Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 1714-1721
Received: 15-09-2017
Accepted: 17-10-2017

RC Jagessar
(a) Department of Chemistry, University of Guyana, Turkeyen Campus, Guyana
(b) Department of Biology, University of Guyana, Turkeyen Campus, Guyana

Correspondence
RC Jagessar
(a) Department of Chemistry, University of Guyana, Turkeyen Campus, Guyana
(b) Department of Biology, University of Guyana, Turkeyen Campus, Guyana

Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of *Passiflora edulis* and *Vicia faba* L. (Fabaceae)

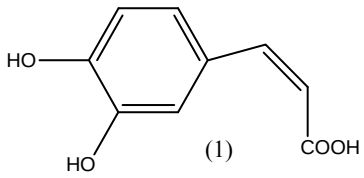
RC Jagessar

Abstract
Phytochemical screening provides first hand knowledge of plants chemical constituents. Phytochemical Screening of the ethanolic extract of *Passiflora edulis* indicates the presence of the following phytochemicals: Cardiac Glycosides, Tannins, Flavanoids, Emodols, Anthracenosides, Alkaloids, Glycosides, Sterol and Triterpenes, Saponins, Phlobatinins. Cholesterol wasn't detected. In contrast, phytochemical screening of the ethanolic extract of *Vicia faba* L. (Fabaceae) indicates the presence of all phytochemicals with the exception of Anthracenosides and Sterols and Triterpenes. The aqueous extract of both plants showed fewer phytochemicals in comparison to the ethanolic extract. For *Passiflora edulis*, these include Tannins, Emodols, Anthracenosides, Glycosides and Saponins, reducing sugars. For *Vicia fabia*, these include Tannins, Alkaloids, Glycosides, Sterol and Triterpenes and Saponins. Reducing sugars were also detected for the ethanolic extract only. The other phytochemicals weren't detected. The selective presence of phytochemicals were also evident.

Keywords: Phytochemicals, *Passiflora edulis*, *Vicia faba* L. Tannins, flavonoids, emodols, anthracenosides, alkaloids, glycosides, sterol and triterpenes, saponins, phlobatinins

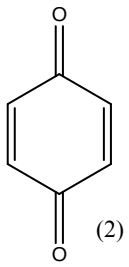
Introduction
Phytochemical screening is a process of evaluating a plant's phytochemical constituents using standard established tests [1-4] and provides first hand knowledge of a plant's phytochemical constituents where instrumentation such as NMR spectroscopy is lacking. It's a prerequisite for chromatographic separation of plant extracts for known and unknown natural products constituents whose structure would then be elucidated using ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMBC and HMQC spectroscopy [4]. Natural products are secondary metabolites of plants and animals which are of restricted occurrence and are classified into major groups such as sterols, triterpenes, flavones aglycones, emodols (anthracenosides aglycones), coumarins, coumarins lactone derivatives, tannins (gallic), reducing compounds, sterols glycosides, cardenolides, saponins and sapogenins [5-6]. Some of these are shown in Fig. 1.0 and Fig. 2.0. They vary widely in both type and concentrations in different parts of the plant and are found as minor components of plant tissues. They are synthesized via metabolic pathways [5-6] and can be either novel or known and are isolated from crude plant extracts whose medicinal activity can be compared with that of the isolates [6-15].

Simple Phenols and phenolic acids **Quinones** **Flavones and Flavonoids**



(1)

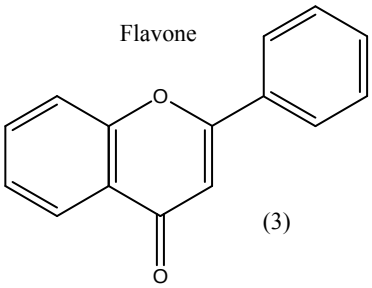
Caffeic acid



(2)

