SCREENIG OF PHYTOCHEMICALS AND ANTIOXIDANT POTENTIALS OF SOME WILD EDIBLE FRUITS

CHANDRA M

Abstract: Aim of the present study was to evaluate the *in vitro* antioxidant activity of four different wild edible fruits viz., *Physalisminima, Cucumis trigonus, Cordia dichotoma* and *Pithecellobium dulce.*The methanol and aqueous extracts of the fruits were assessed using three different assays like DPPH, reducing power and total antioxidant activity at different concentrations. In all the three assays, *Pithecellobium dulce*has exhibited highest antioxidant activity compared to other three wild edible fruit extracts irrespective of the solvents. Results of the antioxidant potentiality of various fruits are discussed.

Keywords: Antioxidant activity, DPPH, Wild edible fruits, Phytochemicals, Reducing power.

Introduction: Fruits are important sources of minerals, fibre and vitamins which provides essential nutrients for the human health. Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other ageing - related pathologies. The wild underutilized edible fruits can also play an important role as food supplement. Fruits offer protection against free radicals that damage lipids, protein and nucleic acids. Polyphenols, carotenoids, vitamins present in fruits have antioxidant and free radical scavenging activities and play a significant role in the prevention of many diseases [1].Wild edible fruits are known to possess beneficiary nutrients like vitamins minerals and polyphenols which provide health benefit in addition to their nutritional value. Several varieties of locally available wild edible fruits are commonly consumed and are considered an integral part of ethno-culture globally. Many of them have been used traditionally as medicines and also made into sauces, jellies, jam or pickles for human consumption [2].The main objective of the present studies was to screen and evaluate the antioxidant potential of some commonly used wild edible fruits growing in India. Therefore, four most potentially utilized wild edible fruit species belongs to different family viz., *Cordia dichotoma* (Boraginaceae), *Pithecellobium dulce* (Fabaceae), *Cucumis trigonus* (Cucurbitaceae) and *Physalis minima(*Solanaceae)are selectedfor present investigation.

Materials And Methods

Extraction: The fresh wild edible fruits are collected and each fresh fruit was washed with running tap water followed by washing with distilled water to remove the surface debris. Exactly 50 g of fruit pulp were weighed and were minced using a mixer grinder for the maceration. After homogenization, it was extracted in two different solvents, 500 ml methanol and 500 ml chloroform water (1.25 ml $CHCl_3$ and volume made up to 500 ml with distilled water) kept for 7 days in dark under room temperature with intermittent shaking. After 7 days, the whole extracts are filtered using muslin cloth at first and then through filter paper. 300 ml fresh solvent was added and refluxed for 90 min followed by filtration and finally both the filtrate were mixed together and concentrated. The yield of crude extracts were noted and stored in desiccators for maximum of 3 days and stored in a deep freezer (-20 $^{\circ}$C) for further use.

Qualitative Phytochemical Analysis: The preliminary qualitative phytochemicals studies were performed for testing the different chemical groups present in methanol and aqueous extracts of four different wild edible fruits [3]-[4].

General Chemicals and Instruments: All chemicals and solvents used in the study were of analytical grade. 2,2-diphenyl -1-picryl hydrazyl (DPPH), methanol, trichloro acetic acid (TCA) were purchased from Himedia, India. Ascorbic acid, monobasic and dibasic sodium phosphate, potassium ferricyanide, ferric chloride, sulphuric acid, sodium phosphate, ammonium molybdate is procured from Sd Fine chem. Ltd, India. UV-Vis Spectrophotometer (Elico SL 159, India), centrifuge (Remi RM12C, India), low deep freezer (Modern Industrial Corporation, India), vacuum rotary evaporator (Shivam Industruments, India), weighing balance (Sartorius, India) an pH meter (Systronics, India) were the instruments used for the study.

DPPH (2, 2-diphenyl-1-picryl hydrazyl) Radical Scavenging Activity: DPPH free radical scavenging assay was measured using the method of [5]. The different concentration of each extracts prepared in methanol were added to 3 ml of 0.1mM methanol solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in dark. Changes in absorbance of samples were measured at 517 nm and methanol was used as a control. Ascorbic acid was used as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula, Percentage Inhibition (%) = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$

Results are expressed as IC_{50}, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%.

