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Preliminary phytochemical screening of Ankola seed

Dr. Poonam Sharma

Abstract
Phytochemical screening is very important for identifying the quality as well as medicinal importance of particular plant. Ankola (*Alangium lamarckii*. Thwaites) an ancient plant described in Vedas, Samhitas and in various nighantus is a very important medicinal plant. Ethnobotanically it is used in many diseases viz. wound healing, diabetes, skin diseases, constipation and various other diseases. It is described in Susruta Samhita in vrana chikitsa for removing the slough of wound. So here we are using it for treatment of vrana and so we are standardizing it for its identity, purity and to prove therapeutic action as well. Research work was carried out to find the phytochemical constituents and its wound healing property. Dried seeds of Ankola were taken and then grinded to make fine powder of it, later its petroleum ether extract made in Soxhlet apparatus along with water extract. Phytochemical screening was done for identifying the bio active substances as such flavonoids, alkaloids, carotenoids, tannin, antioxidants etc. TLC study was done with petroleum ether extract and water soluble extract.

Keywords: Phytochemical, flavonoids, carotenoids

Introduction
The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug [1]. It defines the screening, extraction and identification of the medicinally important active ingredients in the plant. Plants contain various chemicals. These are produced either by primary or secondary metabolism [2]. They help in plant growth and protect them from pathogens, predators and enemy [3].
Mostly they are termed as research compounds because their medicinal importance depends on their phytochemicals [4, 5]. They are classified into two major categories – 1) Carotenoids, [6] 2) Polyphenols, it contains flavonoids, phenolic acid and lignans. For phytochemical screening firstly their extract is extracted in various solvents, here we are collecting extract in petroleum ether and water.
General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them.
"The concept of standardization and quality control of drug can be found in ancient Ayurvedic texts. Assessment of complete and accurate physicochemical value of Ayurvedic herbs not only provides scientific basis of its quality but also helps in globalization of Ayurveda. Under these circumstances, pharmacognosy, pharmacology and phytochemistry are necessary for authentication of crude drug and to prove therapeutic action as well. Ankola (*Alangium lamarckii* Thwaites.) is well known plant in Ayurveda. It has been mentioned in Brihatrayi and later it has been described in Nighantu. So a preliminary phytochemical study on Ankola was done.

Materials and methods
1. Alkaloids
a) Dragendorff's test: Dissolve a few mg of alcoholic or aqueous until an acid reaction occurs, then add 1 ml of Dragendorff's reagent, an orange or orange-red precipitate is produced immediately.
b) Hager's test: 1 ml of alcoholic extract of the drug was taken in a test tube, adding a few drops of Hager's reagent. Formation of yellow precipitate confirms the presence of alkaloids.
c) Wagner's test: Acidifying 1 ml of alcoholic extract of the drug with 1.5% v/v of hydrochloric acid and adding a few drops of Wagner's reagent. A yellow or brown precipitate is formed.
d) Mayer's test: Adding a few drops of Mayer's reagent to 1 ml of acidic aqueous extract of the drug. White or pale yellow precipitate is formed.

2. Carbohydrates

a) **Anthrone test:** To 2 ml of anthrone test solution, adding 0.5 ml of aqueous extract of the drug. A green or blue colour indicates the presence of carbohydrates.
b) **Benedict's test:** To 0.5 ml of aqueous extract of the drug adding 5 ml of Benedict's solution and boiling for 5 mins. Formation of a brick red coloured precipitate is due to the presence of carbohydrates.
c) **Fehling's test:** To 2 ml of aqueous extract of the drug adding 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boiling the contents of the test tube for few mins. A red or brick red precipitate is formed.
d) **Molisch's test:** In a test tube containing 2 ml of aqueous extract of the drug adding 2 drops of a freshly prepared 20% alcoholic solution of β- naphthol and mix, pouring 2 ml conc. sulphuric acid so as to from a layer below the mixture. Carbohydrates, if present, produce a red-violet ring, which disappears on the addition of an excess of alkali solution.

3. Flavonoids

Shinoda's test: In a test tube containing 0.5 ml of alcoholic extract of the drug, adding 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

4. Triterpenoids

Liebermann-Burchard's test: Adding 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A violet colour coloured ring is formed indicating the presence of triterpenoids.