Quinone

Flavone



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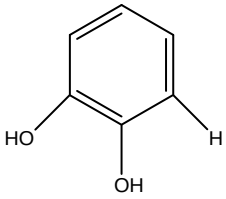
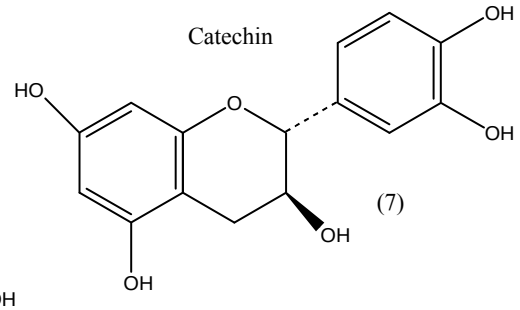


(4)

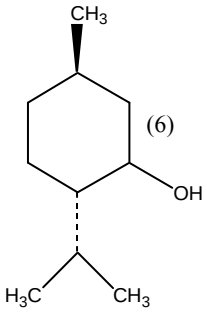
Catechol

Catechin



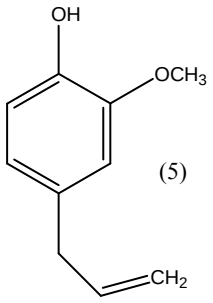
(7)

Terpenoids



(6)

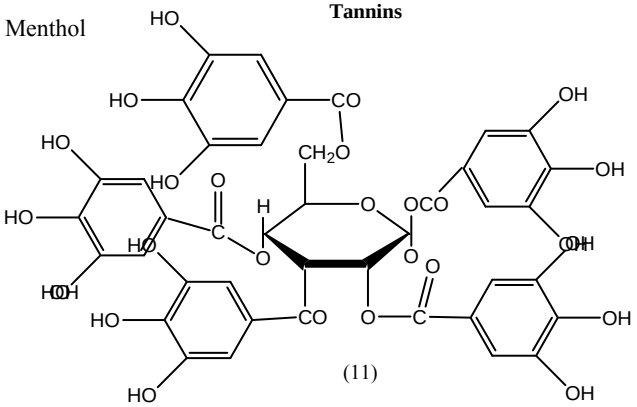
Menthol



(5)

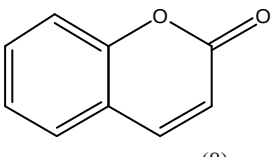
Eugenol

Tannins



(11)

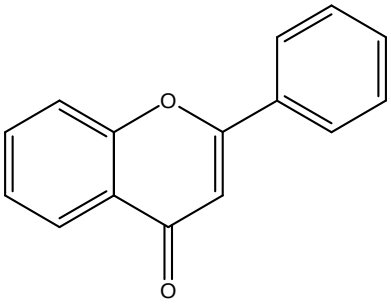
Pentagalloylglucose (hydrolyzable tannins)



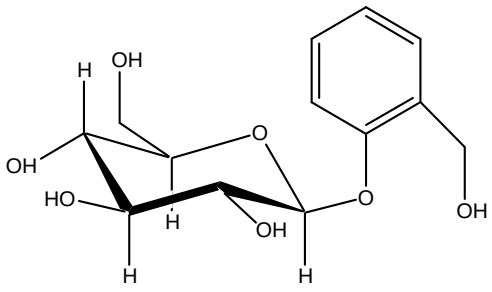
(8)

Coumarin

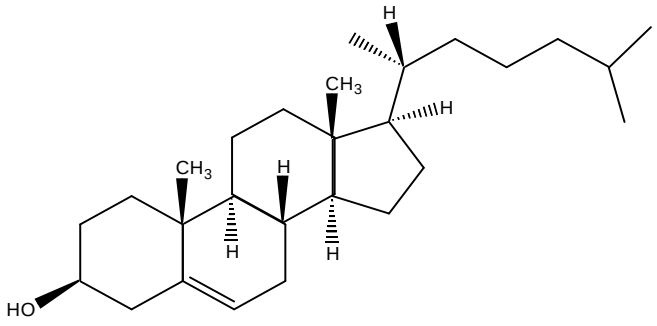
Fig 1: Some types of natural products



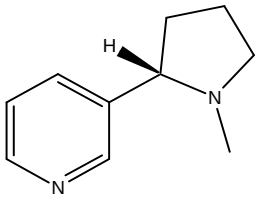
Flavone backbone(2-phenyl-1,4-benzopyrone)



