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Superoxide Dismutase Content and Antioxidant Potential of Some Selected Locally Available Fruits in Bauchi Town, North-eastern Nigeria

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Abstract

The evidences of the link between oxidative stress and diseases are becoming clearer, hence, the need for natural antioxidants against the numerous artificial ones to ensure safety and efficacy. Superoxide dismutase (SOD) is a natural enzymatic antioxidant which can be found in fruits. In this project, Watermelon(*Citrulluslanatus*), Mango(*Mangiferaindica*), Tomatoes (*Solanumlycopersicum*), Guava(*Psidiumguajava*) and Pawpaw (*Carica papaya*) were screened for the presence of SOD and some phytochemicals using standard methods. The total antioxidant potential of the fruits was determined using 2, 2 - diphenyl-1-picryl hydrazyl method. The results of the SOD content determination shows that Guava has the highest SOD content (27.94 ± 0.45 U/ml), followed by Watermelon, Tomatoes, Mango and Pawpaw which have SOD content of 18.95± 0.09, 9.14 ± 0.05, 6.27 ± 0.11 and 4.8 ± 0.27 U/ml respectively. Phytochemical analysis reveals that all the fruits contain Flavonoids with Pawpaw having the highest Flavonoids content (37.88± 0.29 mg/100g). Saponins were found in Watermelon, Mango and Pawpaw. Tannins were found in Mango, Tomatoes and Pawpaw, while only Watermelon contains Anthraquinones. None of the fruits contains Alkaloids or Cardiac glycosides. The result of the antioxidant potential shows that Pawpaw has the highest antioxidant potential with an EC_{50} of 170.00 ± 1.00 µg/ml, followed by Watermelon (270.00 ± 2.00 µg/ml), Mango (320.00 ±5.56 µg/ml), Tomatoes (510.00 ± 4.35 µg/ml) and Guava (620.00 ± 8.66 µg/ml) respectively. These results suggest that each fruit may exert pharmacological function associated with the phytochemicals it contains. The fruits, most especially Pawpaw and Watermelon could be used as general antioxidants. Most importantly, the results reveal that Guava and probably, Watermelon are good sources of SOD, and it is suggested that SOD be purified from them and enhanced for possible application in cosmetics, food and health sectors so as to ameliorate the effects of oxidative stress.

Keywords: Oxidative stress, Antioxidants, Superoxide dismutase, Fruits, Phytochemicals.

Introduction

The production of Reactive Oxygen and Nitrogen Species (RONS) is a normal physiological process. Examples of RONS are H_2O_2, $O_2^{\cdot -}$, $^{\cdot}OH$ and NO (Kroncke, 2003).In limited concentration, RONS are beneficial to the living system. When produced in excess, the antioxidant machinery of the body converts the excess into less harmful or harmless species.

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However, oxidative stress is a condition that arises when the antioxidant machinery of the body cannot efficiently convert the excess RONS into less harmful or harmless species. This condition necessitates for the complementation of the endogenous antioxidant system by exogenous supplementation of antioxidants, otherwise, the excess RONS in the body will react with and destroy proteins, lipids, DNA and other biomolecules, thus resulting to mutations, diseases, disease complication, cell death and by extension, the death of the organism (Rahman *et al.*, 2012).

Exogenous antioxidants naturally occur in plants and the food we eat. They are also synthesized artificially. However, the artificial antioxidants have recently raised safety concern as many of them produce free radicals and other species that are also harmful to the body. Also, many of the exogenous antioxidants are nonspecific (Ghezzi*et al.*, 2017). This calls for the use of specific antioxidants. Enzymes are specific in their actions, therefore, they best suit this need. Superoxide dismutase (EC 1.15.1.1) is a metalloenzyme that belongs to the oxidoreductase class of enzymes. It is an antioxidant enzyme that catalyzes the dismutation (disproportion) of superoxide radical into H_2O_2, which is further converted into H_2O and O_2 by catalase or glutathione peroxidase (Vats *et al.*, 2015). Superoxide radical is a reactive oxygen species (ROS) produced by different oxidases (such as NADPH oxidase, Xanthine oxidase, Cycloxygenase) as well as by mitochondrial electron transport chain during the course of normal oxidative phosphorylation which is essential for generating ATP (Evans *et al.*, 2005). It is a highly reactive cytotoxic ROS, and it is implicated in so many diseases (Rahman *et al.*, 2012). If left in excess without been dismutated by superoxide dismutase, superoxide radical may oxidize or reduce targets to form$^{\cdot}$OH, which initiate free radical chain reaction resulting to the destruction of macromolecules. Superoxide radical may also react with NO to form peroxynitrite, which also has a devastating effect (Linchov and Fridovich, 1999). Thus, external supplementation of superoxide dismutase will help in specifically counteracting excess $O_2^{\cdot-}$, thereby averting the consequences of oxidative stress due to the production of excess $O_2^{\cdot-}$.

