WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 793-801. Research Article ISSN 2277–7105

PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT ACTIVITIES OF *PASSIFLORA EDULIS* (PASSION FRUIT) LEAVES FROM UKHRUL DISTRICT, MANIPUR, INDIA.

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

[1]Research Scholar, Department of Home Science, Women's Christian College, Chennai - 600006 India.

[2]Associate Professor, Department of Home Science, Women's Christian College, Chennai-600006 India.

Article Received on
11 Sep. 2017,
Revised on 01 October 2017,
Accepted on 22 October 2017
DOI: 10.20959/wjpr201714-9978

***Corresponding Author**
Phamiwon ZAS
Research Scholar,
Department of Home Science, Women's Christian College, Chennai -600006 India.

ABSTRACT

Plants have been widely used throughout the world for their beneficial medicinal benefits. *Passiflora edulis* (passion fruit) belongs to the genus Passiflora, is widely distributed in sub-tropical and tropical regions around the world. The present study was evaluated to assess the antioxidant activities of *Passiflora edulis* leaves using three solvents – hexane, methanol and Aqueous. The antioxidant potential of *Passiflora edulis* leaves was determined by DPPH, ABTS and FRAP assay. Qualitative analysis of *Passiflora edulis* leaves confirms the presence of phytochemicals like steroids, alkaloids, flavonoids, tannins, polyphenols and terpenoids. The extracts of *Passiflora edulis* showed potent antioxidant activity in which methanol extract showed better radical scavenging activity with IC_{50} values of 26.23µg/mL DPPH assay and 20.27µg/mL for ABTS assay. Methanol extract of *Passiflora edulis leaves* has the highest FRAP value of 711.01µm/g indicating strongest antioxidant activity. *Passiflora edulis* can be considered as a potential source of natural antioxidant.

KEYWORDS: *Passiflora edulis*, phytochemicals, antioxidant, methanol.

INTRODUCTION

Developed and developing nations of the world used medicinal plants as source of drugs or herbal extracts for various chemotherapeutic purposes.[1] *Passiflora edulis* (passion fruit)

belongs to the genus Passiflora, comprising about 500 species distributed in sub-tropical and tropical regions around the world. It is grown mostly as vine with a shallow root system, a vigorous climber. The leaves are evergreen and alternate 3 lobed leaves when matured. The vine grows to a length of15-20 feet and the life cycle seems to be short in 5-7 years.[2]

Alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds are known in genus. Tannins are present in leaves, saponins were present in leaves and stem.[3] The pulp of the fruit is stimulant and tonic, fruit has anti carcinogenic effect.[4, 5] Identified constituent in plant includes glycosides[6] passiflorine[7] and Harman alkaloids.[8] Recent studies have demonstrated that polyphenols possess antioxidant properties, its role in the prevention of pathophysiological processes associated with oxidative stress, such as cancer, neurodegenerative and cardiovascular diseases. Polyphenols may act as antioxidants by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions in vitro.[9,10]

In Nagaland fresh leaves of *Passilfora edulis* is boiled in little amount of water and extract is drunk for the treatment of dysentery and hypertension.[11] The pigments present in the purple fruit juice are mostly carotenoids, among which β-carotene predominates.[12] The leaves of *Passiflora edulis* has been used traditionally by American countries to treat anxiety and nervousness, rich in polyphenols correlating its antioxidant activity.[13] The present study was undertaken to investigate presence of phyto-constituents and antioxidant activity of the extracts of *Passiflora edulis* leaves. The study would be helpful in development of *P. edulis* as a phyto pharmaceuticals. Antioxidant compounds, such as flavonoids can help in protection against various diseases in which the oxidative species are involved.

MATERIALS AND METHODS

Collection of Plant material

The leaf of *Passiflora edulis* was collected from Ukhrul district, Manipur. The leaves were shade dried for 15 days, when dried properly the leaves were powdered using a mixer. The powdered plant was reduced to size with sieve. The reduced size fine powdered was packed in airtight container to avoid the effect of humidity and stored at room temperature for further use.

