*Available online www.jocpr.com*

**Journal of Chemical and Pharmaceutical Research, 2015, 7(7):152-159**


**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Antioxidant quality ranking of some plant materials based on multiple *in vitro* radical scavenging assays

**Gite S. S., Yadav S. A., Nilegaonkar S. S. and Agte V. V.***

*Agharkar Research Institute, G. G. Agarkar Road, Pune, India*

---

**ABSTRACT**

*Food based phytochemicals have a varying degree of free radicals scavenging capacity depending upon their structure, types of functional groups and their position. In order to understand the relative potency of foods for their nutraceutical activity, a need is felt to develop some scoring system. In this study, we report the results of 24 food samples for antioxidant capacity analyzed through 9 different assays viz., inhibition of lipid peroxidation (TBARS), superoxide radical scavenging activity (SOSA), nitric oxide radical scavenging activity (NOSA), hydroxyl radical scavenging activity (OHSA), ABTS radical scavenging activity (TEAC), DPPH radical scavenging activity, ferrous ion chelating activity (FICA) in addition to their total polyphenols and vitamin C content. The entire data was used to rank the foods using 3 different scoring systems viz., Arithmetic ranking, statistical ranking and Point system or potency based ranking. The point based system could better discriminate the foods quantitatively for their overall antioxidant potential than the arithmetic or statistical ranking. Secondly, present results indicated that estimation of polyphenols alone could be sufficient as biomarker from which TBARS, TEAC, SOSA, OHSA and DPPHSA could be predicted.*

**Key words:** Arithmetic ranking, Point system or Potency based ranking, statistical ranking.

---

## INTRODUCTION

Plant based diets contain large number of naturally occurring antioxidants like carotenoids and phenolics [1]. Each of these phytochemicals has a varying degree of scavenging free radicals activity depending upon the structure viz. types of functional groups and their position. Secondly, there are varieties of *in vitro* protocols for the assessment of antioxidant potential of plant materials such as inhibition of lipid peroxidation, inhibition of superoxide radicals, inhibition of reactive nitrogen species, inhibition of ferrous ion chelating activity and the qualitative and quantitative estimation of known antioxidant molecules by using spectrophotometric methods and HPLC [2].

Existing nutritional rating systems are techniques of ranking individual foods, food products or food categories to evaluate the nutritional quality of food. These systems give points to rank or rate the foods for general nutritional value or rate specific food attributes such as cholesterol content. A healthy eating index (HEI) has been developed as a measure of overall diet quality by Kennedy et al., 1995 [3] which is computed using the recommended servings consumed from five food groups (grains, vegetables, fruits, milk and meat); recommended consumption of fat, saturated fat, cholesterol and sodium; and a measure of dietary variety. A Spanish study used a Mediterranean Diet Quality Index tool that included a 16-item Mediterranean Diet Quality Index (KIDMED index) [4]. Lower frequency of high diet quality was observed in low socio-economic groups, as compared with middle and upper income cohorts (42.8%, 47.6% and 54.9%, respectively). However, there is a need for a nutraceutical ranking

system such as antioxidant potential. The problem becomes complex because of large number of assays developed and used by different research groups [5]. A need was therefore felt to develop a tool to assess the antioxidant quality index (AQI) which accommodates the different free radical scavenging activities and the one that can discriminate between rich and poor sources of fruits and vegetables.

No single antioxidant capacity assay is able to reveal completely the "total antioxidant capacity" of any sample. For complete elucidation of an antioxidant capacity, further tests assessing effectiveness against a variety of reactive oxygen species as well as reactive nitrogen species would be desirable. Considering the huge diversity in the contents and the bio-accessible antioxidants, it is implied that all the plant materials are not equal in their antioxidant quality [6]. A need was therefore felt to develop a tool to assess the antioxidant quality index (AQI) using multiple antioxidant activity assays which can discriminate rich and poor sources among these healthy ingredients. This tool would help in emphasizing specific fruits /vegetables rather than general advice to consume more fruits and vegetables. The data collected for 9 antioxidant indices was used for this purpose.

In this study, we used the results of 24 food samples for antioxidant capacity analyzed through 7 different assays viz. TBARS, SOSA, NOSA, OHSA, TEAC, DPPH, FICA along with their vitamin C and total polyphenols content. The entire data was then used to rank the foods using 3 different scoring systems viz., arithmetic ranking, statistical ranking and point system or Potency based ranking.

## EXPERIMENTAL SECTION

### Chemical reagents

Ascorbic acid, 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy -2, 5, 7, 8-tetramethyl chroman-2-carboxylic acid (Trolox), were obtained from Sigma, Germany. 2-deoxy-2-ribose, mannitol, nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), sodium nitroprusside (SNP), sulfanilamide, naphthylethylenediamine dihydrochloride (NED), quercetin pepsin, pancreatin were from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Hydrogen peroxide, Folin-Ciocalteu reagent and gallic acid were from MP Biomedicals, France.