Superoxide dismutase has been produced commercially from bovine and marketed. However, it was withdrawn from market in Europe due to allergic reaction, and thus limited to veterinary use in the USA (Rosa *et al.*, 2021). This emphasizes need for an alternative source of superoxide dismutase for human use. Thus, plant-based SOD could be the alternative. Plant-based SODs were reported to have enhanced antioxidant circulation and reduced oxidative stress in animals and human models (Buettner, 2011; Romao, 2015). Infact, the promising effect of gliadin-SOD in multiple diseases led to the formulation of clinically commercialized health supplement GliSODin (Menvielle-Bouge, 2005). Although, certain humans and animals with gluten/gliadin intolerance may tend to develop allergic reactions or intestinal inflammation when treated with gliadin-complexed SOD, this cannot rule out the use of plant-based SOD, because other encapsulation methods can be employed to produce a healthy nutritional supplement without side effect (Stephenie*et al.*, 2020). Rani *et al.* (2004) emphasized that fruits could be very good source of Superoxide dismutase.Therefore, in this research, some fruits - Watermelon(*Citrulluslanatus*), Mango(*Mangiferaindica*), Tomatoes(*Solanumlycopersicum*), Guava(*Psidiumguajava*) and Pawpaw(*Carica papaya*), which are locally available in Bauchi town, North-eastern Nigeria, were analyzed for Superoxide dismutase content, as well as, phytochemical content and antioxidant potential with a view of identifying those with reasonable Superoxide dismutase content in particular and antioxidant potential in general.

Materials and Methods

Experimental Fruits

Watermelon(*Citrulluslanatus*), Mango(*Mangiferaindica*), Tomatoes(*Solanumlycopersicum*), Guava(*Psidiumguajava*) and Pawpaw(*Carica papaya*) were bought from MudaLawal market, Bauchi, Nigeria. The fruits were identified and authenticated by Mr. Musa Muhammad of the Biological Science Department, Abubakar Tafawa Balewa University, Bauchi, Nigeria. The fruits were washed and stored under refrigerated condition until use.

Preparation of Crude Enzyme

The fruits - Watermelon(*Citrulluslanatus*), Mango(*Mangiferaindica*), Tomatoes(*Solanumlycopersicum*), Guava(*Psidiumguajava*) and Pawpaw(*Carica papaya*)were labeled A, B, C, D and E respectively. One gram (1g) each of specimen A to E were ground separately in 2ml of 50% ethanol in pre-chilled mortar and pestle. The extracts of specimen A to E were centrifuged separately at 10000 rpm at 4^{o}C for 10min. The supernatant of extract A to E were pipetted into corresponding sample bottles labelled A to E, and then kept under refrigerated condition till use (Rani *et al.,* 2004).

Assay for Superoxide Dismutase (SOD) Activity

SOD was determined using Nitrite method according to procedure of Das *et al.*(2000) as reported by Rani *et al.*(2004). Briefly, 1.4ml of aliquots of the reaction mixture (consisting of 1.11ml of 50mM Phosphate buffer of pH 7.4, 0.075ml of 20mM L-Methionine, 0.04ml of 1% (v/v) Triton X-100, 0.075ml of 10mM hydroxylamine hydrochloride, and 0.1ml of 50mM EDTA) were added to 100µl of the sample, and incubated at 30^{o}C for 5min. 80µl of Riboflavin was added, and the tubes were exposed for 10min to 200W-philips fluorescent lamps. 1ml of Greiss reagent was then added, and absorbance was read at 543nm. SOD activity was calculated according to the formula presented by Giannopolitis and Ries (1977).

Fruits Extracts Preparation

The aqueous extract of each fruit was prepared as reported by Azwanida (2015). Each of the selected fruits was sliced and air-dried under shade. It was then pulverised into powder. 100g of the powdered sample was dissolved in 500 ml of distilled water in a stoppered container for 3 days with frequent agitation. After the 3 days, the mixture was filtered. The filtrate was evaporated at 40 ^{o}Cto obtain the extract.

Phytochemical Analysis

Qualitative and quantitative determination of Flavonoids, Tannins, Saponins, Alkaloids, Cardiac glycosides and Anthraquinones in the samples were carried out using standard procedures as reported by Sofowora (1993) and Harborne (1973).

