International Journal of Scientific Research and Engineering Development-– Volume 6 Issue 1, Jan-Feb 2023

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

Quantitative Tests for Preliminary Phytochemical Analysis

Priyanka Panth[1], Dr. Mohammad Hashim Mansoori[2]

Department of Pharmaceutical Chemistry & M.S. College of Pharmacy,
Email: panthpriyanka29@gmail.com
Department of Pharmaceutical Chemistry & M.S. College of Pharmacy,
Email: hashim.m@mscollegeofpharmacy.in

Abstract:

Medicinalplantshavebeenusedinthetreatmentofvariousdiseasesastheypossesspotentialpharmacologicalactivitiesincludingantineoplastic,antimicrobial,antioxidant,anti-inflammatory,analgesic,anti-diabetic,anti-hypertensive,antidiarrhealandotheractivities.Phytoconstituentsindividually or in combination, determine the therapeutic value of a medicinal plant. Alkaloids,flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the importantphytochemicalswithdiversebiologicalactivities.Thepharmacologicalactivityofaplantcanbepredictedby theidentification of thephytochemicals. Currently, phytochemicals aredetermined byvarious modern techniques, but conventional qualitative tests are still popular for the preliminaryphytochemicalscreening ofplants.

Keywords:Herbal MedicinalPlants,PhytochemicalAnalysis,QualitativeTests, Reagent Preparation

INTRODUCTION

Phytochemical are chemicals of plant origin. Phytochemicals(from Greek:*phyton*= plant) arechemicalcompounds naturallypresentintheplants attributing to positive or negative health effects [1]. Medicinal plants used in differentdiseases and ailments are the richest bio reservoirs of various phytochemicals. The medicinalproperties of the plants are determined by the phytochemical constituents [2]. Some of theimportant phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids,glycosides, terpenes, etc. which are distributed in various parts of the plants [3]. Nature is aunique 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 avital role in survival by mediation of ecological interactions with competitors, protect themfromdiseases,pollution,stress,and UVraysandalsocontributeforcolour,aromaandflavour with respect to the plant. The metabolites produced by the plants to protect themselves againstbiotic and abiotic stresses have turned into medicines that people can use to treat variousdiseases[5,6].

Phytochemicals can be separated from the plant material by various extraction techniques. Themostcommonlyusedconventionalmethodsincludemaceration,percolation,infusion,digestion, decoction, hot continuous extraction (Soxhlet extraction) etc., recently, eco-friendlytechniques such as Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction(MAE), Supercritical Fluid Extractions (SFE) and Accelerated Solvent Extraction (ASE) havealso been introduced [10,11].

Different types of solvents viz. water, ethanol, methanol, acetone,ether,benzene,chloroformetc.areusedintheextractionprocess[12].Extractionofphytochemicals 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.) andextraction-related factors (extraction method adopted, solvent chosen, solvent to sample ratio,pH and temperatureof thesolvent,and lengthof extraction)[10,12].

Previously, the plant parts were directly used as such for the treatment, but now-a-days, theactive principles are identified and isolated in pure form and also synthetically produced withthe help of advance techniques [6]. In developing new synthetic drugs, the chemical structures derived from these phytoconstituents can be used as models [7]. Identification ofphytoconstituentsintheplantmaterialhelpstopredictt predict that plant's potential pharmacological activityterizationandevaluationofp evidence to support those plants' therapeutic claimsncestosupporttherapeutic claims of those plants against various ailments [12].Advanced techniques like Gas Chromatography (GC), LiquidChromatography(LC),High-PerformanceLiquidChromatography(HPLC),High-PerformanceThinLayerChromatography (HPTLC) etc. are very helpful for detectionofphytoconstituentsbothqualitativelyaswellasquantitatively[1].However,whenthesetechniques are unavailable or unaffordable, the conventional phytochemicaltests which are economic, easy and require fewer resources,remainthegoodchoiceforpreliminaryphytochemicalscreening[2].Thepresentcommunicationd ealswiththecollection and compilation of maximum possible qualitativephytochemical tests from various published literatures.

