Short Communication

JYP

Antioxidant Ability of Some Common Indian Vegetables

Kumkum Agarwal[1*], Ranjana Varma[2]

[1]*Research Scholar, Department of Botany, Sarojini Naidu Govt. Girls P.G. College, Shivaji Nagar, Bhopal-462016, Madhya Pradesh, India.*

[2]*Professor, Department of Botany, Sarojini Naidu Govt. Girls P.G. College, Shivaji Nagar, Bhopal-462016, Madhya Pradesh, India*

ABSTRACT

Objective: The aim of this research work is to investigate antioxidant ability and analyse phytochemicals in some of the common Indian vegetables. **Methodology:** Different concentrations of the extracts were screened for estimating their DPPH scavenging ability by UV-spectrophotometry. Methanol was used as control while ascorbic acid was used as a standard antioxidant. IC50 and percentage inhibition were calculated. **Results:** *Chenopodium album* was found to show higher ability with IC_{50} at 82.93 ± 0.17 µg/ml while maximum % inhibition of 68.74 ± 0.17% was obtained at 100 µg/ml. The R^2 values were greater than 0.9 indicating that the relationship between extract concentration and % inhibition was extremely strong. Qualitative biochemical analysis of both the extracts showed the presence of carbohydrates, reducing sugars, alkaloids, flavonoids, triterpenoids, steroids, tannin and phenolic compound. **Conclusions:** The *in vitro* study showed that *Portulaca oleracea* and *Chenopodium album* proved to be good scavengers of free radicals like DPPH.

Key words: DPPH scavenging, IC_{50}, *In vitro,* Qualitative analysis, R^2 values, UV-spectrophotometry.

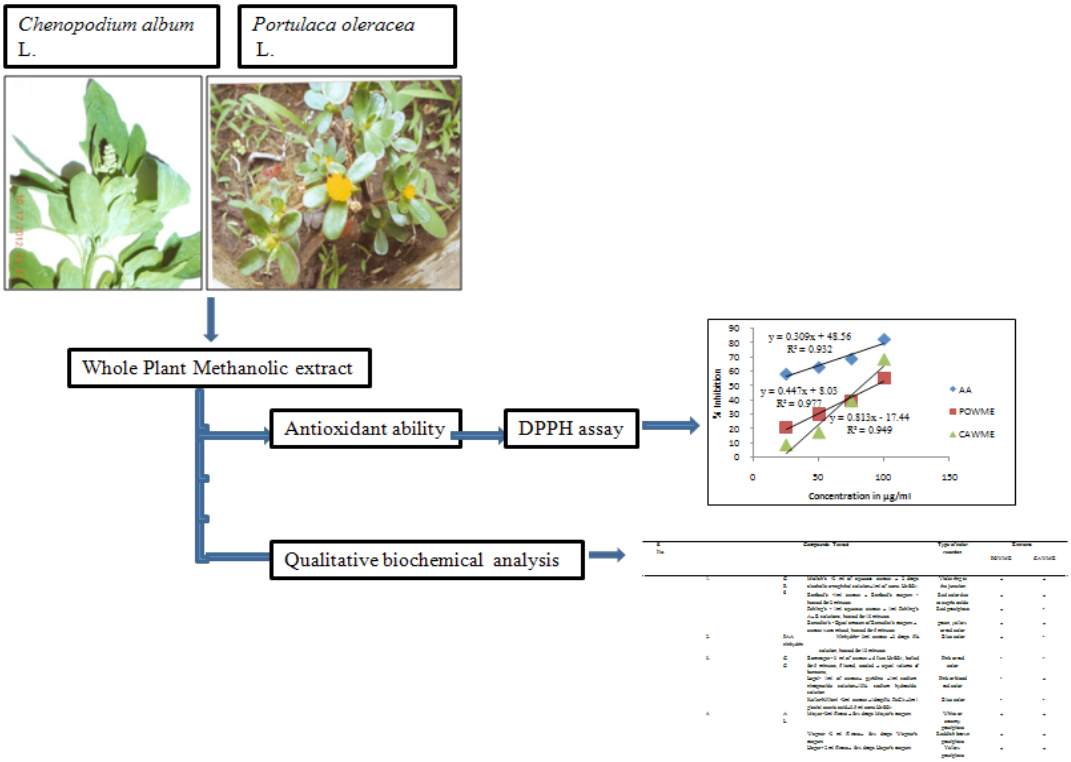
Key message: The two commonly eaten vegetables has shown potent antioxidant activity, thus it could be suggested for use in oxidative stress related diseases and can be eaten normally for prevention. Thus this article justifies the importance of vegetables and their use in traditional medicines for curing various diseases. Literature survey has shown that DPPH scavenging potential in methanolic extract of whole plant of *Chenopodium album* was not found to be reported in earlier studies.

