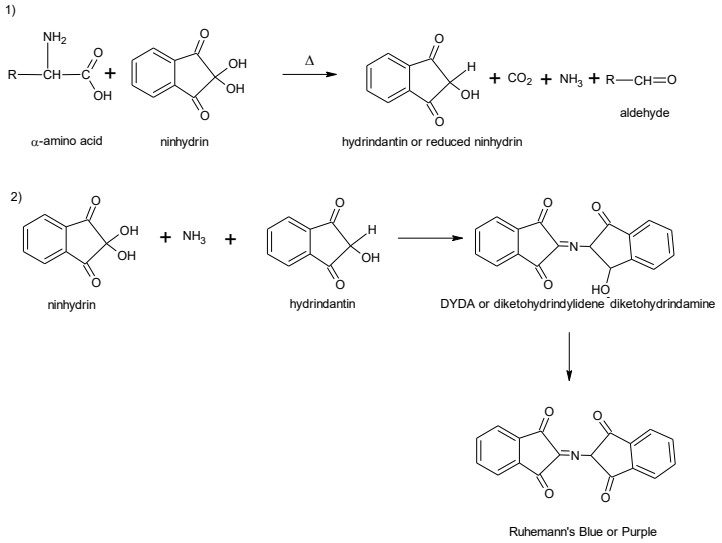
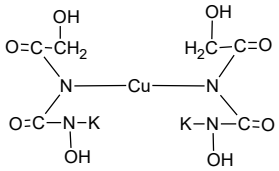
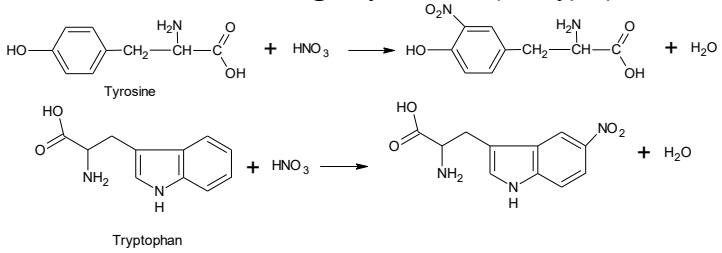
PHYTOCHEMICAL SCREENING

Department of Biochemistry and Molecular Biology, College of Medicine
University of the Philippines Manila

PRIMARY METABOLITES

Test	Principle involved
1. Proteins and amino acids	
Use albumin as positive control.	
1.1. Ninhydrin test To 0.5 mL of the sample, add 1-2 drops of 0.25% (w/v) Ninhydrin reagent. Mix and heat to boiling for 1-2 minutes. **(+)** Development of purple-blue or blue color (Oser, 1966, p. 181; Cabatit, 1988, p. 142; Bettelheim and Landesberg, 2000, p. 456) or deep-violet / Ruhemann's purple (Wade, 1991, p. 1117)	
1.2. Biuret test To 0.5 mL of the sample, add 0.5 mL of 10% NaOH. Add 0.5% $CuSO_4$ dropwise. **(+)** Development of blue color (Oser, 1966, p. 179) pink color (Oser, 1966, p. 179) purplish violet or pinkish-violet color (Oser, 1966, p. 179; Abaya, et al, 1980, p. 55) rose pink to violet to purple color (Cabatit, 1988, p. 142; Bettelheim and Landesberg, 2000, p. 455)	Formation of copper-potassium-biuret compound (cupripotassium biuret or biuret potassium cupric hydroxide) from compounds containing 2 carbamyl (-CONH2) groups joined either directly together or through a single atom (e.g., nitrogen). 
1.3. Xanthoproteic test To 0.5 mL of the sample, add 0.25 mL of concentrated nitric acid. Heat carefully under the hood and observe whether the precipitate turns yellow and finally dissolves. Cool and add enough 4-5mL 10% NaOH to make the solution definitely alkaline. **(+)** formation of orange to yellow color	Nitration of the benzene ring of tyrosine and/or tryptophan. 

2. Carbohydrates and reducing substances

Use 10% glucose as positive control for Fehling's test and Molisch's test. Use 10% fructose as positive control for Seliwanoff's test.

2.1. Benedict's test for reducing substances

To 0.5mL of sample, add 0.5mL of Benedict's reagent. Heat (boiling water bath, 2min).

(+) brick red precipitate (but color may range from green, yellow, orange to brick red)

Cu_2O formation under basic conditions via the reduction of Cu^{2+} to Cu^{+} and oxidation of reducing substances (e.g., reducing sugars).

2.2. Fehling's test for reducing substances

Boil (in a boiling water bath) 1mL of sample with 1mL each of Fehling's solutions A and B.

(+) brick red precipitate (but color may range from green, yellow, orange to brick red)

Cu_2O formation under basic conditions via the reduction of Cu^{2+} to Cu^{+} and oxidation of reducing substances (e.g., reducing sugars).