Reducing Power Assay: The reducing power of the extracts was evaluated according to [6]. Different amounts of methanol and aqueous extracts were prepared in respective solvents and mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% $K_3Fe(CN)_6$. This mixer was incubated at 50° C for 20 min: 2.5 ml of 10% TCA was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was assorted with methanol (2.5ml) and $FeCl_3$ (0.5ml, 0.1%). The absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicated increased reducing power. The reducing power was expressed as equivalents of ascorbic acid (100µg/mg of extract.

Total Antioxidant Capacity by Phosphomolybdenum Method: The total antioxidant capacity was measured by spectrophotometer method of [7]. At different concentration ranges, methanol and aqueous extracts were prepared in their respective solvents and mixed in an eppendorf tube with 1ml of reagent solution (0.6M H_2SO_4, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90 min at 95°C. The mixture was cooled to room temperature and the absorbance was read at 695 nm against blank. The values are expressed as equivalent of ascorbic acid µg/mg of extract.

Results

Qualitative Phytochemical Analysis: Both the methanol and aqueous crude extracts of fruit were subjected to preliminary qualitative phytochemical evaluation to test the presence of various constituents present in these extracts and the results were shown in Table 1 and 2. Analysis revealed that none of the fruits under study showed negative results for phytochemicals in the methanol and aqueous extracts.

DPPH Radical Scavenging Activity: In this study, DPPH was used to determine the proton scavenging activity by both methanol and aqueous fruit extracts and the results are expressed in their IC_{50} values. The IC_{50} value, a measure of the extract concentration which is required for 50% inhibition of the free radical DPPH, was determined. The methanol extracts showed appreciable free radical scavenging activities in terms of IC_{50} values.

The IC_{50} values for methanol extracts were found to be highest in *Pithecellobium dulce* (8.50µg/ml) followed by *Cucumis trigonus* (276.20µg/ml), *Physalis minima* (785.70mg/ml) and *Cordia dichotoma* (1.44mg/ml) .The activity shown by the standard was 2.45µg/ml. The results revealed that dose dependent radical scavenging activity in terms of IC_{50} values. The IC_{50} values for aqueous fruit extracts were found to be highest in *Pithecellobium dulce* (5.87µg/ml) followed by *Cucumis trigonus* (883.54µg/ml), *Cordia dichotoma* (2.38mg/ml) and *Physalis minima* (3.31mg/ml) and the activity shown by standard was 2.45µg/ml. The results revealed that dose dependent radical scavenging activity in terms of IC_{50} values.

Table 1: Results of qualitative phytochemical analysis of four aqueous fruit extracts

Tests	Fruits			
	Cucumis trigonus	*Cordia dichotoma*	*Pithecellobium dulce*	*Physalis minima*
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Amino acids	+	+	+	+
Steroid	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Tannins and polyphenols	+	+	+	+
Glycosides	+	+	+	+

Reducing Power Assay: The reductive capabilities of the four methanol fruit extracts were compared with standard ascorbic acid and the results were 595.00, 23.60, 11.90 and 11.60µg/ml of extract in Pithecellobium dulce followed by Cucumis trigonus, Physalis minima and Cordia dichotoma respectively. In aqueous fruit extracts, reducing power was found to be high in Pithecellobium dulce followed by Cucumis trigonus,Cordia

dichotoma and Physalis minima and the values were 615.00, 12.60, 5.16 and 2.64 and 1.00µg of ascorbic acid/mg of extract respectively.

