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In silico investigation of biochemical and pharmacological activity of the oxidation products of ascorbic acid

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Abstract

The greatest challenge facing the pharmacological use of phytochemicals is the fact that they are subject to modifications by the several processes they may be exposed to. Thus, pharmacological activities of the new compounds become a matter of scientific interest. Scientists are further faced with the challenge of not knowing the direction of research since the activity of some modified products can differ greatly from the primary compounds. In this study, *in silico* approach was used to determine the pharmacological and biochemical activity of ascorbic acid and its oxidation products. The probable activity (Pa) was set at equal to or greater than (≥) 0.900 and probable inactivity (Pi) was set at equal to or less than (≤) 0.03. The oxidation products of ascorbic acid were observed to have lesser antioxidant activity. Other possible pharmacological activities such as antidiabetic and respiratory analeptic were revealed. This approach could guide research on new compounds and their processing induced modified products that will greatly enhance drug discovery and formulation of functional foods.

Keywords: *In silico,* L-ascorbic acid, dehydroascorbic acid, dehydroascorbic acid dimer, 2,3-L-diketogulonate, threonic acid.

Introduction

Phytochemicals have severally been reported to have antioxidant property making them useful in the management of oxidative stress (Danesi and Bordoni, 2008; Halliwell, 2008; Zhang *et al.*, 2013; Jegadeewari *et al.*, 2014). Oxidative stress is implicated in the onset and amplification of pathological conditions such as cancer, cardiovascular disease, neurological disorders, diabetes, ischemia/reperfusion as well as ageing (Dhalla *et al.*, 2000; Jenner, 2003; Dalle-Donne, 2006). The use of products of oxidative damage as biomarkers in the monitoring and management of oxidative stress related diseases substantiates this fact (Valko *et al.*, 2007).

The mechanism of action of antioxidants may be direct, making them extremely important in defense against reactive oxygen species [ROS] or indirect including such action as binding to redox active metals to prevent initiation of free radical reactions (Nimse and Pal, 2015). As a result, antioxidants have the capacity to stabilize or deactivate free radicals to protect cells even at low concentrations hence their relevance in biological systems (Monaghan *et al.*, 2009; Zhang *et al.*, 2013; Jegadeewari *et al.*, 2014).

The toxic effect of synthetic antioxidants has resulted in the increased focus on the antioxidant potential of herbs and vegetables that are commonly part of our diet and also used in traditional medicine. These phytochemicals have complex organic structures that are subject to modifications when exposed to various reactions especially during processing. Such structural modifications can lead to loss or enhanced antioxidant activity that may be attributed to either the primary compounds or their modification products (Ebhohimen *et al.*, 2017).

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Ascorbic acid, a non-enzymatic antioxidant with broad biological activity and essential role in human health, is an example of phytochemical that is readily oxidized during processing and storage (Lee and Labuza, 1975; Steskova *et al.,* 2006; Herbig and Renard, 2017). This water soluble antioxidant is easily oxidized to dehydroascorbic acid and then to 2,3-diketogulonic acid, oxalic acid and threonic acid with reduction of antiscorbutic or reducing properties (Steskova *et al.,* 2006; Lloyd, 2011; Herbig and Renard, 2017). It must be stated further that the availability of several studies on vitamin C have not been able to provide a complete understanding of its degradation (Herbig and Renard, 2017).

The inability of *in vitro* and *in vivo* methods to clearly decipher the possible pharmacological relevance of such derived compounds underscores the need for new approaches. The use of computational methodologies in the study of diverse biological activities of phytochemicals and the discovery of potential pharmaceuticals and nutraceuticals as well as predicting their possible activity and reaction mechanisms is becoming a useful tool with several public databases of phytochemicals available (Sharma and Sarkar, 2012; Tung, 2014; Surakha *et al.,* 2015; Powers and Setzer, 2016; Yi *et al.,* 2017). These processes have been introduced into the Nigerian research space with only modest accomplishments (Setzer and Ogungbe, 2012; Fatumo *et al.,* 2014).

