

## Qualitative & Quantitative Phytochemical analysis on the Leaves & fruits of *Passiflora foetida*.

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**Abstract:** The Phytochemical analysis provides preliminary insight into the major phytoconstituents in the plant species. The earlier study characterized the macroscopic and microscopic characteristic of the plant followed by this present study which revealed presence of Alkaloids, Glycosides, Saponins, Tannins, Flavanoids. The quantification of Alkaloid, Phenolic, Glycosides and Saponin content helped were performed which would also help in the screening of the plant drug for various pharmacological activities.

**Keywords:** Phytoconstituents, Alkaloids, Phenols, Saponins, Flavanoids

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### I. Introduction

#### Aim

To carry out the Phytochemical Analysis and Quantification of the Alkaloid, Phenolic, Flavonoid and Saponin content of the Leaves, fruits of *Passiflora foetida*.

#### Materials

Bromocresol green, Atropine, Di-sodium hydrogen phosphate, Citric acid, Chloroform, Folin Ciocalteu's reagent, Sodium hydroxide, Gallic acid, Sodium carbonate, Aluminium Chloride, Sodium nitrite, Di-ethyl ether, n-butanol, Separating funnel, heater, UV-visible spectrophotometer (Jasco).

### II. Methods

#### Phytochemical Analysis<sup>1</sup>

As per the general method phytochemical screening was carried out.

#### Total Alkaloid content of the Leaves and Fruits of *Passiflora foetida*<sup>2-3</sup>

The total alkaloid content was estimated using Bromocresol Green reagent using Atropine as standard and the Chloroform as the primary solvent.

#### Preparation of the Standard curve of Atropine

From 0.1mg/ml stock solution 0.4 ml, 0.6ml, 0.8 ml and 1.0 ml of the solution were taken in different separating funnels and added 5.0 ml of pH 4.7 phosphate buffer (71.6 g of Na<sub>2</sub>HPO<sub>4</sub> was dissolved in 0.2 M Citric Acid and volume made to 100.0 ml with the same citric acid solution and distilled water to get the desired pH). 5.0 ml of BCG solution (1.0 ml of 0.04% BCG diluted to 10.0 ml) and then shaken the mixture with 1.0 ml, 2.0 ml, 3.0 ml and 4.0 ml Chloroform respectively. Collected the Chloroform extracts in 10.0 ml volumetric flask and made up the volume with Chloroform. The absorbance of this solution was measured at 470 nm.

#### Preparation of the Extract for alkaloid content estimation.

A definite quantity of the extract was dissolved in 10.0 ml of 2.0 N Hydrochloric acid and filtered. 1.0 ml of the filtrate was transferred into a separating funnel and the pH was adjusted to neutral with 0.2 N sodium hydroxide solution. 5.0 ml of phosphate buffer and 5.0 ml of BCG was added followed by 4.0 ml of chloroform and shaken vigorously. The Chloroform layer was collected in a 10.0 ml volumetric flask and made up the volume with Chloroform. The absorbance was measured at 470 nm.

#### Total Phenolic content of the Leaves and Fruits of *Passiflora foetida*.<sup>4</sup>

The total Phenolic content was determined using Folin Ciocalteu's method

#### Preparation of Standard curve of Gallic acid

10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml dilutions were prepared from 1 mg/ml stock solutions. 1.0 ml from each of these solutions and a blank were taken in a test tube and 0.5 ml Folin Ciocalteu's reagent was added and shaken. 1.5 ml of 20% w/v sodium carbonate was added after 5 minutes and volume made up to 10 ml with distilled water. The blue color developed was measured at 750 nm.

#### Preparation of Extract for Phenolic content estimation

1.0 ml of the extract solution taken in respective test tubes and 0.5 ml Folin Ciocalteu's reagent, 1.5 ml of 20% w/v Sodium carbonate was added after 5 minutes and volume made to 10 ml with distilled water. The color developed was measured at 750 nm.

