Quantitative analysis of phenols and flavonoids in different parts of *Passiflora edulis* Sims

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Abstract- The current study was carried out to assess the antioxidant property based on the amount of phenolic compounds in the leaf, stem, fruit pulp and fruit peel of Passiflora edulis. Free radicals can trigger various diseases, such as cancer, cardiovascular, arthritis, diabetes, and neurological disorders, anti-aging. Thus, antioxidants are needed to reduce these free radicals. Passion fruit (Passiflora edulis) is a healthy tropical fruit from the Passifloraceae family. The passion fruit is high in antioxidants, flavonoids, and antiaging compounds. Passiflora edulishas recently gained a lot of attention because it is a source of powerful antioxidants and may also have other health benefits. The leaf, stem, fruit pulp and fruit peel of the passion fruit (Passiflora edulis) has many phenolic compounds that indicate antioxidant capacity. This study aims to determine the total phenolic contents and total flavonoids contents in the leaf, stem, fruit pulp and fruit peel of the Passiflora edulis.Using leaf, stem, fruit pulp, and fruit peel phenolic compounds were determined. The fruit pulp had the highest concentration of total phenol and total flavonoids, while the least amount of flavonoids was determined from the stem of Passiflora edulis. Passion fruit is rich in vitamin C, which is an antioxidant that helps to protect the body from damage caused by free radicals. Vitamin C boosts the immune system by helping the body to absorb more iron from plant-based foods, and may improve the body's ability to fight off infections in the body.

Keywords: antioxidant, phenolic compounds, Passiflora edulis and spectrophotometer.

INTRODUCTION

Reactive oxygen group free radicals (ROS) and other free radicals present in the human body in excess can seriously harm cells and initiate a number of illnesses. Examples include rheumatoid arthritis, inflammation, asthma, bronchitis, kidney failure, cataract and retinal disease, arteriosclerosis, hypertension, ischemia, cardiomyopathy, heart failure, cancer, ageing, diabetes, inflammation, infection, Alzheimer's, Parkinson's, memory loss, depression, stroke, and fetal disorders.(Labola et al., 2018) Antioxidants and electron-giving substances that attach to highly reactive molecules and free radicals to limit oxidation processes can be used to decrease the activity of these free radicals and avoid cell damage. Eating foods high in antioxidants can help prevent the body from experiencing increased oxidative stress. (Isdamayani L.et al., 2015). With over 500 species, the genus Passiflora is the biggest in the Passifloraceae family. Of these, Passiflora edulis is particularly notable due to its significance in both medicine and the economy. (Dhawan et al., 2004). It is widely planted in tropical and subtropical regions in several parts of the world, especially in South America, Caribbean, south Florida, South Africa, and Asia (Zhang et al., 2013; Yuan et al., 2017; Hu et al., 2018).Another name for the plant Passiflora is Krishna kamal, or passion fruit crop. The plants are mostly cultivated for their delicious fruit juice and beautiful blossoms. A fruit's inside is juicy and tender, and it may include a few seeds. Because of the ideal growth circumstances, plants have been grown for aesthetic purposes in gardens in the Satara region. Opportunities for local farmers and growers will arise if the demand for passion fruits increases in the local market. Growing public awareness has led to a major growth in the value of natural plant products as a source for therapeutic development. Since plant-based medications are of interest to the pharmaceutical industry today, over half of all medications contain natural components. Given the extensive study that has been done on P. edulis development in recent years, there has been a growing interest in using passion fruit for human consumption because of its desirable eating qualities, juiciness, appealing nutritional contents, important health advantages, and popularity.(Cazarin et al.,

2016; Lima et al., 2016; Pereira et al., 2019). Passion fruit, commonly referred to as "the king of fruits," "maracujá," "love fruit," and "fruitlover," is commonly consumed raw or juiced. In the meanwhile, a variety of goods have been created using passion fruit, such as yoghurt, cake, ice cream, jam, jelly, complex beverages, tea, wine, vinegar, soup stock, condiment sauce, and more. In several nations, passion fruit is also utilised as a moisturizing component in cosmetics and as a traditional folk remedy. The purple passion fruit has been domesticated and cultivated in China (Xu et al., 2016).Literature revealed that extensive work has been performed on Passiflora edulis Sims. And it has been found to possess therapeutics properties. Flowers of Passiflora edulis contain alkaloids and flavonoids and are used in phytotherapy to treat pain, insomnia, anxiety, nervous tension (Wolfman., et al.1994).