The Prediction Activity Spectra for Substances [PASS] software is used to predict biochemical and pharmacological activities of simple molecules. PASS predictions are presented with appropriate probability values. The value that defines the probability of an activity to be observed in the laboratory is Pa. The probability for the compound to be inactive is reported as Pi. The higher the Pa the higher the probability for the activity to be demonstrated (Sadym *et al.,* 2003; Lagunin *et al.,* 2010).

Materials and Methods

In this study, the Prediction Activity Spectra for Substances [PASS] software (Sadym *et al.,* 2003; Lagunin *et al.,* 2010; Iqbal *et al.,* 2015) was used to analyze L – ascorbic acid and some of its oxidation products to reveal possible biochemical and pharmacological activities as well as their mechanism of action.

External files of chemical compounds

Chemical structures (2D) of L-ascorbic acid, dehydroascorbic acid, dehydroascorbic acid dimer, 2,3-L-diketogulonic acid and threonic acid were obtained with .SDF extension from PubChem Database [https://pubchem.ncbi.nlm.nih.gov] and Sigma Aldrich [http://www.sigmaaldrich.com/catalog]. Perkin Elmer Chem3D software was used to re-orient chemical structures of ascorbic acid derivatives to enhance view. The biochemical and pharmacological spectra for the compounds were obtained *in silico* using PASS 2014 Refined. In this study, the PASS prediction results were set at Pa>0.900 and Pi<0.300 to obtain highest probable activity (Sadym *et al.,* 2003; Lagunin *et al.,* 2010; Iqbal *et al.,* 2015).

Results and Discussion

L-ascorbic acid is the L-enantiomer of ascorbic acid and conjugate acid of L-ascorbate, commonly called Vitamin C. In this study, the predicted pharmacological effects of L – ascorbic acid was obtained at Pa > Pi; with Pa set at 0.900. This is a high probable limit compared to the default limit of demonstrable Pa>0.500 of this software (Sadym *et al.,* 2003; Lagunin *et al.,* 2010). The analysis revealed ascorbic acid to have a probable respiratory analeptic, antioxidant, and antiarthritic potency (Table 1). These possible pharmacological activities have been previously reported (Arrigoni and De-Tullio, 2002; Nimse and Pal; 2015). This antioxidant property is of immense biological significance in cells including animals where it is an important exogenous non-enzymatic antioxidant; it has also been reported to be a suitable standard for antioxidant studies in aqueous *in vitro* assays (Simpson and Ortwerth, 2000; Wilson, 2002).

Table 1: Predicted Pharmacological Activities of Ascorbic Acid

Pa>0.900	Pi<0.300	Activity
0.975	0.002	Respiratory analeptic
0.959	0.002	Antioxidant
0.925	0.004	Acute neurologic disorders treatment
0.919	0.003	Analeptic
0.905	0.004	Antiarthritic

Ascorbic acid is modified upon oxidation (Kennedy *et al.,* 1989; Lloyd, 2011). In our previous study on the effect of heat treatment on phytochemicals in *Aframomum angustifolium* (Ebhohimen *et al.,* 2017), ascorbic acid used as standard in the *in vitro* antioxidant assay was not thermally treated due to the fact that thermal treatment reduces this activity (Nwanchi, 2013). The derivatives of such treatment as thermal and metabolic processes include mainly dehydroascorbic acid, and dehydroascorbic acid dimer. Dehydroascorbic acid can further undergo irreversible hydrolysis to 2,3-diketogulonic acid, threonic acid, L-erythrulose and oxalic acid (Simpson and Ortwerth, 2000). Oxalate and l-erythrulose have been reported to contribute to the formation of kidney stones in susceptible individuals and to the glycation as well as crosslinking of proteins respectively (Steskova *et al*., 2006; Herbig and Renard, 2017).

