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Qualitative tests for preliminary phytochemical screening: An overview

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Abstract
Medicinal plants have been used in the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. Phytoconstituents individually or in the combination, determine the therapeutic value of a medicinal plant. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the important phytochemicals with diverse biological activities. The pharmacological activity of a plant can be predicted by the identification of the phytochemicals. Currently, phytochemicals are determined by various modern techniques, but the conventional qualitative tests are still popular for the preliminary phytochemical screening of plants.

Keywords: Medicinal plants, phytoconstituents, phytochemical screening, qualitative tests

Introduction
Phytochemicals (Greek: *phyton* = plant) are chemical compounds naturally present in the plants attributing to positive or negative health effects [1]. Medicinal plants used in different diseases and ailments are the richest bio reservoirs of various phytochemicals. The medicinal properties of the plants are determined by the phytochemical constituents [2]. Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are distributed in various parts of the plants [3]. Nature is a unique source of structures of high phytochemical diversity representing phenolics (45%), terpenoids and steroids (27%) and alkaloids (18%) as major groups of phytochemicals [4]. Although, these compounds seem to be non-essential to the plant producing them, they play a vital role in survival by mediation of ecological interactions with competitors, protect them from diseases, pollution, stress, UV rays and also contribute for colour, aroma and flavour with respect to the plant. The metabolites produced by the plants to protect themselves against biotic and abiotic stresses have turned into medicines that people can use to treat various diseases [5,6].

Phytochemicals can be separated from the plant material by various extraction techniques. The most commonly used conventional methods include maceration, percolation, infusion, digestion, decoction, hot continuous extraction (Soxhlet extraction) etc., recently, eco-friendly techniques such as Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extractions (SFE) and Accelerated Solvent Extraction (ASE) have also been introduced [10,11]. Different types of solvents viz. water, ethanol, methanol, acetone, ether, benzene, chloroform etc. are used in the extraction process [12]. Extraction of phytochemicals from the plant materials is affected by pre-extraction factors (plant part used, its origin and particle size, moisture content, method of drying, degree of processing etc.) and extraction-related factors (extraction method adopted, solvent chosen, solvent to sample ratio, pH and temperature of the solvent, and length of extraction) [10, 12].

Previously, the plant parts were directly used as such for the treatment, but now-a-days, the active principles are identified and isolated in pure form and also synthetically produced with the help of advance techniques [6]. In the development of new synthetic drugs, the chemical structures derived from these phytoconstituents can be utilized as models [7]. Identification of phytoconstituents in the plant material helps to predict the potential pharmacological activity of that plant [8].

Characterization and evaluation of plants and their phytoconstituents can explore the evidences to support therapeutic claims of those plants against various ailments [12]. Advanced techniques like Gas Chromatography (GC), Liquid Chromatography (LC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC) etc. are very helpful for detection of phytoconstituents both qualitatively as well as quantitatively [1]. However, when these techniques are unavailable or unaffordable, the conventional phytochemical tests which are economic, easy and require fewer resources, remain the good choice for preliminary phytochemical screening [2]. The present communication deals with the collection and compilation of maximum possible qualitative phytochemical tests from various published literatures. The preliminary qualitative phytochemical tests for the detection of different phytoconstituents have been summarized in table 2.

Table 1: Reagent Preparation for Phytochemical Screening

	Reagents/Solutions	Composition
1.	Dragendroff's reagent	**Stock solution:** 5.2gm Bismuth carbonate + 4gm sodium iodide + 50mL glacial acetic acid, boiled for few min., After 12 hr. precipitated sodium acetate crystals are filtered by sintered glass funnel; 40mL filtrate + 160mL ethyl acetate + 1mL distilled water, (stored in amber-coloured glass bottle). **Working solution:** 10mL stock solution + 20mL acetic acid + distilled water to make final volume 100mL.
2.	Hager's reagent	Saturated aqueous solution of picric acid
3.	Mayer's reagent	**Solution A :** 1.358gm mercuric chloride + 60mL distilled water **Solution B :** 5gm potassium iodide + 10mL distilled water **Working solution:** solution A + solution B + distilled water to make final volume 100mL
4.	Wagner's reagent	1.27gm iodine + 2gm potassium iodide + distilled water to make final volume 100mL
5.	Barfoed's reagent	30.5gm copper acetate + 1.8mL glacial acetic acid
6.	Seliwanoff's reagent	0.05 resorcinol + 100mL dilute HCl
7.	Benedict's reagent	**Solution A:** 173gm sodium citrate + 100gm sodium carbonate + 800mL water, dissolve & boil to make solution clear **Solution B:** 17.3gm of copper sulphate dissolved in 100mL distilled water **Working solution:** Mix solution A and solution B
8.	Fehling's solutions	**Solution A:** 34.66gm copper sulphate + distilled water to make final volume 100mL. **Solution B:** 173gm potassium sodium tartarate + 50gm NaOH + distilled water to make 100mL.
9.	Baljet's reagent	95mL 1% picric acid + 5mL 10% NaOH
10.	Millon's reagent	1gm mercury + 9mL fuming nitric acid + equal amount of distilled water (after completion of reaction)