Table 2: Results of qualitative phytochemical analysis of four methanol fruit extracts

Tests	Fruits			
	Cucumis trigonus	*Cordia dichotoma*	*Pithecellobiumdulce*	*Physalis minima*
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Amino acids	+	+	+	+
Steroid	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Tannins & polyphenols	+	+	+	+
Glycosides	+	+	+	+

Total Antioxidant Capacity: Among the methanol fruit extracts, total antioxidant capacity was found to be highest in *Pithecellobium dulce* followed by *Cucumis trigonus, Physalis minima* and *Cordia dichotoma* and the values are 248.50, 59.30, 57.30 and 26.30 µg of ascorbic acid/mg of extract respectively. Similarly, in aqueous fruit extracts, total antioxidant capacity was found to be high in *Pithecellobium dulce* followed by *Cucumis trigonus*,*Cordia dichotoma* and *Physalis minima* and the values were 197.50, 70.00, 52.00 and 23.90 and 1.00µg of ascorbic acid/mg of extract respectively. From the results it is clear that among the two solvents, methanol extracts found to be better since the results indicated higher values of equivalents of ascorbic acid when compared to aqueous extracts.

Discussion: Phytochemical compounds may be related not only to the variety, but also to influences of the extraction methods and conditions, the stage of maturity and fruits harvest, storage conditions, the dosage of reagents. Highest phytochemical contents corresponded to highest antioxidant activity. The total phenol content varied from fruit to fruit and indicated that they could be utilized as potential source of natural antioxidant in food or in pharmaceutical industry [8].A correlation between the phenolics and flavonoids content and the antioxidant activity has been established for many herbs [9]-[10].

DPPH assay is one of most widely used method for screening of antioxidant activity of plant extracts. DPPH assay is based on the concept that a hydrogen donor is an antioxidant and it is one of the few stable and commercially available organic nitrogen radicals [11]-[12]. The antioxidant effect is proportional to the disappearance of DPPH in test samples. A freshly prepared DPPH solution exhibit a deep purple color with absorption maximum at 517 nm. The purple color generally fades or disappears when an antioxidant is present in the medium [13]. In the present study the methanol fruit extracts were evaluated for DPPH radical scavenging activity. Among four methanol fruit extracts,*Pithecellobium dulce* exhibited the highest radical scavenging activity whereas*Cordia dichotoma* showed lowest activity in terms of IC_{50}. Among aqueous fruit extracts, *Pithecellobium dulce* exhibited highest radical scavenging activity whereas *Cucumis trigonus* showed lowest activity in terms of IC_{50}.The reducing capacity of extracts Fe^{3+}/ ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity [14]-[15]. The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. The reduction of the fe^{3+}/ ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution. Absorbance of fe^{3+} can be observed by measuring the OD at 700 nm the reduction power of the extract increase with increase in concentration [16]. In the present study the methanol fruit extracts were evaluated for the reducing power ability. Among the four methanol fruit extracts, *Pithecellobium dulce* exhibited the highest reducing activity whereas *Cordia dichotoma* showed lowest activity in terms of ascorbic acid equivalents. Among aqueous extracts, the reducing power was found to be highest in *Pithecellobium dulce*, and *Physalis minima* had lowest activity. Total antioxidant capacity by phosphomolybdenum method is based on the reduction ofMo(VI) to Mo (V) by the sample analyse and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdemun method is quantitative since the total antioxidant activity is expressed as the number of equivalents ofascorbic acid[7]. In the present study the methanolfruit extracts were

evaluated for the total antioxidant capacity.Among the four methanol fruit extracts, total antioxidants capacity was found to be high in *Pithecellobium dulce* extract and low in *Cordia dichotoma* extract in terms of ascorbic acid equivalents. Whereas in aqueous extracts, *Pithecellobium dulce* exhibited highest antioxidant activity and *Physalis minima* exhibited lowest activity.It is clear from the study that the tested fruits manifested differential expression of antioxidant capacity due to their phytoconstituents. Particularly in fruits, natural antioxidants can be phenolic compounds, nitrogen compounds, or carotenoids as well as ascorbic acid [17]. Tannins are known to inhibit lipid peroxidation and lipoxygenases *in vitro*and information has been accumulated over the past few years demonstrating their ability to scavenge free radicals which are known to be important in cellular prooxidant states [18]-[19].

Acknowledgement : Author is thankful to the Department of Biosciences, Mangalore University for providing laboratory facilities to carry out this work.

