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Phytochemical analysis and thin layer chromatographic studies of *Passiflora edulis* leaf extracts

[*1] Phamiwon ZAS, [2] Sheila John

[1] Research Scholar, Department of Home Science, Women's Christian College, Chennai, Tamil Nadu, India
[2] Associate Professor and Head, Department of Home Science, Women's Christian College, Chennai, Tamil Nadu, India

Abstract
Plants have been used as traditional medicine for treating and preventing diseases due to the presence of various bioactive compounds. The present study was carried out to examine the presence of phytochemical constituents and perform thin layer chromatography studies of *Passiflora edulis* leaf extract using two solvents namely Chloroform and Ethyl acetate. The phytochemical analysis of *Passiflora Edulis* leaf extract reflects the presence of flavonoids, tannins, polyphenols, terpenoids, steroids and alkaloids. Thin layer chromatographic studies of *Passiflora edulis* leaf extracts constituted different colored phytochemical compounds with different R_f values. The presence of the bioactive compounds may be responsible for antioxidant, hypoglycemic and hypotensive activity thereby justifying its use of the plant as traditional medicine in health treatments.

Keywords: *passiflora edulis*, phytochemical, bioactive compounds

1. Introduction
For thousands of years, medicinal plants has been used to treat health disorders and to prevent diseases. Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment. Active compounds produced during vegetal metabolism are responsible for the biological properties [1, 2]. 65% and 80% of the populations of developing countries currently use medicinal plants as remedies [3].
Passion fruit, a native of tropical America (Brazil), belonging to the family Passifloraceae. Passion fruit stands for its exotic and unique flavor, aroma and its amazing nutritional and medicinal properties [4, 5]. Two types of *Passiflora edulis* are grown commercially, the purple form (passiflora edulis sims) and a yellow form (passiflora edulis var. flavicarpa) [6, 7]. *Passiflora edulis* leaf is boiled in little amount of water and extract is drunk for the treatment of dysentery and hypertension. Fruits are eaten to get relief from constipation [8]. *Passiflora edulis* has been used as a sedative, diuretic, anthelmintic, anti-diarrhoeal, stimulant and also in the treatment of hypertension, menopausal symptoms and colic of infants in different regions around the world [9, 10, 11, 12]. Alkaloids, phenols, glycosyl flavonoids and cynogenic compunds are known in the genus [13]. The present study was conducted to investigate the phytochemical constituents of *Passiflora Edulis* leaf using chloroform and ethyl acetate extracts and to carry out thin layer chromatography studies.

2. Materials and Methods
2.1 Collection of Plant material
The leaf of *Passiflora edulis* was collected from Ukhrul district, Manipur. The leaves were shade dried for 15 days and powdered using a mixer and stored in an airtight container.
The powdered leaves (100 g) was weighed and soaked in 300 ml of ethyl acetate and chloroform respectively in a separate conical flask. The conical flask containing the powdered leaves was shaken vigorously, corked and left to stand for 48 hrs by maceration techniques at room temperature. After 48 hrs, the mixture was filtered and the extract was collected and concentrated by evaporation to dryness in evaporating dish (Trease and Evans, 1997) [14].

2.2 Fractionation of different extract
The different extract of *Passiflora edulis* (2 g) was placed at the top of a silica gel (28 g) wet packed in a chromatographic column and eluted with gradient of Hexane: Ethyl acetate (9:1, 1:1) chloroform: methanol (9:1 ratio).The elutes were collected in separate respective small bottles and labeled successively [15].

2.3 Qualitative phytochemical screening of *Passiflora edulis* leaf
The phytochemical test was carried out to screen the presence of phyto-constituents in chloroform and ethyl acetate extracts of *Passiflora edulis* leaf. The screening of phytochemical analysis was performed as described by Evans, 2002; Yusuf *et al*., 2014).

Test for Flavonoids
Alkaline Reagent Test: 1 g of the powdered dried leaves of each specimen was boiled with 10 mL of distilled water for 5 minutes and filtered while hot. Few drops of 20 % NaOH solution were added to 1 mL of the cooled filtrate. A change to yellow color which on addition of acid changed to colorless solution depicted the presence of flavonoids. (Evans, 2002).

