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A photometric permanganate reduction assay for evaluating antioxidants

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Abstract: A photometric assay has been optimized for assaying antioxidants on the basis of their permanganate reducing activity (PRA), expressed as µmole permanganate reduced µmole^{-1} test agent. Incubating a standard mass of acidified potassium permanganate with varying masses of test antioxidant for 20 through 70 minutes causes a linear reduction in absorbance at 540 nm. The regression analysis of permanganate reduced for each mass of antioxidant provides PRA of the test agent in terms of its regression coefficient. The assay p*er se* has been linear over 0.2 through 2 µmole permanganate (b ± se, 0.464 ± 0.003, ca. 30 min; 0.451 ± 0.003, ca. 60 min) with improved regression over 0.2 through 1 µmole correspondingly as, 0.471 ± 0.004 and 0.461±0.007 ; ((r^2 = 0.999, n=6) showing overall mean COV 1.5 per cent. The assay has been employed to evaluate flavonoids, phenolics, organic acids including ascorbic acid, sulfur-containing compounds, curcumin, DMSO, glucose, sucrose, mannitol, and water soluble solvents viz., ethanol, methanol and acetone for their PRA. The assay provides an inexpensive, simple tool to screen test agents for their antioxidant activity.
Key words: Permanganate reduction, Antioxidants, Flavonoids, Phenolics, Ascorbic acid, Curcumin.

Introduction

Implications of reactive oxygen species including free radicals in health and disease[1], and the methods for finding antioxidants for their amelioration are well recognized[2-7]. Cost-effective and simple methods are preferable to complex and sophisticated procedures as former are well within the operational reach of common laboratories. Acidified potassium permanganate constitutes a strong redox system[8] capable of detecting potential antioxidants. It has been employed as a spot-test for all reducing agents, forming a yellow spot against a pink background[9]. The titrimetric assay has been employed for assaying reducing agents such as iron (II) compounds and oxalates; or for determining reducing potential of otherwise oxidizing agents viz., hydrogen peroxide and nitrite[10, 11]. The titrimetric assay has been used to assay catalase by measuring consumption of hydrogen peroxide[12-14] and to screen plant extracts for their antioxidant potentials[15]. The titrimetric procedure has been advocated as a simple and reliable method for determination of total antioxidants and antioxidant capacity of human serum[16]. Spectrophtometric assay, based on electronic signaling protocol recorded at 535 nm at varying concentrations of acidified permanganate has been used for assessing antioxidant activity of plant extracts using ascorbic acid as a standard reductant[17-18]. Permanganate based simple[19] to advanced titrimetry[20], spectrophotometry[21] and chemiluminescence signal detection (CSD) methods[22] have been employed for assaying ascorbic acid in vegetables[19] and in pharmaceuticals[20-22]. The results obtained have shown good agreement to those of iodometric titration[22], offering advantages of fastness and cost-effectiveness[19, 20]. The

greater focus in the recent past has been on using acidified permanganate based CSD coupled to HPLC. The technique has proved a versatile tool to detect not only antioxidants but also pharmaceuticals, pesticides and pollutants[23]. The method has been used to measure total antioxidant capacity of fruit juices and teas[24], to monitor antioxidant potential of polyphenols in cultured cells[25] and for screening antioxidants in complex matrices derived from plant samples[26]. However, permanganate based conventional spectrophotometric assay has not been employed beyond assaying of ascorbic acid.

In view of foregoing observations, and on the basis of experience of having satisfactorily used permanganate titrimetry in assaying antioxidant potential of some flavonoids and dog rose extracts[27], the present work was mooted. The experiments were designed to optimize spectrophotometric method using acidified KMNO4 for assaying and comparing chemically diverse test agents for antioxidant potentials. The assay employed a standard test parameter, permanganate reduction activity expressed with unit as µmoles permanganate reduced µmole^{-1} test agent as test criterion.

