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EVALUATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF SOME WILD FRUITS BY *IN-VITRO* ASSAYS

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ABSTRACT: The present study aimed to evaluate bioactive compounds and total antioxidant activity of different fruits viz *Phyllanthus emblica, Limonia acidissima, Syzygium cumini, Artocarpus hirsutus, Carissa congesta, Anacardium occidentale*. Phytochemical study confirms the presence of carbohydrates, proteins, amino acids, cardiac glycosides, steroids, terpenoids and flavonoids in all the fruit extract. Ascorbic acid was used as a standard antioxidant. The reducing power and total antioxidant activity were high in *Phyllanthus emblica* and low in *Carissa congesta*. In DPPH assay *Phyllanthus emblica* exhibited highest activity whereas *A.hirsutus* showed lowest activity. Thus the study suggests that *Phyllanthus emblica* fruit is a better source of natural antioxidant, which might be helpful in preventing oxidative stress related damage

INTRODUCTION: Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen ions and peroxides, which are formed as a natural product of the normal metabolism of oxygen and play important roles in cell signaling and homeostasis [1].

Plant materials such as fruits, vegetables, leaves, stem, barks, and roots are Potential sources of antioxidant compounds [2]. Phenolic compounds, Flavanoids, flavanols and tannins are especially common in fruits, leaves, stem and barks. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection against infections and degenerative diseases.

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They can either directly scavenge or prevent generation of ROS [3]. Antioxidant studies have shown that increased consumption of fruits and vegetables has been associated with protection against various forms of cancer, a number of chronic diseases, such as neoplasm, cardiovascular diseases, inflammation, neurodegenerative pathologies, cataracts, diabetes as well as the ageing process [4-6]. Fruits and vegetables are major sources of dietary antioxidant such as vitamin C, vitamin E (tocopherol), precursors of vitamin A i.e., carotene, phenolic compounds [7]. The present study aimed to evaluate bioactive compounds and total antioxidant activity of different *wild* fruits viz *Phyllanthus emblica, Limonia acidissima, Syzygium cumini, Artocarpus hirsutus, Carissa congesta, Anacardium occidentale*.

MATERIALS AND METHODS:

Plant materials:

Samples of fresh ripe fruits were collected from the local forest of Udupi, and Kanakapura town of

Ramanagar district, Karnataka, during the year 2013. The fruits comprised of *Phyllanthus emblica, Limonia acidissima, Syzygium cumini, Artocarpus hirsutus, Carissa congesta, Anacardium occidentale.* The fruit samples were authenticated by the taxonomist, Dept of Botany, Poornaprajna College, Udupi, Karnataka, India.

Extraction procedure:
Each Sample of fresh fruit was washed under running tap water followed by washing with distilled water to remove the surface debris. 100g of edible portions of the fruit were weighed and minced using a kitchen blender.

After homogenization, it was extracted in methanol for 72 hours in dark at 37^{o}C incubator shaker. After 3 days, the whole extracts are filtered and then centrifuged to obtain clear extract. The filtrate was concentrated in Rotary vacuum evaporator. The resultant extract was lyophilized to obtain dry powder. The yield of crude extracts were noted and later preserved in a deep freezer (-20° C) for further use.

Qualitative phytochemical screening:
Phytochemical analysis was carried out for all the fruit extracts as per the standard methods [8-12].

Bioactive compounds
Total phenolic content:
Total phenolic content was determined by the method described by Singleton and Rossi[13]. 0.1 ml of sample was made up to 0.25 ml with distilled water and mixed with 0. 25ml of Folin Ciocalteu's phenol reagent. After 3 min, 0.5 ml of sodium carbonate solution was added to the mixture and made up to 5 ml by adding distilled water. The reaction was kept in the dark for 30 min, after which its absorbance were read at 760 nm. The results were expressed as ug of Ferulic acid equivalents/mg of extract.

Flavonoid content:
This was assayed as described by Jia et al [14]. 0.1 ml of the sample is added into a test tube containing 0.4 ml of distilled water. Then added 0. 075ml of sodium nitrite solution and allowed to stand for 5 min. Added 0. 15 ml of aluminium chloride, after 6 min 1 ml of 1.0 M sodium hydroxide were added and the mixture were diluted with another 2.275 ml of distilled water. The absorbance of the mixture at 512 nm was measured immediately. The flavonoid content was expressed as ug catechin equivalents /mg sample.

