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Full Length Research Paper

Antioxidant potentials and degrees of polymerization of six wild fruits

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Aqueous methanolic extracts of six wild fruits, *Ximenia caffra (*sour plum), *Sclerocarya birrea (marula*), *Parinari curatellifolia* (*mobola* plum), *Vitex payos* (chocolate berry), *Bridelia molis* (velvet sweet-berry) and *Berchemia zeyheri* (red ivory) were assayed for radical scavenging effect on DPPH radical, reducing power, superoxide anion radical scavenging effect and the inhibition of phospholipids peroxidation using colorimetric method. The peels and pulps of sour plum exhibited high activity compared to the peels and pulps of the other fruits. Sour plum showed high reducing capacities both in the peel and pulp compared to all the other fruits. At high concentrations of extract over 75% superoxide anion scavenging effect was observed for velvet sweet-berry whilst at concentrations of 40 and 60 mg sample equivalent/µl red ivory fruits showed a high anion scavenging capacity. Velvet sweet-berry, sour plum peel and pulp and chocolate berry peel showed high capacities to inhibit lipid peroxidation at high concentrations. The degrees of polymerization varied between 7 to 16 monomer units of catechin per polymer of phenolic compounds in the different portions of the six fruits.

Key words: Phenolic acids, antioxidant, wild fruits, peroxidation and polymerisation.

INTRODUCTION

There is some evidence that wild plant foods, including fruits and their products have a protective effect against cancer, stroke and coronary heart diseases, which may relate to the antioxidants in these foods. Although the majority of the evidence emphasizes the role of vitamins E, C and β-carotene, the presence of phenolic antioxidants may also play contributory role. It is useful to measure the antioxidant capacity of fruits (Tosun and Ustun, 2003, Wang et al., 1996; Kalt et al., 2000; Cao et al., 1996; Miller and Rice-Evans, 1997).

Recently, consumers have been concerned about the consumption of synthetic food additives, including the two most commonly used antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). There is, therefore, an increasing interest in natural food additives, such as plant extracts which can function as natural antioxidant besides seasoning the food (Wangesteen et al., 2004). However, a natural origin is not a guarantee of safety.

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The several analytical methods that have been developped to estimate the antioxidant activities of natural substances may be grouped into two major types, assays for radical-scavenging ability and assays for oxidative inhibittory effects. The total antioxidant activity can not satisfactorily be evaluated using one method because of the complex composition of phytochemicals as well as of oxidative processes. It is usually prudent to use at least two methods (Wangesteen et al., 2004; Böhm et al., 2001).

Antioxidant actions of polyphenolic compounds and simple phenolic compounds, from a thermodynamic point of view, depends on well defined parameters such as bond energies and standard reduction potentials from which from which it can be deduced whether a given radical can be quenched thus the degree of polymerization plays a crucial role in the antioxidant properties of phenolic compounds (Becker et al., 2004).

We have used convectional colourimetric methods to determine the total phenolic composition, flavonoid concentration, condensed tannins, hydrolysable tannins and degrees of polymerisation. The purpose of the study reported here was to investigate the comparative antioxidant potentials of six fruits commonly found in Zimbabwe.

MATERIALS AND METHODS

Fresh ripe samples of *Ximenia caffra (*sour plum), *Sclerocarya birrea (marula*), *Parinari curatellifolia* (*mobola* plum), *Vitex payos* (chocolate berry), *Bridelia molis* (velvet sweet-berry) and *Berchmia zeyheri* (red ivory) fruits were obtained from various areas around Zimbabwe.

Preparation of fruit samples

Upon arrival at the laboratory, sour plum, *marula*, *mobola* plum and chocolate berry fruits were cleaned under running tap water, manually separated into a peel fraction and pulp fraction and dried in the sun. Velvet sweet-berry and red ivory were obtained in a dried form in which it was not possible to separate the peel from the pulp.

Chemicals

All the reagents used were of analytical grade. Nitroblue tetrazolium salt (NBT), 1, 1- diphenyl – 2 picrylhydrazyl radical (DPPH•), phenazine methosulphate (PMS), ascorbic acid, trichloroacetic acid (TCA), vanillin, catechin and potassium ferricyanide were obtained from Sigma – Aldrich Chemie (Steinheim, Germany). Reduced nicotinamide adenine dinucleotide (NADH) was obtained from Boehringer, Manheim, Germany.