Extraction of the powdered *Passiflora edulis* leaf plant: The samples were extracted using hexane, methanol and aqueous. The powdered leaves (100 g) was weighed and soaked in 300

ml of hexane, methanol and distilled water in a conical flask separately. The conical flask containing the powdered leaves and solvent was shaken vigorously, corked and left to stand for 72 hrs. at room temperature. After 72 hrs the leaf extract was filtered using Whatmann no. 1 filter paper and filtrate was evaporated to dryness in petri dish. (Trease and Evans, 1997). The extracts was used for phytochemical screening and analysis of antioxidant potential.

Preliminary phytochemical screening

The extracts of *Passiflora edulis* were subjected to preliminary phytochemical analysis in order to detect the presence of phyto-constituents. The screening of phytochemical analysis was performed as described by Evans, 2002; Yusuf *et al*., 2014.[14,15]

DPPH radical scavenging assay

The method described by Oyedemi *et al* (2011)[16] was used to determine DPPH scavenging activity of the plant extract. The solution of 0.135mM DPPH was prepared in methanol. Different concentration of extract (0.1ml) was mixed with 1.9ml of DPPH solution. The reaction mixture was vortexed thoroughly and left in the dark are room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference drug. The ability of plant extract to scavenge DPPH radical was calculated from the following formula.

$$\% \text{ DPPH inhibition} = [(Abs_{control} - Abs_{test})/ (Abs_{control})] \times 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + solvents, Abs_{test} is the absorbance of DPPH radical + sample extract / standard.

ABTS radical scavenging assay

A stock solution of ABTS radical cation was prepared by dissolving ABTS (7 mM, 25 mL in deionized water) with potassium persulfate (K2S2O8) (140 mM, 440 μL). The mixture was left to stand in the dark at room temperature for 15-16 h (the time required for formation of the radical) before use. For the evaluation of ABTS radical scavenging activity, the working solution was prepared by the previous solution and diluting it in ethanol to obtain the absorbency of 0.700 ± 0.02 at 734 nm. The solvent extracts and purified compounds (0.1 mL) at different concentrations were mixed with the ABTS working solution (1.9 mL) and the reaction mixture was allowed to stand at Room temperature for 20 min, then the absorbance was measured by using a UV-visible spectrophotometer at 734 nm. The radical scavenging activity is given as ABTS radical scavenging effect that is calculated by equation.

ABTS radical scavenging effect (%) = [(A0-A1)/A0] × 100

Where AO is the absorbance of ABTS radical + solvents, A1 is the absorbance of ABTS radical + sample extract / standard.

Ferric Ion Reducing Antioxidant Power (FRAP Assay)

FRAP assay was performed according to the methods of Rabeta et al., 2013[17] with slight modification. An amount of 200µl extracted samples were mixed with 3 mL FRAP reagent in test tubes and undergoes vortex. Blank samples were prepared for both methanol and deionized water extracted samples. Both samples and blank were incubated in water bath for 30 minutes at 37^{o}C and the absorbance of the samples was determined against blank at 593 nm. Series of stock solution at 200, 400, 800, 1200 and 1600µ M were prepared using aqueous solution of $FeSO_4$.7H2O as standard curve. The values obtained were expressed as µM of ferrous equivalent Fe (II) per gram of freeze dried sample.

RESULTS

Preliminary phytochemical screening of *Passiflora edulis* shows the presence of steroids, alkaloids, flavonoids, Tannins, polyphenols and terpenoids.

DPPH and ABTS free radical scavenging activities

The results of DPPH and ABTS scavenging activities in three different extracts of *Passiflora edulis* leaves is shown in table 1. The scavenging activities of the *Passiflora edulis* leaves extracts were compared to IC_{50} of ascorbic acid as standard. The lower the IC_{50} value the higher the antioxidant activity. The Hexane and methanol extract showed very strong antioxidant activity. The aqueous extract of *Passiflora edulis*also showed strong antioxidant capacity with IC_{50} values of 51.13µg/mL in 50% of DPPH radical and 93.25µg/mL in 50% of ABTS radical. The IC_{50} values of ascorbic acid was 8.25µg/mL in 50% of DPPH radical and 11.05µg/mL in 50% of ABTS radical. The present study indicates that the methanol extract of *Passiflora edulis* leaves showed better radical scavenging activity with IC_{50} values of 26.23µg/mL in 50% of DPPH radical and 20.27µg/mL in 50% of ABTS radical. Fig.1 and fig.2 shows the percentage of DPPH and ABTS inhibition scavenging proportional to the concentration of each extract.