PREPARATION OF REAGENT FOR PHYTOCHEMICAL ANALYSIS:

1. **Dragendroff's Reagent:**
   Stock Solution: 5.2gm Bismuth Carbonate + 4gm Sodium Iodide + 50ml Glacial Acetic acid, boiled for few min., after 12hr. precipitated Sodium Acetate crystals are filtered by sintered glass funnel; 40ml filtrate + 160ml Ethyl Acetate + 1ml distilled water**, (stored in Amber – Colored 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: 1.36gm 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:**0.01gm Resorcinol + 6.6ml Concentrated HCL + Distilled water to make final volume 20ml
7. **Benedict's Reagent:**
   Solution A: 173gm Sodium Citrate + 100gm Sodium Carbonate + 800ml water, dissolve and boil to make solution clear

Solution B: 17.3gm of Copper Sulphate Dissolve in 100ml distilled water
Working Solution: Mix solution A and Solution B

8. **Fehling Solution:**
   Solution A: 34gm Copper Sulphate + Distilled water to make final volume 100ml
   Solution B: 173gm Potassium Sodium Tartrate + 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 reaction)

QUALITATIVE TESTS FOR PHYTOCHEMICAL ANALYSIS:

Detection of Quinones:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	AlcoholicKOHtest	1mLplantextract +a fewmL alcoholic potassium hydroxide	Redtoblue colour	[21, 26]
2	Conc. HCltest	Plantextract + conc. HCl	A greencolour	[17]
3	Sulphuricacidtest	10mgextract +dissolved inisopropylalcohol+adrop of conc.H2SO4	A redcolour	[32]

Detection of Reducing Sugar:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Benedict'stest	0.5mLfiltrate[b]+0.5mL*Benedict'sreagent* +Boiledfor2min.	Green/yellow/redcolour	[7, 21]
2	Fehling'stest	1mLeachof *Fehling'ssolutionA &B*+ 1mLfiltrate[b]+ boiledinwaterbath	Aredprecipitate	[7, 21]

Detection of Proteins and Amino acids:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Biurettest	2mLfiltrate+1drop of2%copper sulphatesol.+ 1mLof 95%ethanol+KOHpellets	Apink-colouredsol. (inan ethanoliclayer)	[1, 7]
2	Millon'stest	2mLfiltrate+a fewdropsof*Millon'sreagent*	Awhiteprecipitate	[1, 7]

3	Ninhydrintest	2mLfiltrate+2dropsofNinhydrin solution(10mg ninhydrin+200mLacetone)	Apurplecolouredsol. {Aminoacids}	[1, 7]
4	Xanthoproteictest	Plantextract+ Fewdrops ofconc. Nitric acid	Ayellow-coloured sol.	[1, 12]

Detection of Alkaloids:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Dragendroff's Test	Few ml Filtrate[a]+ 1-2 ml Dragendroff's reagents	Areddish-brownprecipitate	[1, 21]
2	Hager'stest	FewmLfiltrate[a]+1-2mL *Hager'sreagents*	Acreamy whiteprecipitate	[1, 7]
3	Mayer's/Bertrand's/ Valser'stest	FewmLfiltrate[a]+1-2dropsof*Mayer'sreagent* (Alongthe sidesof testtube)	Acreamy white/yellowprecipitate	[7, 12, 13]
4	Wagner'stest	FewmLfiltrate[a]+1-2dropsof*Wagner'sreagent* (Along the sidesof testtube)	Abrown/reddishprecipitate	[7, 21]
5	Picric acid test	FewmLfiltrate[a]+3-4dropsof2%picric acidsolution	Anorangecoloure	[14, 15]
6	Iodine Test	3mLextractsolution+fewdrops ofiodinesolution	Abluecolour,whichdisappearsonboilingandreappears oncooling	[16, 17]
7	Bouchardat's test	6mLplantextract,evaporatedcompletely+6mLethanol (@60°C)+fewdropsofBouchardat'sreagent (dilute iodinesolution)	A reddish browncolour	[18]
8	Tannicacidtest	Acidifiedextract+ 10%tannicacid solution	Abuffcolour precipitate	[30, 38]