Experimental

The experiments were carried out at an ambient temperature of 18.0 ± 0.8 ^{0}C. The drugs and chemicals used were of standard purity and quality obtained from reputed sources in India. The samples developing discolorations or turbidity during incubation were centrifuged at 8000 rpm for 5 minutes, and transparent centrifugate was monitored for absorbance at 540 nm. The centrifugation was initiated about 10 minutes prior to monitoring. Spectrophtometric measurements were made with UV-Visible Spectrophotometer, Model UVmini-1240 (Shimadzu Corporation, Japan).

Reagents and test agents

Potassium permanganate solution: $KMNO_4$ 800 mg was dissolved in 250 ml water, boiled for 15 to 30 minutes, cooled to room temperature, filtered vide glass-wool, and then standardized against standard oxalic acid 0.05 M (prepared by dissolving 700 mg oxalic acid dihydrate in 111 mL water) by titrimetry while kept warmed to about 70^{o}C as per recommended procedure[8,10,11]. The stock solution was frequently checked for change in molarity, and diluted appropriately in water at the time of assay.

Dilute sulfuric acid solution: 10 % (v/v) concentrated sulfuric acid in water.

Rutin trihydrate, quercetin dihydrate and morin hydrate: The requisite dilutions were made from their respective stock solutions made in methanol containing respectively as 2.0, 5.0 and 2.0 µmole of the flavonoid mL^{-1} methanol. The minimum labeled purity for the flavonoids has been respectively as 90, 98 and 95 per cent.

Diosmin and daflon: Laboratory standards for synthetic diosmin and for daflon were prepared from their respective pharmaceutical tablets: Venex-500 (Elder Pharmaceuticals Ltd., Mumbai) (labeled mass 500 mg synthetic diosmin per tablet) and Daflon (Serdia Pharmaceuticals Ltd., Mumbai) (labeled contents 90 % diosmin and 10 % hesperidin) as per standard procedure[28] to the strength of 0.25 % (w/v) equivalent to 4.1µmole flavonoid mL^{-1} 0.1 M NaOH. The requisite dilutions were made in 0.1 M NaOH from these stock solutions.

Ascorbic acid, thioglycollic acid (TGA), thiourea (TU), dimethyl sullfoxide (DMSO), phenol, resorcinol, hydroquinone, pyrogallol, guaiacol, catechol, and gallic acid solutions: The requisite dilutions were made in water from respective stock solutions made in water as 100 (TU, DMSO), 20 (TGA) and 25 µmole mL^{-1} (others).

Curcumin: Working solution contained 0.1 µmole curcumin mL^{-1} methanol.

Hydrogen peroxide solution: Working solution of hydrogen peroxide contained 1 µmole hydrogen peroxide mL^{-1} water was prepared from stock solution of 55 µmole mL^{-1} that has been standardized against standard potassium permanganate solution.

Glucose, sucrose, mannitol solutions: The stock solutions were made in water to provide 100 µmole sucrose mL^{-1} each.

Citric, tartaric and oxalic acid solutions: These were prepared in water as 0.05 M stock solutions. The working solutions were made by dilution in water at the time of use to provide respectively 0.5, 10 and 5 µmole organic acid mL^{-1} water.

Analytical technique

The assay for linearity setting of permanganate was standardized in aqueous medium using 0, 0.2 through 2 µmole of potassium permanganate in 4 mL water. Each sample was added 1 mL of dilute sulfuric acid, mixed well and allowed to stand at room temperature for about 30 and 60 minutes. The absorbance was monitored at 540 nm. Typical test assay included a mixture of 1 mL each of potassium permanganate (1 µmole) and dilute sulfuric acid. The samples were added test sample in appropriate aliquot of diluent made 3 mL with water. A simultaneously run standard matched in diluent contained potassium permanganate as 0, 0.2, 0.6 and 1 µmole. The samples were incubated at room temperature for appropriate time period as demanded by response of test agents from trial experiments. The discolored or opalescent samples were centrifuged. The transparent samples or centrifugates were monitored at 540 nm at about 30 minutes in general, 20 to 25 minutes in case of oxalic acid and 60 to 70 minutes in case of glucose, sucrose, mannitol and citric acid. Permanganate reduction in test samples was measured in terms of simultaneously run calibration curve. Standard samples were matched for appropriate aliquot of solvent that was used as a diluent for the test substance.