Extraction of phenolic compounds

Total phenolic compounds were extracted from the peel and the pulp as described by Makkar, (1999). The sample of peel or pulp (2 g) was extracted twice with cold 50% aqueous methanol (10 ml). The two extracts were combined, made up to 20 ml with 50% aqueous methanol, centrifuged at 3000 rpm for 10 min and transferred into small sample bottles for analysis.

DPPH radical scavenging activity

The radical scavenging activity was determined following the method by Kuda et al. (2005). Methanolic solutions of DPPH (1.5 ml, 1 mM) containing up to 80 µl sample were incubated at room temperature. After 20 min absorbance at 517 nm was read on a Spectronic 20® Genesys™ Spectrophotometer. Ascorbic acid was used as a positive control. The scavenging activity was calculated as:

Scavenging activity (%) = (Absorbance of sample / Absorbance of control) X 100

Reducing power effects

Reducing power effects were determined following the method by Kuda et al. (2005). Up to 80 µl Sample or ascorbic acid control solution was mixed with phosphate buffer (0.2 mL, 0.2 M pH 7.2) and 1% potassium ferricyanide (0.2 mL). The mixture was incubated at 50°C for 20 min. After which TCA (0.2 mL, 10%) was added. After transferring an aliquot of the mixture (0.125 mL) into microtitre plate, distilled water (0.125 mL) and $FeCl_3$ (0.02 mL, 0.1%) was added. The absorbance at 655 nm was measured on a Spectra MAX 340 (USA, Sunnyvale, California) microtitre plate spectrophotometer.

Superoxide anion radical scavenging activity

Anion radical scavenging activity was determined following the method by Kuda *et al*., (2005). The sample up to 80 µl was mixed with phosphate buffer (0.1 mL, 0.1M, pH 7.2), NADH (2mM, 0.025 mL) and NBT (0.5 mM, 0.025 mL). An aliquot (0.025 mL) was transferred into a microtitre plate and after incubation with PMS (0.03 mM, 0.025 mL) for 3 min, absorbance at 550 nm was read on a Spectra MAX 340 (USA, Sunnyvale, California) microtitre plate spectrophotometer. Ascorbic acid was used as a positive control. The anion scavenging activity was calculated as:

Anion scavenging activity (%) = (Abs sample / Abs control) X 100

Inhibition of phospholipid peroxidation

Female Sprague Dawley rats (*Rattus norvegicus*) were obtained from the Animal House, University of Zimbabwe and dissected in the Physiology Department to obtain rat brain. The rat brains were stored at -85°C until used. Homogenization of rat brain (2 g) was done in a chloroform:methanol mixture (2:1, v/v) followed by centrifugation at 3000xg for 5 min. The supernatant obtained was used as the source of phospholipids.

The blank contained the phospholipid solution (50 µl) mixed with distilled water instead of the sample (0.5 ml) and 50% methanol (0.2 ml). The test run contained the phospholipids solution (50 µl), the vegetable extract (0.5 ml), 50% methanol (0.2 ml), $FeSO_4$ (0.5 ml), thiobarbituric acid (0.5 ml) and trichloroacetic acid (4 ml). Ascorbic acid (5%) was used as the positive control. Incubation at 37°C was followed by the addition of TBA and TCA and the solution was then heated in a boiling water bath for 15 min. After cooling the sample on ice, absorbance was read at 532 nm on a Spectronic 20® Genesys™ Spectrophotometer.

Degrees of polymarisation

The sample (5 µl) was placed in a test tube before adding 2.5 ml methanol-HCl and finally 2.5 ml of the vanillin reagent was added. The mixture was shaken and initial absorbance values were read using a spectronic 20® genesys™ spectrophotometer at 500 nm then followed by incubation at 30°C in a water bath, taking absorbance values at 5 min intervals until no absorbance changes were noted and this point was termed absorbance maximum (Amax).

In another test tube, the sample (5 µl) was mixed with 2.5 ml acetic acid-HCl and finally 2.5 ml of the vanillin reagent was added. The mixture was shaken and initial absorbance values were read using a spectronic 20® genesys™ spectrophotometer at 500 nm then followed by incubation at 30°C in a water bath, taking absorbance values at 5 min intervals until no absorbance changes were noted and this point was termed absorbance maximum (A max).