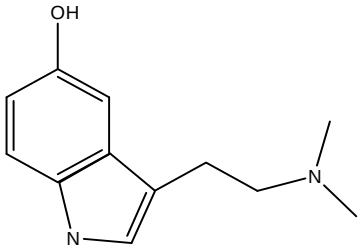
Salicin, a glycoside



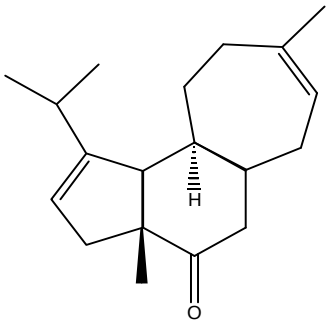
Cholesterol, a sterol



Nicotine, an alkaloid



Bufotenin



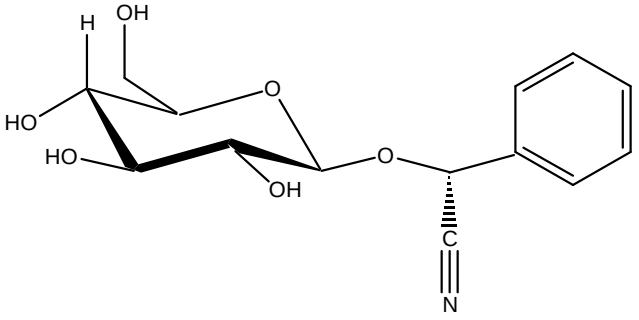
Cyanthiwigin F

Fig 2: Some types of Natural Products Continued

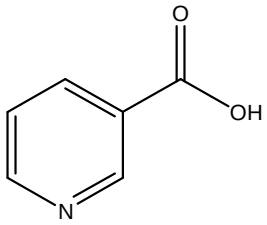
Medicinally, isolated natural products after subjected to clinical trials can be used as drugs for the treatment of cancer [7-8], antimicrobial agent [9], antitumor [10], anti-inflammatory [11], antioxidant agents [12-14], Glucosidase Inhibitors [15]. Also, isolated natural products have been the impetus for the design and synthesis of many pharmaceutical drugs to date. For example, *Cyanthiwigin F*, a complex *bis* molecule active against tumours was first isolated from the sea sponge *Myrmekioderma styx* [16] Fig. 2.0. Crude plant extracts have also been a source of antimicrobial agents [17-24].

Tannins are large polyphenolic compound containing sufficient hydroxyls and other suitable groups, such as carboxyls to form strong complexes with various macromolecules. Flavonoids have similar structures to that of flavones. Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety. Cholesterol have the steroidal nucleus. Sterols have the third carbon of the steroidal nucleus hydroxylated. Sterols may be found either as free sterols, acylated (sterol esters), alkylated, sulfated (sterol sulphate) or linked to a glycoside moiety (steroidal glycosides). Carotenoids are tetraterpenoid (C_{40}) organic pigments. Coumarins are lactones, cyclic esters, Fig. 1.0 and Fig. 2.0. Saponins are amphipathic glycosides noteworthy for their soap like foamimg property. Cardenolides are a special type of steroid. Cardenolide may be linked to a sugar moiety to form a cardenolide glycoside. Alkaloids are a diverse group of plant natural products that contain mostly basic nitrogen atoms. They are usually divided into the true alkaloids, protoalkaloids, polyamine alkaloids, peptide and cyclopeptide alkaloids, pseudoalkaloids [5-6].

Passion fruit is rich in polyphenols [24-27]. The fruit also contain prunasin and other cyanogenic glycosides in the peel and juice [21]. Passion fruit oil is composed mainly of linoleic acid (77%) with smaller amounts of oleic acid (15%) and palmitic acid (10%). It also contains vitamin C (36%), dietary fiber (42%), B vitamins riboflavin (11%) and niacin (10%), iron (12%) and phosphorus (10%) in significant percentages of the daily value [22].