Table 2: Qualitative Tests for Phytochemical Screening

	Test	Procedure	Observations (Indicating Positive Test)	References
		Detection of alkaloids		
1)	Dragendroff's/ Kraut's test	Few mL filtrate[a] + 1-2 mL *Dragendorff's reagents*	A reddish-brown precipitate	[1, 21]
2)	Hager's test	Few mL filtrate[a] + 1-2 mL *Hager's reagents*	A creamy white precipitate	[1, 7]
3)	Mayer's/ Bertrand's/ Valser's test	Few mL filtrate[a] + 1-2 drops of *Mayer's reagent* (Along the sides of test tube)	A creamy white/yellow precipitate	[7, 12, 13]
4)	Wagner's test	Few mL filtrate[a] + 1-2 drops of *Wagner's reagent* (Along the sides of test tube)	A brown/reddish precipitate	[7, 21]
5)	Picric acid test	Few mL filtrate[a] + 3-4 drops of 2% picric acid solution	An orange coloure	[14, 15]
6)	Iodine Test	3mL extract solution + few drops of iodine solution	A blue colour, which disappears on boiling and reappears on cooling	[16, 17]
7)	Bouchardat's test	6mL plant extract, evaporated completely + 6mL ethanol (@60 °C) + few drops of Bouchardat's reagent (dilute iodine solution)	A reddish brown colour	[18]
8)	Tannic acid test	Acidified extract + 10% tannic acid solution	A buff colour precipitate	[30, 38]
		Detection of Carbohydrates		
1)	Barfoed's test	1mL filtrate[b] + 1mL *Barfoed's reagent* + Heated for 2 min.	A red precipitate {monosaccharides}	[7, 19]
2)	Molish's test	2mL filtrate[b] + 2 drops of alcoholic α–naphthol + 1mL conc. H_2SO_4 (along the sides of test tube)	A violet ring	[7, 21]
3)	Seliwanoff's Test	1mL extract solution + 3mL *seliwanoff's reagent* + heated on water bath for 1 min.	A rose red colour {ketoses}	[17, 19]
4)	Resorcinol test	2mL aq. extract solution + few crystals of resorcinol + equal volume of conc. HCl + heated	A rose colour {ketones}	[13, 9]
5)	Test for pentoses	2mL conc. HCl + little amount of phloroglucinol + equal amount of aqueous extract solution + heated over flame	A red colour	[13]
6)	Test for starch	Aqueous extract + 5mL 5% KOH solution	A cinary colouration	[20]
		Detection of Reducing sugars		
1)	Benedict's test	0.5mL filtrate[b] + 0.5mL *Benedict's reagent* + Boiled for 2 min.	Green/yellow/red colour	[7, 21]
2)	Fehling's test	1mL each of *Fehling's solution A* & *B* + 1mL filtrate[b] + boiled in water bath	A red precipitate	[7, 21]
		Detection of Glycosides		
1)	Borntrager's test	2mL filtrated hydrolysate[c] + 3mL Chloroform + shaken well + chloroform layer is separated + 10% Ammonia solution	A pink coloured solution	[7]