Table 1: Antioxidant activity of *Passiflora edulis* (IC_{50} value).

Extracts	Antioxidant activities	
	$IC_{50}$of DPPH (µg/mL)	IC_{50} of ABTS (µg/mL)
Hexane	48.34	41.43
Methanol	26.23	20.27
Aqueous	51.13	93.25
Ascorbic acid	8.25	11.05

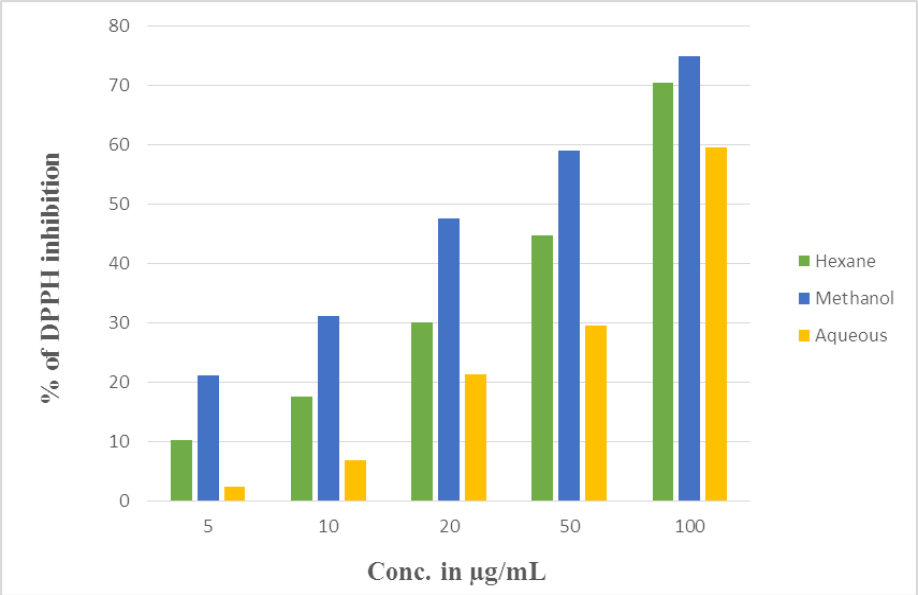

Fig 1: Percentage of DPPH inhibition.

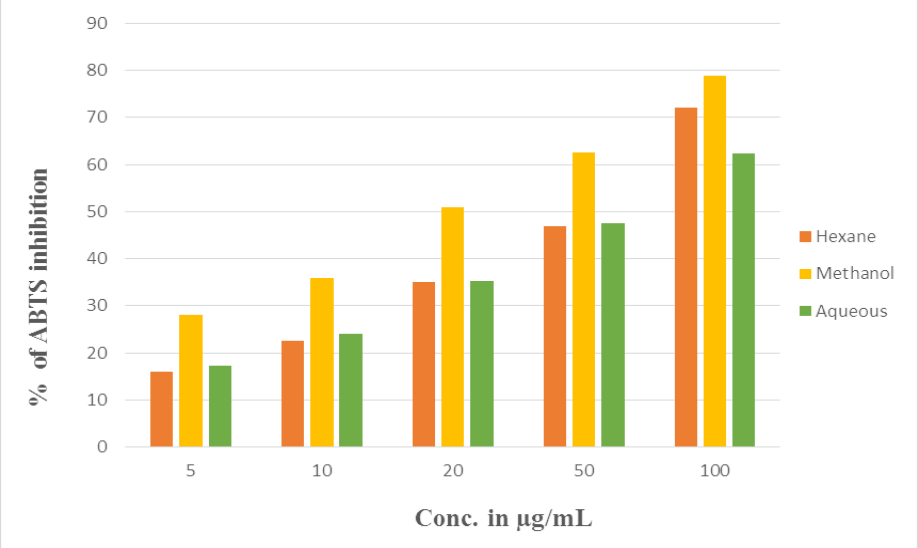

Fig 2: Percentage of ABTS inhibition.

