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"ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF PASSIFLORA EDULIS"

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DECLARATION

I hereby declare that, the minor research project entitled "**ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF PASSIFLORA EDULIS" [MRP (S) – 0714/1314/KLKE020/UGC – SWRO]** is an authentic record of research work carried out by me during the year 2014 – 2016. Further certify that this work is original and carried out according to the plan in the proposal and guidelines of the university grants commission.

Dr. Jubaira Beevi Y

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CHAPTER 1

INTRODUCTION

Passion Fruit (Passiflora Edulis)

Passion fruit belongs to the family of Passifloraceae, Various species of the genus passiflora encompasses approximately 400 species, and most of them are native of Brazil. Within this species, there are two distinct forms, the standard yellow and the purple, differing in P^H and starch content. Purple variety is mainly grown for fresh juice and its flavor is a vital attribute which make the juice a desirable ingredient for many formulated beverages (Farid et al, 2010). In India, passion fruit cultivation is confined to Kerala, Tamil Nadu, Karnataka and North Eastern States (Jamir and Sharma 1999). P. edulis is a vigorous climber. They cling to anything they can grab. They grow quickly and 15-20 feet per year once established. They should have strong support. Their life cycle seems to be short in 5-7 years, but new plants can be planted and fruit can happen the same year. Several species are grown in the tropics for edible fruits, the most widely grown being P. edulis (McGuire, 1999).

Passion fruit is a highly nutrient responsive perennial crop, grows mostly as vine with a shallow root system (Janick and Paull 2008). The leaves are tri-lobed, long and deep green. The fruit has circular or oval shape and about 5 – 8cm in diameter with membranous sac. The sac is filled with pulps, seeds and juice (Johnson et al 2008). The edible part of the passion fruit (40%) consist of pulp with seeds, and 60% of the peel consists of mesocarp and epicarp. They contain many seeds surrounded by gelatinous yellow pulp that has an intense aroma and a sweet-acid taste (Lopez-Vargas et al 2013).

Passiflora edulis is popular not only because of its pulp, but also because of the infusions made with the leaves. It has been largely used in American and European countries as sedative or tranquilizers (Coleta et al 2006). Passiflora edulis has been

used as a traditional medicine as sedative, anxiolytics, diuretics and analgesics for a long time

Plants have been the basis of many traditional medicines throughout the world for thousands of years and have continued to provide new remedies to mankind. Passion Fruit plays an important role in human nutrition and health because of their nutritional and medicinal properties (Chankvetadze et al 2006). Passiflora Edulis plants are the richest source of phyto constituents like flavonoids, tannins, phenol glycosides, fatty acids and alkaloids (ZAS and John 2016). Plants stand for anti oxidant, anti microbial, anti cancerous, anti diabetic and anti hypertensive properties. Anti- diabetic properties of passion fruit could be observed in passion fruit juice, pectin, peel flour, seeds and mesocarp fiber (Hai-Xia et al 2016). The nutrient content of Passion Fruit which consist mainly of vitamin A, vitamin C, pottasium, carotenoids and polyphenolics (Yapo and Koffi 2008). Certain non-nutritive phyto chemicals also present in the passion fruit, carotenoids and polyphenols have been found to inhibit cell proliferation of leukemia (Cindy Marie De Neira 2003) Glycosides, phenols and alkaloids are major constituents in P. edulis (Dhawan et al 2004)

Passion fruit is rich in polyphenols. Poly phenolic compounds containing more than one aromatic hydroxyl group are widely distributed in plant kingdom. The fruit also contain prunasin and other cyanogenic glycosides in the peel and juice. Passion fruit oil is composed mainly of linoleic acid with smaller amounts of oleic acid and palmitic acid. It also contains vitamin C, dietary fiber, vitamin B riboflavin and niacin, iron and phosphorous in significant percentages of the daily value. (Chassagne D, 1996). Dietary fiber from passion fruit peel can be added to a variety of foods, including meat products, breakfast cereals, bakery products and dairy products for different functions (Viuda Martos, 2010).

Scientific name	**: Passiflora Edulis**
Local name	**: Passion Fruit**
Family	**: Passifloraceae**
Kingdom	**: Plantae**
Order	**: Malpighiales**
Genus	**: Passiflora**
Species	**: P. edulis**
Class	**: Magnoliopsida**
Division	**: Magnoliophyta**

Health benefits of Passion fruit

Strengthens the bones: presence of magnesium, copper, iron and phosphorus in P. edulis are beneficial to our bones because it contains vitamins and minerals that maintain healthy bone cells and speeds up bone cell repair.

Enhance metabolic rates: single serving can amount to the daily vitamin C requirement of the body, passion fruit is perceived to be the wonder fruit because of its immunity boosting effects. It also battles serious illnesses such as cancer, heart ailments and body organ malfunction.

Relief from Insomnia: passion fruit provides alkaloids which are effective sedatives that help to sleep easily.

Lowers blood pressure: It can regulate normal blood pressure because of its potassium content, potassium acts as a vasodilator that keeps the veins and blood vessels relaxed. With sufficient potassium level, the blood is able to flow in the blood stream without strain and tension. The potassium content of P. edulis is vital for the proper functioning of the kidneys and muscle contractions and particularly beneficial for smokers vegetarians and athletes. Potassium regulates electrolyte balance and controls the muscle function of our entire body including heart muscles that creates heart beat.

Helps in skin nourishment: passion fruit consumption protects our skin from harmful UV rays and pollution. Passion fruit is abundant in antioxidants, specifically vitamin C which stimulates collagen production. Collagen is responsible for the structure and firmness of the skin.

Fight against cancer: passion fruit has carcinogen killing properties, antioxidants such as vitamin C, vitamin A, phenolic compounds and flavonoids in passion fruit support healthy development of cells and helps cell repairing activities.

Delicious passion fruit is a rich source of antioxidants, minerals, vitamins and fiber. 100 gm fruit contains about 97 Kcalories.

The fruit is a very good source of dietary fiber. 100g of fruit pulp contains 10.4g or 27% of dietary fiber. Good fiber in the diet helps to remove cholesterol from the body. In addition, dietary insoluble fiber by acting as bulk laxative helps protect the colon mucous membrane by decreasing exposure time to toxic substances in the colon as well as binding to cancer causing chemicals in the colon (Hammer et al; 1987)

Nutritive Value of Passion Fruit per 100 gm

Nutrients	Nutritional value per 100 gm	Nutrients	Nutritional value per 100 gm
Energy	97 kcal	Thiamine	0.0 mg
Carbohydrate	23.38 g	Vitamin A	1274 IU
Protein	2.20 g	Vitamin C	30 mg
Total fat	0.7 g	Potassium	348 mg
Cholesterol	0.0 g	Calcium	12 mg
Dietary fiber	10.4 g	Iron	1.60 mg
Folates	14μg	Magnesium	29 mg
Niacin	1.5 mg	Phosphorus	68 mg
Pyridoxine	0.1 mg	Carotene	743 μg
Riboflavin	0.130 mg	Crypto-xantene	41 μg

(source: USDA National Nutrient data base)

Passion fruit is good in vitamin C provides about 30mg per 100g. Vitamin C (ascorbic acid) is a powerful water soluble anti-oxidant and it helps the body prevent and fight against diseases and infections. It also speeds up wound healing by facilitating the repair of tissues. (Hardin et al; 1986). Consumption of fruits rich in vitamin C helps body develop resistance against flu-like infectious agents and scavenge harmful, pro-inflammatory free radicals. Free radicals have very short half life, high reactivity and damaging activity towards macro molecules like proteins, DNA and lipids. Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis,

ischemic heart diseases, ageing, diabetes mellitus, immunosuppression, nuero degenerative diseases, cancer and others. (NYI Mekar Saptarini et al, 2013)

It contains very good levels of vitamin A and flavonoid antioxidants such as β-carotene and β-cryptoxanthin. Current research studies suggest that these compounds have antioxidant properties and along with vitamin A are essential for vision. Single serving of passion fruit has more than 100% of the total required intake of vitamin C for a healthy diet added with vitamin A, Beta carotene and potassium. Beta carotene that shields against cancer and can kill the cancerous cells

Vitamin A is also required for maintaining healthy mucus membranes and skin. Consumption of natural fruits rich in vitamin A and flavonoids helps to protect from lung and oral cavity cancers.