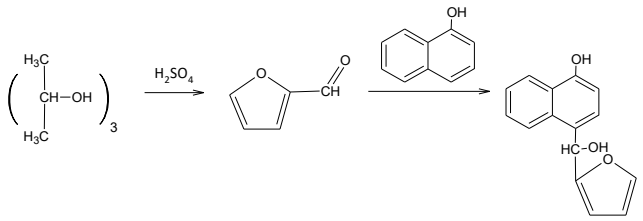
2.3. Molisch's test for carbohydrates

To 2mL of sample, add 1mL 1% α-naphthol in 95% ethanol. Add concentrated H_2SO_4 slowly, down the sides of the tube.

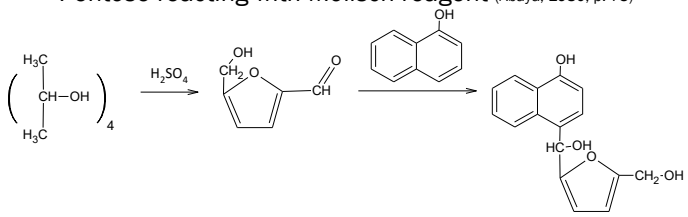
(+) Purple ring at the junction of two liquids
(Nucum and Santiago, 2005, p. 36)

Violet ring at the junction of two liquids
(Cabatit, 1988, p. 65)

Dehydration of carbohydrates by strong mineral acids to form furfural or furfural derivatives.



Pentose reacting with Molisch reagent (Abaya, 1980, p. 73)



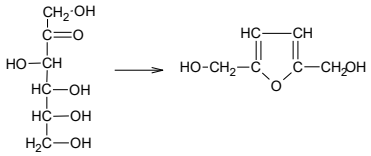
Hexose reacting with Molisch reagent (Abaya, 1980, p. 74)

2.4. Seliwanoff's test for ketoses

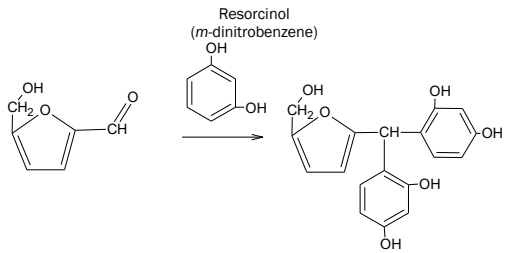
To 1mL of sample previously diluted with an equal amount of distilled water, add a crystal of resorcinol. Add 1 mL conc. HCl then heat on a boiling water bath for 1 minute. Continue heating and observe the color change at one minute intervals for 4 minutes.

(+) bright cherry red coloration
(Nucum and Santiago, 2005, p. 36; Cabatit, 1988, p. 88)

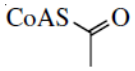
The furfural derivative further undergoes condensation with resorcinol.

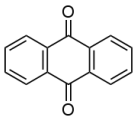


Fructose to hydroxymethylfurfural

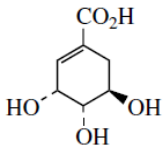


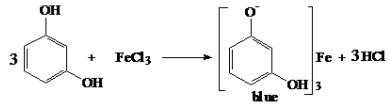
Reaction of 5-hydroxymethylfurfural with resorcinol (Abaya, 1980, p. 76)



Test	Principle involved
3. Anthraquinone glycosides	
Use rutin hydrate as positive control.	
3.1. Hydrochloric acid test To 1mL of sample, add few drops of 2% HCl. **(+)** red precipitate	Precipitation of anthraquinones. 
3.2. Bornträger test (Free anthraquinone test) To 2mL of sample, add 3mL each of chloroform and ammonia TS (10%). **(+)** formation of rose-pink to cherry red color	Extraction of anthraquinones with chloroform, and subsequent washing of the aqueous layer with ammonia. (Evans, 2009)
3.3. Modified Bornträger test Evaporate 5mL of sample to dryness in water bath. Add a mixture of 10mL 5% $FeCl_3$ and 5mL conc. HCl. Heat (water bath, 10min). Filter, then shake the filtrate with 10mL chloroform and 5mL ammonia TS (10%). **(+)** formation of rose-pink to cherry red color	Oxidative hydrolysis to release anthraquinones. Extraction of anthraquinones with chloroform. (Evans, 2009)
3.4. Shouteten reaction To about 1g of concentrated sample, add 20mL boiling water and shake. Cool and add 1g talc. Add 0.25g borax, then heat. Dilute 2mL of the heated solution with water to make 20mL. View under long wavelength (365nm) UV light. **(+)** yellowish green which fluoresce	Strong greenish fluorescence is exhibited in the presence of borax by anthranols, which are readily formed from anthrones by isomerism. (Evans, 2009)