2)	Modified Borntrager's test	Plant extract + ferric chloride solution + boil for 5min. + cooled + equal volome of benzene + benzene layer is separated + Ammonia solution	A rose-pink to blood red coloured solution	[12, 33]
3)	Legal's test	Dissolve 50gm plant extract in pyridine + Sodium nitroprusside + 10% Sodium hydroxide	A pink coloured solution	[7, 12]
4)	10% NaOH test	1mL dil. H_2SO_4 + 0.2mL extract + boiled for 15min. + allowed cooling + neutralize with 10% NaOH + 0.2mL *Fehling's solution A* & *B*	A brick red precipitate	[21]
5)	Aqueous NaOH test	Alcoholic extract + dissolved in 1mL of water + few drops of aqueous NaOH solution	A yellow colour	[22]
6)	Concentrate H_2SO_4 test	5ml plant extract + 2mL glacial acetic acid + a drop of 5% $FeCL_3$ + conc. H_2SO_4	A brown ring	[3]
7)	Raymond's test	Extract solution + dinitrobenzene in hot methanolic alkali	A violet colour	[38]
Detection of Cardiac Glycosides				
1)	Keller-Killani test	1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H_2SO_4 (along the side of test tube)	A blue coloured solution (in acetic acid layer)	[21, 38]
2)	Kedee's test	4mL extract evaporated to dryness + 1-2 mL methanol + 1-2 mL alcoholic KOH + 3-4 drops of 1% alcoholic 3,5-dinitrobenzene + heated	A disappearing violet colour {Cardenolides}	[22]
3)	Test for Cardenolides	Extract + pyridine + Sodium nitroprusside + 20% NaOH	A red colour, fades to brownish yellow	[20]
4)	Bromine water test	Plant extract + few mL of bromine water	A yellow precipitate	[30]
5)	Baljet test	2mL extract + a drop of *Baljet's reagent*	A yellow-orange colour	[39, 40]
Detection of Proteins and Amino acids				
1)	Biuret test	2mL filtrate + 1 drop of 2% copper sulphate sol. + 1mL of 95% ethanol + KOH pellets	A pink coloured sol. (in ethanolic layer)	[1, 7]
2)	Millon's test	2mL filtrate + few drops of *Millon's reagent*	A white precipitate	[1, 7]
3)	Ninhydrin test	2mL filtrate + 2 drops of Ninhydrin solution (10mg ninhydrin + 200mL acetone)	A purple coloured sol. {Amino acids}	[1, 7]
4)	Xanthoproteic test	Plant extract + Few drops of conc. Nitric acid	A yellow coloured sol.	[1, 12]
Detection of Flavonoids				
1)	Alkaline reagent test	1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid	[20, 21, 23]
		Plant extract + 10% ammonium hydroxide sol.	A yellow fluorescence	[7]
2)	Lead acetate test	1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate	[1, 21, 12]
3)	Shinoda's test/ Mg-hydrochloride reduction test	Plant extract is dissolved in 5mL alcohol + Fragments of magnesium ribbon + few drops of conc. HCl	A pink to crimson coloured solution {flavonal glycosides}	[7, 38]
4)	Shibata's reaction/ Cyanidin test	1gm Aq. extract + dissolved in 1-2 mL 50% methanol by heating + metal magnesium + 5-6 drops of conc. HCl	A red colour {flavonols}, orange colour {flavones}	[13, 22]
5)	Ferric chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	A green precipitate	[20]
6)	Pew's test	Few mL aqueous extract solution + 0.1gm metallic zinc + 8mL conc. H_2SO_4	A red colour {flavonols}	[13]
7)	Zinc-hydrochloride reduction test	Plant extract + pinch of zinc dust + conc. HCl along the side of test tube	Magenta colour	[24, 25]
8)	Ammonia test	Filtrate + 5mL dil. Ammonia solution + conc. H_2SO_4	A yellow colour	[26]
9)	Conc. H2SO4 test	Plant extract + conc. H_2SO_4	An orange colour	[35]
Detection of Phenolic compounds				
1)	Iodine test	1mL extract + few drops of dil. Iodine sol.	A transient red colour	[21]
2)	Ferric chloride test	Extract aqueous solution + few drops 5% ferric chloride sol.	Dark green/bluish black colour	[7, 12]
3)	Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	[7]
4)	Lead acetate test	Plant extract is dissolved in 5mL distilled water + 3mL of 10% lead acetate sol.	A white precipitate	[7]
5)	Ellagic Acid Test	Plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution	Solution turns muddy / Niger brown precipitate	[3, 16]
6)	Potassium dichromate test	Plant extract + few drops of potassium dichromate solution	A dark colour	[27]
7)	Hot water test	Warm water in beaker + mature plant part is dipped + warmed for a min.	Black or brown colour ring at the junction of dipping	[34]
8)	Test for Cartenoids	(1gm extract + 10mLchloroform, vigorously shaken and filtered). Filtrate + conc. H_2SO_4	A blue colour at the interface	[35]
Detection of Tannins				
1)	Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	[12, 33]
2)	Braymer's test	1mL filtrate[d] + 3mL distilled water + 3 drops 10% Ferric chloride solution	Blue-green colour	[21, 28]
3)	10% NaOH test	0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion {Hydrolysable tannins}	[21]