Detection of Carbohydrate:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Barfoed'stest	1mLfiltrate[b]+1mL *Barfoed'sreagent*+Heatedfor2min.	Aredprecipitate{monosaccharides}	[7, 19]
2	Molish'stest	2mL filtrate[b]+2dropsofalcoholic α–naphthol+1mLconc.H_2SO_4(alongthesidesoftesttube)	A violet ring	[7, 21]
3	Seliwanoff's Test	1mLextractsolution+3mL *seliwanoff'sreagent*+heated onwaterbath for1min.	Arose redcolour {ketoses}	[17, 19]

4	Resorcinol test	2mLaq.extractsolution+fewcrystalsofresorcinol +equal volumeof conc.HCl +heated	Arosecolour {ketones}	[13, 9]
5	Testforpentoses	2mLconc.HCl+ littleamountofphloroglucinol+equalamountof aqueousextractsolution+ heatedoverflame	A redcolour	[13]
6	Testforstarch	Aqueousextract+ 5mL5%KOH solution	Acinarycolouration	[20]

Detection of Glycosides:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Borntrager'stest	2mLfiltratedhydrolysate[c]+3mLChloroform +shakenwell +chloroformlayer isseparated+10%Ammoniasolution	Apink colouredsolution	[7]
2	ModifiedBorntrager's test	Plantextract+ferric chloridesolution+boil for5min. + cooled + equal volome of benzene + benzene layer isseparated+ Ammoniasolution	Arose-pink toblood redcolouredsolution	[12, 33]
3	Legal'stest	Dissolve 50gmplantextractinpyridine+ Sodiumnitroprusside +10%Sodiumhydroxide	Apink colouredsolution	[7, 12]
4	10% NaOHtest	1mL dil. H2SO4+ 0.2mLextract + boiled for 15min.+allowedcooling+neutralize with10% NaOH+0.2mL*Fehling'ssolutionA&B*	Abrickredprecipitate	[21]
5	Aqueous NaOH test	Alcoholicextract+dissolvedin 1mLofwater +few drops of aqueousNaOHsolution	Ayellowcolour	[22]
6	ConcentrateH2SO4test	5mlplantextract+2mLglacial aceticacid +adrop of5% FeCL3+conc.H2SO4	A brownring	[3]
7	Raymond'stest	Extractsolution+dinitrobenzeneinhotmethanolicalkali	Aviolet colour	[38]

Detection of Cardiac Glycosides:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE

1	Keller-Killanitest	1mLfiltrate+1.5mLglacialaceticacid+ 1 drop of 5% ferric chloride + conc. H2SO4 (along the sideoftesttube)	A blue coloured solution(inacetic acidlayer)	[21, 38]
2	Kedee'stest	4mLextractevaporatedtodryness+1-2mLmethanol+1-2mL alcoholic KOH + 3-4 dropsof 1% alcoholic 3,5-dinitrobenzene+heated	Adisappearing violetcolour {Cardenolides}	[22]
3	TestforCardenolides	Extract+pyridine+Sodiumnitroprussiide +20%NaOH	Aredcolour, fades to brownishyellow	[20]
4	Brominewater test	Plantextract+fewmL ofbrominewater	Ayellowprecipitate	[30]
5	Baljettest	2mLextract+adropof*Baljet'sreagent*	Ayellow-orangecolour	[39, 40]

Detection of Flavonoids:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Alkalinereagenttest	1mLextract+2mLof2% NaOHsolution (+fewdrops dil.HCl)	Anintenseyellow colour,becomes colourlessonadditionof dilutedacid	[20, 21, 23]
2	Hager'stest	Plantextract+10%ammoniumhydroxidesol.	Ayellowfluorescence	[7]
3	Leadacetatetest	1mLplantextract+fewdropsof10%leadacetate solution	Ayellowprecipitate	[1, 21, 12]
4	Shinoda'stest/Mg-hydrochlorideductiontest	Plantextractisdissolved in5mLalcohol+Fragmentsof magnesium ribbon+fewdrops ofconc.HCl	Apinktocrimsoncoloured solution {flavonalglycosides}	[7, 38]
5	Shibata'sreaction/ Cyanidintest	1gmAq.extract+dissolvedin1-2mL50% methanol by heating+metal magnesium+5-6 dropsofconc. HCl	Aredcolour{flavonols}, orange colour {flavones}	[13, 22]
6	Ferricchloridetest	Extractaqueoussolution+fewdrops 10%ferricchloride solution	Agreenprecipitate	[20]
7	Pew'stest	FewmLaqueousextractsolution+0.1gmmetalliczinc+ 8mLconc.H2SO4	Aredcolour {flavonols}	[13]
8	Zinc-hydrochloride	Plantextract+ pinchofzinc dust+ conc.HClalongthe side	Magentacolour	[24, 25]