INTRODUCTION

Oxidative stress is the imbalance between the production of reactive oxygen species and the antioxidant defense molecules present in the living system i.e. when the former exceeds the latter. Antioxidants are substances that either delay or inhibit the oxidation of molecules like lipids by inhibiting the initiation or propagation of oxidative chain reactions thus, preventing and repairing the damage caused by the reactive oxygen species to various cells.[1] The various enzymatic components include glutathione peroxidase, catalase (CAT) and superoxide dismutase (SOD), etc., while the non-enzymatic antioxidants can be phenolic compounds, nitrogenous compounds, carotenoids, vitamins including ascorbic acid, vitamin E and b-carotene etc.[2] A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517nm. The purple color fades when an antioxidant is present in the medium and results are reported as IC_{50}. Antioxidant activity in *Portulaca oleracea*[3-5] and *Chenopodium album*[5] has been reported by various workers. In this piece of research work the DPPH scavenging ability and the

Access this article online	
Journal Sponsor	Website: www.jyoungpharm.org
www.phcog.net	DOI: 10.5530/jyp.2015.3.18

Address for correspondence:
Miss. Kumkum Agarwal, Research Scholar, Department of Botany, Sarojini Naidu Govt. Girls P.G. College, Shivaji Nagar, Bhopal-462016, Madhya Pradesh, India. E-mail : atharva72013@gmail.com



Graphical Abstract

qualitative phytochemical analysis of both these vegetables was undertaken.

MATERIALS AND METHODS

All chemicals used were of high purity Merck grade. DPPH was purchased from Sigma Aldrich. The whole plant of *Portulaca oleracea* and *Chenopodium album* were procured from Vitthal market, Bhopal during the months of May and November, 2012 respectively. The plants were identified with the help of the herbarium of Botanical Survey of India (BSI), Allahabad, with voucher specimen numbers1114-23.01-52 and 1310-131.01-648 respectively. Fresh plant was shade dried and grinded then it was extracted with alcohol by Soxhlet apparatus for 72 hours and concentrated in vacuum to dryness at 30-40°C temperature, obtaining dried extract.

Experimental Work

DPPH radical scavenging assay

In this procedure UV-spectrophotometric (Systronics spectrophotometer 166) determination of antioxidant ability by DPPH assay[6] was undertaken. Methanol was used as the solvent for preparing different solutions (25-100 µg/ml) of ascorbic acid and plant extracts (25–200 µg/ml) and as control.

To 1.5 ml of each of the solutions of standard, test and control, 1.5 ml of 200 µM DPPH solution was mixed and incubated for 30 minutes. Absorbance of each was read at 517 nm.[7-9]

Percentage of antioxidant activity was calculated as:

$$I\% = \frac{Ac - (At - Ab)}{Ac} x\ 100$$

Where, I%=Percentage inhibition, Ac=Absorbance of control, At=Absorbance of sample with DPPH solution, Ab=Absorbance of sample without DPPH solution.

Qualitative Biochemical analysis

Qualitative biochemical analysis of the two extracts was undertaken by using standard procedures.[7-10]

Carbohydrates & Reducing Sugars (CRS)

Molish, barfoed's, fehling's and benedicts tests were applied.[9,10]

Proteins and amino acids (PAA)

Ninhydrin test was applied.[9,10]

Glycosides (GC)

Borntrager's, legal's and keller-killiani test were applied.[9,10]

Alkaloids (AL)

To the extract, add dilute HCl then shake and filter it. With this filtrate standard mayer's, wagner's and hager's tests were performed.[9,10]

Flavonoids (FN)

Alkaline reagent, shinoda and lead acetate tests were applied.[9,10]

Saponin (SN)

Foam test was applied.[9,10]

Triterpenoids and Steroids (TS)

Salkowski's and libermann-burchard's tests were applied.[9,10]

Tannin and Phenolic compounds (TPC)

Ferric chloride, lead acetate, dilute iodine solution tests were applied.[9,10]

Statistical analysis

In statistical analysis of results regression was calculated for knowing the relationship among variables (through excel) using regression equation: y=a + bx, Where y=estimated y value for given x value, a=intercept on the y axis, b=the slope (the average change in y for each change of 1 unit in x), R^2=Correlation coefficient.