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**Total Flavonoid content of the Leaves and fruits of *Passiflora foetida*.<sup>6</sup>**

**Preparation of standard curve of Quercetin**

The different concentrations of Quercetin (50, 100, 200, 400, 600, 800) µg was pipetted with the help of micropipette from 1mg/ml solution of Quercetin in Methanol and was used as standard. 0.3 ml of Sodium Nitrite (5% w/v) was added and after 5 minutes 0.3 ml Aluminium Chloride (10% w/v) was added and 2.0 ml sodium hydroxide (1 M) added and volume made up to 10.0 ml with distilled water. The absorbance was read thereafter at 510 nm using water as blank.

**Preparation of extract for Total Flavonoid estimation.<sup>6</sup>**

1 ml of the extract stock solution was taken in a 10.0 ml standard flask and 4.0 ml of distilled water was added followed by 0.3 ml of 5% w/v Sodium nitrite solution was added. Kept it at room temperature for 5 minutes and added 0.3 ml 10 % w/v Aluminium chloride. Then after a minute added 2.0 ml of Sodium hydroxide solution (2M) and volume made up with distilled water. The color developed was measured at 510nm.

**Total Saponin content of the Leaves and Fruits of *Passiflora foetida***

5 g of the powdered plant material was taken and heated at 55°C with 100 ml of 20%v/v Ethanol for 4 hours and filtered and the marc again treated with 100 ml of 20%v/v Ethanol and combined the filtrate which was concentrated to 40 ml by heating in a water bath at 90°C. The filtrate treated with 20 ml diethyl ether shaken vigorously in a separating funnel. Discarded the ether layer and the aqueous layer was mixed with 60 ml n-butanol and the solution was evaporated to dryness followed by heating the residue to constant weight in an oven. The Saponin content was calculated as percentage of the crude drug taken.

**III. Results**

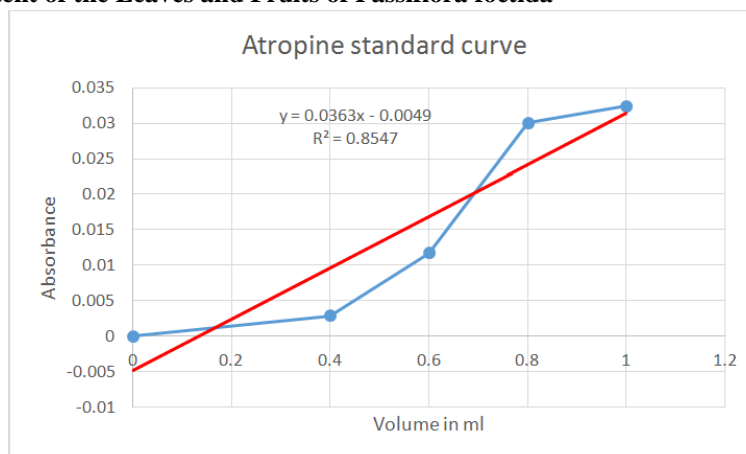
**Phytochemical analysis of the Ethanolic extracts of the fruits and leaves of *Passiflora foetida*.**

