

# SCREENING OF PHYTOCHEMICAL CONSTITUENTS, ANTIMICROBIAL, LARVICIDAL ACTIVITY AND ANTIOXIDANT ACTIVITY OF *PASSIFLORA EDULIS*.

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## ABSTRACT

Passion fruit leaves, botanically classified as *Passiflora edulis*, grow on a fast climbing vine that can spread 4-6 meters a year and are members of the *Passifloraceae* family. Passion fruit leaves contain fiber, vitamin A, vitamin C and niacin. The present study was intended to screen the phytochemical constituents, antimicrobial, larvicidal activity and antioxidant activity of Acetone, Methanol, Hexane, Chloroform, Ethyl acetate and Aqueous extracts of were studied in this work. The preliminary screening of the various extracts revealed the presence of Tannin, Saponin, Terpenoids, Phenol, Flavonoid, Amino acid, Alkaloid, Carbohydrate, Glycosides and Cardiac Glycosides. The antimicrobial screening was carried out using the following organisms; *E. coli*, *Pseudomonas*, *Salmonella*, *Klebsiella*, *Enterobacter* and *Staphylococcus*. The chloroform extract showed the highest antibacterial activity against; *E. coli* with a zone of inhibition is 1.1cm. The hexane extract of *Passiflora edulis* showed highest level of larvicidal activity against *Culex* species of mosquito compared to other extracts. Hexane extract showed highest antioxidant activity compared to other solvent extracts and standard antioxidants. Thus, the results revealed the potential of the *Passiflora edulis* as a source for natural antibacterial and antioxidant agent.

Keywords: *Passiflora edulis*, phytochemical, antibacterial, antioxidant and larvicidal activity.

## 1. INTRODUCTION

Modern searches for bioactive molecules typically make use of sophisticated bioassays and bioassay-guided fractionation of medicinal plants used by traditional healers. This has led to the isolation of several new therapeutically important compounds. A good number of potent drugs and a large number of therapeutic leads

and many new pharmacologically active constituents have been developed from herbal drugs due to the dedicated efforts of researchers (Philipson., 1990). The genus *Passiflora*, comprising about 400 species, is the largest in the family *Passifloraceae* (Montanher *et al.*, 2007).

The plants of genus *Passiflora* are shrubs and herbs, mostly climbers with auxillary tendrils. Leaves alternate, sometimes simple, entire, lobed or palmate, sometimes compound, imparipinnate; stipules germinate at the base of petioles, rarely absent; tendril axillary, arising from sterile pedicels (Dhawan *et al.*, 2004).

Phytochemical analysis of *Passiflora edulis* revealed the presence of carbohydrates, glycosides, flavonoids, resins, alkaloids and phenolic compounds. Tannins were present in the leaf and fruit, saponins were present in the leaf and stem. Organic extract (methanol, ethanol) of *Passiflora edulis* leaves were reported to possess tannins, flavonoids, terpenoids, steroids and saponins (Bolaji *et al.*, 2011).

The antibacterial activity of the *Passiflora* species extracts was studied by the disc diffusion method as reported by (Lalitha., 2012). The chloroform extracts of leaf inhibited growth of bacteria compared to petroleum ether, extracts. *P. edulis* showed that the methanol extract showed more inhibitory activity against human pathogenic bacteria when compared to other extracts. A similar result was reported the secondary metabolites extracted in methanolic extract also found to be more potent as compared to ethyl acetate in *Passiflora* species (Patil, 2010). The methanolic extract of leaves of *P. edulis* showed positive results of antimicrobial activity on *S. aureus*, *Staphylococcus faecalis*, *B. subtilis*, *E. coli*, *P. vulgaris* and *S. typhi* at 200 µg/disc (Kannan *et al.*, 2011).

A larvicide is an insecticide that is specifically targeted against the larval life stage of an insect. Their most common use is against mosquitoes. Larvicides may be contact poisons, stomach poisons, growth regulators, or biological control agents (Baguvan *et al.*, 2011).