**Table-1: List of plant resources used in this study**

| Code | Scientific name | Family | Plant part |
|---|---|---|---|
| M-1 | *Zizipus jujuba* Lam. | *Rhamnaceae* | Dry powder of edible part of fruit |
| M-2 | *Ficus carica* Linn. | *Moraceae* | Dry powder of edible part of fruit |
| M-3 | *Emblica officinalis Gertan.* | *Euphorbiaceae* | Dry powder of edible part of fruit |
| M-4 | *Psidum guajava* Linn. | *Myrtaceae* | Dry powder of edible part of fruit |
| M-5a | *Vitis venifera* Linn. | *Vitaceae* | Dry powder of edible part of fruit |
| M-5b | *Vitis venifera* Linn. | *Vitaceae* | Dry powder of edible part of fruit |
| M-7 | *Carica papaya* Linn. | *Caricaceae* | Dry powder of edible part of fruit |
| M-8 | *Punica granatum* Linn. | *Punicaceae* | Dry powder of edible part of fruit |
| M-9 | *Tamarindus indica* Linn. | *Leguminosae* | Dry powder of edible part of fruit |
| M-13 | *Anona squamosa* Linn. | *Anonaceae* | Dry powder of edible part of fruit |
| M-14 | *Achras sapota* Linn. | *Sapotaceae* | Dry powder of edible part of fruit |
| M-19 | *Dioscorea bulbifera* Linn. | *Dioscoreaceae* | Dry powder of edible part of fruit |
| M-20 | *Limonia acidissima W. & A.* | *Rutaceae* | Dry powder of edible part of fruit |
| M-22 | *Moringa oleifera* Linn. | *Moringaceae* | Dry powder of edible part of fruit |
| M-24 | *Aegle marmelos* Corr. | *Rutaceae* | Dry powder of edible part of fruit |
| M-25 | *Ficus racimosa* Linn. | *Moraceae* | Dry powder of edible part of fruit |
| M-26 | *Coccinia indica* W. & A. | *Cucurbitaceae* | Dry powder of edible part of fruit |
| M-28 a | *Malus sylvestris* Mill. | *Rosaceae* | Dry powder of peel |
| M-28 b | *Malus sylvestris* Mill. | *Rosaceae* | Dry powder of pulp |
| M-29 | *Citrus paradisi* Macf. | *Rutaceae* | Juice |
| M-30 | *Citrus sinensis* Linn. | *Rutaceae* | Juice |
| M-31 | *Madhuca latifolia* Linn. | *Rutaceae* | Dry powder of edible part of fruit |
| M-32 a | *Citrus aurantium* Linn. | *Rutaceae* | Juice |
| M-32 b | *Citrus aurantium* Linn. | *Sapotaceae* | Dry powder of fruit peel |

**Sample preparations and extraction**
Twenty four types of edible plant materials were procured from the local market of Pune (India) and were screened for their antioxidant potential. All plant materials were authenticated by a recognized taxonomist from Department of Botany; University of Pune. Each plant material was cleaned with distilled water and the edible parts of respective plants were used (e.g. whole fruit, peel, pulp, juice) as shown in Table 1. Except juices, all the plant materials were homogenized in a mixer and the puree was dried in oven at 60 degrees Celsius for first 12 h, 50 degrees Celsius for next 12 h and 40 degrees Celsius for last 24 h. After that, the semi dried samples were freeze dried at -20 degrees Celsius and ground to the fine powder. The fine powders were immediately transferred in the seal-able bags and were stored at -20 degrees Celsius. The juice samples were filtered through the muslin cloth and were stored in polypropylene bottles at -20 degrees Celsius.

For antioxidant assay 0.1 g of sample was suspended in 10 ml of specific solvent and kept for overnight extraction. For extraction of total polyphenols, NOSA and OHSA distilled water was used as a solvent. Various solvents used for the extraction with respect to the antioxidant assays were as follows Vit-C: 6% meta-phosphoric acid; for SOSA: 0.2 M phosphate buffer (pH 7.5); for FICA: 1 % sodium dodecyl sulphate; for TBARS: 20 mM sodium phosphate buffer pH 7.4 and for ABTS and DPPH radical scavenging activity: methanol.

**Thio barbituric acid reactive species (TBARS)**
For estimation TBARS, liposomes were prepared according to the method of Tsuda et al [7]. The extent of oxidation was subsequently determined by TBARS measurement.