The possible pharmacological property of these compounds as predicted by the PASS software is different from that of ascorbic acid, the primary compound (Table 2). The antioxidant property of dehydroascorbic acid was lower than that observed for ascorbic acid [Pa:Pi; 0.728:0.003, antioxidant activity]. Dehydroascorbic acid is commonly called oxidized ascorbic acid (Cisternas *et al*., 2014). It has been reported to play important function in many cell types because it can be used to generate ascorbic acid (Wilson, 2002). The structural similarity with ascorbic acid (Figs. 1 and 2) may suggest that the antioxidant property of ascorbic acid is attributed to the hydrogen bonding at C-6 and C-7 positions (Chloe and Min, 2009).

Table 2: Predicted Pharmacological Activity of Dehydroascorbic Acid

Pa>0.900	Pi<0.300	Activity
0.981	0.002	Neurodegenerative diseases treatment
0.975	0.002	Alzheimer's disease treatment
0.970	0.003	Antidiabetic
0.942	0.002	Stroke treatment
0.902	0.004	Respiratory analeptic

Dehydroascorbic acid dimer is an organic heteropentacyclic compound obtained by cyclodimerisation of ascorbic acid [Fig. 3]. It currently does not have any indication as a pharmaceutical compound. PASS analysis revealed a Pa=0.728 which is below the standard set for this study. However, pharmacological activity that may be of interest in the laboratory for this compound include use as an agent to treat neurodegenerative diseases, Alzheimer's disease, diabetes, stroke and respiratory disorders since Pa>0.700 indicate probable activity (Iqbal *et al*., 2015).

Table 3: Predicted Pharmacological Activity of 2,3-Diketogulonic Acid

Pa>0.900	Pi<0.300	Activity
0.953	0.002	Gluconate 2-dehydrogenase (acceptor) inhibitor
0.946	0.001	D-xylulose reductase inhibitor
0.943	0.002	Feruloyl esterase inhibitor
0.936	0.001	Transketolase inhibitor
0.923	0.002	Mucinaminylserine mucinaminidase inhibitor
0.920	0.003	Fucosterol-epoxide lyase inhibitor
0.911	0.002	Peptide agonist
0.910	0.004	Glucose oxidase inhibitor
0.905	0.002	Manganese peroxidase inhibitor
0.903	0.001	Quinoprotein glucose dehydrogenase inhibitor
0.907	0.009	Membrane integrity agonist

2,3-diketogulonic acid is non-enzymatic hydrolysis-product of dehydroascorbate and is devoid of antiscorbutic activity (Steskova *et al.,* 2006). The structural difference between ascorbic acid and 2,3-diketogulonic acid may account for the variance in the predicted activity.

Table 4: Predicted Pharmacological Activity of Threonic Acid

Pa>0.900	Pi<0.300	Activity
0.961	0.002	Feruloyl esterase inhibitor
0.958	0.001	Manganese peroxidase inhibitor
0,952	0.000	Levansucrase inhibitor
0.950	0.001	Mucinaminylserine mucinaminidase inhibitor
0.950	0.002	Fucosterol-epoxide lyase inhibitor