Test agents

Test agents employed during the study have included diverse chemical agents with known antioxidant effects, and those most likely to come in the extracts obtained from Indigenous medicinal plants. Appropriate mass of test agent was chosen by conducting trial experiment so as to get measurable absorbance within the linear range of potassium permanganate under test conditions.

Besides, organic solvents such as ethanol, methanol and acetone were also screened for their actions on the conduct of the assay. The same were necessitated as these are commonly employed as solvents for test extracts, and in particular in the present context methanol served as a solvent for curcumin, morin, quercetin, rutin and guaiacol.

Calculations

The data were subjected to routine statistical analysis. Permanganate reduction is a redox reaction that involves participation of five electrons from permanganate, and consequently potential antioxidants cause quantitative reduction of acidified standard permanganate solution. Therefore test substances were evaluated for antioxidant potential by their permanganate reducing activity (PRA) expressed as µmole permanganate reduced $\mu mole^{-1}$ test agent. Estimation of permanganate reduction for each mass level of test agent used was made with respect to regression parameters obtained from simultaneously run calibration assay (A). The regression analysis was performed on these values to obtain regression coefficient that indicated permanganate reducing activity of test agent. This regression estimate was denoted as calibrated estimate. At the end of the assay, estimations for various masses of test agent were also made with respect to absorbance of standard at single mass level i.e. 1 µmole from calibration data (B). The mean of the values obtained was taken as direct estimate. The calibrated and direct estimates for each test agent, excluding solvents and very weakly acting antioxidants, were analyzed by paired-t test to appreciate difference if any between the two estimates.

The following formulae have been used for estimating permanganate reducing activity:

PRA, µmole permanganate reduced $\mu mole^{-1}$ test agent

$= ([C - ((T-c)/b)]/M)*Factor$ (A)

$= ([C*(1 - (T/S)] /M)*Factor$ (B)

Where

T is absorbance due to standard mass of permanganate in presence of test substance

S is absorbance due to standard mass of the permanganate in absence of test substance

C is mass of permanganate used in µmole with test agent; M is mass of test substance used in µmole(s); c and b are respectively y-intercept value and regression estimate from regression analysis data; Factor = 1000, to convert µmoles into nmoles in case of very weak test agents else its value is unity. PRP, permanganate reducing potential refers to permanganate mass reduced in a given sample at a given mass of test agent. This is provided by the same formulae without making any division by M.

Results and Discussion

Photometric permanganate assay

Table 1 Linear relation between absorbance with varying mass of potassium permanganate

$KMNO_4$, µmole	Absorbance at 540 nm		Per cent decrease[a]
	30-35 min	60-65 min	
0.2	0.083 ± 0.001	0.070 ± 0.001	15.7
0.6	0.268 ±0.001	0.247 ±0.001	7.8
1.0	0.460 ± 0.002	0.439 ± 0.002	4.6
2.0	0.917 ± 0.002	0.880 ± 0.003	4.0
Statistical features			
r ± S.E.	0.9999 ± 0.0001		
b ± S.E.[b]	0.464 ± 0.003	0.451 ± 0.003	2.8
c	-0.0084	-0.0193	-
COV (%)	1.4	1.6	-

[a] $p<0.01$; COV, coefficient of variation;
[b] The regression coefficient unit, absorbance $\mu mole^{-1}$ permanganate
The values are mean ± S.E. of six observations each