Ferric reducing antioxidant power (FRAP) assay

The result indicates that the FRAP values were higher in methanol extracted samples compared to hexane and aqueous extraction as shown in table 2. Methanol extract of

Passiflora edulis leaves has the highest FRAP value of 711.01µm/g. The FRAP values in hexane extract of *Passiflora edulis* was 195.28µm/g and in aqueous extract was 628.14µm/g. It can be concluded that methanol extraction of *Passiflora edulis* leaves has higher antioxidants potential.

Table 2: Antioxidant activity of *Passiflora edulis* (FRAP).

Extracts	FRAP value (µm/g)
Hexane	195.28
Methanol	711.01
Aqueous	628.14

DISCUSSIONS

DPPH and ABTS are stable free radicals and their colors show characteristics absorption at wavelength 516 nm and 734 nm respectively. The color would change when the free radicals were scavenged by antioxidant.[18] IC_{50} value indicates the concentration of the sample that can inhibit 50% of DPPH, 50% of ABTS free radicals. The lowest IC_{50} means the highest antioxidant capacity. The IC_{50} were categorized as, with IC_{50} < 50µg/mL is very strong antioxidant, IC_{50} of 50-100µg/ml is strong antioxidant, IC_{50} of 101-150µg/mL as medium antioxidant while IC_{50}> 150µg/mL is weak antioxidant.[19]

The ethanolic extract of *Passiflora edulis* showed antioxidant activity ($EC_{50:}$ 0.096mg/mL) at the concentration of 0.1mg/mL.[20] The petroleum ether and chloroform extracts of *Passiflora edulis* of leaf showed antioxidant activity with IC_{50} of 58.88µg/mL and 56.85µg/mL, showing strong antioxidant capacity.[1] A concentration of 1100µg/mL of *Passiflora edulis* aqueous extract was able to scavenge 50% of DPPH radical.[21] Leaves of passion fruit have higher antioxidant potential than the pulp. The methanolic extract of *Passiflora edulis* were able to scavenge 50% of ABTS radical at 19.2µmol TE g^{-1}.[22,1]

Hydroalcoholic extracts of *Passiflora edulis* obtained by maceration in a lower drug / solvent ratio of 1:8 were able to significantly (IC_{50} value 84.23µg/mL) reduce in-vitro DPPH[23] implying strong antioxidant activity. Studies have also reported the antioxidant potential of ethanolic extracts of *Passiflora edulis* with IC_{50} of 875µg/mL for DPPH assay.[24]

The aqueous extract of *Passiflora edulis* leaves showed FRAP values of205.7 ± 4.12µmol TE g^{-1} exhibiting high antioxidant potential (Juliana et al., 2013)]. The antioxidant potential of *P.edulis* extract showed highest ferric reducing ability (1.89µmol TE g^{-1}) by maceration

method.[23] Higher FRAP values gives higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the reducing agents. Antioxidants are compounds capable of donating electron or hydrogen ion for reduction.[25]

CONCLUSION

The present study indicates the leaf extracts of *Passiflora edulis* has profound antioxidant activity. The different leaf extracts of *Passiflora edulis* showed strong antioxidant activity of which methanol extract showed a better radical scavenging activity in all the assays. The presence of phytochemical compounds like tannins, flavonoids and tannins may be responsible for its antioxidant activity. Isolation and identification of bioactive compounds will be helpful in understanding its use in traditional medicines. Further examination of *Passiflora edulis* leaves extract in prevention and treatment of diseases where oxidative stress damage to proteins seem to play a role and it can be considered as source of natural antioxidant.

REFERENCE

1. Farhana AR, Mahmuda H, Laizuman N, Monirul I. Antibacterial, Cytotoxic and Antioxidant Activity of Passiflora Edulis Sims. European Journal of Scientific Research, 2009; 31(4): 592-598.
2. Akali S, Maiti CS. Status & Prospects of Passion Fruit Industry in Northeast India, 2006. Available:from:http://www.researchgate.net/publication/266285579_Status__Prospects_of_Passion_Fruit_Industry_in_Northeast_India.
3. Sita Sharan Patel. Morphology and pharmacology of Passiflora edulis: a review. Journal of Herbal Medicine and Toxicology, 2009; 3(1): 1-6.
4. Neira D, Cindy M. The Effects Of Yellow Passion Fruit, Passiflora Edulis Flavicarpa, Phytochemicals On Cell Cycle Arrest and Apoptosis of Leukemia Lymphoma Molt-4 Cell Line", Working Paper, 2003. University of Florida.
5. Capasso A, Sorrentino L. Pharmacological studies on the sedative and hypnotic effect of Kava kava and Passiflora extracts combination. Phytomedicine, 2005; 12: 39-45.
6. Seigler DS, Pauli GF, Nahrstedt A, Leen R. Phytochemistry, 2002; 60: 873–882.
7. Bombardelli E, Bonati A, Gabetta B, Martinelli E, Mustich G. Phytochemistry, 1975; 14: 2661–2665.
8. Dhawan K, Dhawan S, Sharma A. Passiflora: a review update Journal of Ethno-pharmacology, 2004; 94: 1–23.