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals (Patel *et al.*, 2013). This study aims to evaluate medicinal importance of *Passiflora edulis* the extract (Acetone, Methanol, Hexane, Chloroform, Ethyl acetate and Aqueous) were aimed for screening of phytochemical compounds, detection of antimicrobial activity, larvicidal activity and antioxidant activity.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Sample.

*Passiflora edulis* leaves were collected from Kulathoor in Trivandrum District, Kerala.

## 2.2 EXTRACTION

### **Methanol extract**

An amount of 5gm of fresh leaves was weighed and grind using motor and pestle with 5ml of methanol solution. Then the solution was kept for centrifuge at 5000 rpm for 15 minutes. Supernatant was collected and filtered through whatman number 1 filter paper and kept it under UV for 1 hour to prevent contamination and then stored at 5°C for further use.

### **Acetone extract**

An amount of 5gm of fresh leaves was weighed and grind using motor and pestle with 5ml of acetone solution. Then the solution was kept for centrifuge at 5000 rpm for 15 minutes. Supernatant was collected and filtered through whatman number 1 filter paper and kept it under UV for one hour to prevent contamination and then stored at 5°C for further use.

### **Hexane extract**

An amount of 5gm of fresh leaves was weighed and grind using motor and pestle with 5ml of Hexane solution. Then the solution was kept for centrifuge at 5000rpm for 15 minutes. Supernatant was collected and filtered through whatman number 1 filter paper and kept it under UV for one hour to prevent contamination and then stored at 5°C for further use.

### **Ethyl acetate extract**

An amount of 5gm of fresh leaves was weighed and grind using motor and pestle with 5ml of Ethyl acetate solution. Then the solution was kept for centrifuge at 5000rpm for 15 minutes. Supernatant was collected and filtered through whatman number 1 filter paper and kept it under UV for one hour to prevent contamination and then stored at 5°C for further use.

### **Chloroform extract**

An amount of 5gm of fresh leaves was weighed and grind using motor and pestle with 5ml of Chloroform solution. Then the solution was kept for centrifuge at 5000rpm for 15 minutes. Supernatant was collected and filtered through whatman number 1 filter paper and kept it under UV for one hour to prevent contamination and then stored at 5°C for further use.

### **Aqueous extract**

An amount of 5gm of fresh leaves was weighed and grind using motor and pestle with 5ml of Distilled water. Then the solution was kept for centrifuge at 5000rpm for 15 minutes. Supernatant was collected and

filtered through whatman number 1 filter paper and kept it under UV for one hour to prevent contamination and then stored at 5°C for further use.

## 2.3 PHYTOCHEMICAL ANALYSIS

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts with Acetone, Methanol, Hexane, Chloroform, Ethyl acetate and Aqueous using standard procedures (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Okwu, 2001).

## 2.4 ANTIMICROBIAL ACTIVITY OF *PASSIFLORA EDULIS*

The antibacterial assay was determined using Kirby-Bauer disc diffusion method. Pathogenic bacterial strains were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. The bacterial strains were *E. coli*, *Pseudomonas*, *Salmonella*, *Klebsiella*, *Enterobacter* and *Staphylococcus* are spread over the medium. Filter paper disc of uniform size (5 mm) are impregnated with specified concentrations of plant extract and then placed on the surface of Muller Hinton agar plates that has been seeded with organism to be tested. Bacterial colonies were allowed to grow overnight at 37°C, then the inhibition zone around the disc was measured (Bauer *et al.*, 1966).

## 2.5 LARVICIDAL ACTIVITY

Larvae of mosquito species were exposed to test samples of Methanol, Hexane, chloroform, ethyl acetate, Acetone, Aqueous extracts of *Passiflora edulis* leaves. 10ml of tap water was taken in china dish. 1ml of extract was dissolved in the water. A control was maintained by adding 1ml of respective solvents to 10ml of tap water. 5 to 10 per concentration were used for all experiments. Then the time taken for the death of larvae was recorded.

