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Research article

Enzymatic antioxidant activities in eight wild edible fruits of Odisha

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Abstract: Fruits are considered to be rich in antioxidants but the total antioxidant potential is yet to be unveiled systematically in case of wild edible fruits. The present paper attempts to focus on assessing the levels of enzymatic activity of Superoxide dismutase (SOD), Catalase and Peroxidase enzymes in selected eight wild edible fruit species of Odisha *viz. Aegle marmelos, Calamus guruba, Limonia acidissima, Phyllanthus acidus, Phyllanthus emblica, Syzigium cumini, Ziziphus mauritiana* and *Ziziphus oenoplia*. As per the results inferred, SOD enzyme activity of *Phyllanthus acidus* (2.66 $\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein) was found to be the highest and almost equal to *Ziziphus oenoplia* (2.15 $\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein). Catalase enzyme activity was found to be very high in *Calamus guruba* (4.2×10^4 I.E.U.) followed by *Phyllanthus acidus* (4.08×10^4 I.E.U.), *Aegle marmelos* (3.77×10^4 I.E.U.) and *Syzigium cumini* (3.65×10^4 I.E.U.). Peroxidase enzyme activity was highest in *Calamus guruba* (0.975 $OD^{-1}.min^{-1}.gm^{-1}$ tissue wt.) and at par with *Aegle marmelos* (0.645 $OD^{-1}.min^{-1}.gm^{-1}$ tissue wt.). From this study, it could be concluded that wild edible fruits like *Aegle marmelos, Calamus guruba, Phyllanthus acidus, Syzigium cumini* and *Ziziphus oenoplia* can be identified as beneficial fruit species that possess significant free radical scavenging levels. Exploration and higher intake of such fruits with functional attributes could impart promising therapeutic potential.
Keywords: Antioxidant - Catalase - Peroxidase - ROS - SOD.

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INTRODUCTION

Antioxidants are compounds that undergo oxidation terminating the chain reaction by reacting with free radicals and chelating catalytic metals (Patel *et al.* 2010) and results in neutralization of free radicals and simultaneous inhibition of oxidation of other vital molecules (Sies 1996). Free radicals are mainly derived from oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS) (He & Hader 2002, Apel & Hirt 2004), and generated in our body by various endogenous systems, exposure to different physicochemical conditions (such as exposure to ultraviolet light and toxic chemicals), inflammatory or pathophysiological states (Kagan *et al*. 2002, Devasagayam *et al.* 2004). Deleterious effects of these radicals include lipid peroxidation (Jacob & Burri 1996) causing damage of the cell membrane of phospholipids, lipoprotein (Devasagayam *et al.* 2003), proteins oxidation (Stadtman *et al.* 1992), loss of enzyme activity etc (Halliwell & Gutteridge 1997) resulting in various diseases such as cancer, alcoholic liver cirrhosis, arteriosclerosis, arthritis, neurodegenerative disorders etc (Lee *et al.* 2000, Middleton *et al.* 2000, Yoshikawa *et al.* 2000).

Antioxidants play a curative role in chronic ailments such as heart disease, diabetes, hypertension, stroke, gastritis, Alzheimer's disease, AIDS (Pourmorad *et al.* 2006) and decreasing the risk of cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation (Ramana *et al.* 2011). Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters show low solubility and moderate antioxidant activity (Barlow 1990) along with negative health impact. Hence, scientists have now focused on isolation and characterization of natural antioxidants from herbs, spices, seeds, cereals, fruits and vegetables by extraction, fractionation and purification (Dillard & German 2000, Wang & Linn 2000). Natural antioxidants are basically of two types *i.e.*, enzymatic and non-enzymatic antioxidants. Antioxidant enzymes include catalase, superoxide dismutase (SOD), peroxidase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate

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dehydrogenase and ascorbate oxidase etc (Bandyopadhyay *et al.* 1990) while non-enzymatic antioxidants includes α-Tocopherol (vitamin E), Ascorbic acid (vitamin C), carotenoids, flavonoids and related polyphenols, α-lipoic acid, glutathione etc which all together work in synergy to counterbalance oxidative stress (Thiele *et al.* 2001).