0.943	0.001	Transketolase inhibitor
0.941	0.001	Polygalacturonase inhibitor
0.940	0.002	Glucose oxidase inhibitor
0.938	0.001	D-xylulose reductase inhibitor
0.938	0.001	Peptide agonist
0.936	0.004	Membrane integrity agonist
0.932	0.001	Shikimate O-hydroxycinnamoyltransferase inhibitor
0.932	0.002	Sulfite oxidase inhibitor
0.931	0.001	Glycopeptide alpha-N-acetylgalactosaminidase inhibitor
0.928	0.001	Quinoprotein glucose dehydrogenase inhibitor
0.927	0.001	Galacturan 1,4-alpha-galacturonidase inhibitor
0.927	0.001	Aspartate 4-decarboxylase inhibitor
0.924	0.001	Endo-1,3(4)-beta-glucanase inhibitor
0.926	0.003	NADPH peroxidase inhibitor
0.926	0.004	Sphinganine kinase inhibitor
0.923	0.002	Alcohol dehydrogenase (NADP+) inhibitor
0.920	0.003	Lipid metabolism regulator
0.917	0.003	Fragilysin inhibitor
0.916	0.003	Alkylacetylglycerophosphatase inhibitor
0.911	0.001	Mannonate dehydratase inhibitor
0.914	0.004	Acrocylindropepsin inhibitor
0.914	0.004	Chymosin inhibitor
0.914	0.004	Saccharopepsin inhibitor
0.909	0.002	Xylan endo-1,3-beta-xylosidase inhibitor
0.904	0.001	Trehalose-phosphatase inhibitor
0.905	0.002	3-Phytase inhibitor
0.904	0.002	Pyruvate decarboxylase inhibitor
0.906	0.004	Beta-adrenergic receptor kinase inhibitor
0.906	0.004	G-protein-coupled receptor kinase inhibitor
0.902	0.003	2-Dehydropantoate 2-reductase inhibitor

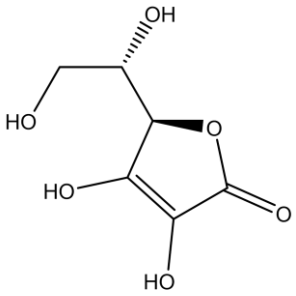

Fig 1: Ascorbic acid

Source:
https://pubchem.ncbi.nlm.nih.gov/compound/54670067

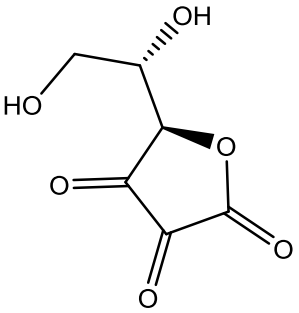

Fig. 2: Dehydroascorbic acid

Source:
https://pubchem.ncbi.nlm.nih.gov/compound/440667

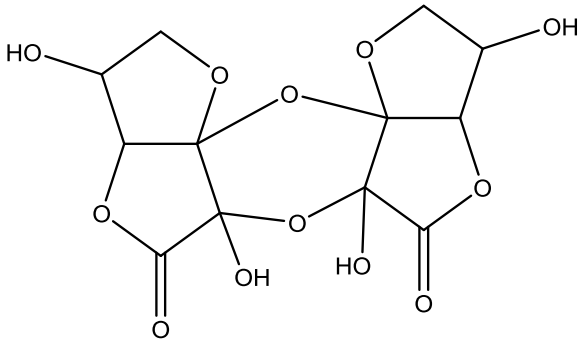

Fig. 3: Dehydroascorbic acid dimer

Source:
http://www.sigmaaldrich.com/catalog/product/sigma/d8132?lang=en&region=NG

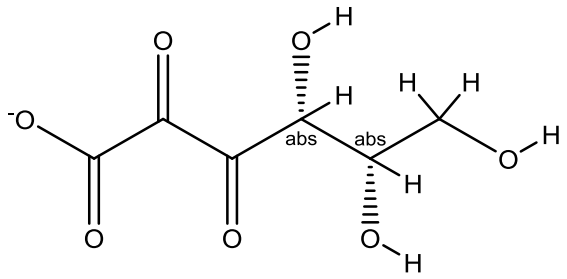

Fig. 4: 2,3-L-diketogulonate
Source:
https://pubchem.ncbi.nlm.nih.gov/compound/21145097