	reductiontest	oftesttube		
9	Ammonia test	Filtrate+5mLdil.Ammonia solution+conc.H2SO4	Ayellowcolour	[26]

Detection of Phenolic Compounds:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Iodine test	1mLextract+few dropsofdil. Iodine sol.	Atransientredcolour	[21]
2	Ferricchloridetest	Extractaqueoussolution+fewdrops 5%ferricchloridesol.	Darkgreen/bluishblack colour	[7, 12]
3	Gelatintest	Plantextractis dissolvedin5mL distilledwater+ 1% gelatinsolution+10%NaCl	Awhiteprecipitate	[7]
4	Leadacetatetest	Plantextractis dissolvedin5mL distilledwater+3mLof 10%lead acetatesol.	Awhiteprecipitate	[7]
5	EllagicAcidTest	Plantextract aqueoussolution+5% glacialacetic acid+5% sodiumnitritesolution	Solutionturns muddy / Nigerbrownprecipitate	[3, 16]
6	Potassiumdichromate test	Plantextract+fewdropsofpotassiumdichromatesolution	A dark colour	[27]
7	Hotwatertest	Warmwaterinbeaker+matureplantpartisdipped + warmedforamin.	Blackor browncolour ringatthe junction of dipping	[34]
8	TestforCartenoids	(1gmextract+10mLchloroform, vigorously shakenand filtered).Filtrate+conc. H2SO4	Ablue colourattheinterface	[35]

Detection of Tannins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Gelatintest	Plantextractis dissolvedin5mL distilledwater+1% gelatinsolution+10%NaCl	Awhiteprecipitate	[12, 33]
2	Braymer'stest	1mLfiltrate[d]+3mLdistilledwater+3drops10%Ferric chloridesolution	Blue-greencolour	[21, 28]
3	10% NaOHtest	0.4mLplantextract+4mL 10%NaOH+shakenwell	Formationofemulsion {Hydrolysabletannins}	[21]

4	Bromine water test	10 mlofbromine water+0.5gmplantextract	Decolorationofbromine	[23]
5	Leadsub acetatetest	1mLfiltrate[e]+ 3dropsof leadsub acetatesolution	Acreamy gelatinousprecipitate	[3]
6	Phenazonet est	(5mLaq.extract+0.5gofsodiumacidphosph ate, heated, allowedtocool+filtered);filtrate+2% solutionofphenazone	Precipitation	[9]
7	Mitchell'st est	Extract solution+iron+sodiumtartarate(+ammoniu m acetatesolution)	A water-soluble iron-tannin complex,whichisinsolu ble in solution of ammoniumacetate	[39]

Detection of Sapnins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Foamtest	0.5gmplantextract+2mL water (vigorously shaken)	Persistentfoamfor10 min.	[12]
2	Braymer'st est	20mLwaterinmeasuringcylinder +50gmextract (vigorously shakenfor15 min.)	Formationof2cmthickl ayer offoam	[7]
3	10% NaOHtest	0.2gmplantextract+5mLdistilled water;shakenwell; heatedtoboiling	Appearanceofcreamy missofsmall bubbles	[29]
4	NaHCO3te st	Plantextract+fewmLsodiumbicarbonatesol ution+ distilledwater(vigorously shaken)	Stablehoneycomblikef roth	[30]

Detection of Phlobatannins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	HCl test	2mLaq.extract +2mL 1%HCl (boiled)	Aredprecipitate	[5, 13]