Test for Steriods
Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 was added. Formation of brown ring at the junction indicated the presence of phytosterols.

Test for Terpenoids
Salkowski test: 5 mL of each extract was mixed in 2 mL of chloroform and Conc. H_2SO_4 (3 mL) was carefully added to form a layer. Presence of reddish brown coloration at the interface indicated the presence of terpenoids.

Test for tannins

Ferric chloride test: About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Evans, 2002).

Test for Saponins

Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Demonstration of frothing: 2.5 mL of filtrate was diluted to 10mL with distilled water and shaken vigorously for 2 minutes (frothing indicated the presence of saponin in the filtrate).

Demonstration of emulsifying properties: 2 drops of olive oil was added to the solution obtained from diluting 2.5 mL filtrate to 10 mL with distilled water (above), shaken vigorously for a few minutes (formation of a fairly stable emulsion indicated the presence of saponins).

Test for alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid (HCl) and filtered.

Mayer's Test: Filtrates were treated with 1mL of Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicated the presence of alkaloids (Evans, 2002).

Wagner's Test: Filtrates were treated with 1ml of Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids (Evans, 2002).

Dragendroff's Test: Filtrates were treated with 1mL of Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Evans, 2002).

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow colored precipitate indicated the presence of alkaloids.

2.4 Thin layer Chromatography

The plant extracts were spotted using capillary tube on TLC plates. The extracts were applied onto the plate about 1.5 cm above the edge and 0.5 cm away from the margin, when the spot was dried, the plate was observed under UV fluorescence light at wavelength 254 nm. The solvent system for the mobile phase was Hexane: Ethyl acetate (9:1, 1:1) chloroform: methanol (9:1) ratios.

3. Results and Discussion

3.1 Qualitative Phytochemical analysis

The result pertaining to phytochemical analysis is presented in Table 1. The result of the present study indicates that phytochemicals such as flavonoids, tannins, polyphenols and terpenoids were present in ethyl acetate whereas only steroids and alkaloids were present in chloroform extract. Razia *et al.* 2014 reported that Aqueous, chloroform and methanolic extracts of Passiflora *edulis* were found to possess tannins, flavonoids, terpenoid, steroids [16, 17]. The alkaloids present are harman harmine, harmalol, harmol and harmaline of which the highest concentration of Harman alkaloids were found in leaves. Edulans I and Edulans II pectins were also present in *Passiflora edulis* [13].

Table 1: Phytochemical screening of *Passiflora edulis* leaf

Extracts	Steroids	Alkaloids	Flavonoid	Saponins	Tannins	Polyphenol	Terpenoids
Chloroform	+	+	-	-	-	-	-
Ethyl acetate	-	-	+	-	+	+	+

+ = Present, - = Absent

Ferreres *et al.,* 2007 charaterized sixteen apigenin or luteolin derivatives which include four mono-C-glycosyl, eight O-glycosyl- C-glycosyl, and four O-glycosyl derivatives exhibiting a deoxyhexose moiety HPLC-DAD-MS/MS [18].

3.2 TLC chromatography

The number of spots observed on TLC plates and their corresponding R_f values are presented in Table II. The R_f value was calculated using the formula:

$$R_f = \frac{\text{Distance moved by the component}}{\text{Distance moved by the solvent}}$$

The TLC analysis of chloroform and ethyl acetate extracts of passiflora edulis leaf was perfromed using various solvent system i.e. Hexane: Ethyl acetate (9:1, 1:1) Chloroform: Methanol (9:1).