In a separate experiment, catechin (5 µl) was placed in a test tube 2.5 ml methanol-HCl and finally 2.5 ml of the vanillin reagent. The mixture was shaken on a votex and initial absorbance values were read using a spectronic 20® genesys™ spectrophotometer at 500 nm then followed by incubation at 30°C in a water bath, taking absorbance values at 5 min intervals. After 35 min, methanol (0.5 µl) was added and absorbance readings were taken for a further 30 min at 5 min intervals.

Amax values were used to estimate the degree of polymerization as the ratio of extinction coefficient of monomer to polymer. The extinction coefficient (E_{500}) of catechin obtained from the formula E_{500} = Amax/lc derived from the Beer-Lambert Law, was found to be 20.8. This being the case, the degree of polymerization of phenolic compounds of the fruits, in the different portions was calculated using Butler's method of dividing the polymer extinction coefficient

by the monomer coefficient for catechin, 20.8 referred above, obtaining estimates of degree of polymerizations.

Statistical analysis

Samples were analysed in duplicate and all results are given as means ± standard deviations. Oneway ANOVA and the student's *t*-test, both packaged in the Statistical Package for Social Sciences (SPSS) for Windows Standard Version 8.0.0 were used for the statistical evaluation with $P< 0.05$ considered statistical significant.

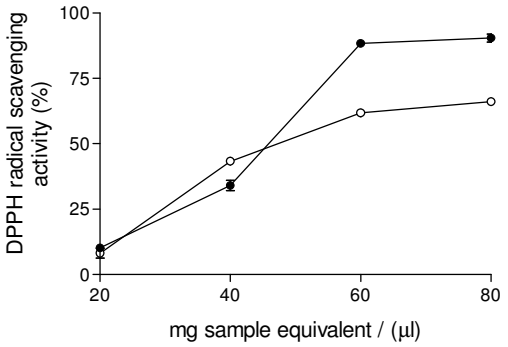


Figure 1. Scavenging effect of the methanolic extracts of velvet sweet-berry (●) and red ivory (○) extracts on DPPH radical.

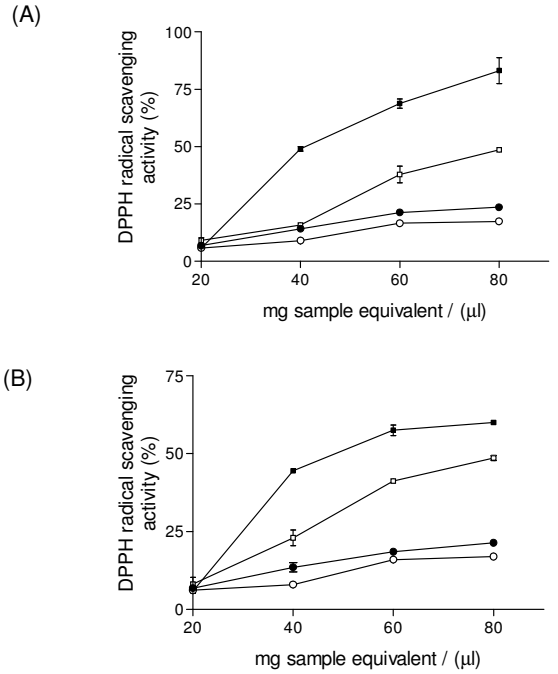


Figure 2. Scavenging effect of the methanolic extracts of *mobola* plum (○), *marula* (●), sour plum (■) and chocolate berry (□), peel (A) and pulp (B) extracts on DPPH radical.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The radical scavenging effects of velvet sweet-berry and red ivory fruits are shown in Figure 1 and Figure 2 (A) and (B) represents those of sour plum, *marula*, *mobola* plum and chocolate berry fruits. The radical scavenging activities were dose dependent for all the four fruits and at higher concentrations, the scavenging effects reached a maximum and then leveled off with further increase of the sample of the methanolic extracts. Velvet sweet-berry had the highest scavenging activities compared to red ivory. The result of these two fruits could not be compared to the other four fruits because they represent both the peel and pulp whilst the other fruits were separated into peel and pulp portions.