Detection of Phytosterols:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Salkowski'stest	Filtrate[f]+ few drops of conc. H2SO4(Shakenwelland allowedtostand)	Redcolour (inlowerlayer)	[21, 12]
2	Libermann-	50gmextract isdissolved in2mL	Anarrayofcolou	[7]

	Burchard's test	aceticanhydride +1-2 dropsof conc.H2SO4(alongthe side of testtube)	r change	
3	Aceticanhydride test	0.5mLplantextract+2mLofaceticanhydride+2 mLconc. H2SO4	Changein colourfromviol etto blue/green	[26]
4	Hesse'sresponse	5mLaq.extract+ 2mLchloroform+2mL conc.H2SO4	Pinkring/Red colour (inlower chloroformlaye r)	[23, 40]
5	Sulphur test	Extract solution+pinchofsulphurpowder	Sulphursinks tothe bottom	[34]

Detection of Cholesterol:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1		2mLextract+ 2mLchloroform+ 10dropsof acetic anhydride+2-3dropsof conc.H2SO4	Ared-rose colour	[22]

Detection of Terpinoids:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1		2mlchloroform+5mLplantextract,(evaporatedonwater bath)+3mLconc. H2SO4(boiledonwaterbath)	A grey colouredsolution	[23]

Detection of Triterpinoids:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Salkowski'stest	Filtrate[f]+ few drops of conc. H2SO4(Shakenwelland allowedtostand)	Goldenyellowlayer (at the bottom)	[21]

Detection of Lignins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Labattest	Extract solution+gallicacid	Anolive greencolour	[16, 38]
2	Furfuraldehydetest	Extract solution+2%furfuraldehydesolution	A redcolour	[16, 38]

Detection of Diterpenes:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Copperacetatetest	Plantextract isdissolved indistilled water+3-4dropsof copperacetatesolution	Emeraldgreencolour	[12, 33]

Detection of Carotenoids:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Carr-Pricereaction	10mLextractevaporated todryness +2-3dropsofsaturated solutionofantimony trichloride inchloroform	Ablue-greencoloureventuallychangingto red	[22]

Detection of Anthraquinones:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Borntrager'stest	10mL10%ammonia sol.+fewmlfiltrate^{g}(shaken vigorously for30sec.)	Apink,violet, orredcoloured solution	[5, 23, 28]
2	Ammoniumhydroxide test	10mgextract isdissolvedinisopropylalcohol+adropof conc.ammoniumhydroxide solution	Formationofredcolourafter 2min.	[32]

Detection of Anthocyanins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	HCl test	2mLplantextract+ 2mL 2NHCl (+Few mLammonia)	Pink-redsol.whichturnsblue-violetafter additionofammonia	[18, 31]

Detection of Leuconthocyanins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Isoamylalcohol test	5mLplantextract +5mLisoamyl alcohol	The upperlayerappearsred	[31]

Detection of Carboxylic acid:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Effervescencetest	1mLplantextract +1mLsodiumbicarbonatesolution	AppearanceofEffervescence	[21, 26]

Detection of Coumarins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	NaOHpaper test	0.5gmmoistenedextractistakenina testtube the , mouthoftest tube is covered with 1N NaOH treated filter paper, heatedforfew min. inwater bath	Yellow fluorescence from paper under theUVlight	[21, 26]
2	NaOHtest	Plantextract+10%NaOH+Chloroform	Ayellowcolour	[27]

Detection of Resins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Aceticanhydridetest	1mLplantextract+Aceticanhydridesolution+ 1mLconc. H2SO4	Orangetoyellow	[21,26]
2	Turbiditytest	1mLplantextractdissolvedinacetone,pouredindistilledwater	Turbidity	[34]
		10mLextract +20mL 4%HCl		[36]

Detection of Emodins:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1		Plantextract+2mLNH4OH+3mLbenzene	A redcolour	[29]

Detection of Gums and Mucilages:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE
1	Alcohol test	Dissolve 100mgextractin 10mLdistilledwater+25mL absolutealcohol(constantstirring)	Whiteorcloudyprecipitate	[7]