**Hydroxyl Radical Scavenging Activity (OHSA)**
OHSA was assayed using the 2-deoxyribose oxidation method from Gutteridge [8]. The results were expressed as mg of gallic acid equivalents per 100 g of dry sample.

**Nitric oxide radical scavenging (NOSA)**
NOSA was estimated by Griess Illosvoy reaction, Govindarajan et al [9-10] using spectrophotometer and results were expressed as mg of quercetin equivalents per 100 g of dry sample.

**DPPH radical scavenging activity**
Assay for DPPH radical scavenging activity was done by the method of Ariga and Hamano 1990 [11]; activity was expressed as mg of Trolox equivalent DPPH radical scavenging activity per 100 g of sample.

**Total Polyphenols content**
Total polyphenols content was determined using the Folin-Ciocalteu's colorimetric assay (Singleton and Rossi 1965; Saucier and Waterhouse 1999 [12-13]; results were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample.

**Ferrous ion chelating activity (FICA)**:
FICA was determined using the method Yamaguchi et al., 2000 [14] and was expressed as mM of EDTA equivalents per 100 g of sample.

**Superoxide radical scavenging activity (SOSA)**
SOSA was determined using the nitroblue tetrazolium (NBT) reduction method Nishikimi et al., 1972 [15] and results were expressed as mg of Rutin equivalent SOSA per 100 g of sample.

**$ABTS^+$ radical scavenging activity**
The Trolox equivalent antioxidant activity (TEAC) was estimated using the $ABTS^+$ system as per the method of Zielinska et al[16] and results were expressed as mg of Trolox equivalent $ABTS^{\cdot+}$ radical scavenging activity per 100 g of sample.

**Vitamin-C content**
Determination of vitamin C was done using reduction of *2, 6* dichlorophenol indophenol (DCPIP) as previously reported Agte et al [17]. Vitamin C equivalents as DCPIP dye reducing potential was expressed as mg per 100 g of sample.

**Statistical analysis**

All tests were performed in triplicates. Results were expressed as mean ± SD of the triplicates for each plant material. The data sets were subjected to further analysis using analysis of variance (ANOVA). The values of $p < 0.05$ were considered significant. Correlation of polyphenols content and their respective antioxidant activity was calculated. Statistical analyses were performed using Win- Excel-2007.

**Arithmetic ranking**

Depending upon the antioxidant potential assessed through a set of antioxidant assays, scores were given to each plant material and the pooled score was also computed to indicate the cumulative antioxidant potential of each plant material. Values were arranged in descending order for each parameter and arithmetic ranks were given.

**Statistical ranking**

For statistical ranking, if the difference between the two successive plant materials was more than the SD for both of them, then they were given different ranks, otherwise same rank was given.

**Point system or Potency based ranking**

For computing the point scores, the values under each column were multiplied by a common factor so that they range between 0 and 10 and then rounded up to one decimal point. Thus ranking for all the 9 parameters were done and pooled ranks were arranged in ascending order; minimum sum represent highest rank and vice versa.

## RESULTS AND DISCUSSION

Table-2 gives the summary of antioxidant activity of 24plant materials for 9 different antioxidant assays. Total polyphenols as mg per 100 g of dry sample were found to be ranging from 77.6 ± 4.7 (M-29) to 22682 ± 543 (M-5b). Total polyphenols content of grape samples (M-5b) were similar as that reported in various studies [18, 19, 20]. The reported total polyphenol contents of the water extract of *P. emblica* was 34.22 ± 1.74 g gallic acid/100g extract. Naik et al., 2005 [21] have also reported 33% gallic acid equivalents of the total phenolic content present in the aqueous extract of this fruit. Vitamin C as mg per 100 g content was found to be ranging from zero (M-19) to 2011 (M-3) in all 24 plant materials. The values for M-3 were found to be matching with the previously reported values i.e. 1.28 % [22].