As evident (Table 1), the permanganate assay has been linear over 0.2 through 2 µmole $KMNO_4$ both with 30 and 60 minute incubation period. There has been somewhat better regression over 0.2 through 1 µmole correspondingly as, 0.471 ± 0.004 (0.9999 ± 0.0001) and 0.461±0.007 (0.9997±0.0003). Regression estimate decreased by about 3 % with increase in incubation from 30 to 60 minutes. The mean absorbance values at 30 minutes have been significantly better, and have shown per cent decrease from about 16 to 4 per cent with increase in permanganate mass ($r = -0.81$, $p < 0.01$) with better relation over 0.2 through 1 µmole ($r = -0.97$). The pooled data., based on random experiments throughout the study. has revealed similar pattern with mean absorbance values at 0.2, 0.6, 1 and 2 µmole $KMNO_4$, with incubation periods varying from 20 through 70 minutes, respectively as 0.091 ± 0.001, 0.271 ± 0.002, 0.455 ± 0.002 and 0.938 ± 0.001 (n = 21 to 40) with perfect linearity (r ± S.E. = 0.999±0.001; b ±S.E. = 0.472 ± 0.003).

The standard permanganate assay has shown COV ranging from 0.5 through 2.95 % (mean 1.4 %) for ca. 30 minutes and 0.84 through 3.5 (mean 1.6 %) for ca. 60 minute (grand mean 1.5 %). The randomized assays (n= 40) spread over the study period have shown COV of about 4.5 %. Overall mean COV for test agent assays (n=27) has been 1.62 with range 0.6 through 2.7 pr cent.

Permanganate reduction activity of test antioxidants

Test agents in the study have been selected on the basis of their recognized reducing and antioxidant potentials, and on the consideration of having one or more of the following functional moieties in their structures those being predominantly present in majority of the antioxidants: -OH, -C=O, -COOH, $-OCH_3$, - S with C, O or H. Of test agents, sucrose and phenol have shown no obvious role as antioxidants. Hydrogen peroxide, with no obvious antioxidant role, was incorporated in this study for two reasons: (i)it is being standardized routinely by permanganate reduction , and (ii) it constitutes part of catalase assay by $KMNO_4$. Phenol served as a simplest class prototype of phenolics while sucrose was used as it is comparable for its

hydroxyl functions present in rutinose, a disaccharide present in glycoside flavonoids viz., diosmin, hesperidin and rutin.

Trial experimentations were required for each test agent to choose the appropriate incubation time period and the proper concentration range within the range of permanganate absorbance values. Incubation time period of about 30 minutes was found most appropriate for majority of the test agents**,** and brief incubation for about 20 minutes was found satisfactory for oxalic acid. In fact during one trial experimentation, absorbance with single mass level of oxalic acid was monitored at 5, 10, 15, 30 and 60 minutes. The mean absorbance of standard (1µmole) decreased from 0.416±0.002 (5 minute) to 0.402 ±0.002 (15 minute) to 0.391 ± 0.003 (60 minute). The corresponding absorbance values for the standard in presence of 0.6 µmole oxalic acid were found to be 0.320 ±0.002 (5 minute), 0.306 ±0.002 (15 minute) and 0.311 ±0.002 (60 minute) (n=5 each). Per cent reduction in mean absorbance with respect to standard ranged over the period from 20.5 to 24.4 (23.1 ±0.6 %, n=5). It was found satisfactory to run the assay for limited period of about 20 to 25 minutes. Citric acid, sugars and mannitol have reacted sluggishly. They failed to respond at all by 30 minutes (citric acid) or responded weakly (with others). Therefore, extended incubation for about 60 to 70 minutes was required for these test agents.

Comparison of random data estimates made by calibration data versus those generated by direct estimations revealed no significant difference between the means ($p>0.1$, n= 20 different assays, each assay with 3 to 4 mass points, paired t-test). The calibrated mean with range of PRA data points from 0.4 through 26.4 was 6.8 ±1.6 and directly estimated mean with range of PRA data points from 0.4 to 25 was found to be 6.7 ±1.4 showing only about 1.6 % deviation ($p>0.1$, unpaired t-test). Therefore, one is at liberty to use either procedure for estimation. For the present study the tabulated data is based on calibrated assays. The calibration assay has advantage of serving as a check to the conduct of the test assay for monitoring any sensitivity alterations.

