

Experiment - 1.

Date: 2/5 Preliminary Phytochemical Screening of Aqueous extract.

Page No. 1

Neem - *Azadirachta indica*.

Aqueous extract of *Azadirachta indica* (leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aq. extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannins, amino acids, sterols as per Harborne 1998.

Extraction of crude drug.

- * Take 50gm of powdered drug and macerate it with 500ml of water for 24 hrs.
- * Then occasionally shake with 6hrs time period and allow it to stand for 18 hrs.
- * After filtration evaporate the filtrate to dryness in a tared flat bottom shallow dish.

Preparation of test solution.

- * Take 500mg of extract and dissolve it in 100ml of water stir the sol till the extract is completely soluble in water.
- * The sample solution is then subjected to various qualitative test to reveal the presence or absence of common phyto pharmaceuticals.

Test for Alkaloids.

About 2gm of the powdered material was mixed with 1gm of calcium hydroxide and 5ml of water into a smooth paste and set aside for 5 min.

It was then evaporated to dryness in a porcelain dish on a water bath. To this 200ml of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5ml of dilute hydrochloric acid was added followed by 2ml of each of the following reagents.

Mayer's Test.

A small quantity of the extract was treated with Mayer's reagent. Cream colour precipitate indicates the presence of alkaloids.

Dragendorff's Test.

A small quantity of extract was treated with dragendorff's reagent. Orange brown ppt indicates the presence of alkaloids.

Wagner's test.

A small quantity of extract was treated with Wagner's reagent. Reddish brown ppt indicates the presence of alkaloids.

Hager's Test.

A small quantity of extract was treated with Hager's reagent. yellow ppt indicates presence of alkaloids.

Test for purine group (Murexide test).

The residue obtained after the evaporation of chloroform was treated with 1ml of hydrochloric acid in a porcelain dish and 0.1gm of potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapours of dilute ammonia solution. No purple colour was obtained indicating the absence of purine group of alkaloids.

Test for Indole.

To the test solution, add acetic acid and trace amount of anhydrous FeCl_3 , under H_2SO_4 intense blue at interface.

Test for Quinoline (Thalleioquin test).

To the extract, add 1 drop of dilute sulphuric acid and 1ml of water. Add bromine water drop wise till the solution acquires permanent yellow colour and add 1ml of dilute ammonia solution, emerald green colour is produced. The powder when heated with glacial acetic acid in dry test tube, evolves red fumes, which condense in the top portion of the tube.

The bark when moistened with sulphuric acid and observed under ultraviolet light shows a blue fluorescence due to the methoxy group of quinine and quinidine.

Test for carbohydrates.

Molisch's test.

The extract of the powdered drug was treated with 2-3 drops of 1% alcoholic α naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicating the presence of carbohydrates.

Fehling's Test.

The extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of carbohydrates.

Benedict's test.

The extract of the powdered leaf was treated with equal vol of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugars.

Test for Anthraquinone Glycosides.

Borntrager's Test.

The powdered drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well.

The organic layer was separated to which ammonia solution was added slowly. No pink colour was observed in ammoniacal layer showing the presence of anthraquinone glycosides.

Modified Borntrager's Test.

About 0.1g of the powdered drug was boiled for 2 min with dil. HCl and few drops of FeCl_3 solution. Filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal vol of dil. NH_3 solution was added to the benzene extract. No pink colour was observed in ammoniacal layer showing the presence of glycosides.

Test for Cardiac Glycosides (for Deoxysugars).

Keller Killani Test.

About 1g of the powdered leaf was boiled with 10ml of 70% alcohol for 2 min, cooled and filtered. To the filtrate 10ml of water and 5 drops of sol of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3ml of glacial acetic acid. To these 2 drops of ferric chloride solution was added. Then 3ml of concentrated H_2SO_4 was added to the sides of test tube carefully and observed. No reddish brown layer was observed indicating the absence of deoxy sugars.

Raymond Test.

Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

Legal's Test.

Test solution when treated with pyridine made alkaline by sodium nitro prusside solution gives pink to red colour.

Test for cyanogenetic Glycosides.

Small quantity of the powdered was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the stopper, so that it was suspended in the flask and it was set aside for 2 hrs in a warm place. Brick red colour was produced on the paper indicating the presence of cyanogenetic glycosides.

Test for coumarin glycosides.

with Ammonia.

Take a drop of ammonia on a filter paper, to this add a drop of aq. extract of leaves. Development of fluorescence shows +ve test for coumarins.

With hydroxylamine Hydrochloride.

✓ To ethereal extract, added on drop of alcoholic KOH. It was then heated, cooled and acidified with 0.5N hydrochloric acid - violet col developed upon addition of a drop of 1% w/v FeCl_3 indicated presence of coumarins.

Test for sterols.

The powdered drug was 1st extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

Salkowski's Test :-

A few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in colour indicating the presence of sterols.

Test for Libermann-Buchard's.