Fresh passion fruits are very rich in potassium. 100g of fruit pulp has about 344 mg of potassium (Knight et al; 1972)

Furthermore, passion fruits are very good source of minerals. Iron, copper, magnesium and phosphorous are present in adequate amounts in the fruit.

Evidence from many epidemiological studies suggests that high intakes of fruit and vegetables are associated with the reduced risk of cardiovascular disease. (Wilcox et al, Curb et al, 2008). The association might be partly attributable to the antioxidant content of these foods because oxidative damage, including oxidative modification of low-density lipoproteins, is a major cause of cardiovascular disease. Antioxidants protect the body from free radical damage and boost the immune system.

Some evidence suggest that antioxidants plays a role in Age-related Macular Degeneration (AMD) development, they might help slow AMD progression.

The aim of the study was to characterize the Phyto Chemical constituents, antimicrobial and anti oxidant properties of Passiflora edulis.

Objectives

- **To find out the phytochemical consistuents of leaves and fruits of passiflora edulis.**
- **To estimate the total phenolics in P. edulis.**
- **To find out the antioxidant activity of passiflora leaves and fruits.**
- **To determine the concentration of vitamin C in the passiflora fruit and leaves**
- **To determine the Iron content in leaves before and after flowering.**
- **To determine the antimicrobial activity of passiflora leaves and fruits.**
- **To characterize the components by FT-IR spectroscopy.**

CHAPTER 2

LITERATURE REVIEW

Passion fruit as an edible fruit, it contains several components such as acids and sugars, nutrients and non-nutritive phyto chemicals that make passion fruit a tasteful and healthy addition to the diet.

In a study of female American nurses, increased fruits and vegetable consumption had significantly reduced the risk of certain cancers (Terry et al, 2001)

Passion fruit provides a good source of nutrients such as vitamin C, vitamin A and potassium and non-nutritive phyto chemicals, carotenoids and polyphenols. (Talcott et al 2003), vitamin C functions as an antioxidant that reduces free radical damage by scavenging oxyradicals. Passion fruit is good in vitamin C which provides about 30mg per 100g. Vitamin C (ascorbic acid) is a powerful water soluble antioxidant. Consumption of this fruit helps to develop body resistance against flu like infectious agents and scavenge harmful free radicals. It contains

very good levels of vitamin A and flavonoids, antioxidants like Beta Carotenes. Vitamin A has important roles in the maintenance of vision and skin care, cell growth and reproduction (reviewed by Olson, 1996, Gerster 1997)

Some experiments in rats proved that passion fruit leaves extract had positive influence in wound healing. (Goncalves Filho et al, 2006) tried to see the influence in rat's bladder wound and explored the mechanisms. They concluded that the extract promoted wound healing because it could reduce acute inflammation.

It is used as a nutritional substitute for soft drinks by providing vitamins, trace elements or anti oxidants. The presence of these substances are hence used for various deficiency disorders

In passion fruit, thirteen different carotenoids have been identified (Mercandente etal 1998). Other non-nutritive phyto chemicals found in the passion fruit are polyphenolic compounds, which have been found to have anti oxidant activity (Rice Evans and Miller 1996, Salah et al 1995) as well as anti cancer properties (Yoshida et al 1992; Kang and Liang 1997)

The extracts of P. edulis stem in chloroform and petroleum ether have profound antibacterial, cytotoxic and antioxidant effect than chloroform and petroleum ether extract of leaf (Farhana et al, 2009)

The aqueous and ethanolic extract of passion fruit in the absence and presence of transition metal salts (Zinc Acetate. 2H2O) were subjected to antimicrobial studies using the Disc Diffusion Assay. (Jagessar et al, 2017)

Medeiros reported that a clinical toxicological assay of passion fruit peel flour was performed with a daily intake of 30 gm and showed no acute or sub chronic toxicity, suggesting its use as a dietary supplement, making it feasible to carry out further efficacy studies in people with diabetes because it is a product rich in soluble fiber. Diabetic guinea pigs fed with the passion fruit peel flour presented diabetes control due to its hypoglycemic action.

Brag et al studied the passion fruit peel flour which is rich in soluble fiber and has pectin as one of its components. Pectin is a soluble fiber widely used as an ingredient in pharmaceutical preparations as anti diarrheal and detoxifying substance. It reduces glucose intolerance in diabetic patients and decreases serum

and triglyceride levels by forming a gel which prevents the absorption of cholesterol and glucose derived from the diet.

Diabetes mellitus is a metabolic disease characterized by the abnormal high plasma glucose levels, leading to major complications, such as insulin resistance, obesity, hyperlipidemia and hypertension also with alternations in the immune and neuronal systems.

Cazarin and Colomen characterized leaves of passiflora alata Curtis for their antioxidant capacity. Antioxidant analysis of DPPH, FRAP, ABTS, ORAC and phenolic compounds were made in three different extracts; aqueous, methanol, acetone and ethanol. Aqueous extract was found to be the best solvent for recovery of phenolic compounds and anti oxidant activity, when compared with methanol, acetone and ethanol.

Results suggested that the consumption of aqueous extract of passiflora may be considered a good source of natural antioxidants and compounds found in its composition can act as inflammatory agents, helping in the control of diabetes.

Phamiwon ZAS and Sheila John carried out a study 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 phyto chemical analysis of passiflora edulis leaf extract reflects the presence of flavonoids, tannins, polyphenols, terpenoids, steroids and alkaloids.

Juliana Kelly da Silva et al investigated the In vitro and In vivo antioxidant potential of aqueous extracts of P. edulis leaves and identification of phenolic compounds by HPLC-PDA and ESI-MS/MS analysis. Total phenols and antioxidant potential (DPPH, FRAP, ABTS and ORAC assay) were determined in aqueous extract. P. edulis leaf extract could be an option to enhance the supply of antioxidant and to safe guard against oxidative stress.

Carlos Victor et al determined antioxidant, anti-inflammatory and hypoglycemic effects of leaf extract from passiflora, this study indicates positive effects of passiflora extract and its potential to inhibit metabolic syndrome, oxidative stress and pain

Konta and Almaida (2014) revealed that yellow passion fruit pulp contains phenolic compounds ascorbic acid, carotenoids and flavonoids. The highest dose of passion fruit pulp significantly reduced the systolic blood pressure. The anti-hypertensive effect of yellow passion fruit pulp might be due to the enhancement of the antioxidant status.