Test agents could be categorized on the basis of magnitude of response into four groups:

Antioxidant category	***PRA range, µmole***	***Agents***
Most active	> 4.0	flavonoids, resorcinol, DMSO, curcumin
Moderately active	2.0 through 4.0	phenolics in general
Weakly active	0.2 through 2.0	thiourea, TGA, ascorbic acid, oxalic acid, citric acid, H_2O_2
Poorly active	<0.2	tartaric acid, sugars, mannitol

As evident from Tables (2 and 3)**,** flavonoids as a class and resorcinol from phenolics are most active along with curcumin and DMSO from non-flavonoids. Assuming regression estimate of diosmin as X, the relative potency of most active category with approximate order of potency within parenthesis may be put as morin (3.1X)>rutin (2.4 X)>quercetin (2X)>curcumin (1.4X)> DMSO(1.2X) >diosmin (1X) ≥Daflon (0.95X). Rutin is a glucoside of quercetin (containing disaccharide, rutinose), and therefore increased potency of rutin is presumably contributed by the rutinose component. Test flavonoids are characterized by presence of phenolic residues (resorcinol, catechol and guaiacol) in their structures. Consequently; it was desired to investigate their relative antioxidant potential vis-à-vis flavonoids. Hydroquinone was incorporated as structural analog of catechol and resorcinol (all di-hydroxy benzenes) and recognized for its antioxidant potential[29], pyrogallol and gallic acid for their known reducing and antioxidant potentials. Overall potency of phenolic residues has been in the range of about 40 to 50 % of diosmin with order: resorcinol (0.5X)> phenol (0.46X) ≥guaiacol (0.45X) ≥ gallic acid (0.44X)>pyrogallol (0.41 X)>catechol (0.36 X). Resorcinol is common component in all test flavonoids (one residue in all and two in morin) while catechol is present in quercetin and rutin and guaiacol in diosmin and daflon. These observation indicate their significant contribution to the overall activity of flavonoids. Organic acids as a class are weakly active with order of potency: citric acid (0.23 X)>thioglycollic acid (0.21X)>ascorbic acid (0.15 X)>oxalic acid (0.05 X). The potency of thiourea (0.1X) is intermediate to those of ascorbic and oxalic acids. Citric acid has been about 1.1 times as potent as TGA while TGA has been about twice as potent as TU and about 1.4 times as potent as ascorbic acid.

Table 2: Permanganate reducing potential of flavonoids, phenolics, sulfur- containing agents and organic acids

