

Antioxidant and Phenolic Content of Nuts, Oil Seeds, Milk and Milk Products Commonly Consumed in India

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ABSTRACT

Food provides not only essential nutrients required for life, but also bioactive compounds usefull to maintan good health and disease prevention. Abundant epidemiological evidences suggest that consumption of food rich in antioxidants (non-nutritional components) can prevent degenerative diseases. A total of 26 commonly consumed nuts, oil seeds, edible oils, milk and milk products were chosen for the study. Considering the fact that antioxidant content (AOC) and phenolic contents (PC) of these foods was not established systematically in Indian context. Therefore, we have assessed and correlated the AOC and PC, an important antioxidant constituents of plant foods. AOC was assessed by DPPH (2, 2-Diphenyl-1-picryl hydrazyl) scavenging activity and FRAP (Ferric reducing antioxidant power) methods and phenolic content (PC), using Folin-Ciocalteu reagent. Among the nuts and oil seeds arecanut had the highest phenolic and antioxidant content 10841, 4220341 mg/100g respectively. In milk, edible oils and sugars the values ranged from 336 - 11674 mg/100g. Jaggery had the highest PC and AOC among the foods studied . Although AOC and PC showed wide variation among the foods, AOC was correlated significantly with PC. Indeed the “r” value between PC and AOC (DPPH and FRAP) was 0.99 ($p < 0.01$) among the nuts and oil seeds, while in milk, milk products and sugars, the “r” values ranged from 0.93 and 0.99 ($p < 0.01$) respectively. The overall results indicate that the phenolic compounds may be significant contributors to the AOC of the foods studied.

Keywords: Antioxidant Content (AOC), 2,2'-Diphenyl-1-picryl hydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), Polyphenols, Phenolic Content (PC), 2,4,6-Tripyridyl-s-triazine (TPTZ)

1. Introduction

Antioxidants are essential for human health. Dietary antioxidants play an important role in controlling oxidative stress [1]. During normal metabolism, the oxidants and antioxidants are maintained in equilibrium [2]. Excess free radical production underlies the pathogenesis of diseases like atherosclerosis, carcinogenesis, diabetes, cataract and accelerated ageing [3]. Robust epidemiological evidence suggests the crucial role of diets in prevention of chronic degenerative diseases [4]. Supplementation of natural antioxidants through a balanced diet containing enough antioxidants could be most effective in protecting against various oxidative stressors [5]. In recent times, natural antioxidants have raised considerable interest among nutritionists, food manufacturers and consumers because of their presumed safety and potential therapeutic value. Indeed, recent research trends indicate a shift

towards identifying non-nutritional antioxidants in functional foods [6].

More than 5000 phytochemicals have been identified in plant foods and many more remain to be discovered [7]. Phenolic compounds have been proposed to be the potent and important contributors in reducing oxidative stresses due to their antioxidant activity, which are of great importance predominantly in Indian diets [8]. Therefore, food industry is concentrating on foods containing various bioactive compounds for health promotion and disease prevention [9].

There are few studies from India (including our studies) on antioxidant activity and phenolic content of plant foods commonly consumed in India [10,11]. The foods chosen in this study were not attempted systematically for their AOC and their correlation with phenolic content. Therefore, to the best of our knowledge, we have

determined for the first time the AOC and its correlation with PC in 26 commonly consumed foods in India.

2. Materials and Methods

Chemicals and reagents: 2,2'-Diphenyl-1-picryl hydrazyl (DPPH), Gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ) and Ferric Chloride were obtained from Sigma Chemical Inc, USA. All other reagents and chemicals used were of analytical grade procured from local sources. Milli Q water was used in the study.

2.1. Sample Collection and Extraction

Three samples of each food were purchased from each of the three local markets of Hyderabad and Secunderabad cities (India). Food samples were pooled from each area and represented as a single sample from that particular market. The samples were analyzed separately and data presented as mean value. Each sample was extracted in duplicate according to Sing *et al.* and Zielinski *et al.*, [12,13] with slight modifications. Methanol extraction was adopted as per the procedure described by Matthaues [14].

