World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

ANTIOXIDANT ACTIVITY OF THE SEEDS OF PRUNUS DULCIS USING DPPH ASSAY

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Article Received on 25/11/2024 **Article Revised on 16/12/2024** **Article Accepted on 06/01/2025**

ABSTRACT
Background: Free radicals are produced in normal or pathological cell metabolism. Oxidation is essential to living organism for the production of energy to fuel for biological processes. Uncontrolled production of oxygen from free radicals leads to many diseases like cancer, atherosclerosis, cirrhosis and also aging. An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate. The aim of this study is to evaluate the antioxidant activity of ethyl acetate extract of the seeds of prunus dulcis (rosaceae). **Method:** The ethyl acetate extract of prunus dulcis (rosaceae) were tested against antioxidant activity at various concentration using DPPH assay. **Results:** The results showed that the ethyl acetate prunus dulcis (rosaceae) shows significant antioxidant activity. EC_{50} values for chloroform extract and ethyl acetate extracts are 40.11 mg/ml and 24.10 mg/ml respectively. **Conclusion:** The present work revealed that prunus dulcis (rosaceae) contains some important chemical constituents which can be extracted using ethyl acetate as solvent, that can be used in future for the treatment of cancer, cirrhosis, atherosclerosis and even for aging.

KEYWORDS: Antioxidant activity, prunus dulcis, DPPH assay, Atherosclerosis, Aging, Cirrhosis.

1. INTRODUCTION

An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate.[1] Antioxidants have been traditionally divided into two classes, primary or chain breaking antioxidants and secondary or preventative antioxidants.[2] A number of chemical and physical phenomena can initiate oxidation which proceeds continuously in the presence of a suitable substrate(s) until a blocking defense mechanism occurs. Target substances include oxygen, polyunsaturated fatty acids, phospholipids, cholesterol and DNA.[3] The plant derived compounds have always been an important source of medicine for various diseases.

Prunus dulcis is one of the highly useful plant in indigenous system of medicine, belongs to Rosaceae family. It is known as badam in Hindi and in Malayalam. It was first introduce in "Chark samhita of agnivesha", the epic treatise of the medicine school of thought of the Hindu system of medicine and narrated as an ingredient of a cigar to alleviate cough. Prunus dulcis is a tree can live up to 20–30 years, but in commercial orchards, the economic life is around 12–15 years, due to reduced productivity or cultivar replacement. Fruit production starts from the second to the third year occurring wildly in sub tropical Himalayas and almost all parts of India. Drug is collected from two year old plant. The active compounds that have been reported are flavones, glycosides, steroids, saponins, triterpenoids and other secondary metabolites.[4] The seeds of this plant are sweet, cooling, diuretic, aphrodisiac, viriligenic and tonic which can be used against hemorrhoids, leucorrhoea, pruritis, skin diseases, asthma, bronchitis and jaundice. It is also used as antioxidant, spermatogenic, hepatoprotective, immunostimulant, anticancer, antibacterial, antiosteoporotic and hypoglycaemic.[5]

Based on the literature survey, it is evident that no work has been carried out on the evaluation of antioxidant activity of ethyl acetate extract of the seeds of this plant. Hence in this present study, the antioxidant activity was assessed by DPPH assay.

2. MATERIALS AND METHODS

2.1. Plant material

The plant was collected and authenticated from

Department of Botany, Calicut university, thenhipalam, Malappuram. All the reagents and chemicals were purchased from nice chemicals.

2.2. Preparation of extract

The fresh seeds of *Prunus dulcis* was dried under shade and then powdered to get a coarse powder. A sample extract was prepared with powdered plant (1 KG): chloroform, ethyl acetate and water by continuous hot extraction method for 72 h by using soxhlet apparatus.[6] The extracts were concentrated to a dry mass by vacuum distillation. After complete drying, extracted material was weighed and the extractive value in percentage was calculated with reference to the air dried sample.[7]

2.3 Antioxidant activity

The DPPH assay measures hydrogen atom (or) one electron donating activity and hence provides a measure of free radical scavenging antioxidant activity. DPPH is purple coloured stable free radical, it becomes reduced to the yellow coloured diphenyl picryl hydrazine. A chloroform and ethyl acetate DPPH-solution (0.15%) was mixed with serial dilutions (1 to 50 mg/ml) of extracts and shaken vigorously. The tubes were allowed to stand at 27^0C for 15 min. the change in absorbance of sample was measured at 517 nm, using UV spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage.

Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (5 mg/mL). Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank.

% scavenging of the DPPH free radical was measured using the following equation:

$$= \frac{\text{Absorbance of the control \_ absorbance of the test sample}}{\text{absorbance of the control}} \times 100$$

The inhibition curve was plotted for duplicate experiments and represented as % of Mean inhibition ± SEM. IC50 values were obtained from the graph.

2.4 Statistical analysis

This average was expressed as the mean ± the standard error of the mean (SEM) for six determinations. Experimental data were analyzed statistically by one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

Phenolic compounds are the major group that contributes to the antioxidant activity of vegetables, fruits, cereals and other plant based materials. This study reported that the ethyl acetate extract gave high antioxidant activity (table1) than chloroform extract (table2) and from the qualitative chemical identification (table3) of the phytoconstituents, the ethyl acetate extract were found to be prominent with phenolic components. Ethyl acetate extract shows significant antioxidant activity may be due to the presence of phenolic compounds. Many quercetin derivatives were found to be active in free radical scavenging property. Antioxidant activity depends on EC_{50} values. EC_{50} value was calculated by the concentration in mg/ml versus percentage inhibition. Lower the EC_{50}, higher the antioxidant activity.

Table 1: Antioxidant activity of ethyl acetate extract of *Prunus dulcis*, against DPPH.

No	Sample PD-2 In mg/ml	DPPH ASSAY	
		OD	Percentage Inhibition
1	Control 0.00	3.26	0.00
2	PD -2- 10	2.65	18.71N I
3	PD -2- 20	2.02	38.03
4	PD -2- 30	1.01	69.01
5	PD -2 -40	0.65	80.06

EC 50: 24.10 mg/ml

Table 2: Antioxidant activity of Chloroform extract of *Prunus dulcis*, against DPPH.

No	Sample PD-1 In mg/ml	DPPH ASSAY	
		OD	Percentage Inhibition
1	Control 0.00	3.26	0.00
2	PD-1- 10	3.01	7.66
3	PD -1- 20	2.91	10.73
4	PD -1- 30	2.45	24.84
5	PD -1 -40	1.59	51.22

EC 50: 40.11 mg/ml

Significant anti-oxidant activity shown by the sample-2

Table 3: Preliminary phytochemical studies on rhizomes of *Prunus dulcis.*

Sl.No.	Phytoconstituents	Chloroform	Ethyl Acetate	Distilled Water
1	Carbohydrates	-	+	+
	Molisch's test	-	+	+
	Benedict's test	-	+	+
	Felhing's test	-	+	+
	Barfoed's test	-	+	+
2	Glycosides			
	Cardiac glycosides	-	-	-
	Legal's test	-	+	-
	Baljet test	-	-	-

	3,5-dinitro bezoic acid test Keller killani test	- -	- +	- -
2(b)	Anthraquinone glycosides Brontrager's test Modified brontrager's test	 - -	 +	 - -
2©	Coumarin glycosides Ferric chloride test Fluorescence test	 + +	 + +	 + +
2(d)	Cyanogenic glycosides Sodium picrate test	 -	 -	 -
3	Amino acids & proteins Millon's test Ninhydrin test	 - -	 - -	 + +
4	Oils &fats Stain test Saponification test	 - -	 - -	 - -
5	Gums &mucilage	-	-	-
6	Alkaloids Mayer's test Dragendroff's test Wagner's test Hager's test Tannic acid test	 - - - - -	 - - - - -	 - - - - -
7	Flavanoids Shinoda test Zinc hydrochloride test Sodium hydroxide test Ammonia test	 - - - -	 + + + +	 - - - -
8	Tannic acid & phenolics Gelatin test Ferric chloride test Vanillin hydrochloride test	 - - -	 + + +	 - - -
9	Saponins Foam test Hemolysis test	 - -	 + +	 - -

4. CONCLUSION

From the above results, it may be concluded that the ethyl acetate extract of sees of *Prunus dulcis* is non-toxic and is safe. As the results indicated that the extract possess significant Antioxidant activity, after carrying out a thorough study of clinical trials, the plant can be considered as a low cost, potent, herbal medicine for free radical scavenging.