In passion fruit thirteen different carotenoids have been identified, including zeta, beta and alpha carotein b-cryptoxanthin and lycopene (mercandente et al 1998) other nutritive phytochemicals found in passion fruit are polyphenolic compounds, which have been found to have antioxidant activity (Rice Evans and miller 1996) as well as anticancer properties (Kang and Liang 1997)

Phenolic content can be used as an important indicator of antioxidant capacity and as a preliminary screen for any product intended to be used as a natural source of antioxidants in functional foods. (Viuda-Martos et al, 2011) The total phenol content of dried samples at 60°c ranged from 5.02 ± 0.33 g/kg d.w (first batch) with satistically significant differences (P <0.05) between two batches.

The phenolic compounds were retrained in the process of lyophilization because enzymatic activity was inhibited during the drying time. Prolonged exposure to 60°c was most likely the cause of the degradation of phenolic compounds observed in the dried passion fruit peel. The dried passion sample had higher Total Phenol Content when compared to the freeze-dried samples. (Oliveira, Gurak et al 2016)

In addition to the colorant properties, carotenoids are known to have several other biological functions such as vitamin A activity, cancer preventing effect, protective effects against cardiovascular disease and reduction of the risk of cataracts and age related macular degeneration (Leong 2012)

Polyphenolic compounds are non-nutritive phytochemicals that contribute to the flavor, pigmentation and health benefits of certain plants. The two main classes of polyphenols found in the diet are phenolic acids and flavonoids. Flavonoids represent the largest class of polyphenolics accounting for approximately 2/3 of the identified polyphenolics (Birt et al 2001). The main flavonoid families include flavones, flavonols, flavanones, catechins (flavanols), anthocyanidins and isoflavones. Fruits in general have been found to contain several flavonoids including flavonols (quercetin), flavanones, catechins and anthocyanidins. In

passion fruit, the total flavonoid content has been quatified (435 mg/L) and the phenolic acids have been identified, however, individual flavonoids have not (Talcott et al 2003).

Certain polyphenols, such as quercetin, catechins, resveratrol, gallic acid and anthocyanins exhibit antioxidant activity by inhibiting lipid perioxidation and scavenging oxygen radicals (Terao et al 1994; Miura et al 1995; Sarah et al 1995), thus potentially lowers the risk of coronary heart diseases and cancer. Epidermiological studies have associated food rich in polyphenols provide prevention against diseases such as cancer (Steinmetz and potter 1996)

Passion fruit peel has been reported to contain significant amount of pectin (Kulkarni and Vijayanand, 2010). In this study there was significant difference in the amount of pectin between the two batches of peel dried at 60°c. The amount of pectin powder by freeze dried is greater than that dried with hot air convection. The pectin extract from the peels of passion fruit can be used to enhance the functional properties of different food products.

Reviews focused on the various areas of research on passiflora, which support the use of traditional medicines to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases. (A.G Ingale and A.U Hivrale, 2010).

CHAPTER 3

MATERIALS AND METHODS

EXPERIMENTAL DETAILS

Plant Material

The plant material for the study was collected from Keralapuram, Kollam, Kerala, India

Extraction and Isolation

The plant leaves were cut into pieces, shade dried and grinded. The powdered plant material was used for extraction purpose.

A. PREPARATION OF CRUDE EXTRACTS OF PASSIFLORA EDULIS

The aqueous extract of each sample was prepared by soaking 10g of powdered samples in 200ml of distilled water for 72 hours. The solution was thoroughly shaken intermittently, then filtered using filter paper and then concentrated to ¼ of the original extracts i.e. 50 ml.

B. PREPARATION OF ETHANOL EXTRACT OF PLANT SAMPLES

20 gm of powdered leaves was weighed and poured into a round bottom flask. 300 ml of ethanol poured into the flask. This was allowed to stay for 3 days and thoroughly shaken intermittently. After 3 days, the mixture was filtered into a beaker. The filtrate in the beaker was concentrated to dryness on a water bath at 50°c. The beaker containing the dark brown extract was then covered with aluminium foil and was then stored in the refrigerator at 4°c until required for use.

C. PREPARATION OF CHLOROFORM EXTRACT OF PLANT SAMPLES.

The chloroform extract of each sample was prepared by soaking 10 g of powdered samples in 300ml of chloroform for 72 hours. The solution filtered, then concentrated to 50 ml and stored in airtight container.

1. PHYTOCHEMICAL ANALYSIS

Phytochemical examinations were carried out for all the extracts as per the standard methods

A. Qualitative analysis

Detection of Alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered.

Mayer's Test: filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Wager's Test: Filtrates were treated with Wager's Reagent (Iodine in Potassium Iodide). Formation of brown or reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formations of red precipitate indicate the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's Reagent (Saturated Picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

Detection of Carbohydrate: Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molish's Test: Filtrates were treated with 2 drops of alcoholic alphanaphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugar.

Fehling's Test: Filtartes were hydrolysed with dilute HCI, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of Steroids: 1 ml of extract is treated with 0.5ml of acetic anhydride and cooled in ice. Add 0.5ml of chloroform and 1ml of concentrated sulphuric acid. Formation of reddish brown ring at the separation level of two liquids indicates the presence of steroids.

Detection of Saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of Saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for 10 minutes it indicates the presence of saponins.

Detection of Terpenoids: 1 ml of extract is treated with 20% NaOH. Formation of yellow colouration and it becomes colourless on adding dilute HCL; it indicates the presence of Terpenoids.

Detection of Quinones: Extract is treated with NaOH. Formation of blue green or red colour, it indicates the presence of Quinones.

Detection of Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour, it indicates the presence of Phenols.

Phosphomolybdic acid Test: The extracts were spotted on a filter paper, drop of Phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue coloration of the spot indicates the presence of phenols.

Detection of Tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of Flavonoids:

Alkaline Reagent Test: Extracts were treated with few drops of sodium chloride solution. Formation of intense yellow colour, which becomes colourless on adding dilute acid, indicates the presence of Flavonoids.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of Flavonoids.

The presence or absences of phytoconstituents were represented in the next chapter.

B. QUANTITATIVE ANALYSIS

The phytyochemical screening showed the presence of phenols which is estimated spectrophotometrically.

2. Estimation of Total Phenolics.

The phenolic contents in the extract were determined by the modified Folin Ciocalteu Method. The standard used was Gallic acid.

0.25 gm of dried extract is made up to 50ml with 4:1 (water: alcohol) mixture, in a 50ml standard flask. Shake well the solution for uniform concentration. The extract will contain the phenol.

0.0143 gm of gallic acid is made up to 100 ml using distilled water. This is used as the working standard. From the working standard different volumes were taken (0.2, 0.4, 0.6, 0.8 and 1 ml) in each test tube and labeled as S_1 to S_5 and made up to 1ml using distilled water. A blank was prepared with 1ml distilled water and labeled as B. Then add 2.5ml 10% Folin Ciocalteau reagent, and shaken vigorously. After 3 minutes add 2ml of 2% Na_2CO_3 solution and mixed thoroughly and allowed to stand for half an hour. After required time, read the absorbance at 670 nm against the reagent blank.

The alcoholic extract of the plant is made up to 50ml in a standard flask. From this different volumes were taken (0.2, 0.4, 0.6, 0.8ml) in each test tube labeled as T_1, T_2, T_3 and T_4. Diluted to 1ml using distilled water, then add 2.5ml 10% Folin Ciocalteau reagent and shaken vigorously.

After 3 minutes add 2ml 2% Na_2CO_3 solution and mixed thoroughly and allowed to stand for half an hour, then the optical density was measured at 670 nm.