Its leaves are used in the treatment of high blood pressure. Passiflora edulis and its related species are also used in traditional medicine to treat headache, convulsions, muscular spasms, and as aphrodisiac and antitussive agents (Dhawan.et al., 2002; Dhawan. et al., 2003). Passiflora edulis is widely used in traditional medicine in the treatment of many diseases.Passion fruit has a lot of active ingredients, including polysaccharides, alkaloids, pectin, and flavonoids (Widodo et al., 2021). Numerous phenolic components found in passion fruit suggest that it has antioxidant potential. The majority of plant secondary metabolites are phenolic chemicals. Since this compound's hydroxyl group is affixed to the benzene ring indefinitely, it is included in the class of aromatic alcohols (Pengelly A. 2006). Phenolic chemicals are antioxidants that aid in the treatment of diabetic mellitus (Candra et al.,2021) and prevent and treat degenerative illnesses, cancer, early ageing, and immune system problems in the body (Isdamayani et al., 2015).

Passion fruit contains high amount of vitamin A and vitamin C both of which are strong anti-oxidants. They neutralize free radicals and protects from cancer. In Nagaland fresh leaves of Passiflora edulis is boiled in little amount of water and extract is drunk for the treatment of dysentery and hypertension. The flowering and fruiting portion are dried and preserved and used as a drug in preparation of certain proprietary products. The root extracts are also used in the treatment of ulcers and haemorrhoids (Anonymous, 2001). The root has been used as a sedative and vermifuge in West Indies, Mexico and Netherlands. In Italy the plant has been used as anti-spasmodic and sedative. In Mauritius the tincture and extract of plant has been used as a remedy for insomnia due to various nervous conditions. The root has been used as a diuretic and a decoction of leaves as an emetic. (Patel et al., 2011).

Passion fruit contains a high level of nutrients, eating it as part of a balanced diet can help keep the immune system strong and lower the risk of illness and infection. It has a lot of vitamin C, which is a strong antioxidant that strengthens the immune system. Almost 10% of the Daily Value (DV) of vitamin C is found in one tiny fruit.In order to assist the body battle oxidative stress and inflammation, vitamin C and other antioxidants work against free radicals in the body. It also contributes to tissue development, wound healing, and the synthesis of white blood cells. Additionally, beta-carotene which the body transforms into vitamin A is found in passion fruit. The body's defences against dangerous microorganisms, the skin and mucous membranes, depend on vitamin A to be healthy. The present study can be used as one of the parameters for standardization of medicinal plants. The present study gives an idea about total phenol and total flavonoids content in different parts of Passiflora edulis like leaf, stem, fruit pulp and fruit peel.

MATERIAL AND METHODS

Plant material

The extensive survey, identification and collection of plant from Amravati region was carried out. Plant identification was carried out with the help of floras (Cook, 1957; Dhore, 1986; 1998; Naik, 1998).

Preparation of plant material

Fresh leaf, stem and fruit were collected, dried under shade, finely powdered and stored in airtight container. All plant parts were powdered separately. 1gm each of each plant part was taken for estimation of phenolic compounds gm/ µgm.

METHODS

Estimation of Total phenol, Ortho-dihydric phenols and Total flavonoids were done according to the

methods prescribed by Thimmaiah (1999), which are given below.

Estimation of total Phenols

1gm of sample was grind with the help of mortar and pestle with 10 ml of 80% ethanol. And centrifuged at 10,000 rpm (20minutes). Supernatant was collected and evaporated to dryness after dryness residue was taken into a test tube and makeup the volume with 5ml distilled water. 1ml aliquot was Pipette out in test tube, and make up the volume up to 3ml with distilled water. 0.5mlof Folin- Ciocalteu reagent was added. After 3 minutes, into each tube 2 ml of 20% Na2CO3 solution was added. Mixed thoroughly and tubes was kept in boiling water for 1minute, then allowed to cool and absorbance was measured at 650nm against reagent blank. Reagent blank was prepared similarly without the extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and1ml) of catechol. (ThimmaiahS.R.1999).

Estimation of Ortho-dihydric phenols

1gm sample was grind with the help of mortar pestle with 10ml of 80% ethanol. And centrifuged it at 10,000 rpm (20minutes) and supernatant was collected and evaporated to dryness. After drying residue was taken and volume made up to 5ml with distilled water. 1 ml of aliquot was pipette out in a test tube, in this test tube 1ml of 0.05 N HCL, 1 ml of Arnow's reagent, and 10 ml of distilled water and 2ml of 1N NaOH was added. Absorbance was measured at 515 nm against a reagent blank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1ml) of catechol. (Thimmaiah S.R.1999)

Estimation of Bound Phenols

1gm sample was grind with the help of mortar and pestle with 5ml of SDS solution and centrifuge for 5 min and supernatant was discarded. The residue was wash with once 5ml SDS solution, twice with 5ml of water, twice with 5ml of ethanol and twice with 5ml of diethyl ether (after each washing centrifuge and the supernatant was discarded). Allow the residue to dry and was suspended in 3ml of 0.5 M NaOH. It was kept overnight at room temperature. In the next morning it was Centrifuge and the supernatant was diluted 1:10 with 0.5 M NaOH (0.5 ml supernatant and 5 ml of 0.5 M NaOH). Absorbance was measured at 290 nm against a reagent blank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol. (Thimmaiah S. R. 1999)

Estimation of total flavonoid

The Aluminium chloride method was used to determine the total flavonoid content. In brief, 1gm sample was grind with the help of mortar and pestle with 10 ml methanol. Centrifuged and supernatant was collected.0.5ml of supernatant was pipette out into test tube. 0.5 ml of each extract (1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water was added. The reaction was left for completion for 30min, and absorbance was measured at 415 nm against a methanolic blank (80% methanol). Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol (Saranya et al, 2017).