The findings indicated that ***Prunus dulcis*** might have a good potential as a source of antioxidants, with their potential use in different fields viz. food, cosmetics, and pharmaceuticals and also can be used in treating many diseases like cancer, cirrhosis, atherosclerosis etc.

5. ACKNOWLEGEMENT

I bowed to god, for his blessings. The authors are sincerely thankful to the Manager, Devaki amma memorial college of pharmacy, chelembra, for providing the facilities and good support for doing the work also thankful to Sabu, Department of botany, Calicut university.

6. REFERENCES

1 Gutteridge, J.M.C. Biological origin of free radicals and mechanisms of antioxidant protection. *Chemico Biological Interactions*, 1994; 91: 133-140.
2 Jadhav, S.J., Nimbalkar, S.S., Kulkarni, A.D. and Madhavi, D.L. Food Antioxidants: Technological, Toxicological and Health Perspectives, Madhavi, D.L., Deshpande, S.S. and Salunkhe, D.K. (Eds.), Marcel Dekker, New York, 1996; 5-64.
3 Ming-Hua, Y. and Schaich, K.M. Factors affecting DNA damage caused by lipid hydroperoxides and aldehydes. *Free Radical Biology and Medicine*, 2000; 20(2): 225-236.
4 A.R. Bafna and S H Mishra. Methanolic extract shows immunostimulatory effect on immunosuppressed mice. Journal of ethnopharmacology, 2006; 104: 1-4.
5 Anuj kumar and sanjaya kumar panda, phytochemical screening of root tubers of curculigo orchioides gaertn .j chem. Pharm., 2010; 107-111.
6 Harborne, J.B. Phytochemical Methods, 3rd ed. Chapman & Hall, London, 1983; 1-39.
7 Wagner, H. and Bladt, S. Plant Drug Analysis: A Thin Layer Chromatography Atlas, second ed.

Springer-Verlag Berlin Heidelberg, New York, 1996; 384.
8 A K Gosh et al.,phytochemical Investigation and Chromatographic Evaluation of the different extracts of tubers of Amorphaphallus Paeoniifolius; International Journal of Pharmaceutical and Biomedical Research, 2010; 150-157.
9 A Sai Ramesh et al; Comparative study on the ratio and bioactive compounds of Terminalia arjuna bark and core-wood, Journal of Pharmacy Research, 2010; 1420-1422.
10 J.B Harbone, Phytochemical methods, 49-52, 3rd edition 2007.
11 Devyani B Rathod and Suman Lahiri, Immunomodulatory and Antioxidant activity of Curculigo orchioides. International Journal of PharmTech Research, 2(2): 1197-1203.
12 Kirtikar and Basu, Indian medicinal plants, 4: 2469.
13 Trease, G.E. and Evans, W.C. Pharmacognosy, 13th ed. Baillière Tinda, London, 1989; 770-771.
14 Udayan.p.s and Indira balachandran, Medicinal plants of Arya vaidya sala herb garden page no 254 Ma C and Zhang J. The effects of curculigoside on immortalized, pre-osteoblastic mouse MC3T3-E1 cells was investigated. Phytother Res., Jun. 2011; 25(6): 922-6. doi: 10.1002/ptr.3449
15 Hari Babu B et al; Phytochemical and Antimicrobial screening of Givotia rottleriformis Griff; Journal of Pharmacy Research, 2011; 2146-2148.
16 Ram P. Rastogi and B.N. Mehrotra, Compendium of medicinal plants, 5: 1990-1994.
17 Dr.Mukul Tailang and Dr.Ashok sharma, Phytochemistry-theory and practical first edition, 67-69.
18 Patel Rajesh M and Patel Natvar J.*In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. *Journal of Advanced Pharmacy Education & Research*, 2011; 1: 52-68.
19 E.Edwin Jarald and Sheeja jarald, Textbook of pharmacognosy and phytochemistry, 52-53, first edition.
20 Fang Y et al., Free radicals, Antioxidants, and Nutrition; Nutrition, 2002; 872-879.