9. Rice-Evans C A, Miller NJ, Paganga G. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 1996; 20: 933–956.
10. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacology and Therapeutics, 2002; 96: 67–202.
11. Jamir TT, Sharma HK, Dolui AK. Folklore medicinal plants of Nagaland, India, Fitoterapia, 1999; 70: 395-401.
12. Razia M, Beulah, Siva Ramakrishnan S. Phytochemical, gc-ms, ft-ir analysis and antibacterial activity of passiflora edulis of kodaikanal region of Tamil Nadu. World Journal of Pharmacy and Pharmaceutical sciences, 2014; 3(9): 435-441.
13. Sita SP, Himesh S, Kaushelendra M, Akhlesh KS. Recent updates on the genus Passiflora: A review. Int. J. Res. Phytochem. Pharmacol, 2011; 1(1): 1-16.
14. Evans WC. Trease and Evans Pharmacognosy, Edn 15, London, 2002; 137-139, 230-240.
15. Yusuf AZ, Zakir A, Shemau Z, Abdullahi M, Halima SA. Phytochemical analysis of the methanol leaves extract of Paullinia pinnata linn. Journal of pharmacognosy and phytotherapy, 2014; 6(2): 10-16.
16. Oyedemi SO, Afolayan AJ. In-vitro and In-vivo antioxidant activitiy of aqueous leaves extract of leonotis leonurus (L) R.Br. International Journal of pharamacology, 2011; 7(2): 248-256.
17. Rabeta and Nur Faraniza. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of Garcinia atrovirdis and Cynometra cauliflora. International Food Research Journal, 2014; 20(4): 1691-1696.
18. Ling LT, Palanisamy UD. Review: Potential antioxidants from tropical plants. In: Valdez B, editor. Food Industrial Processes-Methods. Kuala Lumpur: In Tech, 1999; 64-72.
19. Blois MS. Antioxidant determination by the use of stable free radicals. Nature, 1958; 181: 1199-2000.
20. Ohanny AO, María EM, Nelsy LC, Sandra SA, Patricia L. Antioxidant and anti-proliferative activity of ethanolic and aqueousextracts from leaves and fruits juicePassiflora edulis. perspectivas en nutrición humana. Escuela de Nutrición y Dietética, Universidad de Antioquia. Medellín, Colombia, 2013; 15: 13-25.
21. Juliana KS, Cinthia BB, Talita C, Ângela GB, et al. Antioxidant activity of aqueous extract of passion fruit (Passiflora edulis) leaves: In vitro and in vivo study. Food Research International, 2013; 53(2): 882-890.

22. Pabón et al., L.M.C. Pabón, S. Turbay, B. Rojano, L.M. Álvarez, S. Luz Restrepo, J.M. Álvarez, et al. Algunas especies de Passiflora y su capacidad antioxidante. Revista Cubana de Plantas Medicinales, 2011; 16: 354-363.
23. Ludmilla S, José Wilson, Richard PD, Marisa Cristina, Sonia MF, Maria M, et al. Extraction Parameters Affect Flavonoids Content and Antioxidant Activities in Passiflora edulis. Journal of Chemical and Pharmaceutical Research, 2016; 8(10): 99-107.
24. Sunitha M, Devaki K. Antioxidant Activity of Passiflora edulis Sims Leaves Indian J. Pharm. Sci, 2009; 71(3): 310-311.
25. Schafer F Q, Buettner GR. Redox environment of the cell as viewed through the redox state of the gluthathione disulfide/ gluthathione couple. Free Radical Biology Medicinal, 2001; 30(11): 1191-1212.