In recent years, there has been growing interest in functional foods, i.e., foods that can provide not only basic nutritional and energetic requirements, but also an additional physiological benefit (Savikin *et al.* 2009). Fruits are considered to be major sources of dietary antioxidant compounds. Fruits possess self defense mechanism for protection from oxidative stress by the activation of many antioxidant defense enzymes (Jacob 1995) etc. The consumption of fruits with a high antioxidant composition has been associated with a lowered incidence of chronic, degenerative diseases (Cox *et al.* 2000) including cancer, coronary heart disease, inflammation, arthritis, immune system decline, brain dysfunction, cataracts, altitude sickness (Kumpulainen & Salonen 1999), digestive, stomachic complications, various biological activities like lipid lowering effect (Vijaya *et al.* 2009) etc.

The beneficial effects of fruits are hypothesized to owe, at least in part, to antioxidants (Benzie & Strain 1999). Based on this idea, there has been a strong demand of therapeutic and chemo preventive antioxidant agents with limited cytotoxicity to enhance the antioxidant capacity of the body and help attenuate the damage induced by ROS. One most commendable and sort after strategy is to identify fruits with high content of natural antioxidants that would help increase the body's immunity to many diseases.

Since there is no concrete report regarding the antioxidant potential of wild edible fruits in Odisha, many of them remain undocumented, unexploited and understudied except very few of them (Basak *et al*. 2013). Emphasizing on the possible beneficiary effects of naturally derived antioxidants and recognition of fruits with high antioxidant activities, this study was undertaken to examine and compare the total antioxidant enzyme activity of superoxide dismutase (SOD), catalase and peroxidase enzymes in eight selected wild edible fruits namely *Aegle marmelos, Calamus guruba, Limonia acidissima, Phyllanthus acidus, Phyllanthus emblica, Syzigium cumini, Ziziphus mauritiana* and *Ziziphus oenoplia* for enrolling better understanding of antioxidant properties of these wild edible fruits to therapeutic purposes and signifying the need for domestication and its role for industrial development with a view to popularizing their uses by the wider society.

MATERIAL AND METHODS

Plant material

Eight wild edible fruits namely *Aegle marmelos, Calamus guruba, Limonia acidissima, Phyllanthus acidus, Phyllanthus emblica, Syzigium cumini, Ziziphus mauritiana* and *Ziziphus oenoplia* were shortlisted and selected owing to their immense popularity, widespread consumption and distribution (Table 1).

Sample collection & preparation

Fresh samples (edible ripen stages) were used for enzyme activity analysis. These fruits were collected from botanical garden of the institute and various forest blocks of Odisha during 2011–12 and enzymatic analysis of superoxide dismutase (SOD), Catalase and peroxidase enzymes was performed in the institutional research laboratory.

Superoxide dismutase (SOD) enzyme assay

The extracts were assayed for SOD activity photochemically, using the assay system consisting of methionine, riboflavin, and NBT (Beauchamp *et al.* 1971). The photochemical procedure was chosen as being independent of other enzymes and proteins and, therefore, more reliable in the case of crude extracts than enzymic assay systems (McCord *et al.* 1969). The original assay (Beauchamp *et al.* 1971) was modified (Constantine *et al.* 1977). The reaction mixture was composed of 1.3 µM riboflavin, 13 mM methionine, 63 µM NBT, 0.05M sodium carbonate (pH 10.2), and the appropriate volume of extract. The initial rate of the reaction was determined as increase of absorbance at 560 nm. Under the described conditions, the increase of absorbance in absence of SOD was 0.100 absorbance unit/5 min and was linear up to 15 min. In the presence of SOD, the reaction was inhibited and the amount of inhibition was used to quantitate the enzyme. Each extract was assayed twice and the results varied less than +0.005 absorbance unit/5 min.

Peroxidase enzyme assay

The extracts were assayed for peroxidase enzyme activity using *o*-dianisidine spectrophotometrically (Queseda *et al.* 1992) with slight modifications (Putter 1974, Malik & Singh 1980). The oxidized yellow/orange colored O-dianisidine is measured at 430nm. 0.2ml enzyme extract and 0.1ml freshly prepared O-dianisidine

solution is added to 3.5 ml phosphate buffer in a clean dry cuvette. Water blank is used in the assay. The assay mixture is brought to 28–30 ºC and then 0.2 ml of 0.2M H_2O_2 was added and mixed. The initial absorbance was read at 430nm and then at every 30seconds intervals up to 3minutes. The assay is repeated with diluted extracts if rate of increase is very high. Increase in absorbance was plotted against time. The enzyme activity is expressed in terms of rate of increased absorbance per unit time per mg protein or tissue weight.