## 2.6 ANTIOXIDANT ACTIVITY OF EXTRACTS

### **In-vitro evaluation of antioxidant activity by reducing power method**

Each extracts of *Passiflora edulis* leaf (100µg/ml) were prepared separately in 1ml of distilled water with phosphate buffer (2.5ml, 2M, pH6.6) and potassium ferric cyanide (2.5ml, 1%). The mixture was incubated at 50° c for 20 minutes. A Portion (2.5ml) of Trichloro Acetic Acid (10% TCA) was added to the mixture which was then centrifuged at 1500g for 10 minutes. The upper layer solution (2.5ml) was mixed with distilled water and FeCl<sub>2</sub> (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of reaction mixture indicated reducing power.

## 3. RESULTS

The present study mainly focuses on the Screening of phytochemicals and to check Antimicrobial, Larvicidal and Antioxidant activity.

The result obtained from various analysis is presented below.

### 3.1 PHYTOCHEMICAL ANALYSIS

The result of phytochemical analysis of Acetone, Methanol, Hexane, Ethyl acetate, Chloroform, Aqueous extracts are as follows, (Table 1).

**TABLE 1: Phytochemical Analysis of *Passiflora edulis* leaf extracts.**

Test	Acetone	Methanol	Ethyl acetate	Hexane	Chloroform	Aqueous	Controlled
Tannin	+	+	+	-	+	+	-
Saponin	-	+	-	-	-	+	-
Terpenoids	+	+	+	+	+	+	-
Phenol	+	-	-	-	-	-	-
Flavonoid	-	-	-	-	-	+	-
Amino acid	+	-	-	-	-	+	-
Alkaloid	-	-	-	+	-	-	-
Carbohydrate	-	-	+	+	-	+	-
Glycosides	-	-	-	-	-	+	-
Cardiac Glycosides	+	+	+	+	+	+	-

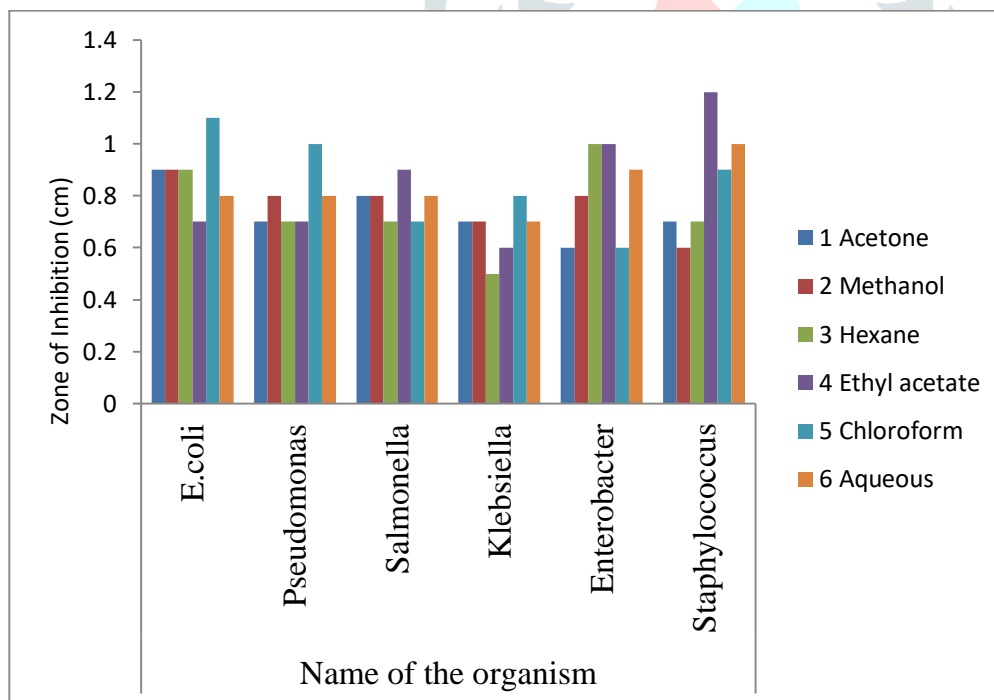
‘+’ indicates positive and ‘-’ indicates negative.