RESULTS

In the present piece of research work DPPH assay was utilized to estimate the antioxidant ability of both the plant extracts. The results for ascorbic acid which was used as a standard reference compound were the same as mentioned in our previous research papers[7-9] i.e. it showed lowest IC_{50} value of 4.69 ± 0.02 µg/ml as well as it showed the highest inhibition of 82.05 ± 0.12% at 100 µg/ml concentration while lowest inhibition of 58.18 ± 0.17% was obtained at 25 µg/ml concentration. When comparing both the methanolic whole plant extracts, the extract of *Portulaca oleracea* showed IC_{50} value at 93.89 ± 0.08 µg/ml concentration. Maximum inhibition of 54.85 ± 0.08% was obtained at 100 µg/ml. The R^2 value obtained from the regression curve was 0.977. The extract of *Chenopodium album* showed IC_{50} value at 82.93 ± 0.17 µg/ml concentrations. Maximum inhibition of 68.74 ± 0.17% was obtained at 100 µg/ml. The R^2 value obtained from the regression curve was 0.949 (Figure 1). In both these extracts the R^2 values were greater than 0.9 indicating that the relationship between extract concentration and % inhibition was extremely strong. Qualitative biochemical analysis of both the methanolic plant extracts showed the presence of carbohydrates, reducing sugars, alkaloids, flavonoids, triterpenoids, steroids, tannin and phenolic compound while proteins and amino acids were only present in *Portulaca oleracea* and glycosides were only found to be present in *Chenopodium album* (Table 1).

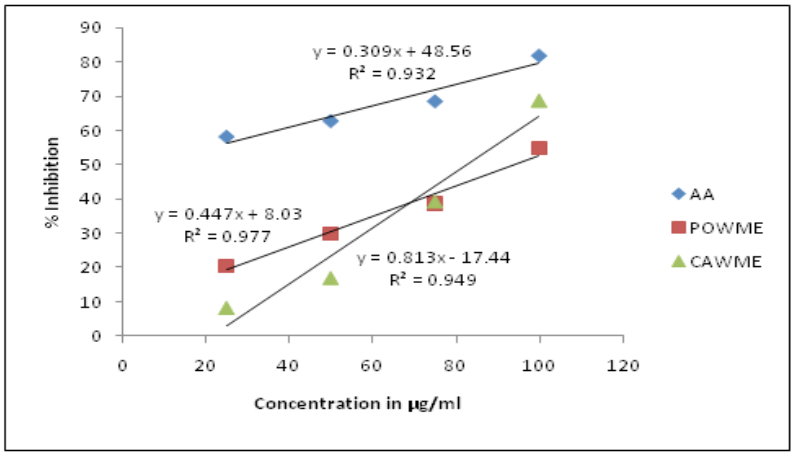


Figure 1: ***Regression curve of standard and plant extracts representing the DPPH inhibiting ability***

AA – Ascorbic acid, POWME: *Portulaca oleracea* whole plant methanolic extract; CAWME: *Chenopodium album* whole plant methanolic extract, DPPH- 1,1, diphenyl 2 picrylhydrazyl