**Table-2: Antioxidant activities per 100 gm of sample (Lowest and highest values are indicated in bold.**

| mg per 100 g of dry matter of edible food | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Plant material** | **Poly phenols** | **Vit-C** | **TEAC** | **OHSA** | **DPPH** | **NOSA** | **FICA** | **TBARS** | **SOSA** |
| M-1 | 716 | 7.6 | 350 | 7672 | 300 | 288 | 292 | 764 | 334 |
| M-2 | 1276 | 16.8 | 460 | 7480 | 328 | **16280** | 1616 | 2160 | 1088 |
| M-3 | 14960 | **2012** | **50920** | 7484 | **10480** | 3336 | 1220 | **11392** | **4788** |
| M-4 | 1108 | 16.4 | 876 | 7472 | 128 | 416 | 1540 | 3868 | 852 |
| M-5a | 19840 | 258 | 3776 | **23280** | 1184 | 204 | 165.2 | 9212 | 1828 |
| M-5b | **22680** | 94 | 3744 | 17368 | 1244 | 316 | 356 | 7748 | 1712 |
| M-7 | 1572 | 64 | 2096 | 8932 | 2372 | 2008 | 2096 | 4740 | 688 |
| M-8 | 1492 | 69.6 | 708 | 9644 | 1096 | 3476 | **7592** | 2808 | 1116 |
| M-9 | 732 | 44.8 | 133.6 | 6648 | 1928 | 4596 | 2536 | 3324 | 780 |
| M-13 | 1408 | 85.2 | 692 | 10232 | 288 | -800 | -15.2 | 3256 | 884 |
| M-14 | 192 | 27.2 | 200 | 9108 | 464 | 696 | 3508 | 1072 | 420 |
| M-19 | 780 | **0** | 128 | 9492 | 72 | -1320 | **26** | 1340 | 388 |
| M-20 | 334.8 | 1.6 | 188 | 3151.6 | 320 | 1044 | 1272 | 3384 | 720 |
| M-22 | 8920 | 12.8 | 8400 | 10264 | 288 | 1472 | 76 | 4252 | 536 |
| M-24 | 7720 | 36.8 | 1924 | 7528 | 2124 | 2464 | 1696 | 4296 | 2260 |
| M-25 | 956 | 25.2 | 268 | 4084 | 292 | 1104 | 828 | 276 | 536 |
| M-26 | 2420 | 45.6 | 428 | 10644 | 296 | -2000 | 68.4 | 1856 | 832 |
| M-28 a | 2000 | 6 | 1692 | 9300 | 1468 | 768 | 208 | 1684 | 984 |
| M-28 b | 160.8 | 10.8 | 143.6 | 8324 | 480 | 672 | 4056 | 312 | 1028 |
| M-29 | **77.6** | 9.2 | 8.4 | 695.2 | **2.8** | **96** | -68 | 716 | 736 |
| M-30 | 278 | 12 | **4.4** | **636** | 20 | 104 | 14.4 | 1116 | 676 |
| M-31 | 960 | 21.6 | 552 | 8280 | 412 | 956 | 176 | 1696 | 420 |
| M-32 a | 111.2 | 11.2 | 67.6 | 700 | 812 | 120 | 48 | 3044 | 1008 |
| M-32 b | 1256 | 8 | 1240 | 5560 | 39.6 | 1152 | 940 | **176** | **208** |

Ferrous ion chelating activity (FICA) ranged from 14.3 ± 0.76 (M-30) to 7593 ± 325 (M-8). Similarly superoxide radical scavenging activity (SOSA) was found to be ranging from 208 ± 4.3 (M-32b) to 4788 ± 32 (M-3). For TEAC also, M-3 (*Embelica officinalis*) showed the maximum 21213 mg of trolox equivalents per 100 g of dry powder followed by M-22, M-5a and M-5b. TEAC reported for *Embelica officinalis* was 17500 mg/100g of dry powder of water extract [19]. TEAC for M-3 was found to be higher than the reported value which might be because of our unique process of drying and preservation of powdered sample which helped in comparatively less loss of phytochemicals such as polyphenols and Vitamin-C. The grape also showed TEAC similar to the reported values [23].

Hydroxyl (OH) radical scavenging activity was ranging from 639 ± 16 (M-30) to 23280 ±100 (M-5a). Nitric oxide (NO) radical scavenging activity was ranging from 49 ± 0.32 (M-29) to 4596 ± 10.4 (M-9). Few plant materials viz., M-13, M-19 and M-26, showed negative NO radical scavenging activity; this might be due to the pro oxidant effect of the extractable phytochemicals from those plant materials. DPPH radical scavenging activity was ranging from7.5 ± 0.82 (M-29) to 26212 ± 64 (M-3). Activity of inhibiting lipid peroxidation (TBARS) was found to be ranging from 11390 ± 364 (M-3) to 175 ± 6.8 (M-32b).