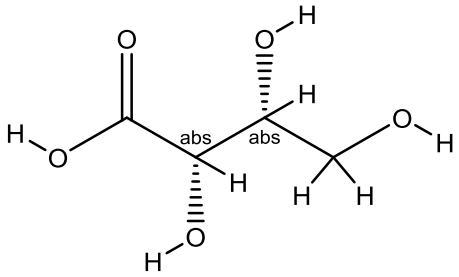

Fig. 5: Threonic acid
Source:
https://pubchem.ncbi.nlm.nih.gov/compound/151152

Other predicted biochemical activicites such as the transport related actions, gene expression regulation, metabolism related action, antitargets and toxicity of the derivatives of ascorbic acid returned values of Pa<0.900 except for the antitarget activity of dehydroascorbic acid as a peroxidase inhibitor [Pa>0.941] which is slightly lower than the Pa value for ascorbic acid as a peroxidase antitarget [Pa>0.975]..

The overall result of this computer – aided study agrees with the reported reduction of the antioxidant capacity of ascorbic acid when oxidized (Steskova *et al.*, 2006; Herbig and Renard, 2017). The information on the possible pharmacological and biochemical properties of phytochemicals is important in discovery of novel drugs. The lead provided will drastically reduce the overall cost and time involved in the drug discovery process (Sharma and Sarkar, 2012; Tung, 2014). This will also enhance the efficiency and expand the application of these medicinal plants.

Conclusion

The introduction of computer-aided appraoches to the drug discovery process in Nigeria with diverse flora holds great hope for the future especially in a time when the global trend is geared towards increasing awareness and use of plant resources. This approach will also enhance understanding and use of modification products. Increased focus on the characterization of the bioactive components of crude extracts from plants is suggested.

Conflict of Interest

There are no competing financial interests.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

Arrigoni, O. and De Tullio, M.C. (2002). Ascorbic acid: Much more than an antioxidant. *Biochimica et Biophysica Acta* 1569:1-9.

Chloe, E. and Min, B.D. (2009). Mechanisms of Antioxidants in the Oxidation of Foods. *Comprehensive Reviews in Food Science and Food Safety* 8:345-358.

Cisternas, P., Silva-Alvarez, C., Martinez, F., Fernandez, E., Ferrada, L., Oyarce, K. et al. (2014). The oxidized form of vitamin C, dehydroascorbic acid, regulates neuronal anergy metabolism. *Journal of Neurochemistry* 129:663-671

Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D. and Milzani, A. (2006). Biomarkers of oxidative damage in human disease. *Clinical Chemistry* 52:601–623.

Danesi, F. and Bordoni, A. (2008). Effect of home freezing and Italian style of cooking on antioxidant activity of edible vegetables. *Journal of Food Science* 73:109-112.

Dhalla, N.S., Temsah, R.M. and Netticadan, T. (2000). Role of oxidative stress in cardiovascular diseases. *Journal Hypertension* 18:655–673.

Ebhohimen, E.I., Edemhanria, L., Ekozin, A. and Okolie, N.P. (2017). Effect of heat treatment on the antioxidant capacity of aqueous and ethanol extracts of *Aframomum angustifolium* seed. *Tropical Journal of Natural Product Research* 1:125-128.

Fatumo, S,A., Adoga, M.P., Ojo, O.O., Oluwagbemi, O., Adeoye, T., Adeoye, T., et al. (2014). Computational Biology and Bioinformatics in Nigeria. *PLoS*

Comput Biol 10: e1003516. doi:10.1371/journal.pcbi.1003516.

Halliwell, B. (2008). Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Archives of Biochemistry and Biophysics* 476:107-112.

Herbig, A.L. and Renard, C. (2017). Factors that impact the stability of vitamin C at intermediate temperatures in a food matrix. *Food Chemistry* 220:444-451.

Iqbal, S.S., Gurumurthy, P., Pravinkumar, P.P. and Pillai, K.S. (2015). GC-MS analysis, heavy metal content and predication of anti-diabetic activity spectra of a novel polyherbal formulation. *International Journal of Advanced Research* 1:276-281.

Jegadeewari, P., Daffodil, E. D. and Mohan, Y. R. (2014). Quantification of total phenolics, flavonoids and *in vitro* antioxidant activity of *Aristolochia bracteata Retz*. *International Journal of Pharmacy and Pharmaceutical Sciences* 6:747-752.