The peels and pulps of sour plum exhibited high activity compared to the peels and pulps of chocolate berry, *marula* and *mobola* plum. We detected high phenolic compounds in sour plum (Ndhlala et al., 2006) and these are responsible for the high antioxidant activity. There were no significant differences in the activities in peels and pulps of the four fruits. The scavenging activities of the fruits were all similar to what we obtained earlier in extracts from *Diospyros mespiliformis* (Jackal Berry), *Flacourtia indica* (*Batoka* plum), *Uapaca kirkiana* (Wild Loquat) and *Ziziphus mauritiana* (yellow berry) fruits (Ndhlala et al., 2006).

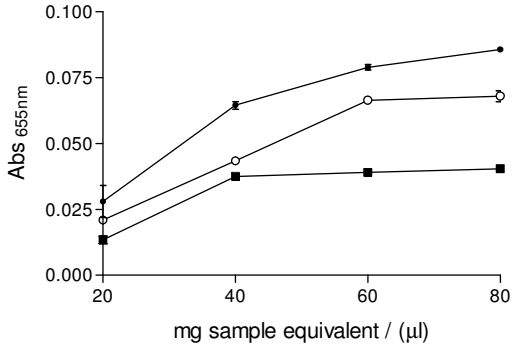


Figure 3. Reducing power effect of the methanolic extracts of velvet sweet-berry (●), red ivory (○) and ascorbic acid control (■).

Reducing power

The reducing powers of the methanolic extracts from velvet sweet-berry and red ivory fruits are shown in Figure 3 and for sour plum, *marula*, *mobola* plum and chocolate berry fruits are shown in Figure 4 (A) and (B). The reducing powers of the extracts increased with increase in concentration of the sample. In all cases, the reducing powers of extracts were greater than the reducing power of the control ascorbic acid solution. At all concentrations, extracts from velvet sweet-berry showed the highest reducing powers compared to red ivory in the current study and was higher than in jackal berry (Ndhla-

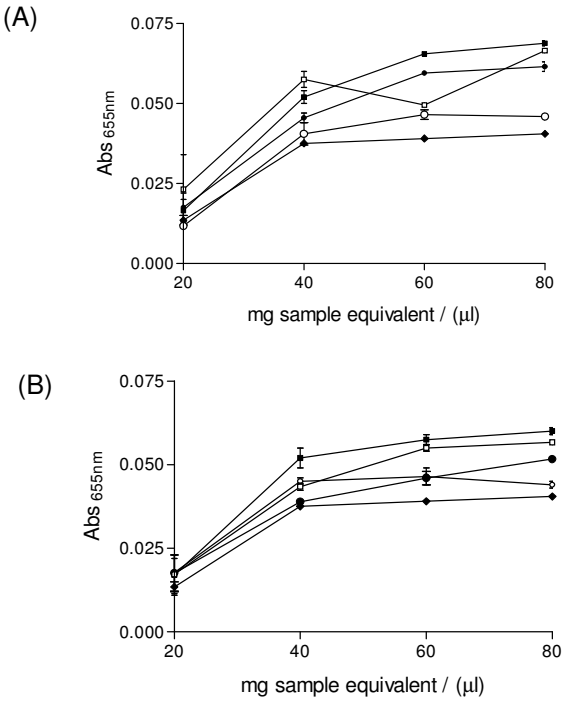


Figure 4. Reducing power effect of the methanolic extracts *mobola* plum (○), *marula* (●), sour plum (■) and chocolate berry (□), peel (A) and pulp (B) extracts and ascorbic acid control (♦).

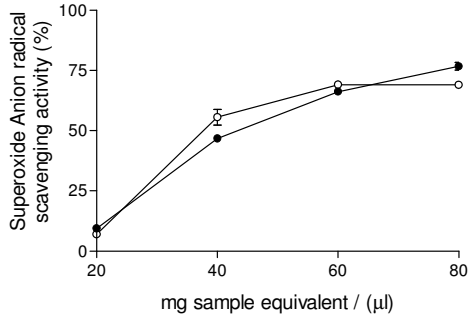


Figure 5. Scavenging effect of the methanolic extracts of Velvet sweet-berry (●) and red ivory (○) on the superoxide anion radical.

la et al., 2006). Sour plum showed high reducing capacities both in the peel and pulp compared to chocolate berry, *marula* and *mobola* plum. No much difference was shown between the activities in the peels and pulps.