Detection of Fixed oils and Fats:

S/N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFERENCE

1	Spottest/ Staintest	Littlequantityofplant extractispressedin betweentofilter papers	Oilstain onthepaper	[7, 38]
2	Saponificationt est	Extract+few dropsof0.5NalcoholicKOH + Adrop of phenolphthalein(Heatedfor 2hr.)	Soapformationorpartialneutralizati onof alkali	[7,[38]
3	Saponificationt est	Extract solutionisappliedonfilterpa per	Atransparentappearance{oilsandre sins}	[35, 36]

Detection of Volatile Oils:

S/ N	TEST	PROCEDURE	OBSERVATION (Indicating Positive Test)	REFEREN CE
1	Fluorescence test	l0mLofextract,filteredtillsaturation,exposed toUVlight	Brightpinkishfluores cence	[37]

[a]=50gm solvent free extract is mixed withfew mL dil. HCl and then filtered

[b]=100mgsolvent free extractisdissolved in5mL of distilledwater and filtered

[c]=50gm ofplant extractis hydrolysedwithconc.HClfor 2hr onwater bath andfiltered

[d]=3gmpowderedsample boiledin50mLdistilledwaterfor3 min.andthenfiltered

[e]=Smallquantityofextractboiledwith5mLof45%ethanol for5min. themcooledand filtered

[f]=Equalquantity ofchloroformis treatedwithplant extractandfiltered

[g]=3mLofaq.The extractisshakenwith3mLofbenzeneandfiltered[5]OR10mLofbenzeneisaddedintheplantsampleandsoaked for10min.thenfiltered [23]OR extract is maceratedwith ether andfiltered[28].

{}=Indicatesthe presenceofspecificphytoconstituents.

Note: Thecompositionsofvariousreagentsandsolutionsdenotedby italicfonthavebeenmentionedin'Preparation of reagent for Phytochemical Analysis'

CONCLUSION:

Thephytochemicalanalysisisverymuchimportanttoevaluate the possible medicinal utilities of a plant and also todeterminetheactiveprinciplesresponsibleforthe knownbiologicalactivitiesexhibitedbytheplants.Further,itprovides the base for the targeted isolation of compounds and toperformmorepreciseinvestigations.Extractionofaphytochemical from the plant material is mainly

dependent onthetypeofsolventused.Similarly,thetestappliedforphytochemical analysis determines the presence or absence ofa phytochemical in the sample. Hence, two or more differenttestsshould beperformed formoreaccurate results.

REFERENCE:

1. SilvaGO,AbeysundaraAT,AponsoMM.Extractionmethods,qualitativeandquantitativetechniquesforscreeningofphytochemicalsfromplants.AmericanJournalofEssentialOilsandNaturalProducts.2017;5(2):29-32.
2. Ezeonu CS, Ejikeme CM. Qualitative and QuantitativeDetermination of Phytochemical Contents of IndigenousNigerianSoftwoods.NewJournal ofScience,2016,1-9.
3. Sheel R, Nisha K, Kumar J. Preliminary PhytochemicalScreeningofMethanolicExtractof*Clerodendroninfortunatum.* IOSR Journal of Applied Chemistry. 2014;7(1):10-13.
4. SaxenaM,SaxenaJ,NemaR,SinghD,GuptaA.PhytochemistryofMedicinalPlants.JournalofPharmacognosyandPhytochemistry.2013;1(6):168-182.
5. Njoku OV, Obi C. Phytochemical constituents of someselected medicinal plants. African Journal of Pure andAppliedChemistry.2009;3(11):228-233
6. KocabasA.EaseofPhytochemicalExtractionandAnalysis from Plants. Anatolian Journal of Botany. 2017;1(2):26-31.
7. RaamanN.PhytochemicalTechniques.NewIndiaPublishingAgency,NewDelhi,2006,19-24.
8. Emran TB, Mir MN, Rahman A, Zia Uddin, Islam M.Phytochemical, Antimicrobial, Cytotoxic, Analgesic andAnti-inflammatory Properties of *Azadirachta indiaca*: ATherapeuticStudy.JournalofBioanalysisandBiomedicine.2015;12:1-7.
9. Evans WC. Trease and Evans Pharmacognosy. 16th Edn.SaundersElsevier,2009,135-415.
10. Azwinda NN. A Review on the Extraction Method Use inMedicinalPlants,Principle,StrengthandLimitation.Medicinaland AromaticPlants.2015;4(3):1-6.
11. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect ofextraction methods on yield, phytochemical constituentsand antioxidant activity of *Withania somnifera*. ArabianJournalofChemistry.2017;10:1193-1199.
12. TiwariP,KumarB,KaurM,KaurG,KaurH.PhytochemicalscreeningandExtraction:AReview.InternationalePharmaceuticaSciencia.2011;1(1):98-106.
13. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu Aand Ibrahim A. Preliminary phytochemical and elementalanalysisofaqueousandfractionatedpodextractsof*Acacianilotica*(Thornmimosa).VeterinaryResearchForum.2014;5(2):95-100.
14. Deshpande PK, Gothalwal R, Pathak AK. PhytochemicalAnalysisandEvaluationofAntimalarialActivityof*Azadirachtaindica*.ThePharmaInnovation.2014;3(9):12-16.
15. IndhumatiV,PerundeviS,VinojaBN,Manivasagan,Saranya K, Ramesh, Babu NG. Phytochemical ScreeningandAntimicrobialActivityofFreshandShadeDriedLeaves of *Azaadirachta indica.* International Journal ofInnovationsinEngineeringandTechnology.2018;11(3):27-32.
16. Bhatt S, Dhyani S. Preliminary Phytochemical