**Table-3: Correlation matrix of polyphenols content of plant materials with their respective antioxidant activity**

| Assay | correlation | Significance |
|---|---|---|
| **Polyphenols** | 1 | *** |
| **TBARS** | 0.842 | *** |
| **OHSA*** | 0.718 | *** |
| **SOSA** | 0.623 | NS |
| **TEAC** | 0.475 | ** |
| **DPPH** | 0.44 | NS |
| **NOSA*** | -0.005 | ** |
| **FICA** | -0.185 | NS |
| *(*** p<0.001, **p<0.01, NS non-significant)* | | |

**Table-4: Arithmetical ranks of plant material with respect to individual antioxidant parameter**

| Plant material ID | Poly phenols | Vit-C | TEAC | OH SA | DPPH | NO SA | FI CA | TBARS | SOSA | Sum of Rank | Final Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M-1 | 18 | 21 | 15 | 13 | 15 | 17 | 14 | 20 | 23 | 156 | 21 |
| M-2 | 11 | 13 | 13 | 16 | 13 | 1 | 7 | 13 | 6 | 93 | 7 |
| M-3 | 3 | 1 | 1 | 15 | 1 | 4 | 10 | 1 | 1 | 37 | 1 |
| M-4 | 13 | 14 | 9 | 17 | 20 | 15 | 8 | 7 | 11 | 114 | 12 |
| M-5a | 2 | 2 | 3 | 1 | 7 | 18 | 17 | 2 | 3 | 55 | 3 |
| M-5b | 1 | 3 | 4 | 2 | 6 | 16 | 13 | 3 | 4 | 52 | 2 |
| M-7 | 8 | 6 | 5 | 10 | 2 | 6 | 5 | 4 | 16 | 62 | 6 |
| M-8 | 9 | 5 | 10 | 6 | 8 | 3 | 1 | 12 | 5 | 59 | 5 |
| M-9 | 17 | 8 | 20 | 18 | 4 | 2 | 4 | 9 | 13 | 95 | 9 |
| M-13 | 10 | 4 | 11 | 5 | 18 | 22 | 23 | 10 | 10 | 113 | 11 |
| M-14 | 21 | 10 | 17 | 9 | 11 | 13 | 3 | 19 | 20 | 123 | 14 |
| M-19 | 16 | 24 | 21 | 7 | 21 | 23 | 21 | 17 | 22 | 172 | 22 |
| M-20 | 19 | 23 | 18 | 21 | 14 | 10 | 9 | 8 | 15 | 137 | 17 |
| M-22 | 4 | 15 | 2 | 4 | 19 | 7 | 18 | 6 | 18 | 93 | 8 |
| M-24 | 5 | 9 | 6 | 14 | 3 | 5 | 6 | 5 | 2 | 55 | 4 |
| M-25 | 15 | 11 | 16 | 20 | 17 | 9 | 12 | 23 | 19 | 142 | 18 |
| M-26 | 6 | 7 | 14 | 3 | 16 | 24 | 19 | 14 | 12 | 115 | 13 |
| M-28 a | 7 | 22 | 7 | 8 | 5 | 12 | 15 | 16 | 9 | 101 | 10 |
| M-28 b | 22 | 18 | 19 | 11 | 10 | 14 | 2 | 22 | 7 | 125 | 15 |
| M-29 | 24 | 19 | 23 | 23 | 24 | 21 | 24 | 21 | 14 | 193 | 24 |
| M-30 | 20 | 16 | 24 | 24 | 23 | 20 | 22 | 18 | 17 | 184 | 23 |
| M-31 | 14 | 12 | 12 | 12 | 12 | 11 | 16 | 15 | 21 | 125 | 16 |
| M-32 a | 23 | 17 | 22 | 22 | 9 | 19 | 20 | 11 | 8 | 151 | 20 |
| M-32 b | 12 | 20 | 8 | 19 | 22 | 8 | 11 | 24 | 24 | 148 | 19 |