Determination of Antioxidant Potential

This was determined using 2, 2 - diphenyl-1-picryl hydrazyl (DPPH) method as reported by He *et al.* (2012) with little modification. In brief: a stock solution (1mg/ml) of each fruit extract was diluted to a dilution series (50, 100, 150, 200 and 1000 µg/ml). 2.7ml of 0.2mM DPPH solution was added to 0.3ml of each of the different extract concentrations. The mixture was shaken vigorously and incubated at room temperature in the dark for 1 hour. The absorbance was taken at 517nm. The radical scavenging activity was calculated as reported by He *et al.* (2012).

Statistical Analysis

Descriptive statistics was used to analyse the data. The values were expressed as Means ± Standard deviation in a tabular form.

Results

Table 1 shows the SOD content of the fruits crude extracts under study. Guava has the highest SOD content (27.94 ± 0.45 U/ml), followed by Watermelon, Tomatoes, Mango and Pawpaw which have SOD content of 18.95± 0.09, 9.14± 0.05, 6.27±0.11 and 4.84 ± 0.27 U/ml respectively.

Table 1: Superoxide dismutase (SOD) content of the fruits crude extracts

Fruit	SOD content (Unit/ml)
Watermelon	18.95± 0.09
Mango	6.27± 0.11
Tomatoes	9.14± 0.05
Guava	27.94± 0.45
Pawpaw	4.84± 0.27

Values are expressed as Mean ± Standard deviation, n = 3.

The results of phytochemical analysis of the fruit extracts are presented in table 2. Qualitative and quantitative analysis reveals the presence of Flavonoids in all the fruits extracts. Saponins are present in Watermelon, Mango and Pawpaw. Among the analyzed fruits, only Watermelon was found to contain Anthraquinones. The analysis also reveals the presence of Tannins in Mango, Tomatoes and Pawpaw. None of the fruits extract contains Alkaloids or Cardiac glycosides.

Table 2: Phytochemical content of the fruits

FRUITS	PHYTOCHEMICALS (mg/100g)					
	Flavonoids	**Saponins**	**Anthraquinones**	**Tannins**	**Alkaloids**	**Cardiac glycosides**
Watermelon	35.67 ± 0.47	21.64 ± 1.38	0.21 ± 0.02	-	-	-
Mango	30.68 ± 5.26	0.34 ± 0.07	-	1.19 ± 0.16	-	-
Tomatoes	25.62 ± 2.98	-	-	0.79 ± 0.02	-	-
Guava	11.61 ± 0.10	-	-	-	-	-
Pawpaw	37.88 ± 0.29	10.06 ± 9.26	-	2.31 ± 0.01	-	-

Values are expressed as Mean ± Standard deviation, n = 3.

Table 3 shows the *in vitro* antioxidant activity of the various fruits extract using DPPH method. Pawpaw has the highest antioxidant activity with an EC50 of 170 ± 1.00 µg/ml, followed by Watermelon (270± 2.00 µg/ml), Mango (320 ± 5.56 µg/ml), Tomatoes (510 ± 4.35 µg/ml) and Guava (620±8.66 µg/ml) respectively.

Table 3: Antioxidant Activity

Fruit	**Antioxidant Activity (EC50 µg/ml)**
Watermelon	270± 2.00
Mango	320± 5.56
Tomatoes	510± 4.35
Guava	620± 8.66
Pawpaw	170± 1.00

Values are expressed as Mean ± Standard deviation, n = 3.

Discussion

Human life is not possible without oxygen (Garba*et al.*, 2022). However, its usage results to the production of superoxide anion among many other RONS. Excess production of these RONS beyond the counteraction of antioxidant machinery of the body results to oxidative stress. In a state of oxidative stress, complementation of the body's antioxidant machinery is necessary (Rahman *et al.*, 2012). Oxidative stress caused by superoxide anion can be ameliorated by exogenous supplementation of superoxide dismutase (EC 1.15.1.1) (Carrilon*et al.*, 2013). Superoxide dismutase from fruits could be a better alternative to the already existing Superoxide dismutase from animal sources (Rosa *et al.*, 2021).