The total content of phenolic compounds in extracts in gallic acid equivalents (GAE) was calculated by the following formula C=c. V/m where: C=total content of phenolic compounds in mg/ml; V=the volume of extract in ml; m=the weight of pure plant ethanolic extract,g.

3. TOTAL ANTIOXIDANT ACTIVITY

A. Phospho molybdenum method

The antioxidant activity of the extract of passiflora edulis was evaluated by the phospo-molybdenum method according to the procedure of prieto et al. 0.3ml of extract was mixed with 3ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695nm using a spectrophotometer against blank. A typical blank solution contains 3ml reagent solution and appropriate volume (0.3ml) of the same solvent in the place of the extract. The total antioxidant capacity was expressed as the number of equivalents of ascorbic acid.

B. DPPH Radical Scavenging Activity Method.

The antioxidant activity of the alcohol extract of leaves of P. edulis was based on the scavenging activity of the stable 1, 1-diphenyl-2picryl hydrazyl (DPPH) free radical and was determined by Braca et al.

The interest in polyphenolic antioxidants has increased because of their elevated capacity in scavenging free radicals associated with various diseases. This property has been evidenced by a large number of tests measuring the antioxidant activity in vitro.

DPPH method

0.01gm of residue is dissolved in 100 ml ethanol and different aliquots (0,1,2….10ml) from the above sample solution were taken, diluted to 10ml with alcohol. Add 1ml of 0.1 milli molar DPPH, allowed to stand for 30 minutes away from light and heat. BHA (Butylated Hydroxyl Anisole) and BHT (Butylated Hydroxyl Toluene) were used as positive controls. Absorbance at 695 nm was determined. The percentage inhibition activity was calculated as:

% inhibition activity = $\{A_o-A_1]/A_o\}100$,

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance of the test compound. IC_{50} is the antioxidant concentration that inhibits the DPPH reaction by 50% under the experimental condition. This was calculated by plotting % inhibition against different concentration.

4. Extraction of ascorbic acid (vitamin C)

20 gm of passion fruit pulp was chosen for the analysis of ascorbic acid. It is mixed with 5-10 ml of 4% of sulphosalicylic acid. Centrifuged and filtered with whatmann no: 41 filter paper. The filtrate is made up to 100ml using 4% sulphosalicylic acid.

Estimation of ascorbic acid – Indo phenol titration method

5ml of made up solution is taken in clean conical flask and 10ml sulphosalicylic acid is added. It is titrated against Indophenole dye from the burette. Titration is done very rapidly to increase the specificity of the reaction. Since ascorbic acid reacts very fast with oxidizing agents where as many of interfering substances like stannous sulphate, ferrous sulphate reacts with indophenole dye very slowly. Dye is standardised against standard ascorbic acid solution. From the titre value, the amount of ascorbic acid present in 100gm pulp was calculated.

Vitamin C in the leaf extract is also determined using the same procedure.

5. Determination of Iron content in leaf extract before and after flowering

Iron present in the sample is released by acid treatment, which undergoes reduction to ferrous state in the presence of thioglycolic acid. The iron complexes with 1, 10-phenanthroline reagent to yield a color, whose intensity is measured at 535nm.

Reagents:

Protein precipitant reagent

Dissolved 100g of trichloroacetic acid in 250-350 ml deionized water. Separately mixed 30ml of thioglycolic acid and 2ml of concentrated HCL, It is added to trichloroacetic acid solution.

Chromogen reagent

Weighed 25 mg phenanthroline, dissolved and made up to 100ml with 2m sodium acetate solution in a volumetric flask

NOTE: Ensured all the glasses used in the experiment were free of any iron contaminations.

Stock solution

Standard Iron Solution

Dissolved 70.22mg of ammonium ferrous sulphate in double distilled water and madeup the volume to 100ml (concentration 100 μg/ml). Diluted the stock solution 1:10 in distilled water to give a final concentration of 10 μg/ml

Procedure

1ml of leaf extract is taken in an Iron free test tube, added 1ml of protein precipitate reagent and vortex for 1 min. Allowed the sample to stand at room temperature for 5min and centrifuge for 15 min.

Run a set of iron standard separately in the range of 1-10 micro g/ml and a reagent blanks along with sample. Added 1ml of protein precipitate reagent to all tubes.

Carefully collected and transferred the supernatant of the test sample into a separate test tube. Added 1ml of chromogen solution to test tube containing blank, standard and test solution. Mixed and allowed the test tubes to stand for 10-20 min at room temperature before recordings the absorbance at 535nm in a photometer.

Constructed a calibration curve and computed the concentration of iron in the leaves. Expressed the value per 100g of the leaf.

6. Antimicrobial activity

Passion fruits and leaves were screened invitro for their antimicrobial activity against different bacterial and fungal strains.

Antimicrobial activity was carried out against 5 micro organisms in Cashew Export Promotion Council of India (CEPC) Kollam.

The micro-organisms were P. aeruginosa, S. flexineri, S. typhi, E. coli and C. albicans

AGAR DISC DIFFUSION METHOD

The antimicrobial activity of ethanolic extract of the fruit, aqueous and methanol extract of the leaves were carried out by Disc Diffusion Method. Using this method 40gm of Mueller Hinton Agar (MHA) was placed into 1000ml of distilled water. This was mixed thoroughly; the mixture was then heated with frequent agitation in a conical flask. The mixture was boiled for 1 minute to completely dissolve the Agar powder; it was then autoclaved at 120°c for 15 minutes. The molten Agar was then poured into 90mm sterile petri dishes to a depth of 4mm, these plates were allowed to cool and refrigerated for use the following day. The plates were labeled and inoculated with the respective bacterial colonies. Discs impregnated with the antimicrobial plant extracts at appropriate concentrations were placed on the MHA plates. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. The inoculated plates were incubated at 37°c for 24 hours. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antimicrobial activity (Jagessar et al, 2017).

Antimicrobial activity of leaf extract and fruit extract are tested against different microbes such as P. aeruginosa, S. flexineri, S. typhi, E. coli and C. albicans

The ethanol leaf extract showed variable degrees of antibacterial activity. Moderate activity was noted in S. flexineri

7. FT-IR STUDY

IR Spectroscopy is the study of interaction of matter with light radiation when electromagnetic waves travel through the medium (matter)

These waves interact with the polarity of the chemical bonds of the molecule, if there is no polarity (dipole moment) in the molecule, then the IR interaction is inactive and the molecule does not produce any IR spectrum.

Infrared spectroscopy is used in chemistry for identification and characterization of molecules. Since an IR spectrum is the finger print of each molecule, IR is used to characterize substances.

Aqueous extract of leaves and ethanol extract of the fruit pulp were considered for FT-IR spectroscopy in the range of 4000 to 400cm^{-1}

Values are given in the table. The FT-IR spectrum (Fig 4 & 5) was recorded using KBr pellets.

RESULTS AND DISCUSSIONS

1. PHYTOCHEMICAL ANALYSIS (QUALITATIVE)

The result of the phytochemical screening of leaf extract and fruit pulp extract of passiflora edulis is presented in the table. Compounds like flavonoid alkaloid, steroid, terpenoid, protein and amino acid are known to have curative activity against several pathogens and therefore could suggest its traditional use for the treatment of various illnesses.

