Analysis of the oxidative stress inhibition potentials of Artemisia annua and its bioactive compounds through in vitro and in silico studies

Stephen Adakole EJEMBI1*, Titilayo Omolara JOHNSON1, Jonathan Dingkwoet DABAK1, Augustina Oduje AKINSANMI1, Jane-Rose Ifuanyachi OCHE1 and Timothy FRANCIS2

1Department of Biochemistry, Faculty of Basic Medical Sciences; 2Department of Pharmacology, Faculty of Pharmaceutical Sciences; University of Jos, P.M.B. 2084, Jos. Plateau State, Nigeria.

Received 21st June 2021; Accepted 3rd August 2021

Abstract
Oxidative stress overwhelms the antioxidant mechanisms of living systems, with active involvement in the pathogenesis of several diseases. Natives of Gangnim in the Plateau State of Nigeria may be unknowingly endowed with some protective advantages against oxidative stress for their habitual consumption of Artemisia annua tea. The antioxidant activities of A. annua extracts were determined using in vitro methods and the inhibitory potentials of twenty-nine (29) bioactive compounds of the plant against oxidative stress target proteins were assessed through molecular docking analysis. These extracts showed significantly high activities in scavenging nitric oxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing ferric (Fe3+) to ferrous (Fe2+) iron. Virtually, none of the bioactive compounds binds to the active site of the antioxidant protein targets. Rather, 72.41, 93.10 and 75.86% of these compounds bind with high binding affinity to the activator binding sites of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) respectively. 7,8-dimethylalloxazine (-8.10 kcal/mol) ranked highest as a prospective inhibitor of xanthine oxidase (XOX). The antioxidant activity exhibited by the extracts of the locally cultivated A. annua and the molecular interactions of its bioactive compounds against the protein targets used predict that oxidative stress inhibition could be effectively achieved with these phytochemicals.

Keywords: Artemisia annua, antioxidant, antioxidant protein targets, molecular docking, oxidative stress

INTRODUCTION
The consumption of locally brewed ‘tea’ and decoction of A. annua has been a common practice among the natives of Gangnim, in Langtang South Local Government Area (L.G.A.) of Plateau State, (North-Central) Nigeria, with the hope of treating malaria. Interestingly, past and recent studies had shown that the Gangnim people are not on the wrong [1-2]. For more than two millennia, A. annua has been in the spotlight as a medicinal plant in Chinese Pharmacopoeia for the cure of malaria [3-5]. Malaria cure efficacy of this medicinal plant has been a trending issue in several studies. There are possibilities, however, that the consumption of A. annua confers more health benefits than just malaria cure.
One of such possibilities could be the potential to prevent or ameliorate the deleterious effect of oxidative stress. This study was carried out, assessing this possibility by the evaluation of the antioxidant activity and oxidative stress inhibition potential of extracts from leaf and seed, as well as some bioactive compounds of the plant. Oxidative stress was coined to mean an oxidant-antioxidant imbalance that results in an overwhelming activity of free radicals against the antioxidant system of living cells [6-7]. The involvement of free radicals (radical generating agents) in a number of chronic diseases [8-9] cannot be overemphasized. The mitochondrial respiratory chain, NAD(P)H oxidase (NOX), xanthine oxidase (XOX) and nitric oxide synthases (NOS) had been reported as the main sources of endogenous free radicals in blood vessels [6]. In short, every system that involves the use of enzymes and oxygen to perform any function in living cells is exposed to free radical reactions. When in excess, they have the potential to cause oxidative damage to DNA, proteins, lipids and other small cellular molecules [10,11] by ‘stealing’ electrons from these molecules [9]. This electron ‘theft’ by free radicals may eventually cause diabetes, cancer, and several degenerative diseases in humans [12]. Inagi [6(p139)] opined that “host cells are endowed with a number of antioxidant systems to limit (free radical) levels. O$_2^-$ may be dismutated by a family of SODs to (a less reactive species) H$_2$O$_2$. H$_2$O$_2$ can be scavenged to water by CAT or by glutathione peroxidase (GPx) in the presence of GSH’’. Studies have shown that antioxidant supplements are in use to boost the activity of the built-in antioxidant system in order to combat oxidative stress [9,13]. Unfortunately, some of these synthetic antioxidants are reported to be involved in several diseases and their use had been discontinued in many developed countries [14], promoting the need for health professionals to search for alternative sources of antioxidants based on natural origin, which may be safer, more effective and economical, preferably from plant materials based on indigenous resources [15].