To the chloroform solution a few drops of acetic anhydride and 1ml of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

Test for Saponins.

Foam test.

0.1g of powder was vigorously shaken with 3ml of distilled water in a test tube for 30 seconds and was left undisturbed for 20 min, persistent foam indicating the presence of saponins.

Test for tannins.

Ferric chloride.

Small quantity of the powdered drug was extracted with water. To the aq. extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

Gold Beates's skin test.

Add 2% hydrochloric acid to all small piece of gold beates's skin, rinses it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a 1% ferrous sulphate solution. A brown or black colour on the skin indicating presence of tannin.

Test for phenolic compounds.

Ferric chloride.

A small quantity of the powdered drug was extracted with water. To the alcoholic extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

Test for Folin coicalteu Reagent.

To a drop of methanolic extract of a few drops of Folin coicalteu reagent was added development of bluish green colour showed presence of phenol.

Biuret test.

The one portion of acidulous - alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution and one drop of dilute copper sulphate solution were added. Violet colour was obtained indicating the presence of proteins.

Ninhydrin Test.

To the test solution add ninhydrin solution and boil, violet colour indicates presence of amino acid.

Test for Sulphur containing amino acid.

5ml of test solution is mixed with 2ml 40% Sodium hydroxide and 2 drops of 10% lead acetate solution. Then boil the solution turned black or brownish due to PbS formation.

Test for terpenoids.

little of the powdered drug was extracted with chloroform and filtered. The filter was washed gently with 4ml and thionyl chloride. pink colour sol appeared which indicating the presence of terpenoids.

Test for carotenoids.

Extract treated with concentrated sulphuric acid and with a chloroform sol of antimony trichloride. Deep blue colour appeared with indicated the presence of carotenoids.

Test For Flavonoids.

Shinoda's test.

Little of the powdered drug was heated with alcohol and filtered. To the test solution Magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for 5 min. Red colour was obtained indicating the presence of flavonoids.

Alkali Test.

To the small quantity of test solution 10% aq. sol Sodium hydroxide solution was added. yellow orange colour was produced indicating the presence of flavonoids.

lead acetate.

To the test solution add a mixture of 10% lead acetate in few drops. It gives white precipitate.

Test For Acid.

To the small quantity of test solution, few drops of concentrated sulphuric acid was added. yellow orange colour was obtained indicates the presence of flavonoids.

Test for protein and amino acids.

Millon's Test.

Small quantity of acidulous - alcoholic extract of the powdered drug was heated with Millon's reagent white precipitate turned.

Test for volatile oil.

Weighted quantity (250 gm) of fresh leaves were extracted and subjected to hydro distillation using volatile oil estimation apparatus.

Test for Fixed oil.

A small amount of powder was pressed in between in the filter paper and the paper was heated in an oven at 105°C for 10 min. A translucent greasy spot appeared indicating the paper.

Test for Gum.

The small quantity of extract was added with few drops of alcohol to form white precipitate which indicates the presence of gum.

Test for anthocyanin.

About 0.2g of plant extract was weighed in separate tube 1ml of 2N sodium hydroxide was added and heated for 5 mins observed for the formation of bluish green colour which indicates the presence of anthocyanin.

Test for leuco anthocyanins.

To 1ml of plant extract, 1ml of 15% alcohol was added, formation of red colour indicated the presence of leuco anthocyanins.

Test for quinones.

To 1ml of extract, 1ml of conc H_2SO_4 was added. Formation of red colour indicated the presence of quinones.

Test for emodins.

The drug extract was added to 25% ammonia solution. The formation of cherry red colour solution indicated the presence of emodins.

Test for coumarins.

To 1ml of plant extract 3ml of $CHCl_3$ and 2ml of benzene was added. Formation of red colour indicated the coumarins.

Test for Resins.

The extracts was treated with acetone. A small amount of water was then added and shaken. Appearance of turbidity indicates the presence of resins.

Test for phlobaphenes.

About 1ml of aq. extract was added to 2ml of 1% HCl and the mixture was boiled. Deposition of red ppt was evidence for the presence.

Result :-

Preliminary phytochemical screening of the aq. extract of ~~Azadirachta~~ *Indica* shows.

Sl. No	Identification Test	Result.
1.	Alkaloids mayers Test	+
2.	Dragendorff's test	+
3.	Wagners test	+
4.	Hangers test	+
5.	Test for quinine mucoside test	+
6.	Test for indole.	+
7.	Test for quindline.	+
8.	Test for carbohydrates; Molish Test	+
9.	Fehlings test	+
10.	Benedicts test.	+
11.	Baumanns test.	+
12.	Modified Baumanns test	+
13.	Test for cardiac glycosides Keller Kiliani test	+
14.	Reyond test	+
15.	Legals test	+
16.	Test for coumarins glycosides.	+
17.	Test for sterols	+
18.	Salkowskis test	+
19.	Libbmann buchards test	+
20.	Test for saponins	+