Approximately 50 g of the edible portion of the food was subjected to grinding in a domestic blender. In case of nuts and oil seeds, 5 grams of the ground sample was taken. Sample extracts were prepared in 20 ml of 70% Methanol containing 0.1% HCl by shaking vigorously for four hours at room temperature. In case of liquid samples like milk and edible oils, 5 ml of the sample was taken directly and extracted as above. The sample suspension was centrifuged at 10,000 g for 15min at 10°C, the supernatant was collected and filtered through Whatman #1 filter paper and the resultant filtrate was stored at -20°C. Analysis was completed within a month of extraction [15].

2.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined according to Yu *et al.* [16]. This method is based on the ability of the antioxidant to scavenge the DPPH cation radical. Briefly, 100 µl of sample extract or standard was added to 2.9 ml of DPPH reagent (0.1 mM in methanol) and vortexed vigorously. The reaction tubes were incubated in dark for 30 min, at room temperature and the discolouration of DPPH was measured against a reagent blank at 517 nm. Percentage inhibition of the discolouration of DPPH by the sample extract was expressed as Trolox equivalents [17].

2.3. FRAP Assay

Ferric reducing antioxidant power (FRAP) was determined according to Benzie and Strain [18]. This method is based on the ability of the sample to reduce Fe⁺³ to

Fe⁺² ions. In the presence of TPTZ, the Fe⁺²-TPTZ complex exhibits blue colour which has absorption maxima at 593 nm. Briefly, 3.0 ml of working FRAP reagent was added to a suitable volume of the sample extract was taken to suit in to standard range. After incubation for 6 min at room temperature the absorbance was measured at 593nm against FeSO₄ as standard.

2.4. Folin-Ciocalteu Assay

Soluble phenolic compounds (PC) were determined in sample extracts using the Folin-Ciocalteu reagent as per the method described by Singleton and Rossi [19] with slight modifications. Briefly, suitable volumes of sample extract to fit into standard concentrations were taken, 1.0ml of 10% Folin-Ciocalteu reagent and 0.8 ml of 7.5% Na₂CO₃ were added, vortexed thoroughly and incubated at room temperature for 90 minutes and the absorbance was read at 725 nm. The values were expressed as equivalents of Gallic acid, which is one of the most commonly, used standards in phenolic estimations. Gallic acid has indeed been shown to be more stable and a pharmacologically active antioxidant, quantitatively equivalent to many other phenolics and gives consistent and reproducible results [20].

2.5. Statistical Analysis

Results are expressed on fresh weight basis and presented as mean ± SD. Statistical analysis was performed to know the correlation between AOC and phenolic content using SPSS 14.0 statistical package.

3. Results and Discussion

Phenols and polyphenols are stronger antioxidants than the vitamins [21]. Several epidemiological studies showed a lower risk with increasing intakes of plant foods [22] and protection against DNA damage [23]. Yet in India, plant foods have received less attention in terms of quantifying their AOC [10]. As such, little data exists on the AOC of plant foods commonly consumed in India, let alone their relationship with the phenolic content [24, 25]. On the other hand, no single method gives a comprehensive estimation of the antioxidant efficacy of the food sample tested. About twenty different AOC indices are currently in use, single index by itself is not considered sufficient to quantify the AOC of foods. Therefore, use of more than one method is recommended for quantifying the AOC of the foods [26]. FRAP and DPPH are widely used and well accepted AOC indices [27,28]. Therefore, these two indices are chosen to determine the AOC of nuts, oil seeds, milk, edible oils and sugars. Considering that phenolic compounds are important antioxidants of commonly consumed foods, soluble phenolic content (PC) was determined by Folin/Ciocalteu assay.

The results among the 26 commonly consumed foods in India are summarized in **Tables 1-4**. In general, the coefficient of variation in the AOC and PC among the three samples collected from three different local markets for a given food sample was less than 10% indicating no significant differences among the market samples collected for the study. However, there was a wide variation in the AOC and PC content of the foods studied.