**Table-5: Statistical ranking**

| Plant material ID | Poly phenols | Vit-C | TEAC | OHSA | DPPH | NOSA | FICA | TBARS | SOSA | Sum of Rank | Final Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M-1 | 4 | 2 | 2 | 3 | 2 | 3 | 4 | 4 | 3 | 27 | 9 |
| M-2 | 4 | 2 | 2 | 3 | 2 | 1 | 3 | 3 | 3 | 23 | 6 |
| M-3 | 2 | 1 | 1 | 3 | 1 | 2 | 3 | 1 | 1 | 15 | 1 |
| M-4 | 4 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 26 | 8 |
| M-5a | 1 | 2 | 2 | 1 | 2 | 3 | 4 | 1 | 2 | 18 | 2 |
| M-5b | 1 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 19 | 3 |
| M-7 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 3 | 3 | 23 | 6 |
| M-8 | 3 | 2 | 2 | 3 | 2 | 2 | 1 | 3 | 3 | 21 | 4 |
| M-9 | 4 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 23 | 6 |
| M-13 | 3 | 2 | 2 | 3 | 2 | 3 | 4 | 3 | 3 | 25 | 7 |
| M-14 | 4 | 2 | 2 | 3 | 2 | 3 | 2 | 4 | 3 | 25 | 7 |
| M-19 | 4 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 3 | 28 | 10 |
| M-20 | 4 | 2 | 2 | 4 | 2 | 3 | 3 | 3 | 3 | 26 | 8 |
| M-22 | 2 | 2 | 2 | 3 | 2 | 2 | 4 | 3 | 3 | 23 | 6 |
| M-24 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 3 | 2 | 22 | 5 |
| M-25 | 4 | 2 | 2 | 4 | 2 | 3 | 3 | 4 | 3 | 27 | 9 |
| M-26 | 3 | 2 | 2 | 3 | 2 | 3 | 4 | 4 | 3 | 26 | 8 |
| M-28 a | 3 | 2 | 2 | 3 | 2 | 3 | 4 | 4 | 3 | 26 | 8 |
| M-28 b | 4 | 2 | 2 | 3 | 2 | 3 | 2 | 4 | 3 | 25 | 7 |
| M-29 | 4 | 2 | 2 | 4 | 3 | 3 | 4 | 4 | 3 | 29 | 11 |
| M-30 | 4 | 2 | 2 | 5 | 3 | 3 | 4 | 4 | 3 | 30 | 12 |
| M-31 | 4 | 2 | 2 | 3 | 2 | 3 | 4 | 4 | 3 | 27 | 9 |
| M-32 a | 4 | 2 | 2 | 4 | 2 | 3 | 4 | 3 | 3 | 27 | 9 |
| M-32 b | 4 | 2 | 2 | 4 | 3 | 2 | 3 | 4 | 3 | 27 | 9 |

*Values were arranged in descending order for each parameter; mean and SD were calculated. If the difference between the two successive plant materials found more than the SD both of them were given different ranks, if there is no difference- same rank was given. Thus ranking for all the 9 parameters were done and pooled ranks were arranged in ascending order; minimum sum represent highest rank and vice versa.*

**Table-6: Point system or Potency based ranking of plant materials based on their 9 antioxidant parameters**

| Plant material | Poly | Vit-C | ABTS | OH | DPPH | NO | FICA | TBARS | SOSA | Pooled points |
|---|---|---|---|---|---|---|---|---|---|---|
| M-1 | 0.3 | 0 | 0.1 | 3.3 | 0.3 | 0.2 | 0.4 | 0.7 | 0.7 | 6.0 |
| M-2 | 0.6 | 0.1 | 0.1 | 3.2 | 0.3 | 10 | 2.1 | 1.9 | 2.3 | 20.6 |
| M-3 | 6.6 | 10 | 10 | 3.2 | 10 | 2 | 1.6 | 10 | 10 | 63.4 |
| M-4 | 0.5 | 0.1 | 0.2 | 3.2 | 0.1 | 0.3 | 2 | 3.4 | 1.8 | 11.6 |
| M-5a | 8.7 | 1.3 | 0.7 | 10 | 1.1 | 0.1 | 0.2 | 8.1 | 3.8 | 34 |
| M-5b | 10 | 0.5 | 0.7 | 7.4 | 1.2 | 0.2 | 0.5 | 6.8 | 3.6 | 30.9 |
| M-7 | 0.7 | 0.3 | 0.4 | 3.8 | 2.3 | 1.2 | 2.8 | 4.2 | 1.4 | 17.1 |
| M-8 | 0.7 | 0.3 | 0.1 | 4.1 | 1 | 2.1 | 10 | 2.5 | 2.3 | 23.1 |
| M-9 | 0.3 | 0.2 | 0 | 2.9 | 1.8 | 2.8 | 3.3 | 2.9 | 1.6 | 15.8 |
| M-13 | 0.6 | 0.4 | 0.1 | 4.4 | 0.3 | 0 | 0 | 2.8 | 1.8 | 10.4 |
| M-14 | 0.1 | 0.1 | 0 | 3.9 | 0.4 | 0 | 4.6 | 0.9 | 0.9 | 10.9 |
| M-19 | 0.3 | 0 | 0 | 4.1 | 0.1 | 0 | 0 | 1.2 | 0.8 | 6.5 |
| M-20 | 0.1 | 0 | 0 | 1.4 | 0.3 | 0.6 | 1.7 | 2.9 | 1.5 | 8.6 |
| M-22 | 3.9 | 0.1 | 1.7 | 4.4 | 0.3 | 0.9 | 0.1 | 3.7 | 1.1 | 16.2 |
| M-24 | 3.4 | 0.2 | 0.4 | 3.2 | 2 | 1.5 | 2.2 | 3.8 | 4.7 | 21.4 |
| M-25 | 0.4 | 0.1 | 0.1 | 1.7 | 0.3 | 0.7 | 1.1 | 0.2 | 1.1 | 5.7 |
| M-26 | 1.1 | 0.2 | 0.1 | 4.6 | 0.3 | 0 | 0.1 | 1.6 | 1.7 | 9.7 |
| M-28 a | 0.9 | 0 | 0.3 | 4 | 1.4 | 0.5 | 0.3 | 1.5 | 2 | 10.9 |
| M-28 b | 0.1 | 0.1 | 0 | 3.6 | 0.5 | 0.4 | 5.3 | 0.3 | 2.1 | 12.4 |
| M-29 | 0 | 0.1 | 0 | 0.3 | 0 | 0.1 | 0 | 0.6 | 1.5 | 2.6 |
| M-30 | 0.1 | 0.1 | 0 | 0.3 | 0 | 0.1 | 0 | 0.9 | 1.4 | 2.9 |
| M-31 | 0.4 | 0.1 | 0.1 | 3.6 | 0.4 | 0.6 | 0.2 | 1.5 | 0.9 | 7.8 |
| M-32 a | 0.05 | 0.1 | 0 | 0.3 | 0.8 | 0.1 | 0.1 | 2.7 | 2.1 | 6.3 |
| M-32 b | 0.6 | 0 | 0.2 | 2.4 | 0 | 0.7 | 1.2 | 0.2 | 0.4 | 5.7 |