In this research, the Superoxide dismutase (SOD) content of Guava, Watermelon, Mango, Tomatoes and Pawpaw were determined. The result is presented in table 1. Among the aforementioned fruits, Guava has the highest Superoxide dismutase content. This indicates that Guava (27.94 ± 0.45 U/ml) and probably Watermelon (18.95 ± 0.09 U/ml) could be very good sources of Superoxide dismutase. Sivaprakasan*et al.* (2004) have reported the purification of SOD from Guava to apparent homogeneity with 47% recovery. Garusamy*et al.*, (2020) also reported that Guava fruit exhibited considerable amount of enzymatic antioxidant activity including SOD. Watermelon was also reported to be good source of SOD (Soumya and Ramana Rao, 2014). Thus, just as Guava and Watermelon in other climes are good source of SOD, equally, according to our results, the Guava and Watermelon locally available in Bauchi town are also good sources of SOD. Thus, SOD could be purified from these fruits and enhanced to be used in ameliorating oxidative stress specifically caused by superoxide anion.

The fruits under study were also assayed for the presence of the following phytochemicals: Flavonoids, Saponins, Anthraquinones, Tannins, Alkaloids and Cardiac glycosides. These phytochemicals are secondary metabolites which are generated by plants to defend themselves or promote their growth under unfavorable conditions (Pietta, 2000). All the fruits under study contain Flavonoids with Pawpaw having the highest amount (37.88 ± 0.29 mg/100g), followed by Watermelon (35.67 ± 0.47 mg/100g), Mango (30.68 ± 5.26 mg/100g), Tomatoes (25.62 ± 2.98 mg/100g) and Guava (11.61 ± 0.10 mg/100g) respectively. Thus, all the fruits could be good source of Flavonoids. Gurusamy*et al.* (2020) have reported the presence of Flavonoids in Guava. Researchers have also revealed the presence of Flavonoids in Watermelon (Ahmed and Majaz, 2022), Mango (Iheagwan*et al.*, 2019) and Tomatoes (Sunmonu*et al.*, 2018). Flavonoids are a class of polyphenolic secondary metabolites found in plants, and thus commonly consumed in the diet of humans. They are recognized for their antioxidant potential (Tungmunnilham*et al.*, 2018; Rasouli, 2017).

Among the fruits, only Watermelon, Mango and Pawpaw contain Saponins. The presence of Saponins in Mango agrees with the report of Iheagwam (2019). Contrary to our findings, Mariya*et al.*(2020) reported the absence of Saponins in Watermelon and Guava. However, in accordance to our findings, Mariya*et al.* (2020) reported the absence of Saponins in Tomatoes. Saponins are reported to possess a variety of biological activity such as antioxidant, immunostimulant and antimicrobial activity (Bawa*et al.*, 2022). Therefore, the fruits that contain Saponins may exert the functions mentioned above.

The results of *in vitro* antioxidant activity of the fruits extract using DPPH method shows that Pawpaw, with an EC50 of 170 ± 1.00 µg/ml has the highest antioxidant activity, then followed by Watermelon (270 ± 2.00 µg/ml), Mango (320 ± 5.56 µg/ml), Tomatoes (510 ± 4.35 µg/ml) and Guava (620 ± 8.66 µg/ml). Surprisingly, Guava that has the highest SOD content is having the lowest antioxidant activity! This could mean that the total antioxidant activity of the fruit is not contributed only by SOD, but also by other phytoconstituents. Thus, Guava could have the highest SOD content and could be the best source of SOD among the fruits, even though it has the lowest total antioxidant activity (which is not due to SOD content only). A careful look at the results of phytochemical analysis presented in table 2 shows that Pawpaw has the highest Flavonoids content, followed by Watermelon, Mango, Tomatoes and Guava respectively. This is the same order (from highest to lowest) of the total antioxidant activity of the fruits extracts. This could mean that the total antioxidant activity of the fruits is majorly (but not exclusively) contributed by Flavonoids. The antioxidant activity of Flavonoids has been reported by many researchers (Rasouli*et al.*, 2017; Tungmunnilhan*et al.*, 2018). Flavonoids are polyphenolic compounds that are very efficient scavengers of free radicals because of their molecular structure which include an aromatic ring with hydroxyl groups containing mobile hydrogen (Halliwel, 1994). More ever, their action can be related to their capacity to reduce and chelate ferric ion which catalyze lipid peroxidation (Sambo *et al.*, 2022).

Conclusion

The present research shows that the fruits under study contain various phytochemicals and thus may serve as sources of these phytochemicals whose role in enhancing human wellbeing is undisputable. The research also reveals that Pawpaw has high antioxidant potential and - either as raw or processed/modified – may be used as source of antioxidants. Finally, the results reveal that Guava and probably, Watermelon are good sources of SOD, and it is suggested that SOD be purified from them and enhanced for possible application in the cosmetics, health and food sectors in order to ameliorate the effects of oxidative stress specifically caused by superoxide anion.

Author's Contribution

All Authors have contributed tremendously during the research.

Conflict of Interest

There is no conflict of interest among the authors in whatever form.

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