References:

1. D. Prakash, G. Upadhyay, and P. Pushpangadan, "Antioxidant potentials of some under-utilized fruits", *Indo Global Journal of Pharmaceutical Sciences*, 2011, 1:25-32.
2. R. S, Glew, J, Dorothy, L. T, Chuang, Y. S, Huang, M. Millson and R. H, Glew "Nutrient content of four edible plants from West Africa", *Plant Foods for Human Nutrition*, 2005,60:187-193.
3. G. E, Trease and W. C, Evans "A Text book of Parmacognosy". 11th Edition, Bailliere Tidall, London, 1978, pp. 530.
4. C. K. Kokate, A. P. Purohith and A. B. Gokhale, Pharmacognosy. Nirali Prakashan, Pune, 1990,pp.120.
5. C. K. Wong, P. L. Lai and H. W. K. Jen, "Antioxidant activities of aqueous extracts of selected plants", *Food Chem.* 99, 2006.
6. M. Oyaizu, "Studies on products of browning reaction prepared from glucosamine", *Jpn J ofNutr.*1986, 44, pp. 307-15.
7. P. Prieto, M. Pineda and M. Anguilar, "Spectrophotometric quantitation of antioxidant capacitythrough the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E", *Anal Biochem*.1999, 269, pp. 337-341.
8. S. Karuppusamy, G. M. Muthuraj and K. M. Rajasekaran, "Antioxidant activity of selected lesserknown edible fruits from Western Ghats of India", 2011, *Indian J. Natural ProductsandResources,* Vol. 2(2), pp. 174-178.
9. S. Y. Wang and K. S. Lewers, "Antioxidant activity and flavonoid content in wild Strawberries",2007, *J Amer. Soc. Hort. Sci*.132, 629-637.
10. N. Rsaissi, E. L. Kamili, B. Bencharki, L, Hilai, and M. Bochache, "Antimicrobial activity of fruit extracts of the wild Jujube "*Ziziphus lotus* (L). Derf", 2013, Vol.4, Issue 9, pp. 1521-28.
11. M. Burits and F. Bucar, "Antioxidant activity of *Nigella sativa* essential oil", 2000, *Phytother. Res.,* 14, pp. 323-28.
12. L. K. MacDonald – Wicks, L.G. Wood M. L. Garg, "Methodology for the determination of biological antioxidant capacity in vitro: a review", *J. Sci. Food Agri*. 2006, 86, pp. 2046.
13. W. Brand – Williams, M. E. Cuvelier, C. Berset, "Use of a free radical method to evaluate antioxidant activity", *Lebensm Wiss Technol*, 1995, 28, pp. 25-30
14. S. Meir and J. Kanner, Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves, 1995, *J. Agric. Food Chem.*, 43, pp. 1813-1819.
15. Yaldrim and A. Mavi, "Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argente*a Desf Ex DC), Sage (*Salvia tribola* L.) and Black tea (*Camellia sinensis*) extracts" *J Agri Food Chem,* 200, 48, pp. 5030-5034.
16. Y. P. Zou and Y. H. Lu, "Antioxidant activity of a flavonoid rich extract of *Hpericum Perforatum* L. *In vitro*", 2004, *J Agri. Food Chem*, 52, pp. 5032-5039.
17. C. A. Hall, S. L. Cuppett, Structure-activities of natural antioxidants, In: Antioxidant Methodology *in vitro* and *in vivo* concepts", O. I. Aruoma and S. L. Cuppett, Eds, AOCS Press, Champaign, IL, 1997, pp. 2-29.
18. D. Prakash, G. Upadhyay, C. Gupta, P. Pushpangadhan and K. K. Singh, "Antioxidant and free radical scavenging activities of some promising wild edible fruits", 2012, *Int. Food Res J,* 19 (3):1109-1116.
19. Mahadkar, Shivaprasad, Jadava, Varsha, Deshmukh and Swathi, "Antioxidant activity of some promising wild edible fruits", 2013, Vol.4, issue 3, pp. 165-16

***

Department of Biosciences, Mangalagangothri, Mangalore University,Dakshina Kannada District-574199, Karnataka, India.E-mail:drchandram1@gmail.com

.