Phytochemical analysis of leaf extract

Table 1.1

Compound	Aqueous Extract	Ethanol Extract	Chloroform Extract
Alkaloids	+ +	+ +	+
Carbohydrate	+	+	+
Steroids	-	+	+
Saponins	-	+	+
Terpenoids	-	+	+
Quinones	+	-	-
Phenols	+ +	+	+
Tannins	+	+	+
Flavonoids	-	+ +	+

Fruit Juice (Ethanolic)

Table 1.2

Serial no:	Compounds	Presence/absence
1	Flavonoids	+
2	Alkaloid	+
3	Steroids	+
4	Terpenoid	+
5	Saponin	+
6	Poly phenols	+
7	Proteins and aminoacids	+
8	Antraquinones	-
9	Quinones	+

Fruit Juice (Aqueous)

Table 1.3

Serial no:	Compounds	Presence/absence
1	Flavonoids	+
2	Alkaloids	+
3	Steroids	+
4	Terpenoid	+
5	Saponin	+
6	Proteins and aminoacids	+
7	Antraquinones	-
8	Quinones	+

Aqueous extract of leaf (After flowering)

Table 1.4

Serial no:	Compounds	Presence/absence
1	Flavonoids	+
2	Alkaloids	+
3	Steroids	+
4	Terpenoid	+
5	Saponin	-
6	Proteins and aminoacids	+
7	Antraquinones	-
8	Quinones	+

Aqueous extract of leaf (Before flowering)

Table 1.5

Serial no:	Compounds	Presence/absence
1	Flavonoids	+
2	Alkaloids	+
3	Steroids	+
4	Terpenoid	+
5	Saponin	-
6	Proteins and aminoacids	+
7	Antraquinones	-
8	Quinones	+

QUANTITATIVE ANALYSIS

2. Total Phenolic Content

Polyphenolics are the major plant compounds with antioxidant activity and they play an important role in absorbing and neutralizing free radicals, quenching singlets and triplet oxygen and decomposing peroxides. The results from the study strongly suggest that the phenolics are important component of plants and some of their pharmacological effects may be due to the presence of phenolics.

Table 2.1

Concentration	Optical density
0 ml	0
200 (0.2ml)	0.23
400 (0.4ml)	0.43
600 (0.6ml)	0.67
800 (0.8ml)	0.89
1000 (1ml)	1.10

The total phenolic content in mg/g Gallic acid equivalent was found to be 14.76mg/100g GAE in the alcohol extract of P. edulis

3. Total Antioxidant activity

A. Phosphomolybdenum method

The antioxidant activity of pulp extract 100, 200, 300, 400, 500 µg/ml concentrations was measured by the phosphomolybdenum method. Total antioxidant capacity of extracts of passiflora edulis was calculated using the standard curve of ascorbic acid ($Y=0.002x+0.176$; $R^2=0.923$) and is expressed as number of equivalent of ascorbic acid per gram of plant extract (AAE). The total antioxidant capacity of ethanol extract was found to be 1.90±0.795, mg/g of plant extract (expressed as ascorbic acid equivalents) as shown in table. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by

the antioxidant compound and the formation of the green phosphate/Mo (V) complex with a maximal absorption at 695nm

Table 3.1

Conc.(µg/ml)	Absorbance at 695nm
100	0.288 ± 0.015
200	0.327 ± 0.02
300	0.831± 0.031
400	1.186 ± 0.031
500	1.260 ± 0.035

Total antioxidant activity of the Ethanolic extract is 1.90 ± 0.795 mg/g

B. DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical is widely used as a model to investigate the free radical scavenging of several plant extract. P. edulis alcoholic extract scavenged the DPPH radical in a concentration dependant manner.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was shown by its absorbance which is induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate antioxidant activity of antioxidants.

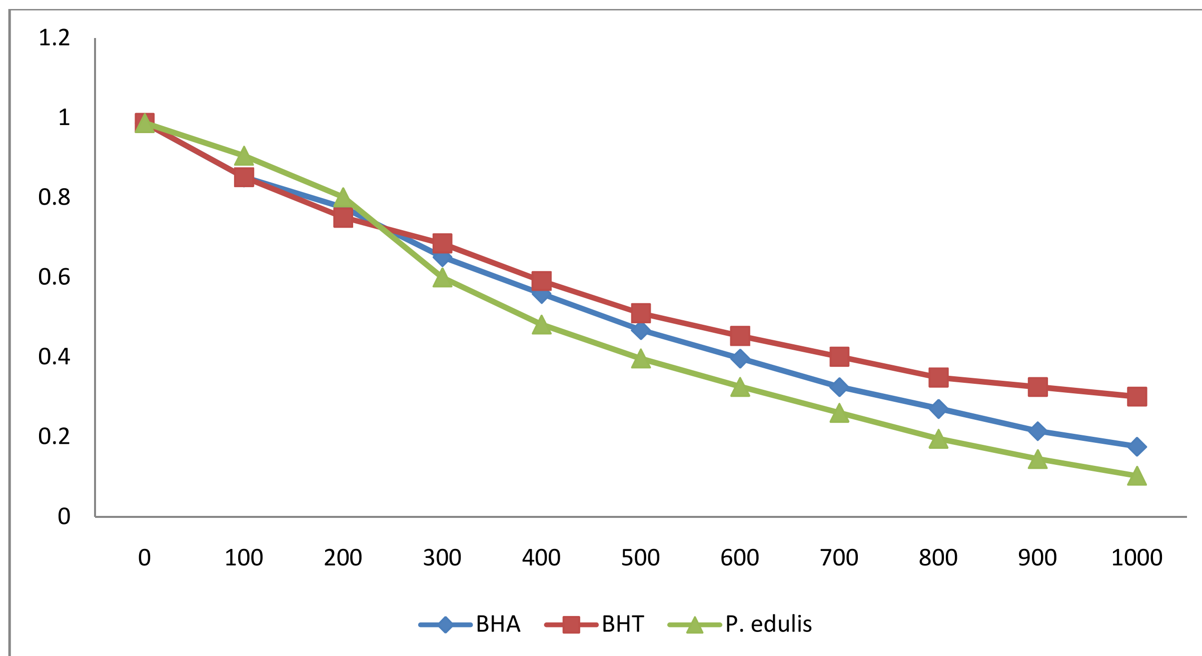
IC_{50} values are calculated according to the absorbance values. This can be explained as the plant concentration which can remove the half of the free radicals from the medium away, so the less IC_{50} value means more antioxidant effect.

Absorbance of P. edulis extract and the standards at different concentration

Table 3.2

STANDARD					
BHA		BHT		P. edulis alcohol extract	
Conc.	Absorbance	Conc.	Absorbance	Conc.	Absorbance
0	0.987	0	0.987	0	0.987
100	0.851	100	0.851	100	0.905
200	0.775	200	0.750	200	0.801
300	0.651	300	0.685	300	0.600
400	0.559	400	0.591	400	0.482
500	0.468	500	0.510	500	0.397
600	0.397	600	0.453	600	0.326
700	0.325	700	0.401	700	0.261
800	0.271	800	0.349	800	0.196
900	0.215	900	0.325	900	0.145
1000	0.176	1000	0.301	1000	0.103