**Table:** Phytochemical analysis of leaf and fruit extract.

<b>Leaves</b>		<b>Fruits</b>	
<b>1) Test for Carbohydrates</b>		<b>1) Test for Carbohydrates</b>	
Molisch's test (General test)	positive	Molisch's test (General test)	positive
Fehling's Test (Reducing sugar)	positive	Fehling's Test (Reducing sugar)	positive
Benedict's Test (Reducing sugar)	positive	Benedict's Test (Reducing sugar)	positive
Barfoed's Test (Monosaccharide's)	positive	Barfoed's Test (Monosaccharide's)	Positive
Pentose sugar	positive	Pentose sugar	positive
Hexose sugar (Selwinoff's Test)	negative	Hexose sugar (Selwinoff's Test)	negative
Non-reducing sugar	negative	Non-reducing sugar	negative
Starch(Iodine Test)	positive	Starch(Iodine Test)	positive
Gums	negative	Gums	positive
Mucilage	positive	Mucilage	positive
<b>2) Test for Proteins</b>		<b>2) Test for Proteins</b>	
Biuret Test(General test)	negative	Biuret Test(General test)	negative
Millon's Test(for protiens)	negative	Millon's Test(for protiens)	negative
Xanthoproteins ( Tyrosine and Tryptophan)	negative	Xanthoproteins ( Tyrosine and Tryptophan)	negative
Proteins containing sulphur	negative	Proteins containing sulphur	negative
Precipitation test using Lead acetate, Copper sulphate, Mercuric chloride, Ammonium sulphate	Negative, positive only for lead acetate	Precipitation test using Lead acetate, Copper sulphate, Mercuric chloride, Ammonium sulphate	Negative, positive only for lead acetate
<b>3) Test for Amino acids</b>		<b>3) Test for Amino acids</b>	
Nin hydrin test (General test)	positive	Nin hydrin test (General test)	positive
Tyrosine	negative	Tyrosine	negative
Tryptophan	negative	Tryptophan	negative
Cysteine	negative	Cysteine	negative
<b>4) Test for Steroids</b>		<b>4) Test for Steroids</b>	
Liebermann-Burchard test	negative	Liebermann-Burchard test	negative
<b>5) Test for Sesquiterpenes</b>		<b>5) Test for Sesquiterpenes</b>	
Salkowski test	negative	Salkowski test	positive
<b>6) Test for Flavonoids</b>		<b>6) Test for flavonoids</b>	
Aluminium chloride test	positive	Aluminium chloride test	positive
<b>7) Test for Cardiac Glycosides</b>		<b>7) Test for Cardiac Glycosides</b>	
Keller killani test	negative	Keller Killani test	Positive
Cardenolides (Legal's test)	negative	Cardenolides	positive
<b>8) Test for Anthraquinone glycosides</b>		<b>8) Test for Anthraquinone glycosides</b>	

Borntrager's test	negative	Borntrager's test	negative
<b>9) Test for Saponin glycosides</b>		<b>9) Test for Saponin glycosides</b>	
Foam test	positive	Foam test	positive
<b>10) Test for Cyanogenetic glycosides</b>		<b>10) Test for Cyanogenetic glycosides</b>	
Guignard reaction	negative	Guignard reaction	negative
<b>11) Test for Coumarin glycosides</b>		<b>11) Test for Coumarin glycosides</b>	
Fluorescence test	positive	Fluorescence test	positive
<b>12) Test for Alkaloids</b>		<b>12) Test for Alkaloids</b>	
Mayer's test	positive	Mayer's test	positive
Dragendroff's test	positive	Dragendroff's test	positive
Hager's test	positive	Hager's test	positive
Wagner's test	negative	Wagner's test	negative
<b>13) Test for Tannins</b>		<b>13) Test for Tannins</b>	
Ferric chloride test	positive	Ferric chloride test	positive
Lead acetate test	positive	Lead acetate test	positive
Acetic acid test	negative	Acetic acid test	negative
Ammonium hydroxide and potassium ferricyanide test	negative	Ammonium hydroxide and potassium ferricyanide test	negative
<b>14) Test for Enzymes</b>		<b>14) Test for Enzymes</b>	
Catalase	positive	Catalase	positive
Dehydrogenase	negative	Dehydrogenase	negative
<b>15) Test for Organic acids</b>		<b>15) Test for Organic acids</b>	
Malic acid	negative	Malic acid	negative
Oxalic acid	positive	Oxalic acid	positive
Citric acid	negative	Citric acid	positive
<b>16) Test for Vitamins</b>		<b>16) Test for Vitamins</b>	
Vitamin A	positive	Vitamin A	positive
Vitamin C	negative	Vitamin C	positive
Vitamin D	negative	Vitamin D	negative
<b>17) Test for Volatile oils</b>		<b>17) Test for Volatile oils</b>	
Ruthenium red test	positive	Ruthenium red test	positive
<b>18) Test for Fixed oils</b>		<b>18) Test for Fixed oils</b>	
Pressing between filter paper	negative	Pressing between filter paper	negative