Jenner, P. (2003). Oxidative stress in Parkinson's disease. *Annals of Neurology* 53: 26–36.

Kennedy, J.F., White, C.A., Warner, F.P., Lloyd, L.L. and Rivera, Z.S. (1989). The identification and analysis of the oxidation products of L-ascorbic acid by HPLC C. *Journal of Micronutrient Analysis* 5:9-109.

Lagunin, A., Filimonov, D and Poroikov, V. (2010). Multi – targeted natural products evaluation based on biological activity prediction with PASS. *Current Pharmaceutical Design* 16:1703-1717.

Lee, S.H. and Labuza, T.P. (1975). Destruction of ascorbic acid as a function of water activity. *Journal of Food Science* 40:370–373.

Lloyd, L. (2011). Identification of oxidation products of L-ascorbic acid by HPLC. *Retrieved from www.agilent.com/chem* November 7, 2017.

Monaghan, P., Metcalfe, N.B. and Torres, R. (2009). Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters* 12:75–9.

Nimse, S.B. and Pal, D. (2015). Free radicals, natural antioxidants and their reaction mechanisms. *RSC Advances* 5:279-286.

Nwanchi, E. O. (2013). Effect of heat treatment on the antioxidant properties of *Tetrapleural tetraptera, Xylopia aethopica and Piper guineense. International Journal of Biotechnology and Food Science* 1:1-5.

Powers, C.N and Setzer, W.N. (2016). An *in silico* investigation of phytochemicals as antiviral agents against dengue fever. *Combinatorial Chemistry & High Throughput Screening* 19: 516-536.

Sadym, A., Lagunin, A., Filimonov, D. and Poroikov, V. (2003). Prediction of biological activity spectra via internet. *SAR and QSAR in Environmental Research.* 14:339-347.

Setzer, W. N. and Ogungbe, I. V. (2012). *In silico* investigation of antitrypanosomal phytochemicals from Nigerian medicinal plants. *PLoS Neglected Tropical Diseases.* 6: e1727.

Sharma, V. and Sarkar, I.N. (2012). Bionformatics opportunities for identification and study of medicinal plants. *Briefings in Bionformatics* 14:238-252.

Simpson, L.W. G. and Ortwerth, B.J. (2000). The non-oxidative degradation of ascorbic acid at physiological conditions. *Biochimica et Biophysica Acta* 1501:12-24.

Steskova, A., Morochovicova, M. and Leskova, E. (2006). Vitamin C degradation during storage of fortified foods. *Journal of Food and Nutrition Research* 45:55-61.

Surakha, S.J., Rakesh, N.R., Priya, V.G.S. and Madabhavi, P. (2015). *In silico* approach and medicinal chemistry study on *Vibrio choleare* species: A review. *International Journal of Advanced Research in Science, Engineering and Technology* 2:714-721.

Tung, C-w. (2014). Public database of plant natural products for computational drug discovery. *Current Computer – aided Drug Design* 10:191-196.

Valko, M., Leibfritz, D., Moncola, J., Cronin, M. T.D., Mazura, M. & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology* 39:44–84.

Wilson, J.X. (2002). The physiological role of dehydroascorbic acid. *FEBS Letters* 527:5-9

Yi, F., Sun, L., Xu, L-j., Peng, Y., Lui, H-b., He, C-n. and Xiao, P-g. (2017). *In silico* approach for anti – thrombosis drug discovery: $P2Y_1R$ structure – based TCMs screening. *Frontiers in Pharmacology* 7:531

Zhang, K., Deng, K., Luo, H., Zhou, J., Wu, Z. and Zhang, W. (2013). Antioxidant properties and phenolic profiles of four Chinese Za wines produced from hull-less barley or maize. *Journal of the Institute of Brewing* 119:182-190.