Test agent	Mass, µmole	PRP[a], µmole	r ± se	b[b] ± se	COV, %
Flavonoids					
Morin	0.005	0.140±0.004	0.995±0.005	21.3 ± 1.1	2.04
	0.01	0.273±0.004			
	0.02	0.536±0.001			
	0.04	0.893±0.003			
Rutin	0.01	0.137±0.002	0.995±0.006	20.6 ± 1.2	1.85
	0.02	0.403±0.006			
	0.04	0.768±0.002			
Quercetin	0.01	0.103±0.002	0.996±0.004	15.6 ± 0.7	1.39
	0.02	0.304±0.002			
	0.04	0.637±0.001			
	0.06	0.887±0.004			
Diosmin	0.02	0.240±0.007	0.999±0.001	8.6 ± 0.2	2.39
	0.04	0.399±0.005			
	0.06	0.582±0.006			
	0.08	0.753±0.003			
Daflon	0.02	0.184±0.005	0.999±0.001	8.2 ± 0.3	1.47
	0.04	0.346±0.002			
	0.06	0.485±0.003			
	0.08	0.683±0.009			
Phenolics					
Phenol	0.05	0.212±0.001	0.997±0.003	3.6 ± 0.1	0.82
	0.10	0.418±0.002			
	0.15	0.604±0.003			
	0.20	0.747±0.004			
Catechol	0.05	0.138±0.001	0.999±0.001	3.1± 0.1	0.75
	0.10	0.283±0.001			
	0.20	0.600±0.002			
	0.30	0.897±0.005			
Resorcinol	0.05	0.302±0.003	0.997±0.003	5.2 ±0.2	0.83
	0.10	0.595±0.002			
	0.15	0.823±0.003			
Hydroquinone	0.05	0.202±0.002	0.999±0.001	3.2 ± 0.1	2.04
	0.10	0.378±0.006			
	0.15	0.519±0.008			
	0.20	0.682±0.004			
Guaiacol	0.04	0.107±0.001	0.998±0.002	3.9 ± 0.1	1.51
	0.08	0.291±0.003			
	0.12	0.431±0.004			
	0.16	0.575±0.006			
yrogallol	0.05	0.172±0.001	0.999±0.001	3.5 ± 0.1	0.61
	0.10	0.338±0.002			
	0.15	0.533±0.001			
	0.20	0.692±0.001			
Gallic acid	0.05	0.235±0.003	0.999±0.001	3.8 ± 0.1	1.55
	0.10	0.405±0.002			
	0.15	0.618±0.005			
	0.20	0.799±0.008			
Sulfur containing agents					
DMSO	0.025	0.278±0.004	0.995±0.005	8.9 ± 0.4	1.19
	0.050	0.533±0.003			
	0.075	0.777±0.005			
	0.100	0.935±0.001			
TGA	0.05	0.107±0.002	0.999±0.001	1.8 ± 0.04	1.41

	0.10	0.192±0.001			
	0.15	0.293±0.001			
	0.20	0.375±0.002			
Thiourea	0.2	0.166±0.001	0.998±0.002	0.82± 0.03	2.43
	0.3	0.253±0.007			
	0.4	0.342±0.006			
	0.5	0.410±0.002			
Organic acids					
Ascorbic acid	0.1	0.119±0.001	0.999±0.001	1.31± 0.03	1.79
	0.2	0.249±0.004			
	0.3	0.387±0.002			
	0.4	0.510±0.006			
Citric acid	0.1	0.359±0.004	0.998±0.002	1.95±0.06	0.66
	0.2	0.563±0.001			
	0.3	0.777±0.001			
	0.4	0.937±0.001			
Oxalic acid	0.5	0.270±0.005	0.998±0.002	0.40±0.01	2.29
	1.0	0.492±0.006			
	1.5	0.670±0.007			
Tartaric acid	3	0.295±0.002	0.992±0.008	87 ± 6[c]	1.96
	4	0.383±0.007			
	6	0.601±0.003			
	8	0.719±0.011			

COV, coefficient of variation; [a] PRP, permanganate reducing potential as, µmole permanganate reduced for given mass of test agent estimated by permanganate calibration standard; [b] Index of permanganate reducing activity (PRA), µmole or nmole[c] permanganate reduced µmole^{-1} test agent;

The values are mean ± S.E. of 3 observations each.

Table 3: Permanganate reducing potential of sugars, mannitol, curcumin and hydrogen peroxide

Test agent	**Mass, µmole**	**PRP[a], µmole**	**r ± se**	**b[b] ± se**	**COV, %**
Glucose	10	0.104±0.002	0.999±0.001	20.3 ± 0.5[c]	2.32
	20	0.274±0.003			
	50	0.908±0.009			
Sucrose	50	0.116±0.001	0.999±0.001	3.4 ± 0.1[c]	1.69
	100	0.295±0.004			
	150	0.452±0.006			
	200	0.566±0.004			
Mannitol	50	0.010±0.001	0.999±0.001	0.67± 0.01[c]	2.71
	100	0.139±0.001			
	200	0.207±0.004			
	300	0.268±0.007			
Curcumin	0.02	0.213±0.001	0.999±0.001	11.4 ± 0.3	0.81
	0.04	0.439±0.001			
	0.06	0.693±0.002			
	0.08	0.890±0.008			
H_2O_2	0.5	0.254±0.003	0.999±0.001	0.40±0.01	1.59
	1.0	0.452±0.006			
	1.5	0.669±0.007			
	2.0	0.854±0.001			