Table 2: Thin Layer chromatography

Extracts	Solvent system	Number of spots	R_f value
Chloroform	Hexane : Ethyl acetate (9:1)	2	0.14, 0.35
	Hexane : Ethyl acetate (1:1)	3	0.58, 0.72, 0.81
	Chloroform : Methanol (9:1)	3	0.74, 0.89, 0.97
Ethyl acetate	Hexane : Ethyl acetate (9:1)	-	-
	Hexane : Ethyl acetate (1:1)	3	0.45,0.39, 0.93
	Chloroform : Methanol (9:1)	8	0.18, 0.24, 0.36, 0.54, 0.69, 0.75, 0.82, 0.89

TLC studies of the Chloroform extract of *Passiflora edulis* leaf: In Hexane: Ethyl acetate (9:1) solvent system, 2 spots were visible with R_f values 0.14, 0.35. In Hexane: Ethyl acetate (1:1), 3 spots were observed with R_f values 0.58, 0.72, 0.81. In Chloroform: Methanol (9:1), 3 spots was detected with R_f values 0.74, 0.89, 0.97.

TLC studies of the Ethyl acetate of *Passiflora edulis* leaf: No spot was detected In Hexane: Ethyl acetate (9:1) solvent system, In Hexane: Ethyl acetate (1:1), 3 spots were obtained with R_f values 0.45, 0.39, 0.93. In Chloroform: Methanol (9:1), 8 spots was visible with R_f values 0.18, 0.24, 0.36, 0.54, 0.69, 0.75, 0.82, 0.89.

TLC analysis for phytochemicals gives different R_f values in different solvent system which helps in understanding their polarity and selection of appropriate solvent system for separation of pure compounds. Mixing Different ratio of solvents with variable polarity can be used for separation of pure compound from plant extract [19].

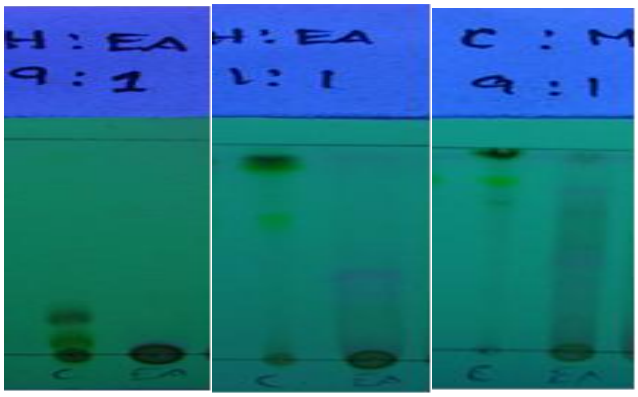

Fig 1: TLC of chloroform and ethyl acetate of *Passiflora edul*is

Phytochemicals constitute an important class of plant-derived compounds with beneficial health properties. Isolate and characterization of luteolin-7-O-[2-rhamnosylglucoside] from *Passiflora edilis* leaf showed an anxiolytic-like activity without compromising motor activity [20]. Ichimura *et al.* [21] reported that the orally administered methanol extract of P. edulis rind (10mg/kg or 50 mg/kg) or luteolin (50 mg/kg), which is one of consistent polyphenols of the extract, significantly lowered systolic blood pressure in spontaneously hypertensive rats (SHRs). The presence of tannins as one of the chemical constituent said to possess anti-helmentic activity [17].

Chloroform and petroleum ether extracts of *Passiflora edulis* stem and leaf have significant cytotoxic potentials with the LC50 value of 6.63 µg/ml, 6.89 µg/ml and 7.91 µg/ml 11.17µg/ml and antioxidant activity with the IC50 value of 51.28 µg/ml and 54.01 µg/ml respectively [22]. The presence of phenols and flavonoids may be responsible for the reduced glycated hemoglobin and hypoglycemic activity of *Passiflora edulis* among Type 2 diabetics and diabetic induced albino rats [23, 24].

4. Conclusion

The study indicated the presence of phytochemicals like steroids, alkaloids, flavonoids, saponins, tannins, polyphenols and terpenoids in leaf extract of *Passiflora edulis* thereby indicating its potential use as a medicinal agent due to its significant activities like antioxidant, antimicrobial and cytotoxic activity. *Passiflora edulis* leaves can also be utilized in the treatment and prevention of non-communicable diseases with an effort in pharmaceutical drug development, a path of light in further research.

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