

CHEMICAL TESTS IN PHARAMCOGNOSY

CHEMICAL TESTS FOR ALKALOIDS

TEST	OBSERVATION	INFERENCE
<u>Mayer's test:</u> To small amount of crude drug, add mayer's reagent (potassium mercuric iodide solution)	Gives cream colour or precipitate	presence of alkaloids
<u>Dragendroff's test:</u> To the small amount of crude drug, add dragendroff's reagent (potassium bismuth iodide solution)	Gives reddish brown colour or precipitate	Presence of alkaloids
<u>Wagner's test:</u> To small amount of crude drug, add Wagner's reagent (iodine-potassium iodide solution)	Gives brown or reddish brown colour or precipitate	Presence of alkaloids
<u>Hager's test:</u> To small amount of crude drug, Hager's reagent (saturated solution of picric acid)	Gives yellow precipitate	Presence of alkaloids
<u>Van-urk's for indole alkaloids:</u> To a 2-3 ml of solution add p-dimethyl amino benzaldehyde.	Gives blue colour	Presence of Indole alkaloids
<u>Vitali morin test for tropane alkaloids:</u> 2-3ml of samples sol is treated with fuming HNO_3 , followed by evaporation to dryness and addition of methanolic KOH solution to an acetone solution of nitrated residue.	White coloration takes place	Presence of Tropane alkaloids
<u>Thalleoquin Test for Quinoline Alkaloids:</u> To the powder drug, when Br_2 water and dilute NH_2 solution	Gives emerald green colour	Presence of Quinoline alkaloids
<u>Modified Born-Trager's Test:</u> Powdered sample+ferric chloride and filtered, to the filtrate add dilute HCl and organic solvents like benzene, ether, chloroform. The organic layer is separated using pipette. To the organic layer add dilute ammonia.	Upon standing pink colour changes to red	Presence of glycosides
<u>Test For Saponin Glycosides:</u> 1. Foam test: The powdered drug is shaken well with water. 2. To the powder add 80% H_2SO_4 .	Foam is formed Shows deep yellow colour	Presence of saponins Presence of saponins
<u>Antimony Trichloride Test:</u> Solution of the glycoside is heated with antimony trichloride and trichloroacetic acid. <u>Liebermann Burchard Test:</u> To the solution of glycosides is added in acetic anhydride followed by concentrated sulphuric acid.	Blue or violet colour is obtained Gives violet to blue colour	Presence of cardiac glycosides Presence of cardiac glycosides

<u>Raymond's Test:</u> A small qty of glycosides is dissolved in 1ml of 50% ethanol followed by addition of 0.1ml of 1% solution of dinitro benzene in ethanol or methano. To this solution, 2-3 drops of 20% NaOH sol is added. (Or) Test solution+hot methanolic alkali.	Appearance of violet color, which changes into blue colour Violet colour is produced.	Presence of cardiac glycosides Presence of cardiac glycosides
<u>Kedde's Test:</u> Extract the drug with chloroform, evaporate to dryness. Add 1 drop of 90% alcohol. Make alkaline with 20% NaOH sol.	Purple colour is produced.	Presence of cardiac glycosides
<u>Baljet's Test:</u> Test solution+picric acid or sodium picrate.	Orange colour is formed.	Presence of cardiac glycosides
<u>Xanthohydrate Test:</u> Test sample is heated with 0.125% solution of xanthohydral in glacial acetic acid containing 1% HCl.	Red colour is produced by deoxy sugars.	Presence of glycosides
<u>Tollen's Test:</u> Glycoside sol is taken in minute of pyridine and ammonial silver nitrate and warmed on water bath.	Formation of silver mirror on the walls of test tube	Presence of glycosides
<u>Test for Coumarin Glycosides:</u> <ol style="list-style-type: none"> 1. Alcoholic extract made alkaline. 2. Cover the test tube containing test sample with filter paper moistened with dilute NaOH sol. Place the covered test tube on water bath for several minutes. Remove the paper and expose to UV light. 	Shows blue or green fluorescence The paper shows green fluorescence	Presence of coumarin glycosides
<u>Test for Cyanogenic Glycosides:</u> <ol style="list-style-type: none"> 1. 200mg of drug is taken in conical flask and moisten with few drops of water. Moisten a piece of picric acid paper with 5% aq sodium carbonate sol. And suspended with by means of cork in the neck of the flask. Warm gently at about 37°C 2. Paper sol of Guaiacum resin in absolute alcohol and allow it to dry on paper. Treat it with CuSO₄ sol. 	Formation of reddish-purple color Paper turns blue colour	Presence of cyanogenic glycosides Presence of cyanogenic glycosides
<u>Test for hydroxyl anthrac quinines:</u> Add KOH sol to the sample.	Red colour is produced	Presence of glycosides
<u>Test for Cyanophoric Glycosides:</u> To the powder in a test tube add little amount of water and suspend the piece of sodium picrate paper above the drug. Trapping a top edge between the cork and the tube wall. Allow it to stand for 30 minutes. Hydrochloric acid gets evolved.	Picrate paper turns to brick red colour	Presence of cyanophoric glycosides
<u>Legal Test:</u> To a sol of glycoside in pyridine. Sodium nitrogen amide solution and NaOH solution are added.	Pink to red colour is formed	Presence of cardiac glycosides
<u>Schonteten's Test:</u> To a solution (5ml), borax (0.2g) is added and it is heated to dissolve completely. Few drops of the liquid	Green fluorescence is produced	Presence of anthraquinone glycosides (aloe)