Prunasin



Niacin

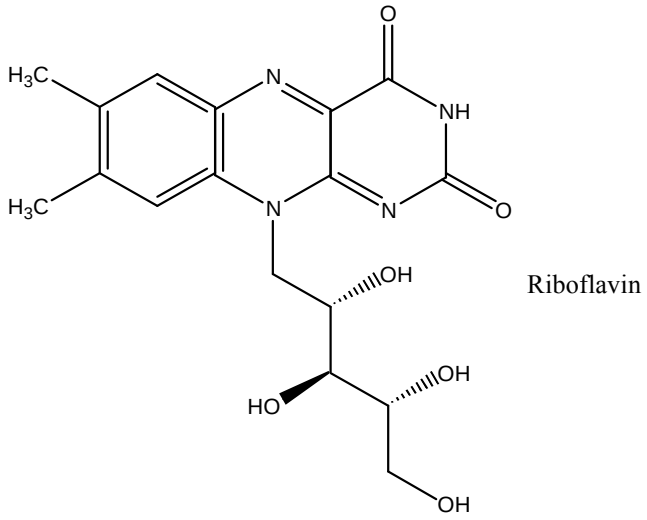
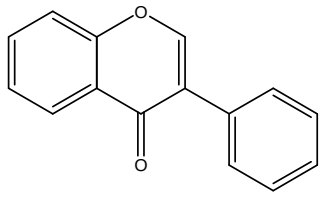
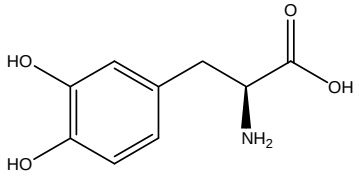


Fig 3: Some of the natural products constituents of passion fruit, *Passiflora edulis Sims* [19-22].

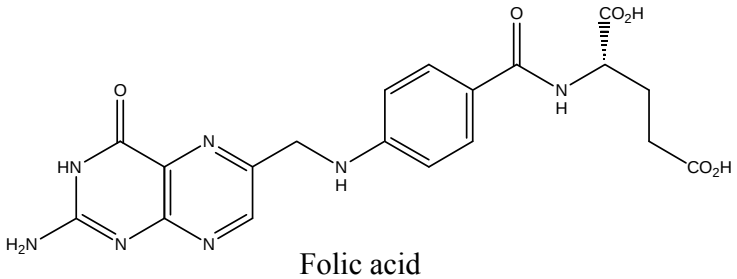
Two new antimicrobial plant peptides related to □-thionine were isolated from *Vicia faba* L. broad beans [29[. Fava beans are very low in saturated fat, cholesterol and sodium. They are also a good source of dietary fiber, protein, phosphorus, copper, manganese, and a very good source of Folate [30-32]. Fava beans are very high in protein, rich source of dietary fiber, high in phyto-nutrients such as isoflavone and plant-sterols. It also contains *Levo*-dopa or L-dopa, a precursor of neuro-chemicals in the brain such as dopamine, epinephrine and nor-epinephrine. For Parkinson Disease, it has also been reported to be a cure. It is also an excellent source of folates. Significant amounts of vitamin-B_6 (pyridoxine), thiamin (vitamin B-1), riboflavin and niacin have also been reported. It is also a fine source of minerals like iron, copper, manganese, calcium, magnesium. At 1062 mg or 23% of daily recommended levels, *Vicia faba L* are one of the highest plant sources of potassium [30-32].



Isoflavone



Levodopa



Folic acid

Fig 4: Phytochemical constituents of *Vicia faba L.*

Material and Methods

Phytochemical screening for secondary metabolites: On each solvent type extract, test for alkaloids, Saponin glycosides, Cardenolides, Bufadionolides, Flavonoids, Tannins, Polyphenolic compounds, Anthraquinones, Cyanogenic glycosides, Carbohydrates, Fixed oils, Fats, and Volatile oils were carried out using standard Phytochemical methods [4].

Cholesterol: To 2 ml of the extract, 2ml of $CHCl_3$ was added in a dry test tube. This was followed with the addition of 10 drops of acetic anhydride and 2 to 3 drops of conc. H_2SO_4. It is anticipated for a positive test that a red-rose colour should be evident.