ScreeningofAilanthusexcelsaRoxb.InternationalJournalofCurrentPharmaceuticalResearch.2012;4(1):87-89.

17. Basumatary AR. Preliminary Phytochemical Screening ofsomecompoundsfromplantstembarkextractsofTabernaemontanadivaricataLinn.usedbyBodoCommunity at Kokrajhar District, Assam, India. ArchivesofApplied ScienceResearch.2016;8(8):47-52.
18. Obouayeba AP, Diarrassouba M, Soumahin EF, KouakouTH.PhytochemicalAnalysis,PurificationandIdentificationofHibiscusAnthocyanins.JournalofPharmaceutical, Chemical and Biological Sciences. 2015;3(2):156-168.
19. Sadasivam S, Manickam A, Biochemical methods. Edn 3,New Age International Limited, Publishers, New Delhi,2005,1-4.
20. AuduSA,MohammadI,KaitaHA.Phytochemicalscreeningoftheleavesof*Lophiralanceolata*(Ochanaceae).LifeScienceJournal.2007;4(4):75-79.
21. Singh V, Kumar R. Study of Phytochemical Analysis andAntioxidant Activity of *Allium sativum* of BundelkhandRegion. International Journal of Life Sciences ScientificResearch.2017;3(6):1451-1458.
22. JagessarRC.Phytochemicalscreeningandchromatographicprofileoftheethanolicandaqueousextract of *Passiflora edulis* and *Vicia faba* L. (Fabaceae).JournalofPharmacognosyandPhytochemistry.2017;6(6):1714-1721
23. GulR,JanSU,SyedF,SheraniF,NusratJahan.PreliminaryPhytochemicalScreening,QuantitativeAnalysis of Alkaloids, and Antioxidant Activity of CrudePlant Extracts from *Ephedra intermedia* Indigenous toBalochistan.The ScientificWorld Journal,2017,1-7.
24. Vimalkumar CS, Hosagaudar VB, Suja SR, VilashV,Krishnakumar NM, Latha PG. Comparative preliminaryphytochemical analysis of ethanolic extracts of leaves of*Oleadioica*Roxb.,infectedwiththerustfungus*Zaghouaniaoleae*(E.J.Butler)Cumminsandnon-infectedplants.JournalofPharmacognosyandPhytochemistry.2014;3(4):69-72.
25. Pant DR, Pant ND, Saru DB, Yadav UN, Khanal DP.Phytochemicalscreeningandstudyofantioxidant,antimicrobial,antidiabetic,anti-inflammatoryandanalgesicactivitiesofextractsfromstemwoodof*Pterocarpusmarsupium*Roxburgh. JIntercultEthnopharmacol.2017;6(2):170-176.
26. KumarRS,VenkateshwarC,SamuelG,RaoSG.Phytochemical Screening of some compounds from plantleafextractsof*Holopteleaintegrifolia*(Planch.)and*Celestrusemarginata*(Grah.) used byGondu tribesatAdilabadDistrict,Andhrapradesh,India.InternationalJournal of Engineering Science Invention. 2013; 2(8):65-70.
27. KumarR,SharmaS,DeviL.InvestigationofTotalPhenolic, Flavonoid Contents and Antioxidant ActivityfromExtractof*Azadirachtaindica*ofBundelkhandRegion.InternationalJournalofLifeSciencesandScientificResesrch.2018,4(4):1925-1933.
28. Uma KS, Parthiban P, Kalpana S. Pharmacognostical andPreliminary Phytochemical Screening of Aavaarai VidhaiChooranam.AsianJournalofPharmaceuticalandClinicalResearch.2017;10(10):111-116.
29. RaufA,RehmanW,JanMR,MuhammadM.Phytochemical,phytotoxicandantioxidantprofileof *Caralluma tuberculata* N.E. Brown. Journal of PharmacyandPharmacology.2013;2(2):021-025.
30. RayS, Chatterjee S, ChakrabartiCS. AntiproliferativeActivity of Allelo chemicals Present in