Catalase enzyme assay

One unit of catalase activity is defined as that amount of enzyme which breaks down I µmol of H_2O_2 min^{-1} under the defined assay conditions (Chance & Maehly, 1955) with slight modifications. Five milliliters of the assay mixture for the catalase activity comprised: 0.2M of phosphate buffer (pH 6.8), 0.4N of H_2O_2, and I ml of the twice diluted enzyme extracted. After incubation at 25ºC for 1 min, the reaction was stopped by adding 10 ml of 2% (v/v) H_2SO_4, phosphate buffer and the residual H_2O_2 was titrated against 0.01N $KMnO_4$ until a faint purple color persisted for at least 15 sec. A control was run at the same time in which the enzyme activity was stopped at "zero" time.

Table 1. Experimental wild edible fruits selected for antioxidant enzyme analysis.

S. N.	Fruit species	Local Name	Fruiting season	Medicinal uses
1.	*Aegle marmelos*	Bela	May–June	The fruit is an astringent and used for treatment of Asthma, anaemia, fractures, healing of wounds, high blood pressure, diabetes mellitus, intestinal tonic, chronic constipation, indigestion, hemorrhoids, intermittent fever, hypochondria, melancholia and for heart palpitation, diarrhea and dysentery.
2.	*Calamus guruba*	Kanta Beta	December–February	The fruit is traditionally used as a tranquilizer and a general "wonder drug." It is a sedative, hypotensive, muscle relaxant, cures cough, cold and pulmonary disorders.
3.	*Limonia acidissima*	Kaitha	November–January	Fruits are refrigerant, stomachic, stimulant, astringent, aphrodisiac, diuretic, cardiotonic, tonic to the liver and lungs; cures cough, hiccup and dysentery; good for asthma, consumption, tumours, ophthalmia and leucorrhoea.
4.	*Phyllanthus acidus*	Nara Koli	June–October	Fruits are a good remedy for different types of ailments like emetic and purgative (hypertension and respiratory). Its hepatoprotective, anti-bacterial, anti-diabetic, anti-nociceptive, cathartic, liver tonic, laxative etc. Cures coughs, asthma, bronchitis, poulticing, soles, rheumatism, sudorific gonorrhea and skin disorders.
5.	*Phyllanthus emblica*	Amla	November–January	Fruits are antioxidant, anti-diabetic, hypolipidemic, antibacterial, anti-inflammatory, antiulcerogenic, hepatoprotective, gastroprotective, and possess chemopreventive properties. Also prevents ulcer and used for treatment of diarrhea, jaundice etc.
6.	*Syzigium cumini*	Jamu Koli	July–August	Fruits are considered to be anti-diabetic, well known antioxidant, digestive, anthelmintic and astringent to bowels. It lowers blood pressure and used for treatment of sore throat, bronchitis, asthma, dysentery, ulcers, chronic diarrhea, enteric disorders, treat cold, cough, fever, skin problems etc.
7.	*Ziziphus mauritiana*	Bara Koli	November–February	The fruits are applied on cuts and ulcers; are employed in pulmonary ailments and fevers; the dried ripe fruit is a mild laxative, halts nausea, vomiting and abdominal pains in pregnancy, liver trouble, asthma and fever, checks diarrhea, burning sensations, cough, wound, skin disease, ulcers, stomatitis, sexual weakness and general debility.
8.	*Ziziphus oenoplia*	Kantei Koli	October–January	Pacifies vitiated pitta, kapha, worms, peptic ulcer, stomach pain and wounds. Also used for sore throats, dysentery and inflammation of the uterus.

RESULTS

In present investigation, eight wild edible fruits were selected for their antioxidant enzyme potential which would prove to be an essential parameter for "free radical scavenging" activity in human health and nutrition owing to their widespread consumption and assimilation in many of the forest blocks of Odisha. The research findings pertaining to the antioxidant enzyme activity of superoxide dismutase, catalase and peroxidase are shown below in table 2.

Table 2. Antioxidant enzyme activity analysis.