### 3.2 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the *Passiflora edulis* were tested against different microorganisms including a range of gram positive and gram negative bacteria (Table 2). The extract such as Acetone, Methanol, Hexane, Ethyl acetate, Chloroform and Aqueous showed varying levels of antibacterial activity with different bacterial species. The chloroform extract showed the highest antibacterial activity against *E. coli* with a zone of inhibition is 1.1 cm. The results are tabulated in table 2 and figure 1.

Table 2: Antibacterial activity of *Passiflora edulis* leaf extract

Sl. NO	Zone of inhibition (cm)	Name of the organism					
		<i>E.coli</i>	<i>Pseudo monas</i>	<i>Salmonella</i>	<i>Klebsiella</i>	<i>Enterobacter</i>	<i>Staphylococcus</i>
1	Acetone	0.9	0.7	0.8	0.7	0.6	0.7
2	Methanol	0.9	0.8	0.8	0.7	0.8	0.6
3	Hexane	0.9	0.7	0.7	0.5	1	0.7
4	Ethyl acetate	0.7	0.7	0.9	0.6	1	1.2
5	Chloroform	1.1	1	0.7	0.8	0.6	0.9
6	Aqueous	0.8	0.8	0.8	0.7	0.9	1

Figure 1: Antibacterial activity of *Passiflora edulis* leaf extracts against selected microbes

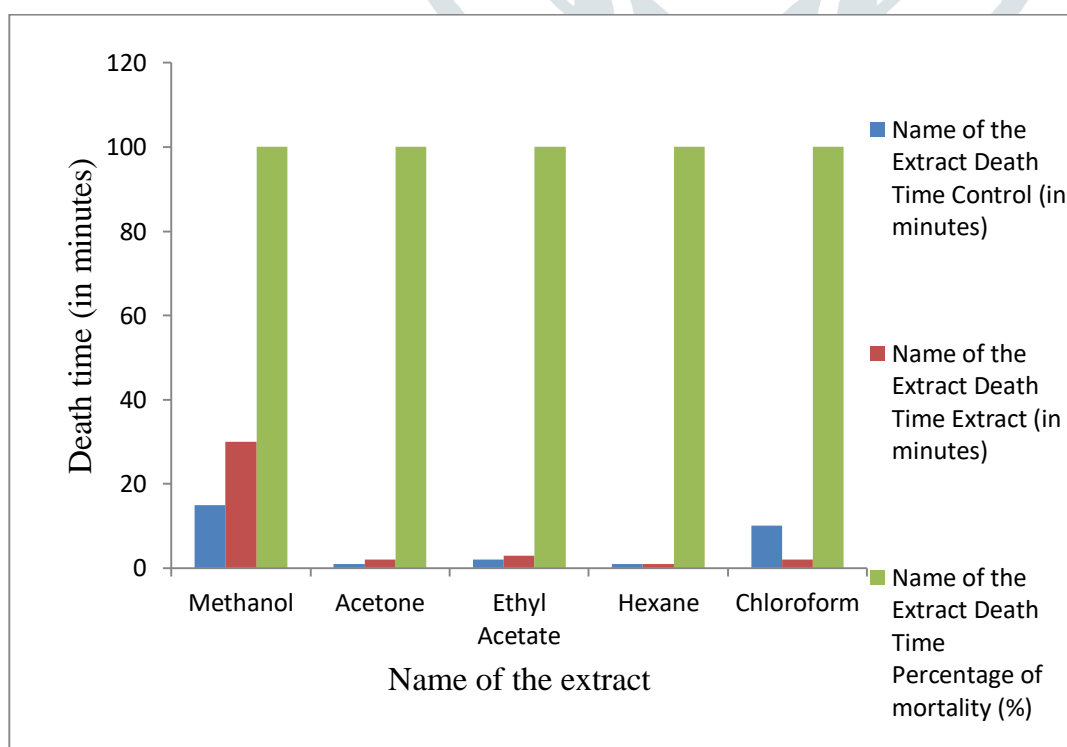
### 3.3 LARVICIDAL ACTIVITY

The Methanol, Acetone, Ethyl acetate, Hexane, Chloroform and Distilled water extracts of *Passiflora edulis* leaves showed high level of larvicidal activity. The Hexane leaf extract of *Passiflora edulis* show highest level of larvicidal activity than other extracts. 100% mortality of larvae was found. The observations are present in the table 3 and figure2.