4)	Bromine water test	10 ml of bromine water + 0.5gm plant extract	Decoloration of bromine	[23]
5)	Lead sub acetate test	1mL filtrate[e] + 3 drops of lead sub acetate solution	A creamy gelatinous precipitate	[3]
6)	Phenazone test	(5mL aq. extract + 0.5 g of sodium acid phosphate, heated, allowed to cool + filtered); filtrate + 2% solution of phenazone	Precipitation	[9]
7)	Mitchell's test	Extract solution + iron + sodium tartarate (+ ammonium acetate solution)	A water-soluble iron-tannin complex, which is insoluble in solution of ammonium acetate	[39]
Detection of Phlobatannins				
1)	HCl test	2mL aq. extract + 2mL 1% HCl (boiled)	A red precipitate	[5, 13]
Detection of Saponins				
1)	Foam test	0.5gm plant extract + 2mL water (vigorously shaken)	Persistent foam for 10 min.	[12]
		20mL water in measuring cylinder + 50gm extract (vigorously shaken for 15 min.)	Formation of 2cm thick layer of foam	[7]
		0.2gm plant extract + 5mL distilled water; shaken well; heated to boiling	Appearance of creamy miss of small bubbles	[29]
2)	$NaHCO_3$ test	Plant extract + few mL sodium bicarbonate solution + distilled water (vigorously shaken)	Stable honeycomb like froth	[30]
3)	Olive oil test	Aq. extract + 5mL distilled water; shaken vigorously + few drops of olive oil + shaken vigorously	Appearance of foam	[23, 26]
4)	Haemolysis test	Drop of fresh blood on glass slide + plant extract	Zone of hemolysis	[3, 16]
Detection of Phytosterols				
1)	Salkowski's test	Filtrate[f] + few drops of conc. H_2SO_4 (Shaken well and allowed to stand)	Red colour (in lower layer)	[21, 12]
2)	Libermann-Burchard's test	50gm extract is dissolved in 2mL acetic anhydride + 1-2 drops of conc. H_2SO_4 (along the side of test tube)	An array of colour change	[7]
3)	Acetic anhydride test	0.5mL plant extract + 2mL of acetic anhydride + 2mL conc. H_2SO_4	Change in colour from violet to blue/green	[26]
4)	Hesse's response	5mL aq. extract + 2mL chloroform + 2mL conc. H_2SO_4	Pink ring / Red colour (in lower chloroform layer)	[23, 40]
5)	Sulphur test	Extract solution + pinch of sulphur powder	Sulphur sinks to the bottom	[34]
Detection of Cholesterol				
1)		2mL extract + 2mL chloroform + 10 drops of acetic anhydride + 2-3 drops of conc. H_2SO_4	A red-rose colour	[22]
Detection of Terpinoides				
1)		2ml chloroform + 5mL plant extract, (evaporated on water bath) + 3mL conc. H_2SO_4 (boiled on water bath)	A grey coloured solution	[23]
Detection of Triterpinoides				
1)	Salkowski's test	Filtrate[f] + few drops of conc. H_2SO_4 (Shaken well and allowed to stand)	Golden yellow layer (at the bottom)	[21]
Detection of Diterpenes				
1)	Copper acetate test	Plant extract is dissolved in distilled water + 3-4 drops of copper acetate solution	Emerald green colour	[12, 33]
Detection of Lignins				
1)	Labat test	Extract solution + gallic acid	A olive green colour	[16, 38]
2)	Furfuraldehyde test	Extract solution + 2% furfuraldehyde solution	A red colour	[16, 38]
Detection of Carotenoids				
1)	Carr-Price reaction	10mL extract evaporated to dryness + 2-3 drops of saturated solution of antimony trichloride in chloroform	A blue-green colour eventually changing to red	[22]
Detection of Quinones				
1)	Alcoholic KOH test	1mL plant extract + few mL alcoholic potassium hydroxide	Red to blue colour	[21, 26]
2)	Conc. HCl test	Plant extract + conc. HCl	A green colour	[17]
3)	Sulphuric acid test	10mg extract + dissolved in isopropyl alcohol + a drop of conc. H_2SO_4	A red colour	[32]
Detection of Anthraquinones				
1)	Borntrager's test	10mL 10% ammonia sol. + few ml filtrate[g] (shaken vigorously for 30 sec.)	A pink, violet, or red coloured solution	[5, 23, 28]
2)	Ammonium hydroxide test	10mg extract is dissolved in isopropyl alcohol + a drop of conc. ammonium hydroxide solution	Formation of red colour after 2 min.	[32]
Detection of Anthocyanins				
1)	HCl test	2mL plant extract + 2mL 2N HCl (+ Few mL ammonia)	Pink-red sol. which turns blue-violet after addition of ammonia	[18, 31]
Detection of Leuconthocyanins				
1)	Isoamyl alcohol test	5mL plant extract + 5mL isoamyl alcohol	Upper layer appears red	[31]
Detection of Carboxylic acid				
1)	Effervescence test	1mL plant extract + 1mL sodium bicarbonate solution	Appearance of Effervescence	[21, 26]
Detection of Coumarins				
1)	NaOH paper test	0.5gm moistened extract is taken in test tube, mouth of test tube is covered with 1N NaOH treated filter paper, heated for few min. in water bath	Yellow fluorescence from paper under the UV light	[21, 26]