S. N.	Fruit sample	Local Name	SOD ($\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein)	Catalase (I.E.U.) in 1gm fresh wt. tissue	Peroxidase ($OD^{-1}.min^{-1}.gm^{-1}$ tissue wt.)
1.	*Aegle marmelos*	Bela	0.6050	3.77×10^4	0.6450
2.	*Calamus guruba*	Kanta Beta	0.9320	4.20×10^4	0.9750
3.	*Limonia acidissima*	Kaitha	0.1800	2.65×10^4	0.2790
4.	*Phyllanthus acidus*	Nara Koli	2.6600	4.08×10^4	0.0087
5.	*Phyllanthus emblica*	Amla	0.0067	1.24×10^4	0.0129
6.	*Syzigium cumini*	Jamu Koli	0.0047	3.67×10^4	0.0081
7.	*Ziziphus mauritiana*	Bara Koli	0.0130	1.8×10^4	0.2100
8.	*Ziziphus oenoplia*	Kantei Koli	2.1500	2.85×10^4	0.0900

Superoxide dismutase enzyme activity

The SOD enzyme activity varied from 0.0047 to 2.66 $\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein as shown in figure 1. Highest SOD enzyme activity was observed in *Phyllanthus acidus* (2.66 $\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein) followed by *Ziziphus oenoplia* (2.15 $\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein) and *Calamus guruba* (0.932 $\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein). The SOD enzyme activity was found to be lowest in *Syzigium cumini* (0.0047 $\Delta OD^{-1}.min^{-1}.mg^{-1}$ protein). SOD enzyme activity was observed in the rest of the four wild edible fruits in the following decreasing trend: *Aegle marmelos* > *Limonia acidissima* > *Ziziphus mauritiana* > *Phyllanthus emblica.*

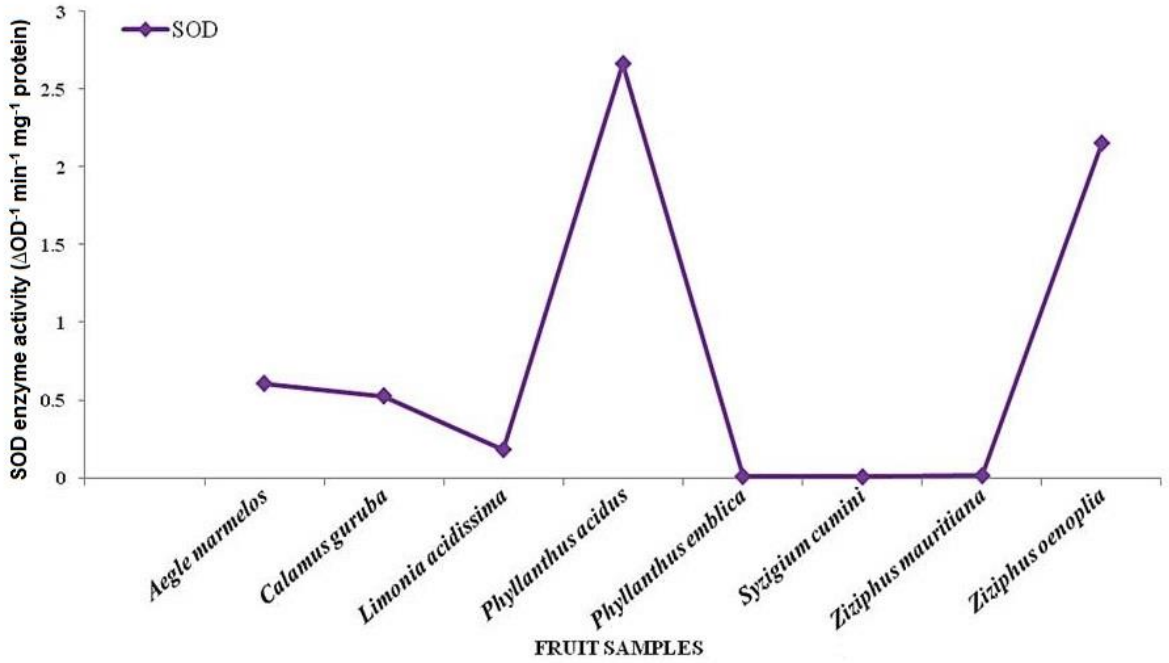

Figure 1. Comparison of SOD enzyme activity in eight wild edible fruits.