Cardiac Glycosides: 5ml of each extract was treated with 2ml of glacial acetic acid, containing one drop of ferric chloride solution. This was underlayered with 1ml of conc. H_2SO_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring, whereas the acetic acid layer, a greenish ring might form just gradually throughout the thin layers.

Glycosides: A small amount of alcoholic extract was dissolved in 1ml of water and a few drops of aqueous sodium hydroxide solution were added. A yellow colour was taken to signify the presence of glycosides.

Tannins: About 0.5g of extract was dissolved in 5 to 10ml of distilled water and was filtered. A few drops of a 5% $FeCl_3$ solution were added to the filtrate. A blue, blueblack, green, or blue-green colour or a precipitate was taken as an indication of the presence of tannins.
***Flavonoids*:** A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red colour was taken as an indication of the presence of flavonoids.

Sterols and Triterpenes
Liebermann-Burchard Reaction: 10ml of the extract was placed in a test tube and evaporated to dryness on a water bath. The residue was dissolved in 1ml of acetic anhydride and 1ml of chloroform. The solution was then transferred to two clean dry test tubes; one served as the reference tube. 1-2ml of conc. H_2SO_4 was added to the other tube using a teat pipette. A violet ring was formed at the two liquids, with the supernatant becoming violet this indicates the presence of sterols and triterpenoids.

Carotenoids: (Carr-Price Reaction): 10ml of the extract was added to a test tube and was evaporated to dryness on a water bath. This was followed with the addition of 2-3 drops of saturated $SbCl_3$ in $CHCl_3$ to the residue. A blue-green colour eventually changing to red indicates the presence of carotenoids.

Flavone Aglycones: *Shibata's Reaction or Cyanidin test*: 3ml of the extract was evaporated to dryness in a water bath. The residue was then dissolved in 1-2ml of 50% CH_3CH_2OH while heating. A piece of magnesium ribbon and 4-5 drops of concentrated HCl were added. A red or orange colour indicates the presence of Flavone Aglycones.

Emodols (Anthracenoside and Anthracenoside Aglycone): Borntrager's Reaction: 1ml of 25% NH_3 was added to 3ml of the extract in a test tube. The mixture was then shaken well. A red colour indicates the presence of Emodols. To 2ml of the extract in a test tube, 1-2ml of 25% NH_3 was added while it was been shaken. A cherry-red colour indicates the presence of anthracenosides.

Test for Steroids: 2 ml of acetic anhydride was added to 0.5g of the ethanolic extract of each sample with 2ml of H_2SO_4. The colour change from violet to blue or green, indicative of the presence of steroids

Test for Coumarin and derivatives: 5ml of ether extract was evaporated to dryness. The residue was dissolved in 1-2ml of water by heating. It was then divided in two equal portions. To the non-reference tube, 0.5ml of 10% NH_3 was added and then the tubes were viewed under UV light. The presence of blue-green fluorescence indicated the presence of coumarin. 4-5 drops of hydroxylamine hydrochloride solution and alcoholic KOH were added to the non-reference test tube until the pH was approximately 8-9. The resulting solution was evaporated to dryness. The residue was dissolved and the pH was adjusted to 3-4 by adding 10% HCl add 1-2 drops 3% $FeCl_3$ while being observed. A fast disappearing violet colour indicates the presence of coumarin derivatives.

Alkaloids: 10ml of the extract was evaporated to dryness. The residue was then dissolved in 1.5ml of 2% HCl. The solution was divided into two equal portions, one served as a reference. 2-3 drops of Mayer's reagent was added to the non-reference tube. The development of opalescence or a yellowish white precipitate indicates the presence of alkaloids.

Mayer's reagent: 1.35g of mercuric chloride was dissolved in 60 ml of water, 5g of potassium iodide was added to 10ml of water and diluted to 100 ml. 0.85g of basic bismuth nitrate was dissolved in a mixture of 40 ml of water and 10 ml of acetic acid. 8g of potassium iodide dissolved in 20 ml of water and homogenize was added. The solution obtained was kept in a dark bottle for 2-3 months.