**Table 3: Shows the larvicidal activity of leaf extract against larvae of *Culex* species of mosquito.**

Name of the Extract	Death Time		
	Control (in minutes)	Extract (in minutes)	Percentage of mortality (%)
Methanol	15	30	100
Acetone	1	2	100
Ethyl Acetate	2	3	100
Hexane	1	1	100
Chloroform	10	2	100
Aqueous	1 day	2 day	100

**Figure 2: Larvicidal Activity of plant extract of *Passiflora edulis*.**





### 3.4 ANTIOXIDANT ACTIVITY

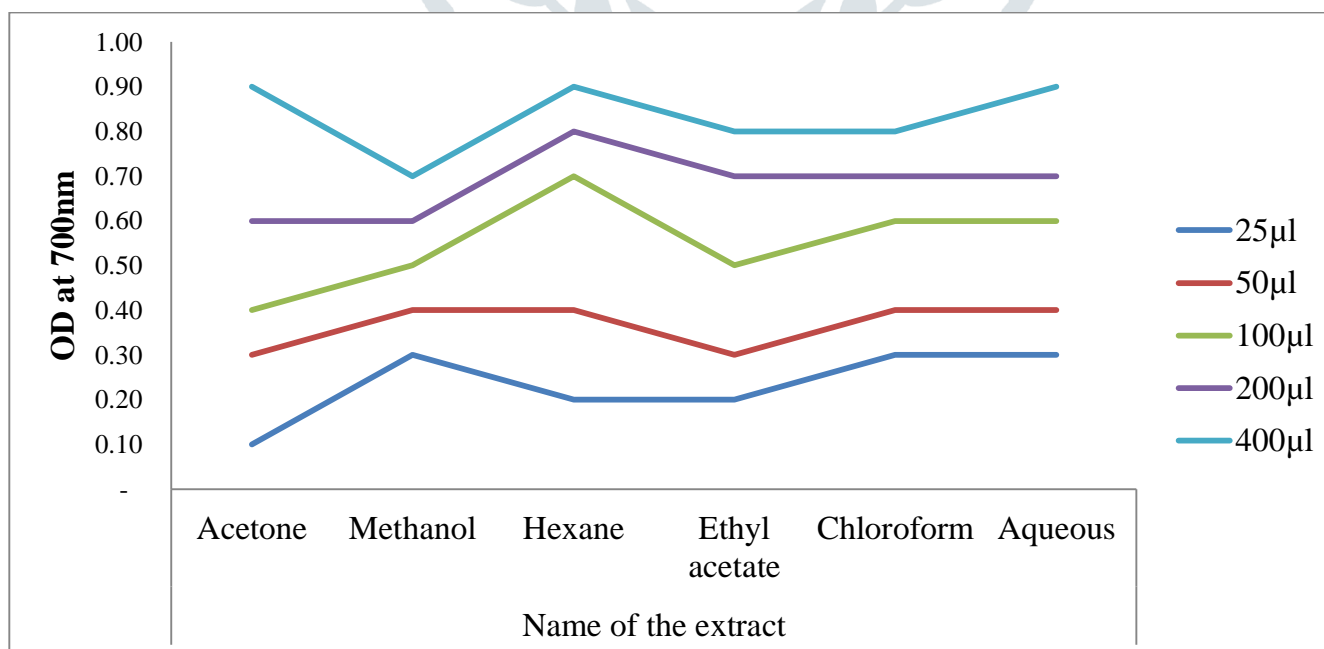
#### In vitro evaluation of *Passiflora edulis* by Reducing power assay.