Catalase enzyme activity

The catalase enzyme activity varied from 1.24×10^4 I.E.U. to 4.2×10^4 I.E.U. per gram of fresh tissue in the selected eight wild edible fruits as shown in figure 2. *Calamus guruba* was recorded to be the fruit with highest catalase enzyme activity (4.2×10^4 I.E.U. per gram of fresh tissue) and *Phyllanthus emblica* was observed to possess the lowest enzymatic activity (1.24×10^4 I.E.U. per gram of fresh tissue). The enzyme activity was observed in the decreasing trend among fruits: *Phyllanthus acidus* > *Aegle marmelos* > *Syzigium cumini* > *Ziziphus oenoplia* > *Limonia acidissima* > *Ziziphus mauritiana.*

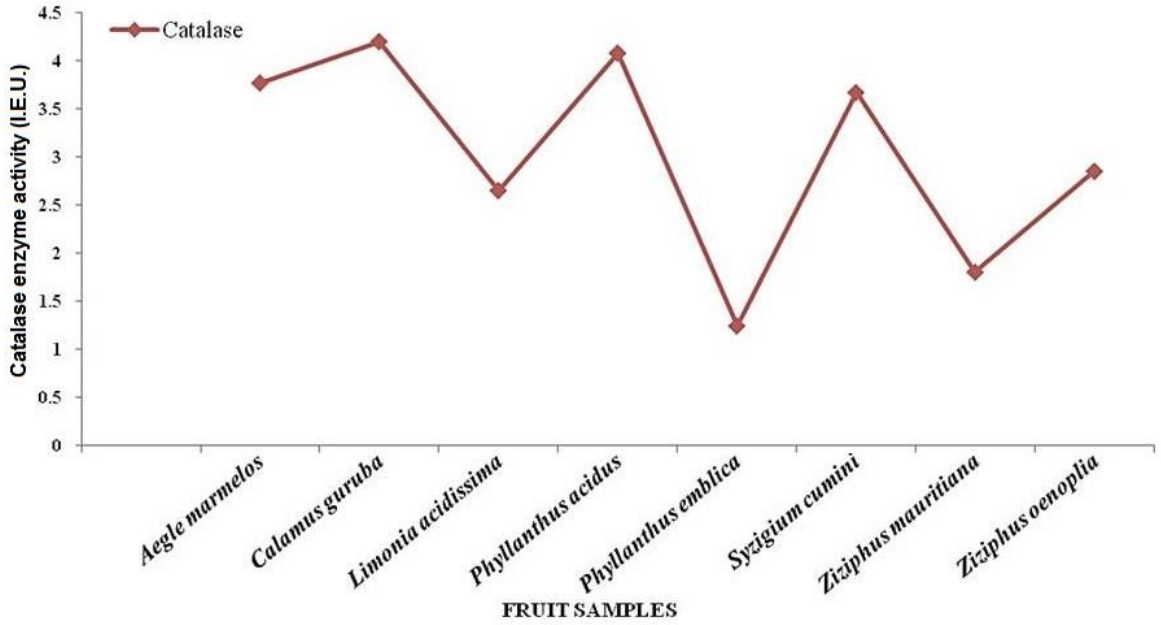

Figure 2. Comparison of catalase enzyme activity in eight selected wild edible fruits.

Peroxidase enzyme activity

Highest peroxidase enzyme activity was observed in *Calamus guruba* with a total enzyme activity of 0.975 $OD^{-1}.min^{-1}.gm^{-1}$ tissue weight while the lowest enzyme activity was recorded in *Syzigium cumini* with enzyme activity observed to be 0.0081 $OD^{-1}.min^{-1}.gm^{-1}$ tissue wt. For rest of the wild edible fruit species the enzyme activity value ranged from 0.645–0.0087 $OD^{-1}.min^{-1}.gm^{-1}$ tissue weight (Fig. 3).