Aqueous Extractof *Synedrella nodiflora* (L.) Gaertn. In Apical MeristemsandWistarRatBoneMarrowCells.IosrJournalofPharmacy.2013;3(2):1-10.

31. SavithrammaN, RaoML, Suhrulatha D. ScreeningofMedicinal Plants for Secondary Metabolites. Middle-EastJournal ofScientificResearch.2011;8(3):579-584.
32. Maria R, Shirley M, Xavier C, Jaime S, David V, Rosa S*et al*. Preliminary phytochemical screening, total phenoliccontentandantibacterialactivityofthirteennativespecies from Guayas province Ecuador. Journal of KingSaudUniversity Science.2018;30:500-505.
33. PandeyAandTripathiS.Conceptofstandardization,extraction and pre phytochemical screening strategies forherbaldrug.JournalofPharmacognosyandPhytochemistry.2014;2(5):115-119.
34. Pooja S, Vidyasagar GM. Phytochemical screening forsecondarymetabolites of*Opuntia dillenii* Haw. JournalofMedicinalPlantsStudies.2016;4(5):39-43
35. Tyagi T. Phytochemical Screening of Active MetabolitesPresent in *Eichhornia Crassipes* (Mart.) Solms and *Pistiastratiotes* (L.): Role in Ethanomedicine. Asian Journal ofPharmaceutical Education and Research. 2017; 6(4):40-56.
36. SanthiK,SengottuvelR.QualitativeandQuantitativePhytochemical analysis of *Moringa concanensis* Nimmo.InternationalJournalofCurrentMicrobiologyandAppliedSciences.2016;5(1):633-640.
37. Mallhi TH, Qadir MI, Khan YH, Ali M. Hepatoprotectiveactivity of aqueous methanolic extract of *Morus nigra*againstparacetamol-inducedhepatotoxicityinmice.Bangladesh Journalof Pharmacology.2014;9:60-66.
38. NannaRS,BanalaM,PamulaparthiA,KurraA,KagithojuS.EvaluationofPhytochemicalsandFluorescentAnalysisofSeedandLeafExtractsof*Cajanus cajan* L. International Journal of PharmaceuticalSciencesReviewand Research.2013;22(1):11-18.
39. Rahman MA, Rahman MA, Ahmed NU. Phytochemicaland biological activities of ethanolic extract of *C. hirsute*leaves. Bangladesh Journal of Scientific andIndustrialResearch.2013;48(1):43-50.
40. KumarV,JatRK.PhytochemicalEstimationofMedicinal Plant *Achyranthes Aspera*Root. InternationalJournalofResearchinPharmacyandPharmaceuticalSciences.2018;3(1):190-193.