Historically, plants are well known to contain a wide variety of free radical scavenging molecules, such as flavonoids, carotenoids, and vitamins [16]. Convincingly, the curative properties of medicinal plants are conceivably due to the presence of these secondary metabolites and Artemisia annua, commonly known as “sweet wormwood” in English, “qinghao” in Chinese and “armoise annuelle” in French [3] is among the potential medicinal plants of antioxidant properties, exhibiting several promising characteristics of which over five hundred (>500) bioactive compounds had been reported to have been characterized [17] including the popular antimalarial drug, artemisinin [5].

The results of our study beep with optimism that oxidative stress chemoprotection with phytochemicals from A. annua plant could probably be one of the most feasible approaches for the cure of diseases for which their pathogenesis and progression are traceable to oxidative stress.

**EXPERIMENTAL METHODS**

**Plant sample collection.** The leaf and seed of A. annua used in this study were harvested near flowering stage from the nursery farm of the Centre for Biotechnology and Genetic Engineering sited in Gangnim, Langtang South L.G.A. of Plateau State, Nigeria. The freshly harvested plant was identified and authenticated by Mr. J.J. Azila of Federal School of Forestry, University of Jos, Jos, Plateau State and assigned a voucher number: No. FHJ 249 of herbarium specimen.

**Aqueous extraction.** The method described by Asuzu [18] was used for the aqueous extraction. The filtrate was concentrated using a hot water bath at 50°C and air-dried. Dried extracts were labeled AAL (aqueous leaf extract of A. annua) and AAS (aqueous seed extract of A. annua).
extract of *A. annua*) and kept in the freezer until it was ready to be used.

**Methanol extraction.** Methanol extraction was carried out, using the method described by Chu, *et al.* [19]. The filtrate was poured on a flat plastic tray and left to air dry. Dried extracts were labeled MAL (methanol leaf extract of *A. annua*) and MAS (methanol seed extract of *A. annua*) and stored in the freezer until it was ready to be used.

**Qualitative and quantitative phytochemical determination.** Qualitative phytochemical analysis was carried out using standard methods [20-22]. The total phenolic and flavonoid contents of extracts were determined using the method described by Singleton, *et al.* [23] and Meda, *et al.* [24] respectively using UV/Vis spectrophotometry.

**In vitro antioxidant analysis of extracts of *A. annua* leaf and seed.** The method by Oyaizu [25] was followed to evaluate the iron-reducing property, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to Gyamfi, *et al.* [26] and nitric oxide radical scavenging activity according to Marcocci, *et al.* [27].

**Molecular docking**

*Crystal structure of target proteins and ligands collection.* Twenty-nine (29) previously identified and characterized bioactive compounds of *A. annua* in a review by Nigam, *et al.* [5] and standard ligands were docked against eight selected protein targets (Table 1) alongside known inhibitors of these targets. *SOD, GSH-Px* and *CAT* were used for the *in silico* evaluation of antioxidant activity while Xanthine oxidase (*XOX*), NADPH oxidase (*NOX*), caspase-1 (*Casp-1*), endothelial nitric oxide synthase (*eNOS*) and induced nitric oxide synthase (*iNOS*) were the other five targets selected as a result of their direct involvement in the generation of free radicals. Crystal structures of these targets were accessed from the protein data bank [28] with their PDB IDs (Table 1). Structure data file (SDF) format of bioactive compounds of *A. annua*, standard ligands reported to be agonists of antioxidant targets (trehalose, metformin and vitamin E), and inhibitors of oxidative stress proteins (asymmetric dimethylarginine (ADMA), indoprofen, 6-mercaptopurine, and VAS2870) were obtained from PubChem database. The PubChem CIDs of ligands are shown in Tables 4 & 5.