The absorbance was found to increase with the dose of extract and standard which is suggested reducing power. In the  $\text{Fe}^{3+}$  reducing assay the reducing power of solvent extract was found to increase with the dose and the result was shown below (Table 4, Figure 3).

**Table 4: Antioxidant activity of *Passiflora edulis* by reducing power assay.**

OD at 700nm	Name of the extract					
	Acetone	Methanol	Hexane	Ethyl acetate	Chloroform	Distilled water
25 $\mu\text{l}$	0.10	0.3	0.2	0.2	0.3	0.3
50 $\mu\text{l}$	0.3	0.4	0.4	0.3	0.4	0.4
100 $\mu\text{l}$	0.4	0.5	0.7	0.5	0.6	0.6
200 $\mu\text{l}$	0.6	0.6	0.8	0.7	0.7	0.7
400 $\mu\text{l}$	0.9	0.7	0.9	0.8	0.8	0.9

**Figure 3: Antioxidant activity of *Passiflora edulis* by reducing power assay.**





#### 4. DISCUSSION

*Passiflora edulis*, is one of the most beautiful plants that had the pleasure of caring for and using medicinally. People who grew/grow up in the Caribbean use this plant to make a refreshing drink, also to treat a number of diseases naturally and spiritually. The leaves, flowers, peels and stems are all used as medicine in different ways. The leaves mainly contain the alkaloids. Harman, mentioned above, lowers blood pressure naturally. The flower can be made into a sedative and antispasmodic. Passion flower is also used to treat nervous disorders, bronchial conditions, arthritis, asthma, insomnia, gastrointestinal disorders and menopausal symptoms. Carotenoids and polyphenols in the yellow fruit extract can also kill cancer cells in vitro.

The extract of *Passiflora edulis* is aimed to know the components present in the plants. Extraction showed the desired phytochemicals. Phytochemical analysis of *Passiflora edulis* revealed the presence of carbohydrate, glycosides, flavonoid, resins, alkaloids and phenolic compounds. Tannin were present in the leaf and fruit, saponins were present in leaf and stem. organic extract (methanol, ethanol) of *Passiflora edulis* leaves were reported to possess tannins, flavonoids, terpenoids, steroids and saponins (Bolaji *et al.*, 2011). The present study shows various phytochemical such as different solvent present are tannin, saponin, terpenoids, phenol, flavonoid, amino acid, alkaloid, carbohydrate, glycosides and cardiac glycosides.

The methanolic extract of leaves of *P. edulis* showed positive results of antimicrobial activity on *S. aureus*, *Staphylococcus faecalis*, *B. subtilis*, *E. coli*, *P. vulgaris* and *S. typhi* at 200 µg/disc (Kannan *et al.*, 2011). The present study shows that the chloroform extract showed the highest antibacterial activity against *E. coli* with a zone of inhibition is 1.1cm.

Ashwani *et al.*, 2017 reported that Hexane, chloroform, ethyl acetate, acetone and methanol extract of *Passiflora* act against the fourth instar larvae of malaria vector, *Anopheles subpictus* and Japanese encephalitis vector, *Culex*. The present study shows the Larvicidal activity of *P. edulis* leaf extract of Hexane showed high larvicidal activity against *culex* species of mosquito as compared to other solvents. 100% mortality of larvae was found.

The strongest antioxidant activity in the stem was recorded from the methanol extracts is (429.6±3.6) µg/ml (Ripa *et al.*, 2009). The present study shows that Acetone extract showed highest Antioxidant activity in an optical density of 400µl at 700nm is 0.9. The Methanol extract showed highest Antioxidant activity in an optical density of 400µl at 700nm is 0.7. The Hexane extract showed highest Antioxidant activity in an optical density of 400µl at 700nm is 0.9. The Ethyl Acetate extract showed highest Antioxidant activity in an optical density of 400µl at 700nm is 0.9. The Chloroform extract showed highest Antioxidant activity in an optical density of 400µl at 700nm is 0.9 The Aqueous extract showed highest Antioxidant activity in an optical density of 400µl at 700nm is 0.9.

## 5. ACKNOWLEDGEMENT

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