Absorbance vs Conc. Fig. 1



CALCULATION OF % INHIBITION ACTIVITY

% inhibition of 100 micro gm of extract=[0.987-0.905/0.987]*100

=8.308

% inhibition of 200 micro gm of extract=[0.987-0.801/0.987]*100

=18.84

% inhibition of 300 micro gm of extract=[0.987-0.600/0.987]*100

=39.20

% inhibition of 400 micro gm of extract=[0.987-0.482/0.987]*100

=51.2

% inhibition of 500 micro gm of extract=[0.987-0.397/0.987]*100

=59.1

% inhibition of 600 micro gm of extract=[0.987-0.326/0.987]*100

=67

% inhibition of 700 micro gm of extract=[0.987-0.261/0.987]*100

=73.6

% inhibition of 800 micro gm of extract=[0.987-0.196/0.987]*100

=80.1

% inhibition of 100 micro gm of BHA standard=[0.987-0.881/0.987]*100

=10.7

% inhibition of 200 micro gm of BHA standard=[0.987-0.775/0.987]*100

=21.5

% inhibition of 300 micro gm of BHA standard=[0.987-0.651/0.987]*100

=34

% inhibition of 400 micro gm of BHA standard=[0.987-0.559/0.987]*100

=43.4

% inhibition of 500 micro gm of BHA standard=[0.987-0.468/0.987]*100

=52.6

% inhibition of 600 micro gm of BHA standard=[0.987-0.397/0.987]*100

=59.8

% inhibition of 700 micro gm of BHA standard=[0.987-0.325/0.987]*100

=67.1

% inhibition of 800 micro gm of BHA standard=[0.987-0.271/0.987]*100

=72.5

% inhibition of 100 micro gm of BHT standard=[0.987-0.851/0.987]*100

=13.8

% inhibition of 200 micro gm of BHT standard=[0.987-0.750/0.987]*100

=24.0

% inhibition of 300 micro gm of BHT standard=[0.987-0.685/0.987]*100

=30.6

% inhibition of 400 micro gm of BHT standard=[0.987-0.591/0.987]*100

=40.1

% inhibition of 500 micro gm of BHT standard=[0.987-0.510/0.987]*100

=48.3

% inhibition of 600 micro gm of BHT standard=[0.987-0.453/0.987]*100

=54.2

% inhibition of 700 micro gm of BHT standard=[0.987-0.401/0.987]*100

=59

% inhibition of 800 micro gm of BHT standard=[0.987-0.349/0.987]*100

=64.6

Table 3.3

STANDARDS				Passiflora edulis	
BHA		BHT			
Conc.	% activity	Conc.	% activity	Conc.	% activity
		0		0	
100	10.7	100	13.8	100	8.308
200	21.5	200	24.0	200	18.84
300	34	300	30.6	300	39.20
400	43.4	400	40.1	400	51.2
500	52.60	500	48.3	500	59.1
600	59.8	600	54.2	600	67
700	67.1	700	59	700	73.6
800	72.5	800	64.6	800	80.1

BAR DIAGRAM REPRESENTING THE % ACTIVITY OF ALCOHOL EXTRACT OF P. EDULIS, BHA AND BHT VS CONCENTRATION

Fig. 2

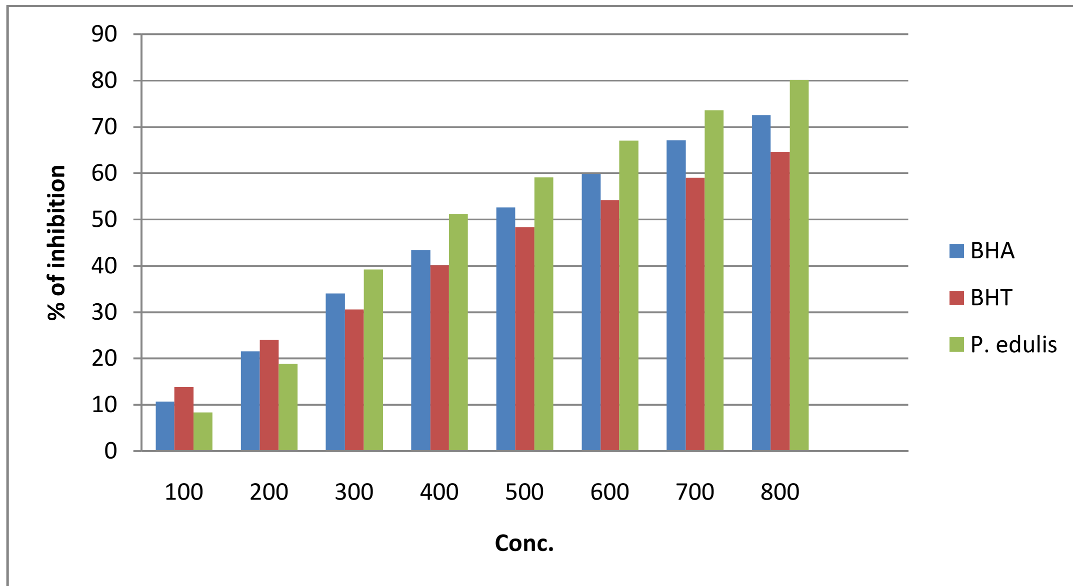
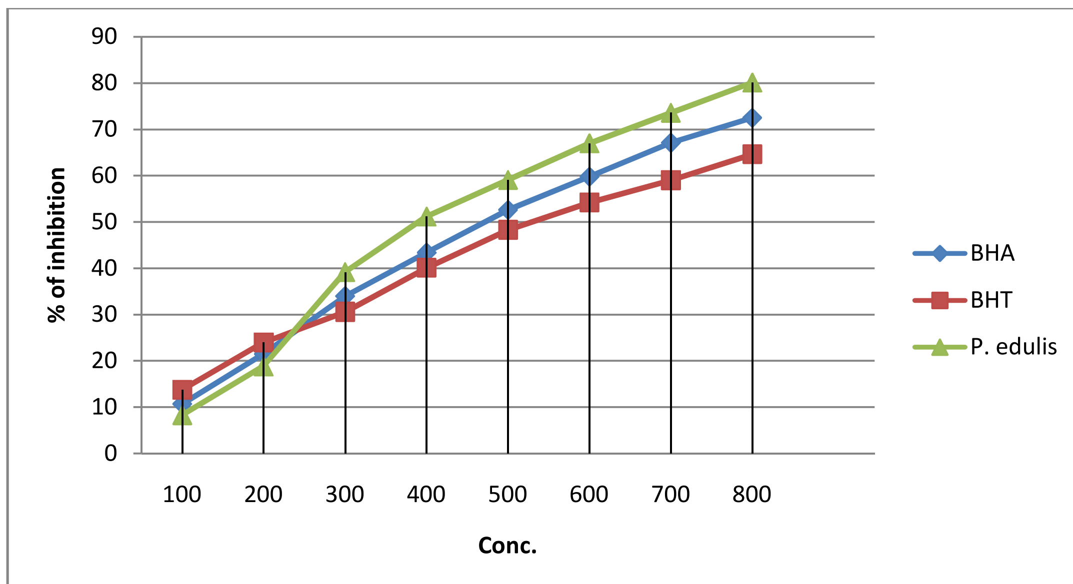


Fig. 3



From the plot of % inhibition activity against conc., IC_{50} can be calculated.

IC_{50} is the concentration of a sample required to decrease the absorbance at 695 nm by 50% compared to the control response. The lower the IC_{50} value, greater is the free radical scavenging activity. Greater free radical scavenging activity of P. edulis leaves confirms its use as strong antioxidants.

4. Estimation of Ascorbic acid

Vitamin C is an essential component of the diet for humans and an adequate intake is important not only for the prevention of scurvy but also to limit the risk of chronic diseases such as heart diseases and cancer (Carr et al 1999).