Tannin content:
The tannin content in sample was estimated by the method of Price and butler[15] with slight modifications. 0.020ml of the sample is added into a test tube containing 0.980 ml of distilled water. 0.5 ml of 1% K_3 Fe (CN) $_6$ and 0.1 ml of 1 % $FeCl_3$ were added and was made up to 3 ml with distilled water. After 10 min the solutions were measured spectrophotometrically at 720nm. The tannin content was expressed as mg of catechin equivalents/ mg of extract.

Evaluation of *in vitro* antioxidant activity
DPPH radical scavenging activity
DPPH free radical scavenging assay was measured using the method of Wong *et al.*2006 [16]. The different concentrations of extract in methanol were taken in a series of test tubes containing 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30min at room temperature in dark. Absorbances of samples were measured at 520nm against control. Ascorbic acid was used as the standard control. All the tests were performed in triplicates.

Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\% \text{ Inhibition} = \left(\frac{\text{Control O.D} - \text{Sample O.D}}{\text{Control O.D}}\right) \times 100$$

Total antioxidant capacity
The total antioxidant capacity was determined by the method described by Prieto *et al.*[17] with slight modification. Methanol extract is added into a series of eppendorf tube containing methanol and mixed with 1ml of phosphomolybdenum reagent solution.

The tubes were incubated for 90min at 95^{o}C in dry bath. The mixture was cooled to room temperature and the absorbance was read at 695nm against blank. The experiment was conducted in triplicates

and values are expressed as equivalents of ascorbic acid (µg) /mg of extract.

Reducing power assay

The reducing power of the extracts was evaluated according to Oyaizu[18]. Different concentrations of methanol extracts were prepared in methanol solvent and diverse with 2.5ml of 0.2M phosphate buffer and 2.5ml of freshly prepared 1% $K_3Fe(CN)_6$. This mixture was incubated at 50°C for 20 min, 2.5ml of 10% TCA was added and centrifuged at 3000rpm for 10min. 2.5ml of the supernatant was assorted with 2.5ml of methanol and 0.5ml of 0.1% $FeCl_3$, and the absorbance was measured at 700nm. The experiment was conducted in triplicates and the reducing power was expressed as equivalents of ascorbic acid (µg) / mg of extract.

RESULTS AND DISCUSSION:

Qualitative phytochemical analysis revealed that all the methanol fruit extracts showed the presence of carbohydrates, proteins, amino acids, glycosides, steroids, terpenoids and Flavonoids. Analysis also revealed negative results for saponins in all the methanol fruit extracts **(Table 1).** The presence of these bioactive compounds in the fruit extracts encourages antioxidant studies.

TABL 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF WILD FRUIT EXTRACTS

FRUIT EXTRACTS						
Tests	***Phyllanthus emblica***	***Limonia acidissima***	***Syzygium cumini***	***Carissa congesta***	***Artocarpus hirsutus***	***Anacardium occidentale***
Carbohydrates	+	+	+	+	+	+
Proteins	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+
Saponins	–	–	–	–	–	–
Cardiac glycosides	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+

Total phenolic content

Total Phenol contents were found to be highest in *Phyllanthus emblica* followed by *Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta* and *Artocarpus hirsutus* and values were 201.10ug, 68.12ug, 51.86ug, 43.3ug, 12.36ug and 7.86ug/mg of the extract respectively. The results of total phenolic content of the extracts were displayed in **Fig.1**.

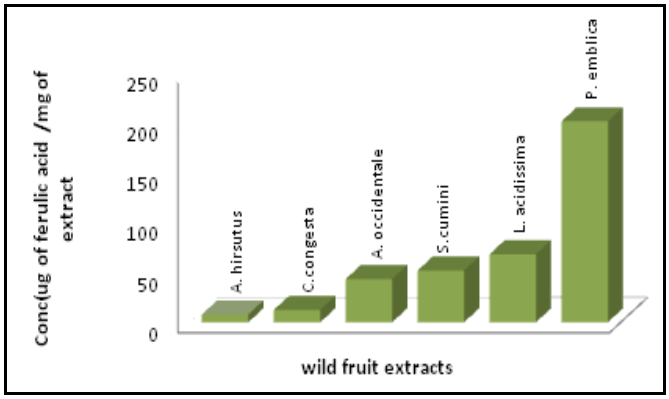

FIG 1: TOTAL PHENOLIC CONTENTS OF SIX WILD FRUIT EXTRACTS.