**Total Alkaloid content of the Leaves and Fruits of *Passiflora foetida***



**Figure:** Calibration curve of Atropine.

**Table:** Data for the calibration curve and for the extract

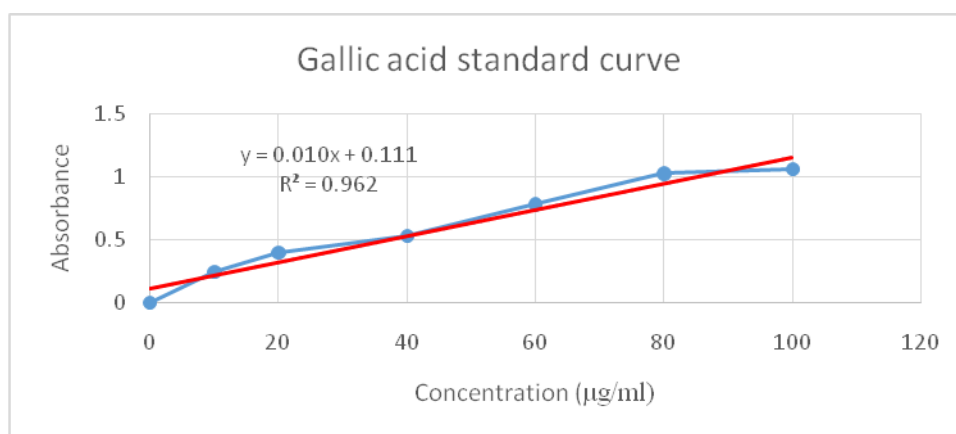
Absorbance	Volume in ml
Atropine( 1000 µg/ml)	
0.0	0.0
0.0029	0.4
0.0117	0.6
0.0301	0.8
0.0324	1.0
0.0045	0.259 (Fruits)
0.0047	0.264 (Leaves)

1 ml of Atropine contains 100µg

The fruits contain 0.260 mg Total alkaloid / 1 g of the extract.

The leaves contain 5.162 mg total alkaloid content/ 1g of the extract.

**Total Phenolic content of the Leaves and Fruits of *Passiflora foetida*.**



**Figure:** Calibration curve of Gallic acid.

**Table:** Data for the calibration curve and extracts

Absorbance	Concentration in µg
Gallic acid	
0	0
10	0.2406
20	0.3941
40	0.5293
60	0.7789
80	1.0198
100	1.0552
Extract of Leaves	
0.7012	56.67307692
Extract of fruits	
0.595	46.46153846

The Total phenolic content of Leaves was found to be 2.464 mg/1g of extract.

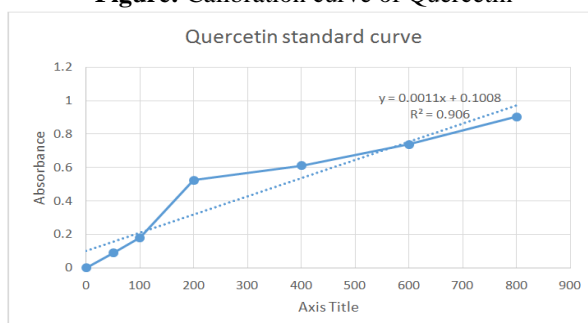
The Total phenolic content of the Fruits was found to be 1.66 mg/1g of the extract.

**Total Flavonoid content of the Leaves and fruits of *Passiflora foetida***

Absorbance	Concentration
Quercetin	
0	0
0.09	50
0.181	100
0.5232	200
0.6112	400
0.7375	600
0.9024	800
Extract of Leaves	
0.1270	23.81818182
Extract of fruits	
0.2409	127.3636364

**Table:** Data for the calibration curve and extract

**Figure:** Calibration curve of Quercetin



The Total Flavonoid content of the fruits was found to be 4.561 mg/1 g of the extract

The Total Flavonoid content of the leaves was found to be 0.238 mg/ 1g of the extract

#### **Total Saponin content of the Leaves and Fruits of Passiflora foetida**

For leaves the weight of the residue= 0.953 g

Percentage Saponin content=  $0.953/5.0 * 100 = 19.06\%$  w/w

For fruits the weight of the residue= 1.442 g

Percentage Saponin content=  $1.442/5.0 * 100 = 28.84\%$  w/w

#### **IV. Conclusion**

The above study revealed the presence of Carbohydrate, alkaloids, Flavonoids, tannins, Enzymes(catalase), Organic acid (oxalic acid), Vitamin A, Coumarin glycosides, Saponin glycoside, mucilage, Volatile oil in both leaves and fruits, cardiac glycosides, Sesquiterpenes, gums, organic acid (oxalic acid), Vitamin D in fruits particularly. Quantification showed more Alkaloid and Phenolic content in leaves whereas fruits showed higher flavonoid and Saponin content.

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