Vitamin C content of the passion fruit pulp and leaves is determined by Indophenol titration method. The absorbance of different extracts of passion fruit are measured at 695 nm to detect the presence of vitamin C

Absorbance of different extract

Table 4.1

Sl. No	Extracts	Absorbance at 695 nm	Mean Absorbance
1	Aqueous passion fruit juice	1.195 1.190 1.193	1.192
2	Alcoholic passion fruit juice	1.862 1.851 1.862	1.858
3	Leaves before flowering	1.755 1.789 1.793	1.779
4	Leaves after flowering	0.452 0.457 0.455	0.454

Total vitamin C content on P.edulis

Table 4.2

Sample	Concentration (mg/100g)
Fruit pulp	62.495 ± 0.742 mg/100gm
Leaf extract	4.325 ± 0.234 mg/100gm

Vitamin C content is:

62.495 ± 0 .742 mg/100gm in fruit pulp.
4.325 ± 0.234 mg/100gm in leaves

5. Determination of Iron content in the leaves of P. edulis before and after flowering

In the aqueous extract of the leaves, the absorbance is greater in leaves before flowering than that in the leaves after flowering at 535 nm. That is, it shows Iron content more in leaves before flowering than the leaves after flowering. Similarly the absorbance is measured at 695 nm to detect the presence of vitamin C. The absorbance is greater in leaves before flowering than that in leaves after flowering

at 695 nm. It indicates the vitamin C is more in leaves before flowering than the leaves after flowering.

Absorbance of Iron at different concentration.

Table 5.1

Sl. No.	Volume of standard (ml)	Volume of distilled water (ml)	Volume of precipitant reagent (ml)	Volume of chromogen reagent (ml)	Conc. Of iron (µg)	Absorbance at 535 nm
B	0.0	2.0	1.0	1.0	0.0	0.00
S_1	0.2	1.8	1.0	1.0	2	0.05
S_2	0.4	1.6	1.0	1.0	4	0.10
S_3	0.6	1.4	1.0	1.0	6	0.14
S_4	0.8	1.2	1.0	1.0	8	0.20
S_5	1.0	1.0	1.0	1.0	10	0.25

The absorbance of different extracts of P. edulis are measured at 535 nm to detect the presence of iron content.

Absorbance of P. edulis fruit juice and leaves.

Table 5.2

Sl. No	Extracts	Absorbance at 535 nm	Mean Absorbance
1	Aqueous passion fruit juice	1.316 1.320 1.320	1.318
2	Alcoholic passion fruit juice	2.763 2.716 2.706	2.649
3	Leaves before flowering	2.726 2.716 2.706	2.716
4	Leaves after flowering	0.329 0.329 0.331	0.329

Iron content in aqueous fruit juice = 1.72 mg/100 gm

Iron content in ethanolic fruit juice = 1.93 mg/100 gm

Iron content in leaves before flowering = 1.92 mg/100 gm

Iron content in leaves after flowering = 1.42 mg/100 gm

6. Antimicrobial activity

In passiflora species many of the chemical components of plants have antimicrobial activity. Methanolic extract of leaves showed higher antimicrobial activity at higher concentration compared to aqueous extract. Passiflora edulis could serve as a better source of antimicrobial agent.

Antimicrobial activity of ethanol extract of passiflora fruit.

Table 6.1

Organism	Fruit extract
P. aeruginosa	6.2 ± 1.2
S. flexineri	5.3 ± 0.4
S. typhi	7.4 ± 1.1
E. coli	6.3 ± 0.6
C. albicans	9.5 ± 1.3

Antimicrobial activity of passiflora edulis leaves

Aqueous extract

Table 6.2

Organisms	100mg	200 mg
P. aeruginosa	4.8 ± 1.0	5.8 ± 0.2
S. flexineri	7.7 ± 1.1	7.9 ± 0.3
S. typhi	5 ± 0.4	7.2 ± 0.4
E. coli	7.1 ± 0.4	9.5 ± 0.1
C. albicans	9.65 ±1.25	10.45 ±0.50

Methanol extract

Table 6.3

Organisms	100mg	200 mg
P. aeruginosa	5.8 ± 0.5	6.3 ± 0.6
S. flexineri	8.9 ± 0.6	9.1 ± 1.1
S. typhi	3.1 ± 0.4	6.9 ± 0.3
E. coli	14.2 ± 0.8	22.2 ± 0.8
C. albicans	12.75± 0.40	13.45 ± 0.60

Methanol extract showed zone of inhibition at a maximum concentration (200µg/disc) against ecoli 22.2mm, S. flexineri 9.1mm, S. typhi 6.9mm, P. aeruginosa 6.3mm and C. albicans 13.45mm.

The aqueous extract showed zone of inhibition E.coli 9.5mm, S. flexineri 7.9mm, S. typhi 7.2mm, P. aeruginosa 5.8mm and C. albicans 10.45mm.

Methanol extract of P. edulis showed more inhibitory activity against human pathogenic bacteria when compared to aqueous extract.

7. FT-IR STUDY

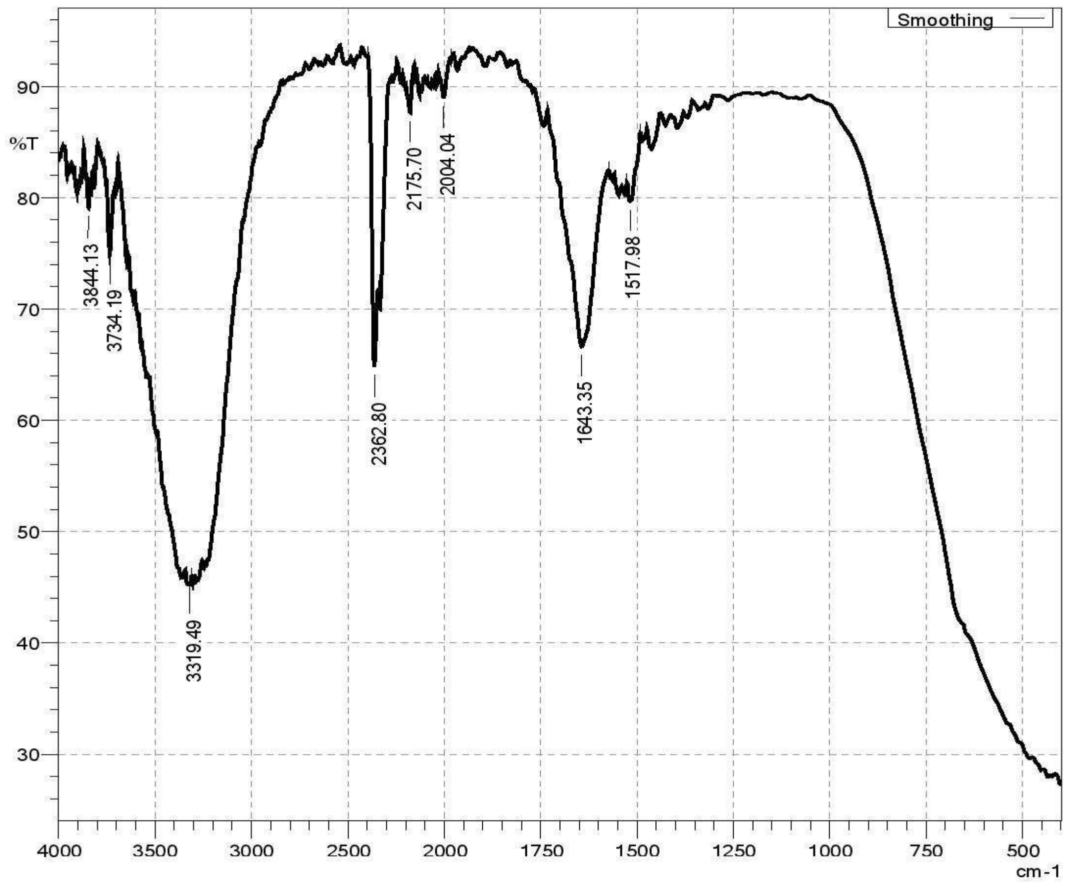
This characterization was used to identify the functional groups of the components based on the peak value in the region of infrared radiation.

