **CARBOHYDRATES**.

- **QUINONES TEST :** A small amount of extract was treated with concentrated HCl and observed. Formation of yellow precipitates or coloration indicates the presence of **QUINONES.**

- **SAPONIN TEST** : A fraction of the extract was taken and filtered with filter paper in a test tube. Five ml of water was added to the test tube and was vigorously shaken. Appearance of froth on the top of the solution indicates the presence of **SAPONINS.**

- **GLYCOSIDE TEST** :A fraction of the extract was taken and 10% Lead acetate was added to it. Appearance of yellow or white precipitates indicates the presence of **GLYCOSIDES.**

- **TANNIN TEST:** 2-4 ml of the extract was taken and treated with 10% alcoholic ferric chloride. Appearance of blue or green precipitates indicates the presence of **TANNINS**.

- **AMINO ACID TEST:** 2-4ml of extract was treated with 2-4 drops of Ninhydrine and then kept in water bath. Appearance of pink colour indicates the presence of **AMINO ACIDS**.

Column chromatography (CC):

Column chromatography is a technique used to isolate chemical compounds from a mixture. It is a method used to separate substances based on differential adsorption of compounds to the adsorbent; compounds move through the column at different rates which allows them to get separated into fractions. This technique is widely applicable because it can be used with a wide range of solvents according to their polarities.

Experimental procedure:

- The packing of column is done by wet method. The extract of Ethyl acetate was concentrated and slurry was made with silica gel (60-120 mesh) and eluent petroleum ether and then kept in water bath for few minutes. The column was prepared by packing the column with cotton at the bottom. The stationary phase silica gel was used by making its slurry with petroleum ether. The silica gel slurry is added first and then the slurry of the extract was added and packed with cotton at the top.

- Petroleum ether is used as the first solvent according to its least polarity.

- Pure petroleum was used as the first solvent followed by mixture of 90% petroleum ether – 10% Benzene. Successive mixtures containing 20%, 30%, 40% followed till 100% Benzene, Ethyl acetate and acetone respectively complete the transition of the polarity.

  Solvents selected were in following order

  Petroleum ether < Benzene < Ethyl acetate < Acetone < Methanol < Water

- The fractions were collected from the column and then distilled. The concentrated samples were collected in the test tube and then TLC for components were done.

- The samples were again concentrated in the water bath and then crystallized with solvents. Melting point of the obtained pure compounds was determined in the laboratory.
- During the column chromatography, chemical compounds were obtained from the different fractions of the plant extracts. They were collected, purified & recrystallized by solvent Ethanol and then further analysis like determination of MP, IR and NMR were done.