5. Proteins

a) **Biuret's test:** To 1 ml of hot aq. extract of the drug adding 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.
b) **Millon's test:** Dissolving a small quantity of aqueous extract of the drug in 1 ml of distilled water and adding 5-6 drops of Millon's reagent. A white precipitate is formed which turns red on heating.

6. Resins

Dissolving the extract in acetone and pouring the solution into distilled water. Turbidity indicates the presence of resins.

7. Saponins

In a test tube containing about 5 ml of an aqueous extract of the drug adding a drop of sodium bicarbonate solution, shaking the mixture vigorously and leave for 3 mints. Honeycomb like forth is formed.

8. Steroids

A) **Liebermann-Burchard's test:** Adding 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish colour is developed which turns to blue.
B) **Salkowski Reaction:** Adding 1 ml of conc. sulphuric acid to 2 ml of chloroform extract of the drug carefully, from the side of the test tube. A red colour is produced in the chloroform layer.

9. Tannins

To 1–2 ml of plant extract, adding a few drops of 5% FeCl3 solution was added. A green colour indicates the presence of gallotannins while brown colour tannins.

10. Starch

Dissolving 0.015g of Iodine and 0.075g of Potassium Iodide in 5 ml of distilled water and adding 2–3 ml of an aqueous extract of drug. A blue colour is produced.

11. Glycosides

Detection of glycoside on paper spray solution No. 1 (0.5 % aqueous sol. of Sodium metaperiodate) & waiting for 10 minutes after then spraying solution No. 2 [0.5% Benzidine (w/v) in solution of Ethanol–acetic Acid (4:1)], white spot with blue back ground shows presence of *glycoside.*

Thin layer chromatography study

In 1958 Sthal *et al*: demonstrated application of Thin Layer Chromatography. At present it is important preliminary analytical tool for analysis of natural product.

Thin Layer Chromatography

The principle underlying the separation of the compounds is their adsorption at the solid-liquid interface. For successful separation the compounds of the mixture should show different degrees of affinity for the solid support (or adsorbent) and the interaction between adsorbent and the component must be reversible. As the adsorbent is washed with the fresh solvent, the various components move down the column and arrange themselves in the order of affinity to the adsorbent. Those with the least affinity move down the column at a faster rate than those with greater affinity.

Apparatus: The equipment consists of

1. Glass plate of uniform thickness throughout entire area, 15-20 cm long, and wide enough to accommodate the required number of test and reference solution.
2. A device for spreading a uniform layer of coating material of desired thickness on the glass plate.
3. A rack to hold the prepared plates, during the drying period.
4. A chromatographic chamber of transparent material, usually glass, with a tightly fitting lid, of suitable size to accommodate the test plates.
5. A suitable spraying implement with a fine spray nozzle.
6. UV device to see the fluorescence of substance.
7. Iodine chamber.

Materials used

All the TLC plates used for the analysis were prepared with silica gel containing binder. Most frequently used binder in silica gel is calcium sulphate (Silica gel 60 F_{254}, Merk). Precoated aluminium sheets were also used which is coated with silica gel 60 F_{254} (Merck).

Preparation of TLC plates

Required quantity of silica gel was mixed in a glass mortar to as smooth consistency with the requisite amount of water and the slurry quickly transferred to the spreader. The mixture has been spreaded over the plates in thickness of 0.2mm and was allowed to set into a thin layer. The plates were transferred carefully to a suitable holder and after 30 minutes, dried and heated at 100–120 °C for at least one hour. The plates were

kept in a desiccator after cooling, until required for further use. The pre coated plates were also activated by heating them for 30 minutes at 100 °C.

Application of sample
A known quantity of sample was dissolved in a known volume of solvent and the sample applied on precoated TLC plates.

Selection of Solvent Systems
The choices of the solvents depend upon the nature of the substances to be separated and also in the material on which the separation is to be achieved. The solvents system was selected on the basis of trial and error method and by elutropic series. It has been found that combination of two solvents gave better separation than with a single solvent.

Chromatographic development (separation)
Development of the chromatogram is effected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.

Visualization: TLC plates were visualized under Iodine solution and after spray of Sulphuric acid reagent and heated at 110 °C for 5 min.

Calculation

$$\text{Rf value} = \frac{\text{Distance from the center of the spot up to the base line}}{\text{Distance of the solvent front from the base line}}$$

Detection of spots
Detection of Rf values of spot done by using Iodine exposure and Sulphuric acid reagent.