Many authors have described the potential antioxidant properties of polyphenols. These compounds act as antioxidants by donation of a hydrogen atom, as an acceptor of free radicals, by interrupting chain oxidation reactions or by chelating metals[19-20].

Flavonoid content

The values of total flavonoid content were 16.73, 4.53, 3.39, 1.01, 0.64 and 0.28ug/mg of the extract in *Phyllanthus emblica* followed by *Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta* and *Artocarpus hirsutus* respectively. The results of total flavonoid content of the extracts were displayed in **Fig.2**.

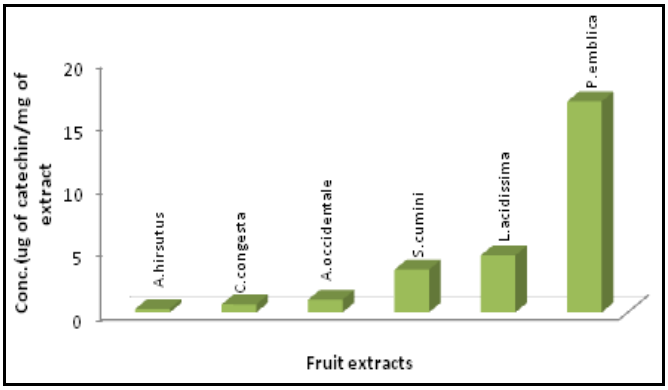

FIG 2: FLAVONOID CONTENTS OF SIX WILD FRUIT EXTRACTS.

Tannin content:

The results of total tannin content of the extracts were represented graphically in **Fig.3.** Tannin

content were found to be highest in *Phyllanthus emblica* followed by *Anacardium occidentale, Limonia acidissima, Syzygium cumini, Carissa congesta* and *Artocarpus hirsutus* and values were 16.82, 15.07, 14.16,12.31, 3.78 and 1.25ug/mg of the extracts respectively. Tannins are an important component of berry fruits. They comprise both condensed non-hydrolysable tannins, known as proanthocyanidins, and esters of gallic acid and ellagic acid—defined as hydrolysable tannins [21-22].

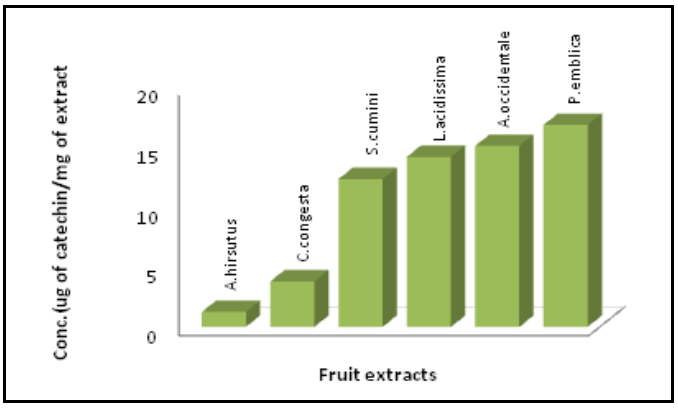

FIG 3: TANNIN CONTENTS OF SIX WILD FRUIT EXTRACTS

DPPH radical scavenging activity

In DPPH free radical scavenging activity, the results are expressed as IC50 which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%. DPPH free radical scavenging activity was found to be highest in *Phyllanthus emblica followed by Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta, Artocarpus hirsutus* and the values were 22.52ug/ml, 115.95ug/ml, 226.73ug/ml, 683.73ug/ml, 2.24 mg/ml and 2.65 mg/ml respectively.

IC50 value for the standard Ascorbic acid was found to be 2.52ug/ml **(Fig.4).**

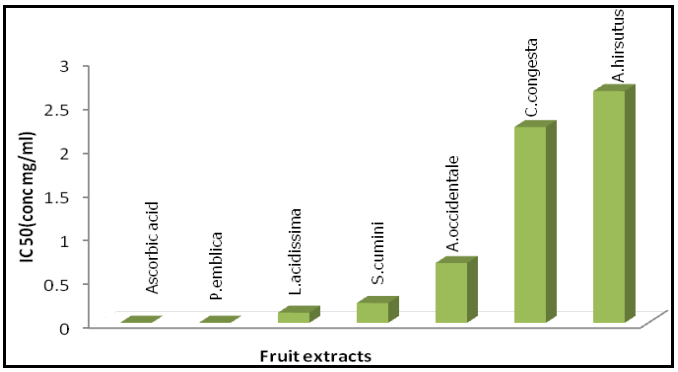

FIG 4: DPPH RADICAL SCAVENGING ACTIVITY OF SIX WILD FRUIT EXTRACTS IN TERMS OF IC_{50} VALUES

DPPH radical has a deep violet color in solution, and it becomes pale yellow when neutralized, which indicates the presence of antioxidant activity in the sample [23]. In DPPH assay the lower the IC_{50} the better it is able to scavenge the radicals, particularly peroxy radicals which are the propagators of the autoxidation of lipid molecules and thereby break the free radical chain reaction[24].