*The values under each column are multiplied by a common factor so that they range between 0 and 10 and rounded up to one decimal point.*

ANOVA for individual antioxidant assay of all 24 plant materials were found to be highly significant ($p<0.001$), which indicated the huge diversity of polyphenols content, vitamin-C content and antioxidant activity of different plant materials. The values of antioxidant activities of M-3 and M-24 are in agreement with other studies [24, 25].

Polyphenol content of plant materials showed significant ($p<0.01$) positive correlations with ITBARS (0.842), SOSA (0.623), OHSA (0.718), TEAC (0.475), DPPH radicals scavenging activities (0.44) whereas, negative but non-significant correlations were given by FICA (-0.185) and NO radical scavenging activity (-0.005) (table-3). Reported *in vitro* studies have also shown significant linear correlation between concentration of total phenolic compounds and antioxidant capacity [26, 27]. This means that total polyphenols content of plant materials, was one of the major determinants for the ITBARS, SOSA, OH, ABTS and DPPH radical scavenging activities. However, polyphenol levels are not seen to be influencing the FICA and NO radical scavenging activity.

In this study, the units of expression for each of the antioxidant parameter were different as also the magnitude of observed values for their assays. Mere pooling of the values or finding average value would not have been appropriate since the final value would be dominated by the assay producing values of bigger magnitude. As a first step, a simple arithmetic approach of arranging the individual members of each food group in increasing order and giving these an increasing rank was made (Table 4). Among the present study materials, *M-29 i.e. Citrus paradise, M-30 i.e. Citrus sinensis* and M-19 i.e. *Dioscorea bulbifera* were the low ranking foods, while grapes (M-5), gooseberry (M-3), bael (M-24), were the top rankers. But, this simple scheme of ranking gives two different ranks for the materials with very close values. Statistical ranking system avoids this error as these are computed considering the standard deviation of the mean (Table 5). However, both approaches were found to be inadequate since these do not offer the score giving quantitative estimate of relative differences in observed values of plant materials. For example, if orange is the poor fruit having rank 23 and gooseberry is the rich fruit having rank 3, it would not give any quantitative estimate as to what is the equivalence of eating one gooseberry in terms of oranges or the relative potency of orange with respect to gooseberry.

Thus the ideal tool needs to have the point system and instead of ranks, individual food needs to be given scores based on point system.

As a next step therefore, each of the values for antioxidant indices were so modified with constant multiplier that highest value would get the score of 10 and all the values would lie between 0 to10. The values of AQI were then rounded to one decimal point. Table-6 gives scores based on point system or potency based ranking of plant materials for 9 antioxidant parameters. This approach could rank the plant materials and also showed their correct relative ratios due to multiplication with constant number to all the values and uniformity in the range of numbers.

To the best of our knowledge, this is the first attempt of ranking nine different antioxidant parameters of the 24 different edible plant materials assayed under similar experimental conditions. Further, the results of ranking are useful to identify relative potency of antioxidant enriched foods to be further used in development of functional food products.

## CONCLUSION

The point based system could better discriminate the foods quantitatively for their overall antioxidant potential than the arithmetic or statistical ranking. Secondly, present results indicated that estimation of polyphenols alone could be sufficient as biomarker from which TBARS, TEAC, SOSA, OHSA and DPPH radical scavenging activities could be predicted.

**Acknowledgments**
The authors wish to thank Director, Agharkar Research Institute, Pune; for making available all necessary facilities to accomplish this work. This work was carried out under the research project funded by Department of Biotechnology, Government of India.