Results
Genuine sample of *Alangium lamarckii* Thwaites. gave the presence of following phytochemicals.

Table 1: Phytochemical screening for petroleum ether extract

S. No.	Chemical test	Decoction of *Ankola* seeds
1.	Carbohydrate Test	-
2.	Protein Test	+
3.	Steroids Test	+
4.	Amino Acid	-
5.	Glycosides Test (Saponin)	+
6.	Alkaloids Test	-
7.	Tannins and Phenolic Compounds Test	-
8.	Flavonoids Test	-
9.	Mucilage Test	-

Table 2: Phytochemical screening for water extract of seeds

S. No.	Chemical Test	Decoction of *Ankola* seeds
1.	Carbohydrate Test	+
2.	Protein Test	-
3.	Steroids Test	-
4.	Amino Acid	-
5.	Glycosides Test (Saponin)	+
6.	Alkaloids Test	-
7.	Tannins and Phenolic Compounds Test	-
8.	Flavonoids Test	-
9.	Mucilage Test	-

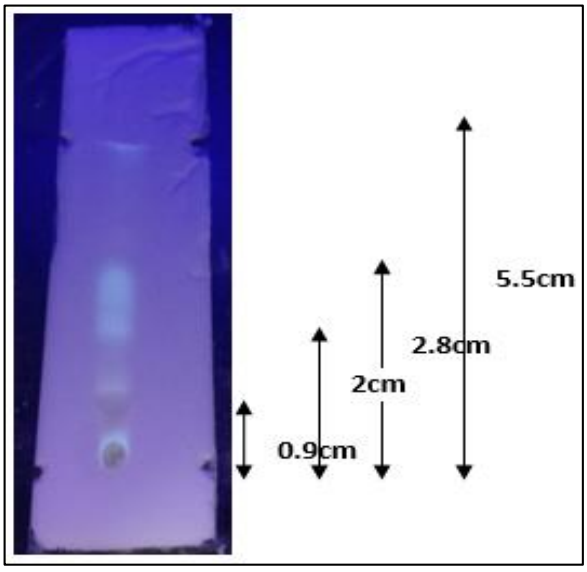

Fig 1: TLC of Petroleum ether extract in UV light

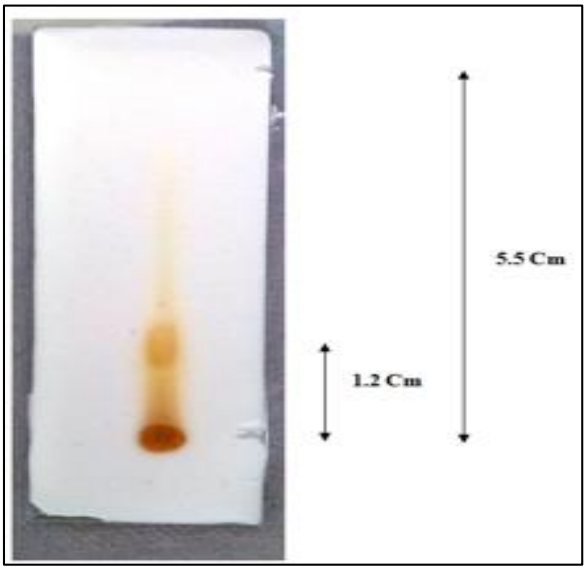

Fig 2: TLC of water extract in visible light

Table 3: Spots on TLC plates

Stationary phase	Glass plate coated with silica gel	
Mobile phase	Toluene : Ethanol : Hydrochloric acid	Chloroform : Methanol : Water
Rf value of spots visualized after spray of Sulphuric acid reagent and heated at 110 °C for 5 min.	0.16, 0.36, 0.51	0.21

Conclusion

In phytochemical screening of Ankola seed with Petroleum ether showed the presence of proteins, steroids and glycosides. While the phytochemical screening of water soluble extract showed presence of carbohydrates and glycosides. So we can say that proteins help in the wound healing along with carbohydrates and glycosides. So it reveals that Ankola (*Alangium lamarckii*. Thwaites) is very important medicinal plant with various therapeutic applications. In TLC study of Petroleum ether extract shows spots at 0.9,2, & 2.8cm & in water extract spot at 1.2 cm.

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