Fig. 3: Elution of column by different solvent systems of Piper betle extract

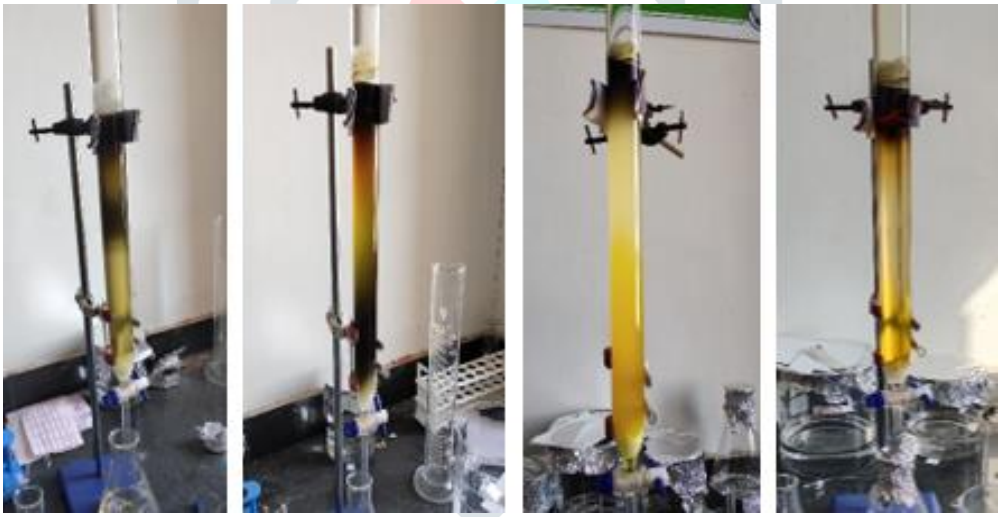

Fig. 4: Elution of column by different solvent systems of Justicia gendarussa extract

V. Result

Table No.1 : Test results of the Ethyl acetate extract

S.N.	Tests conducted	Piper betle	Justicia gendarussa
1.	**Shinoda test**	+	+
2.	**Wagners test**	-	-
3.	**Phenol test**	-	-
4.	**Molisch test**	+	-

5.	**Quinones test**	+	+
6.	**Saponin test**	-	-
7.	**Glycoside test**	-	-
8.	**Tannin test**	-	-
9.	**Amino acid test**	-	-

Table No.2 : Test results of the Methanol Extract

S.N.	Tests conducted	Piper betle	Justicia gendarussa
1.	**Shinoda test**	+	+
2.	**Wagners test**	+	+
3.	**Phenol test**	+	+
4.	**Molisch test**	-	-
5.	**Quinones test**	-	-
6.	**Saponin test**	+	+
7.	**Glycoside test**	-	-
8.	**Tannin test**	-	-
9.	**Amino acid test**	-	-

Table No.3 : Test results of the Water extract

S.N.	Tests conducted	Piper betle	Justicia gendarussa
1.	**Shinoda test**	+	-
2.	**Wagners test**	+	+
3.	**Phenol test**	+	-
4.	**Molisch test**	-	+
5.	**Quinones test**	+	-
6.	**Saponin test**	+	+
7.	**Glycoside test**	-	-
8.	**Tannin test**	+	+
9.	**Amino acid test**	+	+

- During the CC of the Ethyl acetate extract of the plants following chemical compounds were extracted.
- From the column of Piper betle leaves extract, Ascorbic acid and Riboflavin were isolated.
- From the column of Justicia gendarrusa leaves extract, Oleic acid was isolated.

Fig.5 : Ascorbic acid

Fig.6 : Riboflavin

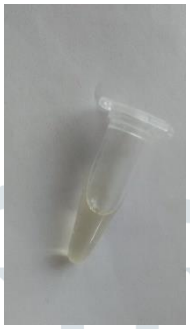

Fig.7 : Oleic acid

VI. Conclusion

- During the phytochemical examination of the Ethyl acetate extract of Piper betle leaves; Flavonoids, Carbohydrate and Quinones were detected while Glycosides, Alkaloid, Phenol, Saponins, Tannins and Amino acids were found to be absent. The tests on Methanol extract of Piper betle leaves shows presence of Flavonoids, Alkaloid, Phenol and Saponins. The tests on Water extract of Piper betle leaves shows the presence of Flavanoids, Alkaloid, Phenol, Quinones, Saponins, Tannins and Amino acids. Ascorbic acid and Riboflavin were separated by column chromatography and their structure was established by comparing it with the original sample.

- During the phytochemical examination of the Ethyl acetate extract of Justicia gendarussa leaves; Flavonoids and Quinones were detected while Glycosides, Alkaloid, Phenol, Saponins, Carbohydrates, Tannins and Amino acids were found to be absent. The tests on Methanol extract of Justicia Gendarussa leaves showed the presence of Alkaloid and Saponins. The tests on Water extract of Justicia Gendarussa leaves shows the presence of Alkaloid, Carbohydrates, Glycosides, Saponins, Tannins and Amino acids. Oleic acid was separated by column chromatography and its structure was established by comparing it with the original sample.

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