## REFERENCES

[1] DG Lindsay; SB Astley. *Mol. Aspects Med.*, **2002**, 23, 1–38.
[2] AM Gómez-Caravaca; M Gómez-Romero; D Arráez-Román; A Segura-Carretero; A Fernandez-Gutierrez. *J. Pharma. Biome. Anals.*, **2006**, 41, 1220–1234.
[3] ET Kennedy; J Ohls; S Carlson; K Fleming. *J. Am. Diet. Assoc.*, **1995**, 95(10), 1103-1108.
[4] SM Lluı´s; R Lourdes; N Joy; MO Rosa; G Alicia; PR Carmen; A Javier. *Public Health Nutrition.*, **2004**, 11, 7(7), 931-935.

[5] AV Badarinath; K Mallikarjuna Rao; CC Madhusudhana; S Ramakanth; TVS Rajan; K Gnanaprakash. *Int. J. Pharm.Tech. Research.,* **2010**, 2(2), 1276-1285.
[6] H Xiuzhen; S Tao; L Hongxiang. *Int. J. Mol. Sci,*. **2007**, 8, 950-988.
[7] T Tsuda; M Watanabe; K Ohshima; SC Norinobu; SW Choi; S Kawakishi; T Osawa. *J. Agric. Food Chem.*, **1994**, 42(11), 2407–2410.
[8] JM Gutteridge. *Biochemical Journal.*, **1984**, 224, 761-767.
[9] DC Garrat. The quantitative analysis of drugs. 3rd Ed., Chapman and Hall ltd Japan, **1964**, 3, 456- 458.
[10] R Govindarajan; M Vijayakumar; AK Rawat; S Mehrotra. *Indian J. Exp. Biol.,* **2003**, 41(8), 875-879.
[11] T Ariga; M Hamano. *Agric. Biol. Chem.*, **1990**, 54, 2499-2504.
[12] VL Singleton; JA Rossi. *Am. J. Enol. Vitic.*, **1965**. 6, 144-158.
[13] CT Saucier; AL Waterhouse. *J. Agric. Food Chem.*, **1990**, 47(11), 4491-4494.
[14] F Yamaguchi; T Ariga; Y Yoshimura; H Nakazawa. *J. Agric. Food Chem.,* **2000**.48, 180-185.
[15] M Nishikimi; N Appaji; K Yagi. *Biochem. Biophys. Res. Commun.,* **1972**, 46, 849-854.
[16] D Zielinska; ND Szawara; A Ornatowska; W Wiczkowski. *J. Agric. Food Chem.*, **2007**, 55(24), 9891-9898.
[17] VV Agte; MK Gokhale; KM Paknikar; SA Chiplonkar. *Plant Foods Hum. Nutr.* (*Formerly Qualitas Plantarum*)**, 1995,** 48, 149-158.
[18] RY Gan; XR Xu; FL Song; L Kuang; HB Li. *J. Med. Plants Res.,* **2010**, 4(22), 2438-2444.
[19] J Charoenteeraboon; C Ngamkitidechakul; N Soonthornchareonnon; K Jaijoy; S Sireeratawong. *J. Sci. Tech.*, **2010**, 32(6), 599-604.
[20] L Fu; BT Xu; XR Xu; RY Gan; Y Zhang; EQ Xia; HB Li. *Food Chem.* **2011**, 129(2), 345–350.
[21] GH Naik; KI Priyadarsini; RG Bhagirathi; B Mishra; KP Mishra; MM Banavalikar; M Hari. *Phytotherapy Res.*, **2005**, 19, 582– 586.
[22] P Scartezzini; F Antognoni; MA Raggi; F Poli; C Sabbioni. *J. Ethnopharmacol.*, **2006**, 104(1-2), 113–118.
[23] Y Yilmaz; RT Toledo. *J Food Compost. Anal.,* **2006**, 19(1), 41–48.
[24] MA Ebrahimzadeh; F Pourmorad; AR Bekhradnia. *Afr.* J. *Biotechnol.,* **2008,** 7(18), 3188-3192.
[25] KV Tarwadi; VV Agte. *J. Sci.* Food *Agric.,* **2005**, 85, 1469-1476.
[26] Z Hodzic; H Pasalic; A Memisevic; M Srabovic; M Saletovic; M Poljakovic; M. *Eur. J. Sci. Res.*, **2009**, 28(3), 471-477.
[27] MP Kahkonen; AI Hopia; HJ Vuorela; JP Rauha; K Pihlaja; TS Kujala; M Heinonen. *J. Agric. Food Chem.*, **1999**, 47, 3954-3962.