DPPH radical scavenging activity (trolox equivalents) and FRAP (ferrous sulphate equivalent), showed marked variation among the 12 commonly consumed nuts and oil seeds studied (**Table 1**). DPPH radical scavenging activity presented in **Table 1**, ranges from 20.0 - 28622.0 mg TE/100g with the highest activity being found in the arecanut followed by the mustard seeds (1155) and the least in the coconut water (20 mg/100g) (**Table 1**). Similarly, their FRAP activity ranged from 220.0 - 4220341.0 mg /100g. In line with the DPPH results, FRAP content was highest in the arecanut, followed by the mustard seeds and the least in the coconut water. The PC of nuts and oil seeds ranged from 10.0 to 10841.0 mg/100g, and in line with their AOC (DPPH and FRAP), arecanut has the highest phenolic content (10841.4) and coconut water the least (10.0).

AOC and PC of milk, milk products, edible oils, sugar, and sugarcane juice findings have been highlighted in **Table 3**. The DPPH activity ranges from 3.4 - 208.0 mg /100g with Jaggery having the highest DPPH content (208) followed by ground nut oil (22.1) and lowest activity found in whole milk (3.4). On the other hand, the FRAP activity ranges from 36 - 11674 mg/100g, with the highest activity in jaggery (11674.0) followed by sugarcane juice (872.0) and lowest was in sunflower oil (36.0 mg/100g). The PC content of milk, milk products, edible oils and sugars (**Table 3**) showed a wide range of values 0.72 - 336 mg/100g. Here again, jaggery had the highest

PC (336.4) followed by honey (140.4) and the least phenolic content was observed in vanaspathi (0.70).

Indeed, the AOC values reported here for the foods studied (**Tables 1 and 3**) are the first of their kind from India. However very scanty data is available from India on the phenolic content of some of the foods studied but not AOC [29]. Available information from other parts of the world on the PC content of sugar (cane) reported ranged from 11 - 41 mg/100g [30] and our findings (12.28 mg/100g) are comparable for sugar. Where as sugarcane juice is reported to have a phenolic content of 16 mg/100g [31], we observed 27.1 ± 6.00 mg/100g. The reported data on the phenolic contents of different brands of honey ranges from 234 - 394 mg/100g [32], whereas our values (140.4 mg/100g) are found to be lower (**Table 3**). This discordance could be due to factors like agronomic, genomic and post—harvesting conditions, which may affect the chemical composition of foods studied [33,34]. However, there is no published data on the AOC of the foods studied from India to compare our findings. Among the 26 foods studied, a few of them were not rich sources of AOC/PC, inspite of that they were included to have reference values from India.

Even though the foods studied belong to different food groups, significant correlation was observed between AOC (as estimated by both DPPH and FRAP methods) and PC (**Tables 2 and 4**). The correlation coefficient “*r*” values between PC and AOC was 0.99 in nuts and oil seeds (**Table 2**), whereas the “*r*” value was 0.93 in other foods given in **Table 4**. The positive correlation between PC and AOC (as assessed by DPPH and FRAP) among all the foods studied, indicate the importance of PC to antioxidant content.

4. Conclusions

The data reported here is the first of its kind from India

Tables 1. Antioxidant and Phenolic contents of nuts and oil seeds.