RESULT AND DISCUSSION

Sr. No	Phenolic compounds	Wt. of sample	Total volume of extract	Volume taken for analysis	µgm/gm			
					Leaf	Stem	Fruit pulp	Fruit peel
1	Total phenol	1 g	10 ml	1 ml	330	220	720	420
2	Ortho-dihydric phenols	1 g	5 ml	1 ml	305	215	775	525
3	Bound Phenols	100 mg	5 ml	1 ml	145	95	825	490
4	Total flavonoid	1 g	10 ml	0.5 ml	1050	700	2450	1650

The results showed that the maximum amount of Total Phenol (720µgm/gm), Bound Phenols (825µgm/gm), Ortho-dihydric phenols (775µgm/gm) and Total Flavonoid (2450µgm/gm) was observed in fruit pulp of Passiflora edulis while the least amount of Total Phenol (220µgm/gm), Bound Phenols (95µgm/gm), Ortho-dihydric phenols (215µgm/gm) and Total Flavonoid (700µgm/gm) was observed in stem. Phenolic compounds and flavonoids have been reported to be

associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. The nitric oxide scavenging activity of flavonoids and phenolic compounds are known (KimH.et al., 2002). Phenols are present in food; they may have an impact on health and most are known to have an antioxidant activity. (Demitrios2006).

The passion fruit pulp is a famous food source of flavonoids, which contains 158.0 µg/ml of total flavonoids, 16.2 µg/ml of isoorientin (Zeraik and Yariwake, 2010) and 0.42 µg/g of quercetin (Rotta et al., 2019). The aerial parts of *P. edulis* extracted by reflux with 40% ethanol contain 0.90% of apigenin. So far, 33 flavonoids have been identified in various parts of *P. edulis* (Lutomski et al., 1975; Mareck et al., 1991; Moraes et al., 1997; Chang and Su, 1998; Xu et al., 2013). Among them, the major flavonoids identified from *P. edulis* are vitexin, isovitexin, isoorientin, apigenin, quercetine, luteolin, and their derivatives, which represent important classes of effective compounds in *P. edulis* regarding their various biological and pharmacological properties (Deng et al., 2010; Xu et al., 2013; Zhang et al., 2013).

Flavonoids are a widely distributed group of phenolics, occurring virtually in all plant parts. They are a major coloring substance in flowers and fruits. They also play a vital role as a secondary antioxidant defense system against different biotic and abiotic stresses. Flavonoids are located within the centers of ROS generation and in the nucleus of mesophyll cell. Flavonoid components have been reported in the leaves, fruit peel, and pulp of passion fruit. Passion fruit has been used as a sedative, diuretic, anthelmintic, anti-diarrheal, stimulant and also treatment for hypertension, menopausal symptoms and colic of infants in South America. The fruit of Passiflora edulis is regarded as a digestive stimulant and used a remedy for gastric carcinoma. Fruits are eaten to get relief from constipation (Patel et al., 2011). The flavonoids further enhance the potency of passion fruit in providing antioxidant to body and protecting from cancer. The studies have also shown that in cancer patients, passion fruit can kill the cancerous cells in vitro. Antioxidants in passion fruit primarily eliminate free radicals, which are known for mutating the DNA of healthy cells into cancerous ones. Passion fruit juice showed anticancer activity on cell cycle, apoptosis and cell viability of the MOLT4 lymphoma cell line. The effect on the cell cycle was due to the presence of organic acids, amino acids and proteins in passion fruit juice (Cindy Marie De Neira, 2003).

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, flavonoids, carbohydrates steroids. (Edoga et al., 2005; Mann1978).

CONCLUSION

The present investigation revealed that the fruit pulp had the highest concentration of total phenol, bound phenol, ortho-dihydric phenols and total flavonoid. Least amount of secondary metabolites was found in stem. The significant amount of secondary metabolites holds up its traditional use in the treatment of various diseases. Phenols and polyphenolic compounds such as flavonoids are widely found in this plant and have been shown to posse's significant antioxidant activities. Even through Passiflora edulis is a perennial vine, it has not been thoroughly studied. As a result, the current study's findings may help the pharmaceutical industry to understand how this plant might be beneficial for human welfare. The use of natural antioxidants has been encouraged because of worries about the safety of synthetic drugs.

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