are poured in a test tube filled with water.		
<u>Bromine Test:</u> To the sample add bromine.	Pale yellow precipitate of tetrabromation	Presence of anthraquinone glycosides (aloe)
<u>Klunge's Isobarbaloin Test:</u> To an aqueous solution (20ml) CuSO ₄ sol (1 drop) is added followed by NaCl (1g) and 90% alcohol (10ml).	A purple colour is formed	Presence of isobarbaloin (aloe)
<u>Test for Flavonoid Glycosides:</u> To the small qty of the residue, add lead acetate solution.	Yellow colour precipitate is formed.	Presence of flavonoid glycosides

CHEMICAL TESTS FOR TANNINS

TEST	OBSERVATION	INFERENCE
<u>Goldbeater's skin test:</u> A small piece of goldbeater's skin is soaked in 2% HCl rinsed with distilled water and placed in a solution of tannin for 5min. The skin piece is washed with distilled water and kept in a solution of FeSO ₄ .	A brown or black colour is produced on the skin	Presence of tannins
<u>Gelatin Test:</u> To a sol of tannin (0.5-1%) aqueous sol of gelatin (1%) and NaCl (10%) are added.	A white buff-colored precipitate is formed	Presence of tannins
<u>Phenazone Test:</u> A mixture of aq extract (5ml) of a drug and sodium and phosphate (0.5g) is heated, cooled and filtered. A sol of phenazone (2%) is added to the filtrate.	A bulky colored precipitate is formed	Presence of tannins
<u>Catechin test (matchstick test):</u> A matchstick is dipped in aq plant extract, dried near burner and moistened with conc HCl.	On warming near a flame the matchstick wood turns pink or red due to formation of phlorogucinol.	Presence of tannins
<u>Chlorogenic acid test:</u> An extract of chlorogenic acid containing drug is treated with aq NH ₃ .	A green color is formed on exposure to air	Presence of tannins
<u>Vanillin-Hydrochloric Acid Test:</u> When the drug is treated with Vanillin-Hydrochloric Acid reagent	Pink or red colour is formed due to formation of phloroglucinol	Presence of tannins
<u>Gambir-fluorescin test:</u> A mixture of alcoholic extract of pale catechu (1g) NaOH SOL (5ml) and petroleum ether (5ml) is shaken and kept for sometime.	The petroleum ether layer shows green fluorescence	Presence of gambir (tannin)
A very dilute FeCl ₃ sol is gradually added to an aq extract of hamamelis leaves	A blue color is produced which is changed to olive green as more FeCl ₃ is added	Presence of tannins

CHEMICAL TESTS FOR RESINS

TEST	OBSERVATION	INFERENCE
To the extract add 5ml of distilled water	Turbidity is formed	Presence of resins
Alcoholic solution of colophony	It turns blue litmus to red	Presence of diterpenic acid
Alcoholic solution of balsam of tolu	Gives green colour with FeCl_3	Presence of toluidine tannols
To a petroleum ether sol of benzene, 2-3 drops of H_2SO_4 is added in a china dish.	Sumatra: reddish brown colour Slam: purple red colour	Presence of resins
0.1g in 10ml $(\text{CH}_3\text{CO})_2\text{O}$ with aid of gentle heat, cool and add 0.05ml of H_2SO_4 .	A bright purplish red colour to violet	Presence of resins (colophony)
0.1g powder in 10ml of $(\text{CH}_3\text{CO})_2\text{O}$ in a test tube and add a drop of concentrated H_2SO_4 .	Purple colour	Presence of resins (colophony)