**Proteins and ligands preparation, docking and Post docking analysis.** Prior to the docking analysis, all non-standard residues (all bound ligands, cofactors, and water molecules) were removed from our target proteins. The Proteins were checked for polar hydrogen, and torsion bonds of the ligands were selected and defined. Gasteiger charges were computed, and the AutoDock atom types were defined using AutoDock version 4.2 as described by Singh, *et al.* [29]. AutoDock vina in PyRx software was used to perform the molecular docking while the post docking analysis was carried out using Chimera 1.14 at 100 steepest minimizations and 10 steps of the conjugate gradient. A relax constraints of 0.4 Angstrom and 20° were used to determine the conventional H+ bond interaction of ligands with the side chains of the amino acids of targets. Chimera 1.14 and Discovery Studio 2020 were used for the 3D (3-dimensional) and 2D (2-dimensional) interactions, respectively, adopting the method described by Rana, *et al.* [30] and Johnson, *et al.* [31].

**Data analysis.** The results in triplicates of the *in vitro* assays were collated and expressed as mean ± standard error mean (SEM). Analysis of Variance (ANOVA) (followed by the Bonferroni post-hoc t-test) was used for result analysis and significance difference at *P* <0.05, using Microsoft Excel 2010. The binding affinity ( - ΔG kcal/mol) of ligands were recorded as generated by the computational tools unaltered.
RESULTS AND DISCUSSION

Phyto constituents of extracts of *A. annua* leaf and seed. The brewing of *A. annua* plant for tea by Gangnim community dwellers is justifiable: the results of our study (Table 2) reveal that flavonoids and phenols were higher in the leaf aqueous extract than the methanol extraction. Emmanuel, et al. [1] reported the presence of similar classes of phytochemicals in their n-hexane extracts of *A. annua* from the same community. The phytochemicals of *A. annua* are predominantly terpenoids (in particular sesquiterpene lactones), flavonoids, coumarins and other shikimate metabolites (Table 3), of which *A. annua* is currently the only commercial source of the sesquiterpene lactone, Artemisinin [17,32,43]. Studies have shown that the majority of these groups of phytochemicals have been established to have significant antioxidant activity [33] and hot water is an effective extraction solvent [3,34]. Phenolics, according to Oboh and Ademosun [35] are capable of scavenging free radicals, chelating metals, activating antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases.

In vitro antioxidant activity of extracts of *A. annua* leaf and seed. Results (Figure 1) reveal a significant difference in the iron-reducing activity of MAS at 27.28 μg/ml, with p < 0.05 measured in ascorbic acid equivalent (AAE). The reduction of ferric iron (Fe$^{3+}$) to ferrous iron (Fe$^{2+}$) and the chelation of excess iron is very crucial in the antioxidant mechanism [32,33,36]. The extracts displayed significantly high iron-reducing activity with MAL showing the highest activity (0.066μg AAE/mg extracts). Phenolic compounds are known to protect against a wide range of diseases including certain types of cancers and part of the antioxidant effects of flavonoids is in their ability to chelate metals such as iron and copper [32].

The results of the DPPH scavenging assay (Figure 2) show a significant difference in the activity of extracts at 83.3μg/ml. The results of nitric oxide scavenging activity (Figure 3) revealed that all the extracts followed a normal curve pattern and their activity is dose-dependent. Reactive peroxynitrite (ONOO$^{-}$), a product from the reaction of nitric oxide (NO) and superoxide anion, is known to produce serious toxic reactions with protein, lipids and nucleic acids. The scavenging of NO by extracts of *A. annua* may prevent the pathological effect caused by excessive generation of ONOO$^{-}$ [37].