Phlobatinins: Each plant extract was boiled with 1% aqueous HCl. A red precipitate is expected to be deposited and is taken as evidence for the presence of phlobatinins.

Proteins: To 2 ml of plant extract, 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet colour indicates the presence of peptide linkage of the molecule.

Amino Acids: To 2ml of the sample in a test tube, 2ml of Ninhydrin reagent was added. The test tube was placed in the water bath for 20 minutes.

Non-Hydrolysed CH_3CH_2OH extract
Tannins: 1-2 ml of water was added to 0.5-1.0ml of the extract and then 2-3 drops diluted (3% $FeCl_3$) solution was added to the mixture. A blackish-blue colour indicated the presence of Gallic tannins, while a greenish-black colour indicates the presence of catechol tannins.
Reducing compounds: 1-2ml of water was added to 0.5ml of the extract then 0.5-1.0ml of Fehling solution (1 and 2) were added. The mixture was then heated in a water bath. A brick red precipitate indicates the presence of reducing compound.

Alkaloids salt: 20 ml of the extract was evaporated to dryness and 5-10ml of 10% HCl was added to the residue. 10% NH_3 was then added until pH of 8-9 was achieved. The solution was placed into a separatory funnel and extracted with a polar solvent. The resulting extract was evaporated to dryness. The residue was then dissolved in 1.5ml of 2% HCl and divided into two portions; one was a reference. 2-3 drops of Mayer's reagent was added to the non-reference tube. Development of opalescence or a yellow-white precipitate indicates the presence of alkaloid salts.

Hydrolysed CH_3CH_2OH extract: 2ml of 10% HCl was added to the 25ml extract to hydrolyze the alcohol extract. The solution was refluxed for 30 minutes and then allowed to cool. 10-12ml of ether was extracted three times and the extracts were combined. A spatula full of anhydrous Na_2SO_4, filter was added and left for the following test.

Cardenolides (Cardiac Glycosides and Aglycones
The Kedee's test: 4ml of the ether extract was evaporated to dryness. The residue was dissolved in 1-2 ml methanol. 1-2 ml of alcoholic KOH was added to the mixture. 3-4 drops of 1% alcoholic 3, 5-dinitrobenzene was added and the solution was heated. A disappearing violet colour indicates the presence of Cardenolides.

Saponins (foam test): 2ml of the ether extract was evaporated to dryness. The residue was dissolved in 1ml water and shaken vigorously. The presence of saponins was indicated by persistent foam (1cm in test tube).

Flavanosides: (Shibata's Reaction): 5ml of ether extract was evaporated to dryness. The residue was dissolved by heating in 1-2 ml of 50% methanol. Metallic magnesium was added to the mixture followed with 5-6 drops conc. HCl. The development of an orange colour indicates the presence of flavanones. (Flavanols gave a characteristic red colour).

Results and Discussion

Table 1: Phytochemical Screening of the Ethanolic Extract of *Passiflora edulis*

Class of Natural Products	Observations (-)	Observations (+)
Cholesterol	(-)	
Cardiac Glycosides		(+)
Tannins		(+)
Flavanoids		(+)
Emodols		(+)
Anthracenosides		(+)
Alkaloids		(+)
Glycosides		(+)
Sterol and Triterpenes		(+)
Saponins		(+)
Phlobatinins		(+)
Reducing Sugars		(+)

Table 2. Phytochemical Screening of the Aqueous Extract of *Passiflora edulis*

Class of Natural Products	Observations (-)	Observations (+)
Cholesterol	(-)	
Cardiac Glycosides	(-)	
Tannins		(+)
Flavanoids	(-)	
Emodols		(+)
Anthracenosides		(+)
Alkaloids	(-)	
Glycosides		(+)
Sterol and Triterpenes	(-)	
Saponins		(+) Foam less
Phlobatinins	(-)	
Reducing sugars		(+)

Table 3: Phytochemical Screening of the Ethanolic Extract of *Vicia faba L*. (Fabaceae)