SECONDARY METABOLITES
THE SHIKIMATE PATHWAY:
AROMATIC AMINO ACIDS AND PHENYLPROPANOIDS



Test	Principle involved
4. Plant Acids	
Use 2% HCl as positive control.	
To 1mL of sample, add 1mL $NaHCO_3$ solution. **(+)** Dense stable froth.	Neutralization to release CO_2.
5. Phenols	
Use gallic acid as positive control.	
5.1. Ferric chloride test To 1mL of sample, add few drops of 5% $FeCl_3$ solution. **(+)** Formation of bluish-black or green color	Complex formation. 
5.2. Lead acetate test To 1mL of sample, add 3mL 10 $Pb(CH_3COO)_2$ solution. **(+)** Bulk white precipitate	Formation of insoluble lead salts.
6. Quinones	
6.1. Sulfuric acid test To 1mL of sample, add 1mL conc. H_2SO_4. **(+)** Formation of red color	
7. Tannins and phenolic glycosides	
Use catechin as positive control.	
7.1. Ferric chloride test To 1mL of sample, add 0.25mL of 5% $FeCl_3$. **(+)** Formation of blue **(hydrolysable tannins)** or green **(condensed tannins)** color.	Complex formation of Fe^{3+} with phenolic moieties.
7.2. Gelatin test To 1mL of sample, add 1% gelatin solution containing 10% NaCl. **(+)** jelly precipitate	Gelatin precipitates under the cross-linking action of tannins.
8. Coumarins	
8.1. Alkaline reagent test To 1mL of sample, add 1mL 10% NaOH. **(+)** formation of yellow color	

9. Flavonoids

Use quercetin as positive control.

9.1. Alkaline reagent test

To 1mL of sample, add few drops of 20% NaOH. Then, add few drops of dil. HCl.

(+) Formation of intense yellow color upon addition of base, and turns colorless upon addition of acid

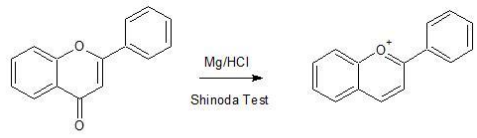
pH-dependent color changes.

9.2. Shinoda test for flavones and flavonols

To 1mL of sample, add few Mg turnings and few drops of conc. HCl.

(+) Formation of pink, scarlet, crimson red, or, occasionally, green to blue color

Reduction of the flavonoid to an anthocyanidin, with the metal acting as electron donor and the acidic condition to provide a supply of protons.



general flavone backbone
yellow color

anthocyanidin skeleton
orange to deep-red color

9.3. Lead acetate test

To 1mL of sample, add few drops of $Pb(CH_3COO)_2$ solution.

(+) white to yellow precipitate

Precipitation as lead salts.

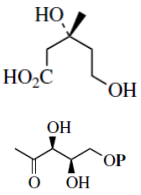
9.4. Alkaline reagent test for cyanins

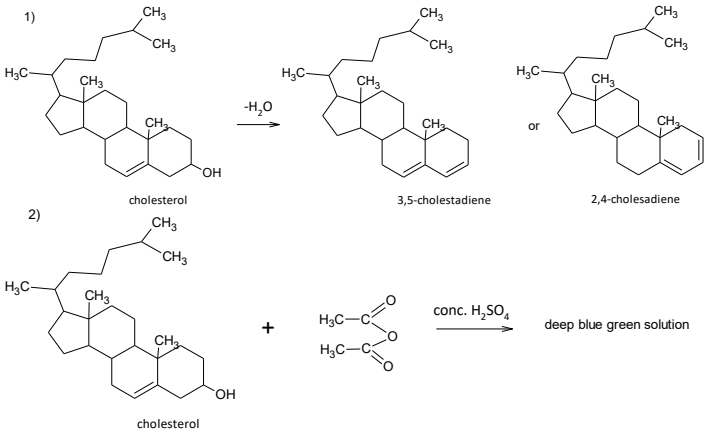
To 1mL of sample, add 0.5mL 2M NaOH. Heat (5min, 100°C).

(+) formation of bluish green color **(anthocyanin)** or
formation of yellow color **(betacyanin)**

pH-dependent color changes of cyanins.