TESTS FOR VOLATILE OILS

TEST	OBSERVATION	INFERENCE
To the section of the drug, add alcoholic solution of sudan III	Red colour obtained by globules	Presence of volatile oil
To the thin section of the drug, add a drop of tincture alkaline.	Red colour is obtained	Presence of volatile oil

TESTS FOR FLAVONOIDS

TEST	OBSERVATION	INFERENCE
<u>Shinoda test:</u> To dry powder extract add 5ml of 90% ethanol, few drops of conc HCl and 0.5g of magnesium turnings.	Pink colour is observed	Presence of flavonoids
To small qty of residue add lead acetate sol	Yellow colored precipitate is formed	Presence of flavonoids
Add increasing amount of NaOH to the residue	It shows yellow coloration, which decolorizes after addition of acid	Presence of flavonoids

CHEMICAL TESTS FOR CARBOHYDRATES

TEST	OBSERVATION	INFERENCE
<u>Fehling's solution test:</u> The substance (0.5g) is treated with dil HCl. The reaction mixture is neutralized by addition of NaOH sol and then Fehling's sols 1 and 2 are added.	Red precipitate of cuprous oxide is produced on heating	Presence of carbohydrates

<u>Molisch test:</u> A sol of carbohydrate is prepared in water containing α -naphthol concentrated H_2SO_4 is added along the side of the test tube	A purple ring is formed on the junction below upper layer	Presence of carbohydrates
<u>Osazone formulation:</u> A sugar is heated with phenyl hydrazine hydrochloride, sodium acetate and acetic acid	Formulation of yellow crystals of osazone	Presence of carbohydrates
<u>Resorcinol test for ketones (selvinoff's test):</u> A crystal of resorcinol is added to the solution and heated with equal volume of concentrated HCl.	Pink colour is produced	Presence of carbohydrates (in case of ketones fructose, honey, hydrolyzed insulin)
<u>Test for pentoses:</u> A solution of materials is heated with equal volume of HCl containing a little phloroglucinol	Red colour is formed	Presence of carbohydrates (in case of pentoses)
<u>Killer-kilani test for deoxy sugars:</u> A deoxy sugar is dissolved in acetic acid containing a trace of $FeCl_3$ and transferred to the surface of concentrated H_2SO_4	A reddish-brown color is formed at the junction which turns blue later on	Presence of carbohydrates (deoxysugars)
<u>Furfural test:</u> The sample is heated in a test tube with a drop of syrupy phosphoric acid to make it into furfural. A disk of filter paper moistened with a drop of 10% solution of aniline in 10% acetic acid is placed over the mouth of the test tube. The bottom of the test tube is heated for 30-60 seconds.	A pink or red stain appears on the reagent paper	Presence of carbohydrates
<u>Benedict's test:</u> To the solution, add benedict's reagent and heated on water bath	Solution appears green, yellow or red depending on concentration of reducing sugar	Presence of carbohydrates
<u>Lead sulphide test:</u> To the alkaline solution of sulphur containing proteins add lead acetate	A black precipitate is formed	Presence of proteins
<u>Heat coagulation test:</u> Heat the test solution in a boiling water bath.	Proteins get precipitated	Presence of proteins

CHEMICAL TESTS FOR FIXED OILS

TEST	OBSERVATION	INFERENCE
<u>Halphen's test/bevan's test:</u> 2ml of oil is mixed with 1ml of amyl alcohol and 1ml of 1% solution of sulphur in CS_2 for 10 minutes in a water bath	Red color is formed (fades when heated to over $200^\circ C$)	Presence of cotton seed oil
<u>Boudouin's test:</u> The oil is shaken with half its volume of concentrated HCl containing 1% of sucrose	Development of pink colour	Presence of sesamol
<u>BP Test for sesamol:</u> The oil is shaken with a furfural sol in acetic anhydride in the presence of H_2SO_4 (mentioned in BP)	Development of bluish-green color	Presence of sesamol

<u>Test for persic oil:</u> The oil is shaken with HNO ₃	Produces color	Presence of persic oil
---	----------------	------------------------

CHEMICAL TESTS FOR OTHER GROUPS

<u>TEST</u>	<u>OBSERVATION</u>	<u>INFERENCE</u>
<u>Test for insulin:</u> To the test solution add solution of α -naphthol and H ₂ SO ₄	Brownish red colour is produced	Presence of insulin
<u>Test for mucilage:</u> 1. To the test solution add ruthenium red 2. To the test solution add thionine solution and after 15 minutes wash with alcohol	Pink color is obtained violet red	Presence of mucilage Presence of mucilage
<u>Test for waxes:</u> To the test solution, add alcoholic alkali solution	Waxes get saponified	Presence of waxes