Class of Natural Products	Observations (-)	Observations (+)
Cholesterol		(+)
Cardiac Glycosides		(+)
Tannins		(+)
Flavanoids		(+)
Emodols		(+)
Anthracenosides	(-)	
Alkaloids		(+)
Glycosides		(+)
Sterol and Triterpenes	(-)	
Saponins		(+)
Phlobatinins		**(+)**
Reducing sugars		**(+)**

Table 4: Phytochemical Screening of the Aqueous Extract of *Vicia faba L*. (Fabaceae)

Class of Natural Products	Observations (-)	Observations (+)
Cholesterol	(-)	--------
Cardiac Glycosides	(-)	
Tannins	(-)	(+)
Flavanoids	(-)	
Emodols	(-)	
Anthracenosides	(-)	
Alkaloids	(-)	(+)
Glycosides	(-)	(+)
Sterol and Triterpenes	(-)	(+)
Saponins	(-)	(+)
Phlobatinins	(-)	
Reducing sugars	(-)	

Table 5: TLC profile of *Passiflora edulis* and *Vicia faba L*. Spots are numbered from the baseline

	Passion fruit (R_f)	*Vicia faba L.* (R_f)
Spot 1	0.19	0.13
Spot 2	0.31	0.89
Spot 3	0.44	0.94
Spot 4	0.63	
Spot 5	0.75	
Spot 6		

Phytochemical screening indicates selective presence of phytochemicals in the ethanolic and aqueous extracts of both fruits. For example, in fruit 1 aqueous extract, Tannins, emodols, Anthracenosides and glycosides are present, whereas cholesterol, cardiac glycosides, flavonoids and sterol and triterpenes are not present.

Likewise, Tannins, alkaloids, glycosides, sterol and triterpenes and saponins are present in the aqueous extract. However, cholesterol, cardiac glycosides, flavonoids, emodols and anthracenosides registered negative presence.

Reducing compounds were observed for both the ethanolic and aqueous extract of *Passiflora edulis* but was only observed for the ethanolic extract for *Vicia faba L*.

On another note, the ethanolic extract seem to contain more phytochemicals than the aqueous. For example, nine different kinds of phytochemicals were detected for the ethanolic extract of *Pasiflora edulis* whereas five different types of phytochemicals are present in the aqueous extract. Likewise eight different types of phytochemicals are present in the ethanolic extract of fruit 2, whereas five (5) different kinds of phytochemicals are present in the aqueous extract of fruit 2.

TLC profile indicates the presence of five spots with R_f values of 0.19, 0.31, 0.44, 0.63 and 0.75 for Passiflora edulis extract, whereas two spots with R_f values of 0.13, 0.89 and 0.94 were observed for *Vicia faba L*. ethanolic extract. The R_f values indicates the chromatographic mobility of the compounds on a stationary phase such as silica gel. It's a parameter that must be taken into consideration when separating plants phytochemical constituents.

Conclusion

The aqueous and ethanolic extract of *Passiflora edulis* and *Vicia faba L* were phytochemically screened for natural products using conventional colorimetric tests. Phytochemical Screening of the ethanolic extract of *Passiflora edulis* indicates the presence of the following phytochemicals: Cardiac Glycosides, Tannins, Flavanoids, Emodols, Anthracenosides, Alkaloids, Glycosides, Sterol and Triterpenes, Saponins, Phlobatinins. Cholesterol wasn't detected. In contrast, phytochemical screening of the ethanolic

extract of *Vicia faba L*. (Fabaceae) indicates the presence of all phytochemicals with the exception of Anthracenosides and Sterols and Triterpenes. The aqueous extract of both plants showed fewer phytochemicals in comparison to the ethanolic extract. For *Passiflora edulis*, these include Tannins, Emodols, Anthracenosides, Glycosides and Saponins, reducing sugars. For *Vicia fabia*, these include Tannins, Alkaloids, Glycosides, Sterol and Triterpenes and Saponins. Reducing sugars were also detected for the ethanolic extract only. The other phytochemicals weren't detected. The selective presence of phytochemicals were also evident.

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