SECONDARY METABOLITES
THE MEVALONATE AND DEOXYXYLULOSE PATHWAY: TERPENES AND STEROIDS



Test	Principle involved
10. Terpenes and terpenoids	
10.1. Copper acetate test for diterpenes To 1mL of sample, add 3-4 drops $Cu(CH_3COO)_2$ solution. **(+)** formation of emerald green color	
10.2. Salkowski's test for triterpenes To 1mL of sample, add 2mL chloroform and few drops of concentrated H_2SO_4. Shake and let stand. **(+)** formation of golden yellow color	Oxidation to yield a colored derivative.
10.3. Salkowski's test for terpenoids To 1mL of sample, add 2mL chloroform. Then, add 3mL of concentrated H_2SO_4 slowly, down the sides of the tube. **(+)** formation of reddish brown coloration at the interphase	Oxidation to yield a colored derivative.
11. Steroids and phytosterols	
11.1. Liebermann-Burchard test for unsaturated sterols (acetic anhydride-sulfuric acid test) To 1 mL of sample, add a few drops of acetic anhydride. Then, add 1 drop conc. H_2SO_4. **(+)** Reddish brown ring at the junction, which may turn into green, blue, or purple Red to blue to bluish green color Lilac color gradually turning to blue and then finally to emerald green color (Nucum and Santiago, 2005, p. 55; Cabatit, 1988, p. 118) Pink color then to lilac and finally to deep green (Bettelheim and Landesberg, 2000, p. 429)	
12. Saponins and sapogenins	
Use saponin standard (from quillaja bark) as positive control.	
12.1. Froth test for saponins Dilute 2.5mL sample to 10mL with distilled water. Shake vigorously (2min). **(+)** Honeycomb froth >3cm stable for at least 30 min.	Reduction of surface tension as a consequence of the amphiphilic nature of saponins
12.2. Cholesterol crystallization test for saponins and sapogenins To 5mL of sample, add few drops of saturated alcoholic solution of cholesterol **(+)** crystal formation	

13. Cardiac glycosides

Use rutin hydrate as positive control.

13.1. Salkowski's test (Sulfuric acid test)

To the concentrated sample, add 0.5mL each of chloroform and H_2SO_4.

(+)
chloroform layer:
bluish red to cherry red and purple solution
acid layer:
green fluorescence
(Cabatit, 1988, p. 105)

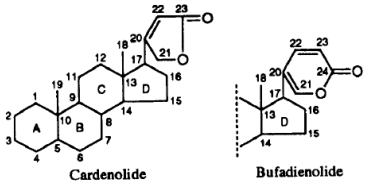
Oxidation yields a red-colored derivative (λ_{max} 560nm). (Evans, 2009)

13.2. Baljet test

Combine 1mL each of 1% picric acid in ethanol and NaOH TS. Add a small amount of evaporated sample.

(+) formation of yellow to orange color

The butenolide moiety forms a red color (λ_{max} 495nm) with alkaline sodium picrate reagent. (Evans, 2009)



13.3. Legal test

Dissolve a small amount of the solid sample in 2-3 drops pyridine. Add 1 drop 0.5% recently prepared sodium nitroprusside. Then add 4 drops 0.2N NaOH.

(+) formation of red color

The butenolide moiety forms a red color (λ_{max} ~470nm) with sodium dinitroprusside. (Evans, 2009)

13.4. Keller-Killiani test

To 1mL of sample, add 3mL ferric chloride reagent, and shake. Then, add 1mL H_2SO_4 slowly, down the sides of the tube.

(+) formation of a reddish-brown layer at the junction of the two liquids and the upper layer slowly becomes bluish-green, darkening with standing (Evans, 2009)

Specific for deoxysugars (e.g., digitoxose). The sugar dissolves in acetic acid with a trace of $FeCl_3$, and transferred at the surface of H_2SO_4.
(Evans, 2009)

SECONDARY METABOLITES

ALKALOIDS, CYANOGENIC GLYCOSIDES

Test	Principle involved
14. Alkaloids	
Use reserpine as positive control.	
To about 4mL of sample, add sufficient 1% HCl until acid to litmus. Divide this into 4, and add the following reagents along the sides of the tube: **14.1. Mayer's test** Mayer's reagent (mercuric-potassium iodide TS) **(+)** white or creamy precipitate **14.2. Valser's test** Valser's reagent (mercuric iodide TS) **(+)** white precipitate **14.3. Wagner's test** Wagner's reagent (iodine in potassium iodide TS) **(+)** reddish brown precipitate **14.4. Hager's test** Hager's reagent (saturated solution of picric acid in water) **(+)** yellow precipitate **14.5. Dragendorff's test** Dragendorff's reagent (bismuth potassium iodide TS) **(+)** orange-red to red precipitate	Halogenation for most alkaloidal reagents. Formation of an insoluble picrate salt with Hager's reagent.
15. Cyanogenic glycosides	
15.1. Grignard's test (Sodium picrate test) Moisten a strip of filter paper with 10% aqueous picric acid, drain then dip into 10% Na_2CO_3, then drain again. Trap this strip with a cork in a 10-mL test tube containing a small amount of the sample. **(+)** brown-red coloration in the filter paper	Hydrolysis, yielding HCN (which oxidizes paper)

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