Sl. No	Common name of the nuts & oil seeds	Botanical name	Phenolic content (Gallic acid Eq mg/100g)	Antioxidant Content (mg/100g)	
				DPPH (Trolox Eq)	FRAP (FeSO ₄ Eq)
1	Areca nut	<i>Areca catechnu</i>	10841 ± 2258	28622 ± 5251	4220341 ± 774374
2	Coconut (dry)	<i>Cocos nucifera</i>	40 ± 1.9	99 ± 0.0	1259 ± 17.2
3	Coconut tender	<i>Cocos nucifera</i>	39 ± 3.7	74 ± 5.0	1126 ± 177.3
4	Coconut milk	<i>Cocos nucifera</i>	31 ± 4.9	129 ± 13.0	920 ± 56.4
5	Coconut water	<i>Cocos necifera</i>	10 ± 1.3	20 ± 1.0	220 ± 22.5
6	Gingelly seeds	<i>Sesamum indicum</i>	148 ± 35.3	154 ± 15.4	5693 ± 165.9
7	Linseed seeds	<i>Linum usitatissimum</i>	119 ± 11.0	135 ± 32.1	5283 ± 1148.6
8	Mustard seeds	<i>Brassicainigra</i>	725 ± 38.4	1155 ± 82.5	27666 ± 3293.5
9	Niger seeds	<i>Guizota abyssinica</i>	143 ± 3.0	154 ± 3.0	5347 ± 312.6
10	Safflower seeds	<i>Carthomus tinctorius</i>	599 ± 51.5	228 ± 33.1	12975 ± 781.6
11	Sunflower seeds	<i>Helianthus annus</i>	207 ± 7.3	850 ± 126.9	8151 ± 199.7
12	Water melon seeds	<i>Citrullus vulgaris</i>	74 ± 11.0	54 ± 4.0	1621 ± 155.8
<i>Range</i>			10-10841	20-28622	220-4220341

Values are Mean ± SD, *n* = 3. Decimal points are not given due to higher values, to keep uniform units higher values are not condensed.

Table2. AOC Vs TPC correlation of nuts and oil seeds.

Correlation	r	r ² %
PC Vs DPPH	0.998	99.71
PC Vs FRAP	0.997	99.51
DPPH Vs FRAP	0.999	99.85

Table 3. Antioxidant and phenolic contents of milk, milk products, edible oils and sugars.

S. No.	Common name	Phenolic content (Gallic acid Eq mg/100g)	Antioxidant Content (mg/100g)	
			DPPH (Trolox Eq)	FRAP (FeSO ₄ Eq)
1	Toned milk (Dairy milk)	3.4 ± 0.4	4.1 ± 0.3	73.9 ± 8.1
2	WHOLE MILK (BUFFALO)	3.0 ± 0.2	3.4 ± 0.3	38.9 ± 1.6
3	Curd (Inoculated from dairy milk)	7.4 ± 0.6	4.1 ± 0.5	117.0 ± 2.7
4	Groundnut oil (unrefined)	3.2 ± 0.3	22.1 ± 0.5	111.1 ± 0.0
5	Groundnut oil (refined)	3.1 ± 0.1	13.2 ± 0.0	36.4 ± 5.5
6	Sunflower oil	1.5 ± 0.2	12.4 ± 0.7	36.1 ± 1.3
7	Vanaspathi (Dalda)	0.7 ± 0.0	13.1 ± 1.8	73.9 ± 3.3
8	Palm oil	3.2 ± 0.2	12.1 ± 0.6	132.3 ± 19.7
9	Til oil	5.1 ± 0.6	19.9 ± 1.7	372.7 ± 78.9
10	Ghee (Vijaya)	10.2 ± 1.3	14.7 ± 0.8	333.0 ± 19.4
11	Honey (Agmark Girijan)	140.4 ± 26.6	19.6 ± 0.3	604.9 ± 86.4
12	Jaggery	336.4 ± 12.8	208.1 ± 27	11674.0 ± 1133
13	Sugarcane juice	27.1 ± 6.0	22.0 ± 6.3	872.6 ± 146
14	Sugar	12.2 ± 3.1	15.1 ± 1.4	389.7 ± 24.2
Range		0.7 - 336	3.41 - 208	36 - 11674

Values are Mean ± SD, n = 3. Decimal points are not given due to higher values, to keep uniform units higher values are not condensed.

Table 4. AOC Vs TPC correlation of milk, milk products, edible oils and sugars.

Correlation	r	r ² %
PC vs. DPPH	0.93	86.52
PC vs. FRAP	0.93	87.55
DPPH vs. FRAP	0.99	99.03

and these findings would be useful to nutritionists and consumers to know and formulate the antioxidant—rich therapeutic diets. In addition, the present study would be a valuable information to the existing knowledge on non—nutrient antioxidant contents of commonly—consumed Indian foods.

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