Total antioxidant capacity

The total antioxidant activity was expressed as (ug) equivalents of ascorbic acid/ mg of the extract. The total antioxidant activity was found to be highest in *Phyllanthus emblica* followed by *Anacardium occidentale, Syzygium cumini, Limonia acidissima, Artocarpus hirsutus and Carissa congesta* and the values were 208.04ug/mg, 44.50ug/mg, 38.95ug/mg, 36.91ug/mg, 31.29ug/mg, and 30.52ug/mg of the extract respectively.

The results are displayed in the **Fig.5.** Phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of green phosphate/Mo (V) complex at acidic pH. Natural antioxidants have some advantages over synthetic ones in that they can be obtained easily, has lesser side effects and cheaply available.

Antioxidant activity may be probably due to phenolic compounds present in the extract. It had been reported that the antioxidant activity of plant extract is correlated with the amount of their phenolic compounds [25].

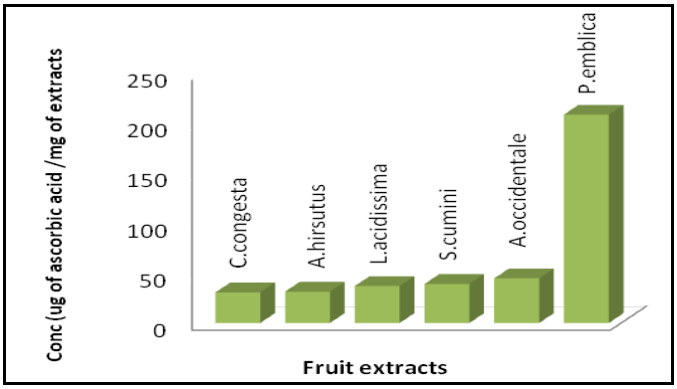

FIG 5: TOTAL ANTIOXIDANT ACTIVITY OF SIX WILD FRUIT EXTRACT

Reducing power assay

Reducing power capacity of different fruit extract was expressed as (ug) equivalents of ascorbic acid/mg of the extract. Reducing power capacity of

fruit extracts were found to be highest in *Phyllanthus emblica* followed by *Syzygium cumini, Anacardium occidentale, Limonia acidissima, Artocarpus hirsutus and Carissa congesta* and values were 318.01±2.168, 38.74±0.208, 35.87±0.179, 20.19 ±0.098, 8.05±0.078 and 5.95±0.190 respectively **(Fig.6).**

The reducing power capacity of the extract may serve as a significant indicator of its potential antioxidant activity. It was reported that the reducing power capacity of mushrooms might be due to their hydrogen- donating ability [26].

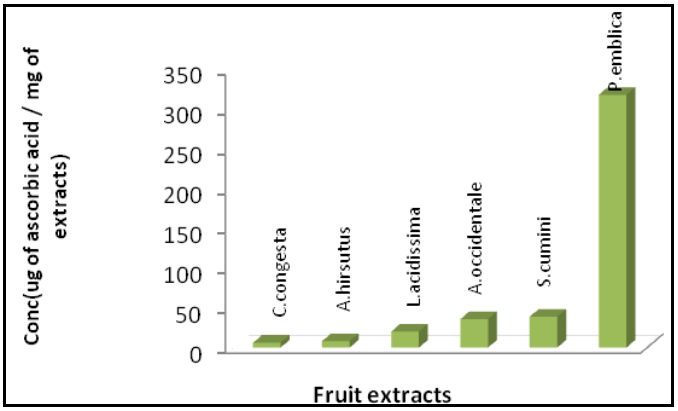

FIG 6: REDUCING POWER CAPACITY OF SIX WILD FRUIT EXTRACTS.