Superoxide anion radical scavenging activity

The superoxide scavenging capacity of from velvet sweet-berry and red ivory fruits are shown in Figure 5 and for sour plum, *marula*, *mobola* plum and chocolate berry fruits the results are shown in Figure 6 (A) and (B). At high concentrations of extract over 75% anion scaven-

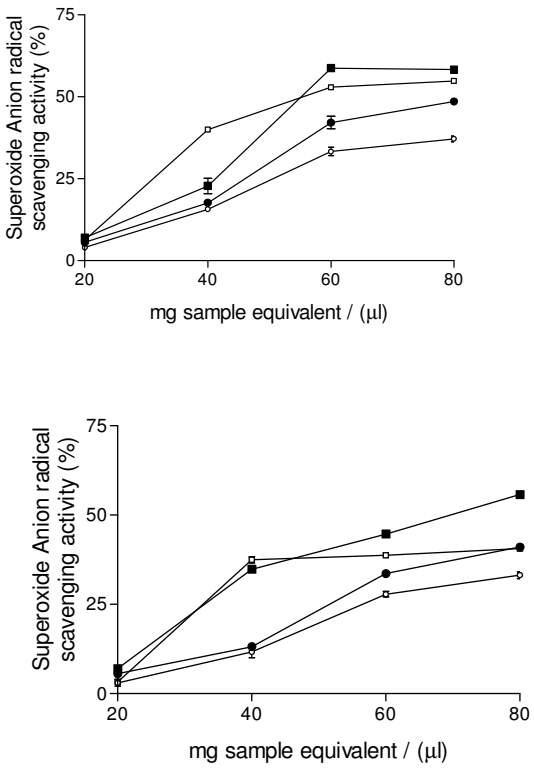


Figure 6. Reducing power effect of the methanolic extracts *mobola* plum (○), *marula* (●), sour plum (■) and chocolate berry (□), peel (A) and pulp (B) extracts.

ging effect was observed for velvet sweet-berry. At concentrations of 40 and 60 mg sample equivalent/µl red ivory fruits showed a high anion scavenging capacity. At 80 mg sample equivalent/µl, velvet sweet-berry showed high capacity of anion scavenging. The pulps of sour plum had higher activities than the peels in contrast to all the fruits where the pulp had had higher activity than the peel though there were no statistical differences. In all the cases, there was a steady increase in the anion scavenging effect which increased in concentration of extract with activity leveling off at high concentration except for the pulp of sour plum which continued to increase between the concentrations of 60 and 80 mg sample equivalent/µl.

Inhibition of phospholipid peroxidation

The capacity to inhibit lipid peroxidation in rat brain by the fruit extracts is shown in Figure 7 and Figure 8 (A) and (B). In biological systems, lipid peroxidation generates a number of degradation products such as malondialdehyde (MDA) and is found to be important cause of cell membrane damage. MDA is thus measured as an index of lipid peroxidation and as a marker for stress (Berker et al., 2004). The ability of all the fruit extracts to inhibit lipid

Table 1. Degrees of polymerization of phenolic compounds in the six fruits.

Sample		Estimated degree of polymerization
Sour plum	peel	10.1
	pulp	10.1
Velvet sweet-berry	peel and pulp	9.8
marula	peel	6.8
	pulp	15.4
Chocolate berry	pulp	12.0
	peel	9.9
Red ivory	pulp and peel	13.0
mobola plum	peel	10.3
	pulp	10.3

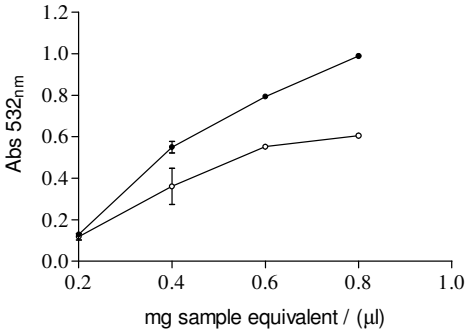


Figure 7. The capacity to inhibit lipid peroxidation of the methanolic extracts of velvet sweet-berry (●) and red ivory (○) in rat brain.