Table 1: Qualitative analysis of biochemicals in plant extracts

Compounds Tested		Type of color reaction	Extracts	
			POWME	CAWME
CRS	Molish's - 2 ml of aqueous extract + 2 drops alcoholic α-naphthol solution+1 ml of conc. H_2SO_4	Violet ring at the junction	+	+
	Barfoed's - 1 ml extract + Barfoed's reagent - heated for 2 minutes	Red color due to cupric oxide	+	+
	Fehling's - 1 ml aqueous extract + 1 ml Fehling's A+ B solutions, heated for 10 minutes	Red precipitate	+	-
	Benedict's - Equal amount of Benedict's reagent + extract were mixed, heated for 5 minutes	green, yellow or red color	+	+
PAA	Ninhydrin- 3 ml extract +3 drops 5% ninhydrin solution, heated for 10 minutes	Blue color	+	-
GC	Borntrager - 3 ml of extract + dilute H_2SO_4, boiled for 5 minutes, filtered, cooled + equal volume of benzene,	Pink or red color	-	-
	Legal- 1 ml of extract+ pyridine +1 ml sodium nitropruside solution+10% sodium hydroxide solution	Pink or blood red color	-	+
	Keller-Killiani -2 ml extract+1 drop5% $FeCl_3$+3 ml glacial acetic acid+0.5 ml conc.H_2SO_4	Blue color	-	-
AL	Mayer -2 ml filtrate + few drops Mayer's reagent	White or creamy precipitate	+	+
	Wagner -2 ml filtrate+ few drops Wagner's reagent	Reddish brown precipitate	+	+
	Hager - 2 ml filtrate+ few drops Hager's reagent	Yellow precipitate	+	+
FN	Alkaline reagent test - extract + few drops NaOH	yellow color	+	+
	Shinoda- extract+5 ml ethanol+ few fragments of magnesium turning+conc. HCl	Pink color	+	+
	Lead acetate - extract+ few drops lead acetate solution	Yellow precipitate	+	+
SN	Foam - extract + distilled water, shaken	Foamy layer	-	-
TS	Salwonski - extract+ chloroform, filtered+ few drops conc.H_2SO_4, shaken	Lower layers turn red (sterols present) or golden yellow (triterpenes present)	+	+
	Liberman and Burchards - extract+ chloroform+ acetic anhydride, boiled, cooled + conc. H_2SO_4	Two layers formed, upper layer turns green (steroids present) or deep red (triterpenoids present).	+	+
TPC	Ferric chloride -extract + distilled water +2 ml of 5% $FeCl_3$	Blue, green or violet color	+	+
	Lead acetate - extract + distilled water + lead acetate solution	White precipitate	+	+
	Dilute Iodine solution -2 ml extract solution+ few drops dilute iodine solution	Transient red color	+	+

POWME: *Portulaca oleracea* whole plant methanolic extract; CAWME: *Chenopodium album* whole plant methanolic extract; + Present, - Absent; CRS: Carbohydrates & Reducing Sugars; PAA: Proteins & amino acids; GC: Glycosides; AL: Alkaloids; FN: Flavonoids; SN: Saponin; TS: Triterpenoids and Steroids; TPC: Tannin and Phenolic compounds, H_2SO_4: Sulphuric acid, HCl – Hydrochloric acid, $FeCl_3$ – ferric chloride, NaOH- Sodium hydroxide.

DISCUSSION

These vegetables after *in vivo* tests can be recommended for consumption in oxidative stress related diseases. *Chenopodium album* was found to show lower IC_{50} value alongwith higher percentage of inhibition. Although several researchers[10] have reported antioxidant potential in its leaves but to the best of our knowledge and in accordance with the literature review DPPH scavenging potential in methanolic extract of whole plant of *Chenopodium album* was not found to be reported in earlier studies.

The antioxidant activity of alkaloids[11] flavonoids[12] phenolic compounds, saponins,[13] glycosides,[14] proteins,[15] tannins,[16] triterpenoids[17] and steroids[18] has been reported. Thus, the presence of these phytochemicals in both the extracts indicate towards the positive relation between these phytochemicals with their antioxidant potential.

CONCLUSION

This *in vitro* study showed that both plants are good scavengers of free radicals like DPPH and when used as vegetables or in medicines they will be beneficial for treating various oxidative stress related diseases. These *in vitro* results should be confirmed *in vivo*.

CONFLICT OF INTEREST

Authors declared there is no conflict of interest.

ACKNOWLEDGEMENTS

The author would like to acknowledge the Principal of Sarojini Naidu Govt. Girls. P.G. College, Bhopal as well as sincere gratitude are also due to the Head of Department of Botany, Teaching and non-teaching staff of the college for their cooperation.