CONCLUSIONS: In DPPH free radical scavenging activity, the IC50 values were found to be highest in *Phyllanthus emblica* followed by *Limonia acidissima, Syzygium cumini, Anacardium occidentale, Carissa congesta* and *Artocarpus hirsutus*. The same trend was seen in Total phenolic content and total flavonoid content of six methanolic fruit extracts. *Phyllanthus emblica* also exhibit highest activity for reducing power assay and total antioxidant assay. *Phylanthus emblica* also contain high amount of tannin content compared to other fruit extracts. Among six wild fruits, *Phyllanthus emblica* exhibit highest activity in all the six assays. Hence author concluded that, *Phyllanthus emblica* fruit is a better source of natural antioxidant, which might be helpful for further studies to unravel novel treatment strategies for diseases associated with free radical induced tissue damage.

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REFERENCES:

1. Devasagayam TPA, Tilak JC, Boloor KK, Sane Ketaki S, Ghaskadbi Saroj S and Lele RD: "Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects". *Journal of Association of Physicians of India (JAPI) 2004;* 52: 796.
2. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R and Fidanza F: Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine 1995; 155: 381–386.
3. Shi HL, Noguchi N and Niki E: Introducing natural antioxidants, In J. Pokorny *et al*., Antioxidants in Food: practical application 2001, Woodhead Publishing Ltd. and CRC Press.
4. Machlin LJ and Bendich A: Free radical damage, antioxidant defenses. Federation of the American Society of Experimental and Biological Journal 1987; 441–445.
5. Young IS, Boxborough HE and Woodside JV: Antioxidants and respiratory disease, CAB International,Wallingford, Oxon, UK, 1999; 293-311.
6. Halliwell B: Antioxidants and human disease: a general introduction. Nutr Rev. 1997; 55:S44–S52.
7. Frei B and Higdon JV: Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr. 2003; 133: 3275S–3284S.
8. Harborne JB: Phytochemical methods, London. Chapman and Hall, Ltd 1973; 49-188.
9. Gahan, PB: Plant histochemistry and cytochemistry: An introduction. Academic press, Florida, U.S.A. 1984; 301.
10. Sofowora, A: Screening Plants for Bioactive Agents,In Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. 1993; 134-156.
11. Trease GE and Evans WC Pharmacognosy. 15th Ed. Saunders Publishers, London.2002; 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
12. Yasuma A and Ichikawa T: Ninhydrin-Schiff and Alloxan-Schiff staining methods for proteins, J. Lab. Clin. Med. 1953; 41(2):296-299.
13. Singleton VL and Rossi JA: Colorimetry of total phenolics with phosphomolybdic-phosphotungtic acid reagent. Am J Enol Viticult 1965; 16:144-158.
14. Jia ZS, Tang MC and Wu JM: The determination of Flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999; 64:555-559.
15. Price ML and Butler LG: Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J Agric Food Chem 1977; 25: 1268-1273.
16. Wong SP, Lai PL and Jen HWK: Antioxidant activities of aqueous extracts of selected plants, Food Chem. 2006; 99: 775-783.
17. Prieto P, Pineda M and Aguilar M: Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to determination of vitamin E. Anal Biochem 1999; 269:337-341.
18. Oyaizu M: Studies on products of browning reaction prepared from glucosamine. Jpn J of Nutr. 1986; 44: 307–15.

19. Hernández-Hernández E, Ponce-Alquicira E, Jaramillo-Flores ME and Guerrero-Legarreta: *Meat Sci.* 2009; *81*(2): 410.
20. Gramza A, Khokhar S, Hes M and Jedrusek-Golinska A: *Polish J. Environm. Stud.* 2004; *13*:143.
21. Shahidi F and Naczk M: Phenolic compounds in fruits and vegetables. In: Phenolics in food and nutraceutical, CRC LLC 2004; 131–156.
22. Puupponen-Pimiä R, Nohynek L, Alakomi HL and Oksman- Caldentey KM: Bioactive berry compounds—novel tools against human pathogens. Appl Microbiol Biotechnol 2005; 67:8–18.
23. Brand-Williams W, Cuvelier ME and Berset C: Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol. 1995; 28: 25-30.
24. Frankel, EN: Recent advances in lipid oxidation. Journal of the Science of Food and Agriculture 1991; 54: 495–511.
25. Velioglu YS, Mazza G, Gao L and Oomah BD: Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agriculture and Food Chemistry 1998; 46:4113-4117.
26. Shimada K, Fujikawa K, Yahara K and Nakamura T: Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agriculture and Food Chemistry 1992; 40:945–948.

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