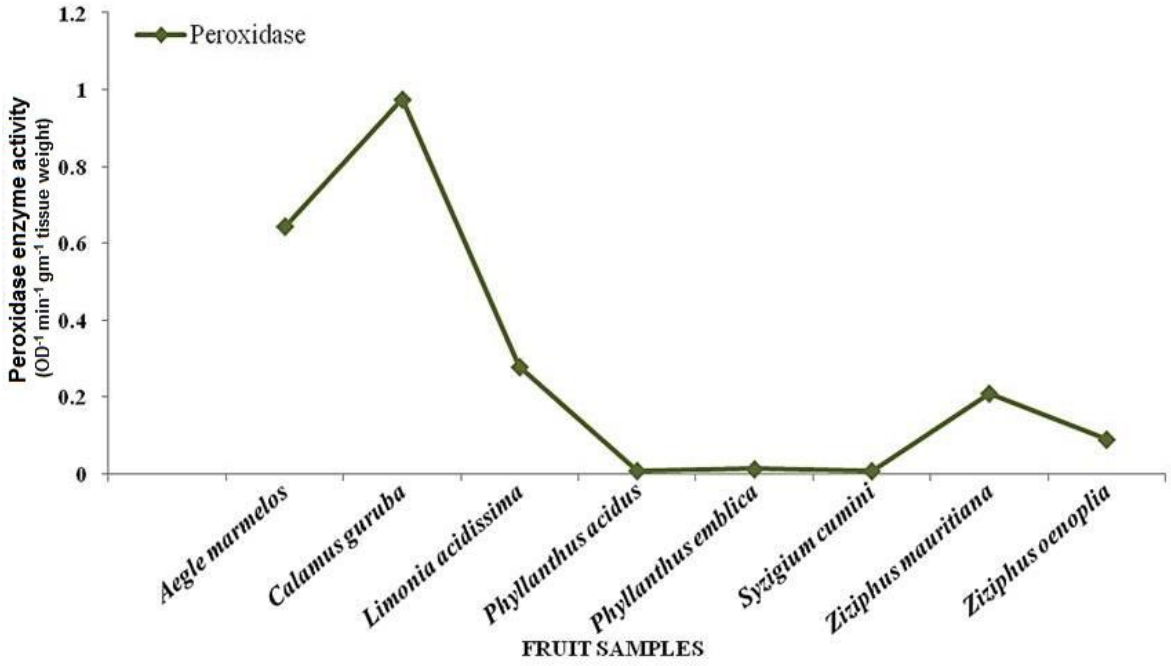

Figure 3. Comparison of peroxidase enzyme activity in eight selected wild edible fruits.

DISCUSSION

The present study was undertaken to assess the free radical scavenging capacity of the selected eight wild edible fruits of Odisha, India. From the results, it was observed that *Calamus guruba* and *Phyllanthus acidus* showed promising activities of catalase and SOD. Similarly, *Aegle marmelos* and *Calamus guruba* showed high peroxidase enzyme activity. From our experiment it was deducted that wild edible fruits like *Aegle marmelos, Calamus guruba, Phyllanthus acidus* and *Ziziphus oenoplia* possessed high enzyme activity levels. Since antioxidant enzymes altogether work in a network, therefore these fruits, possessing high enzymatic activities of

catalase, peroxidase and SOD can efficiently serve as potential antioxidant additives in human diet thereby preventing oxidative damage by reactive oxygen species.

The ability of the fruit extracts to quench superoxide radicals from reaction mixture is reflected in the decrease of the absorbance at λ = 560 nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The observed increase in SOD activity suggests that the *Phyllanthus acidus, Ziziphus oenoplia* and *Calamus guruba* have an efficient protective mechanism in response to ROS. SOD catalyzes the breakdown of endogenous cytotoxic superoxide radicals to H_2O_2 which is further degraded by CAT. Thus, they play a crucial role in maintaining the physiological levels of O_2 and H_2O_2 (Arivazhagan *et al.* 2000).

CONCLUSION

Our results support the possible use of the eight analyzed fruits possessing good antioxidant enzyme activity levels, as natural antioxidants to replace the synthetic additives as well as their use in the production of functional foods with high antioxidant activity that are capable of blocking the action of reactive oxygen species involved in oxidative stress. Evaluation of these fruit extracts has provided interesting results that might be beneficial for the pharmacological use of these plants in clinical trials. All these fruits with their free radical scavenging activity and various extents of antioxidant activity provide a valuable source of nutraceutical supplements, chemo preventive and therapeutic agents. It is suggested that further research needs to be conducted for selecting the fruits with high antioxidant levels and clarify the importance and role of these antioxidants on pathogenesis of various diseases.

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