Highlights of Paper

- Reactive oxygen species (ROS) are considered responsible for causing various diseases.
- Antioxidants prevent and repair the damage caused by ROS.
- Both Portulaca oleracea and Chenopodium album showed antioxidant ability but the latter was found to be more potent antioxidant.
- In both these extracts the R^2 values were greater than 0.9 indicating that the relationship between extract concentration and % inhibition was extremely strong.
- Qualitative biochemical analysis showed the presence of biochemicals that are known to have antioxidant ability.

Author Profile

- Kumkum Agarwal is a Research Scholar in the Department of Botany, Sarojini Naidu Govt.Girls P.G. (Auto.) College Bhopal India. Her research interest is to study various aspects of medicinal plants, including their ethnobotanical and taxonomical studies, in vitro medicinal abilities including antioxidant and calcium oxalate inhibiting ability as well as their phytochemical analysis.

REFERENCES

1. Cadenas E, Packer L. Hand book of antioxidants, 2nd ed.USA: Marcel Dekker AG Publication; 2002. p. 10-5.
2. Sharma P, Jha A, Dubey R, Mohammad P. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal Botany 2012; 20(2012): 1-27.
3. Oliveira I, Valentao P, Lopes R, Andrade P, Bento A, Pereira J. Phytochemical characterization and radical scavenging activity of *Portulaca oleracea* L. leaves and stems. Microchemical J. 2009; 92(2): 129–34.
4. Dkhill M, Moniem A, Quraishy S, Saleh R. Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. J. Med. Plants Res. 2011; 5(9): 1589-63.
5. Ghimire B, Seong E, Kim E, Ghimeray A, Yu C, Ghimire B, *et al*. A comparative evaluation of the antioxidant activity of some medicinal plants popularly used in Nepal. J Med Plants Res. 2011; 5(10): 1884-91.
6. Pandey N, Barve D. Antioxidant activity of ethanolic extract of *Annona squamosa* L. bark'. International Journal Research Pharmaceutical Biomedical Sciences 2011; 2(4): 1692-97.
7. Kumkum A, Ranjana V. Studies on antioxidant activity and phytochemical screening of selected medicinal plant *Ocimum gratissimum* L. International Journal Pharmaceutical Sciences Research 2014; 5(8): 1000-8.
8. Kumkum A, Ranjana V. Reactive oxygen species scavenging ability and phytochemical screening of an asteraceae member-*Xanthium strumarium*. Int J Pharm Phytopharmacol Res. 2014; 3(4): 306-10.
9. Kumkum A, Ranjana V. Antioxidant activity and phytochemical analysis of *Hyptis suaveolens* (L.) Poit. J Adv Pharm Edu Res. 2013; 3(4): 541-9.
10. Gokhale S, Kokate C, Purohit A. Pharmacognosy, India. Nirali Prakashan; 2006. p. 22-5.
11. Olarte E, Herrera A, Villasenor I, Jacinto S. Antioxidant activity of a new indole alkaloid from *Cassia alata* L. Philipp Agric Scientist 2010; 93(3): 250–4.
12. Singh D, Chander V, Chopra K. Protective effect of catechin on ischemia reperfusion induced renal injury in rats. Pharmacol Rep. 2005; 57(1): 70–6.
13. Gulcin I, Mshivdadze V, Gepdiremen A. Antioxidant activity of saponins isolated from ivy: alpha hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F. Planta Med. 2004; 70(6): 561-3.
14. Pujimulyani D, Raharjo S, Marsono Y. The effect of blanching on antioxidant activity and glycosides of white saffron (*Curcuma mangga Val*.). International Food Research Journal 2012; 19(2): 617-21.
15. Elias R, Kellerby S, Dekker E. Antioxidant activity of proteins and peptides. Crit Rev Food Sci Nutr. 2008; 48(5): 430- 41.
16. Zhang S, Lin Y, Zhou H, Wei S, Lin G, Ye G. Antioxidant tannins from stem bark and fine root of *Casuarina equisetifolia*. Molecules 2010; 15(8): 5658–70.
17. Arafat O, Tham S, Sadikun A, Zhari I, Haughton P, Asmawi M. Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats. J Ethnopharmacol. 2008; 118(3): 354-60.
18. Mooradian A. Antioxidant properties of steroids. J Steroid Biochem Mol Biol. 1993; 45(6): 509-11.