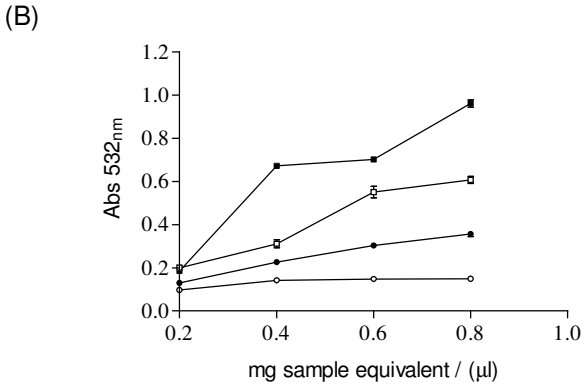


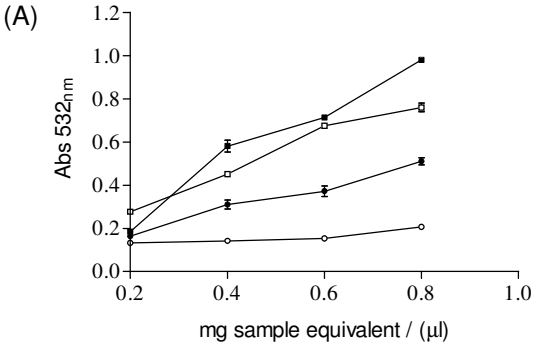
Figure 8. Reducing power effect of the methanolic extracts *mobola* plum (○), *marula* (●), sour plum (■) and chocolate berry (□), peel (A) and pulp (B) extracts.

peroxidation was dose dependant, Figures 7, 8 (A) and (B). Velvet sweet-berry, sour plum peel and pulp and chocolate berry peel showed high capacities to inhibit lipid peroxidation at high concentrations. The highest inhibitory activity was observed in velvet sweet-berry extract.

Degrees of polymerisation

Antioxidant activity has been shown to depend on the number of hydroxyl groups and /or degree of polymerization (Hodnick et al., 1988). The results for the degrees of polymerization are shown in Table 1. We obtained between 7 to 16 monomer units of catechin per polymer of phenolic compounds in the different portions of the six fruits. In a previous study we obtained between 4 and 10 monomer units in *Uapaca kirkiana* and *Ziziphus mauritiana* (Muchuweti et al., 2005). Degree of polymerization of between 5 and 20 are considered to make up a polymer of medium molecular weight (Butler et al., 1982). There were significant differences in the degrees of polymerization of phenolic compounds in the peel and pulp of *marula* fruit. This shows differences in the quality of phenolic compounds in the peel and pulp of the *marula*.

The study of the degree of polymerization is increasing because not much is known regarding the impact of the degree of polymerization of phenolic compounds and their biological properties. Arteel and Sies (1999) and Bearden et al. (2000) looked into the effectiveness of cocoa procyanidins in-*vitro* to scavenge peroxynitrite and inhibit LDL oxidation, respectively, and they both found



that antioxidant activity was influenced by degree of polymerisation. Mao et al. (1999) studied the ability of the procyanidins to modulate interleukin-2 *in vitro* and found the higher oligomers inhibited interleukin-2 expression in stimulated cells, whereas the monomer had no effect.

In general, fruits with high degree of polymerisation should present lower antioxidant activity, and the opposite way around. Apart from a high content of flavan-3-ol monomers and gallic acid, low values of degree of polymerisation corresponded to products rich in procyanidin oligomers (Monagas et al., 2005).

Wang et al. (1996), studied the antioxidant effects of 12 fruits and five fruit juices which included grape fruit and juice, tomato fruit and juice, orange fruit and juice and apple fruit and juice. The antioxidant activities they obtained varied markedly with orange juice having 5-7 folds more activity than the other fruit and their juices.

Conclusions

The six fruits that were studied showed significant reducing power, radical scavenging effects and antioxidant activity. Velvet sweet-berry had high antioxidant activities in all assays, having degrees of polymerization of 10 showing that compounds of medium molecular weight could be the most potent antioxidants.

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