2)	NaOH test	Plant extract + 10% NaOH + Chloroform	A yellow colour	[27]
Detection of Emodins				
1)		Plant extract + 2mL NH_4OH + 3mL benzene	A red colour	[29]
Detection of Gums and Mucilages				
1)	Alcohol test	Dissolve 100mg extract in 10mL distilled water + 25mL absolute alcohol (constant stirring)	White or cloudy precipitate	[7]
Detection of Resins				
1)	Acetic anhydride test	1mL plant extract + Acetic anhydride solution + 1mL conc. H_2SO_4	Orange to yellow	[21, 26]
2)	Turbidity test	1mL plant extract dissolved in acetone, poured in distilled water	Turbidity	[34]
		10mL extract + 20mL 4% HCl		[36]
Detection of Fixed Oils and Fat				
1)	Spot test/ Stain test	Little quantity of plant extract is pressed in between to filter papers	Oil stain on the paper	[7, 38]
2)	Saponification test	Extract + few drops of 0.5N alcoholic KOH + A drop of phenolphthalein (Heated for 2hr.)	Soap formation or partial neutralization of alkali	[7,[38]
3)		Extract solution is applied on filter paper	A transparent appearance {oils and resins}	[35, 36]
Detection of Volatile Oils				
1)	Fluorescence test	l0 mL of extract, filtered till saturation, exposed to UV light	Bright pinkish fluorescence	[37]

[a] = 50gm solvent free extract is mixed with few mL dil. HCl and then filtered
[b] = 100mg solvent free extract is dissolved in 5mL of distilled water and filtered
[c] = 50gm of plant extract is hydrolysed with conc. HCl for 2 hr on water bath and filtered
[d] = 3gm powdered sample boiled in 50mL distilled water for 3 min. and then filtered
[e] = Small quantity of extract boiled with 5mL of 45% ethanol for 5 min. them cooled and filtered
[f] = Equal quantity of chloroform is treated with plant extract and filtered
[g] = 3mL of aq. extract is shaken with 3mL of benzene and filtered [5] OR 10mL of benzene is added in the plant sample and soaked for 10 min. then filtered [23] OR extract is macerated with ether and filtered [28].
{ }=Indicates presence of specific phytoconstituents.
Note: The compositions of various reagents and solutions denoted by italic font have been mentioned in table 1.

Conclusion
The phytochemical analysis is very much important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for targeted isolation of compounds and to perform more precise investigations. Extraction of a phytochemical from the plant material is mainly dependent on the type of solvent used. Similarly, the test applied for phytochemical analysis determines the presence or absence of a phytochemical in the sample. Hence, two or more different tests should be performed for more accurate results.

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