FT – IR peak values of fruit pulp and aqueous extract of leaves.

Table 7.1

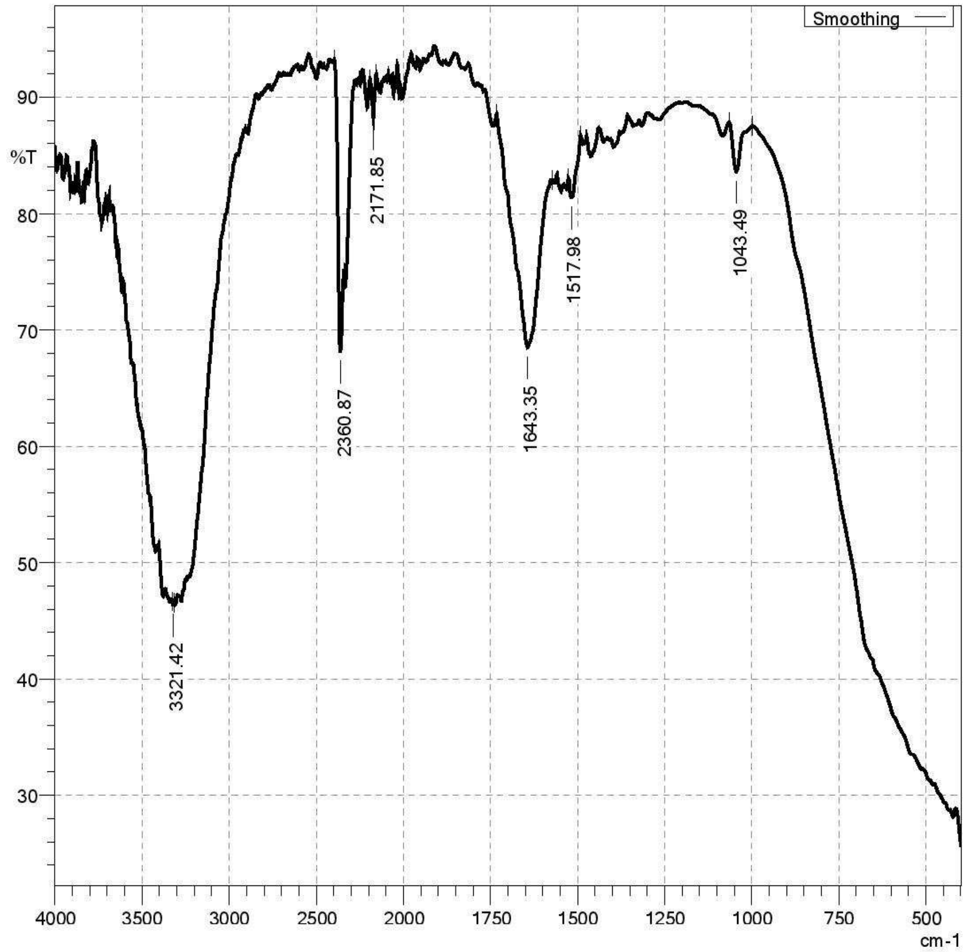
Frequency of Leaf extract	Stretching	Frequency of Fruit pulp
_	Stretching vibration of (C-O)H	1043
1518	C-H-Ph	1518
1643	C=O	1643
2176	Alkanes	2172
2363	C ≡ N	2361
3319	O-H stretching	3321
3734-3844	N-H bond	3700-4000

Fig.4



IR spectra of P edulis leaf extract

Fig.5



IR spectra of P fruit pulp

The leaf extract and pulp extract showed a broad band at 3319 cm^{-1} and 3321 cm^{-1} respectively, matching the O-H stretching frequency; it can originate from intermolecular hydrogen bonds linking the compounds and alcohols, phenols and carboxylic acids. Peaks in the range 3734 – 4000 cm^{-1} may be assigned to the N-H bond.

The fruit extract showed bands at 1643 indicating the presence of the carbonyl group, that is a strong absorption band due to C=O stretching vibration observed in the region 1800-1550 cm^{-1} (Suganya et al). Peak at 1043 cm^{-1} shows the stretching vibration of the C-O group in the carboxylic and alcoholic groups, which is present only in the fruit pulp.

Summary and Conclusion

Passion fruit pulp is found to be a rich source of vitamin C and recommended for use as dietary source. Considering the advantages of passiflora edulis, the presence of phytoconstituents like phenols, alkaloids, flavonoids and its antioxidant and antimicrobial properties can be utilized for the treatment and prevention of many diseases. Passion fruit is considered as a good source of antioxidant.

Phytochemical analysis of P. edulis showed the presence of alkaloids, carbohydrates, flavanoids and phenolic compounds. Saponins were present in the leaf and fruit. Organic extract (ethanol and chloroform) of P. edulis were reported to possess saponons, tannins, terpenoids, flavanoids and steroids. Protein and amino acids were reported in the ethanolic fruit extract.

Compounds like flavanoids, alkaloid, steroid, terpenoid, protein and amino acids are known to have curative activity against several pathogens and therefore could suggest its traditional use for the treatment of various illnesses. The result obtained in the present study demonstrated that Passiflora edulis is a potential source of natural antioxidants.

Vitamin C content of fruits and leaves were found to be 62.495mg/100gm and 4.325mg/100gm respectively suggest that adequate intake of P. edulis fruit prevents scurvy and limit the risk of heart diseases and cancers. Determination of iron content in the leaves of P. edulis before and after flowering indicated that iron content is more in leaves before flowering than in leaves after flowering.

The results regarding the total phenolic content, passion fruit pulp can have a concentration of total phenolics 14.76 mg GAE/100 gm. This shows that the extract has great antioxidant property.

Passiflora edulis is found to be useful as antimicrobial agents. Antimicrobial activity was determined based on the inhibitory zones around the colonies. Methanol extract showed zone of inhibition at a maximum concentration (200µg/disc) against ecoli 22.2mm, S. flexineri 9.1mm, S. typhi 6.9mm, P. aeruginosa 6.3mm and C. albicans 13.45mm.

The aqueous extract showed zone of inhibition E.coli 9.5mm, S. flexineri 7.9mm, S. typhi 7.2mm, P. aeruginosa 5.8mm and C. albicans 10.45mm.

Methanol extract of P. edulis showed more inhibitory activity against human pathogenic bacteria when compared to aqueous extract.

Characterization by FT-IR study revealed that the leaf extract and pulp extract showed a broad band at 3319 cm^{-1} and 3321 cm^{-1} respectively, matching the O-H stretching frequency; it can originate from intermolecular hydrogen bonds linking the compounds and alcohols, phenols and carboxylic acids. Peaks in the range 3734 – 4000 cm^{-1} may be assigned to the N-H bond.

The fruit extract showed bands at 1643 indicating the presence of the carbonyl group, that is a strong absorption band due to C=O stretching vibration observed in the region 1800-1550 cm^{-1} Peak at 1043 cm^{-1} shows the stretching vibration of the C-O group in the carboxylic and alcoholic groups, which is present only in the fruit pulp.

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