COV, coefficient of variation; [a] PRP, permanganate reducing potential as, µmole permanganate reduced for given mass of test agent estimated by permanganate calibration standard; [b] Index of permanganate reducing activity (PRA), µmole or nmole[c] permanganate reduced µmole^{-1} test agent;

The values are mean ± S.E. of 3 observations each.

Tartaric acid, mannitol, glucose and sucrose are very poorly active with PRP in nmoles per µmole test agent (tartaric acid > glucose>sucrose> mannitol).Tartaric acid has been most potent and mannitol least potent (about 5 and 1/30th times that of glucose, correspondingly). Reducing monosaccharide glucose is about 6 times more active than non-reducing disaccharide sucrose. The response of sucrose as reductant in the assay though very weak would deserve some further investigation whether reduction is due to intact molecule or due to its hydrolytic products under test conditions. It is interesting to note that oxalic acid is used to standardize potassium permanganate titrimetrically maintaining titrand at an elevated temperature and potassium permanganate is used to assay hydrogen peroxide. Theoretical stoichiometric value for either is 0.40. The value correlates well with observed regression estimate for oxalic acid (Table 2) and hydrogen peroxide (Table 3)**.** This implies that the present technique is quite suitable for standardization works.

Table 4: Permanganate reducing potential of water soluble organic solvents

Test agent	Mass, mL	PRP, µmole	r ± S.E.	b[a] ± S.E.	COV, %
Ethanol	0.01 0.02 0.03 0.05	0.060±0.002 0.130±0.002 0.172±0.002 0.255±0.003	0.992± 0.008	4.73 ± 0.30	2.03
Methanol	0.05 0.10 0.15 0.20	0.088±0.002 0.144±0.012 0.221±0.002 0.277±0.007	0.998± 0.002	1.29 ± 0.04	2.07
Acetone	1.0 1.5 2.0	0.169±0.008 0.297±0.022 0.391±0.011	0.996± 0.005	0.222 ± .001	1.57

PRP, permanganate reducing potential
[a] PRA, permanganate reducing activity, µmole permanganate reduced per mL test agent; The values are mean ± S.E. of 3 observations each.

Water soluble organic solvents have been found to be capable of affecting permanganate reducing assay. Relative susceptibility of ethanol, methanol and acetone to oxidizing action of acidified potassium permanganate have revealed (Table 4) highest susceptibility of ethanol (about 3.7 times that of methanol) and least in acetone (about 1/6th of methanol). These considerations are necessary to pay attention to while assaying extracts present in such solvents.

Conclusions

The present study has demonstrated usefulness of photometric permanganate assay in screening chemically diverse antioxidants. The assay has responded to flavonoids, phenolics, ascorbic acid, curcumin, DMSO, carboxylic acids, thio-compounds, glucose, sucrose and mannitol enabling their relative ranking as potential antioxidants. The photometric assay has shown its applicability for standardization of oxalic acid and hydrogen peroxide at room temperature obviating necessity of maintaining elevated temperature as is otherwise recommended for titrimetric assay for oxalates. The relative potential can be evaluated by comparison of permanganate reducing potential under standard conditions. The assay has advantages of simplicity, cost-effectiveness and is applicable to chemically diverse antioxidants, enabling to detect even those having weakest reducing potential. The assay can be incorporated as a component in a battery of screening methods for screening potential antioxidants for further evaluation with more sophisticated methods.

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