Binding energies of bioactive compounds of *A. annua* plants. Binding affinity (ΔG kcal/mol) of ligands which had been identified as activators of SOD, CAT and GSH-Px, and those of standard protein target inhibitors are displayed in Table 4 and Table 5 respectively. SOD, CAT and GSH-Px (targets) are involved in antioxidant pathways as described in biochemistry [38] form an excellent choice for this study. The study of Rana, et al. reported that some phytochemicals “act as agonists of (antioxidant enzymes) and increase the activity of SOD, GSH-Px and CAT” [30(p4)].

The results of this study show that 3,5-di-O-cafeoylquinic acid, 5-O-[(E)-cafeoyl]quinic acid, Daucosterol, 7,8-dimethylalloxazine, Methyl-3,4-di-O-cafeoylquinic acid and Methyl-3,5-di-O-cafeoylquinic acid ranked highest with docking score ranging from −7.30 to −6.50 (kcal/mol) compared to −5.50 (kcal/mol) of Trehalose (standard ligand) against SOD. The docking scores of 3,4-di-O-cafeoylquinic acid, 3,5-di-O-cafeoylquinic acid, 4,5-di-O-cafeoylquinic acid, Artemisinin, β-sitosterol, 1,3-di-O-cafeoylquinic acid and Daucosterol were higher than that of standard ligand (Metformin (−5.40 kcal/mol)) with 4,5-di-O-cafeoylquinic being the highest (−9.40 kcal/mol) and closely followed by β-sitosterol (−9.30 kcal/mol) against CAT. Similarly, 3,5-di-O-cafeoylquinic acid, β-sitosterol, 1,3-di-O-cafeoylquinic acid, Daucosterol and Methyl-3,5-di-O-cafeoylquinic acid ranked highest in their binding affinity to GSH-Px.
compared to Vitamin E (-5.80 kcal/mol). Overall, 72.41, 93.10 and 75.86% of these bioactive compounds are likely to be agonists of SOD, CAT and GSH-Px respectively for their high and promising binding energies towards the activator sites of these proteins. Virtually, none of the bioactive compounds of A. annua in this study had significant binding affinity for the active sites of these antioxidant proteins.

Concurrently, 3,4-di-O-cafeoylquinic acid, 3,5-di-O-cafeoylquinic acid, 4,5-di-O-cafeoylquinic acid, β-sitosterol, Daucosterol and 7,8-dimethylalloxazine were ligands with topmost binding affinities for iNOS, eNOS, Casp-1, XOX and NOX compared to standard inhibitors of each protein (Table 5). Only in the docking against NOX is VAS2870 (-7.50 kcal/mol) higher than 4,5-di-O-cafeoylquinic acid (-7.30 kcal/mol). Generally, 93.10, 100.00, 13.79, 96.55 and 17.24% of these bioactive compounds of A. annua have high prospects of being iNOS, eNOS, Casp-1, XOX and NOX inhibitors, respectively.

Nitric oxide is produced in living cells via nitric oxide synthases (NOS). Studies have shown that three isoforms of NOS: eNOS (endothelial NOS), nNOS (neuronal NOS) and iNOS (inducible NOS), had been identified [39]. Our study made use of two of these isoforms with emphasis on the iNOS. “Inducible nitric oxide synthase is a key enzyme responsible for the production of nitric oxide (NO) and it plays an important role in oxidative stress” [37(p398)].

| Table 1: Target proteins and their binding site grid-box dimension |
|---------------------------------------------------------------|
| Protein targets                                               | PDB ID   | Dimension |
| #                | Name               | Abbrev | x       | Y       | z       |
| Blind docking                                             |          |
| 1                | Superoxide dismutase | SOD    | 1PU0   | 33.5431 | 44.5211 | 41.6160 |
| 2                | Catalase            | CAT    | 1QQW   | 68.1248 | 80.4353 | 75.6187 |
| 3                | Glutathione peroxidase | GSH-Px | 2I3Y   | 42.7537 | 48.5262 | 50.4933 |
| 4                | Xanthine oxidase    | XOX    | 3B9J   | 36.9225 | 46.5775 | 52.5419 |
| 5                | NADPH oxidase       | NOX    | 1OEY   | 28.9431 | 45.4195 | 34.4152 |
| 6                | Caspase-1           | Casp-1 | 5MMV   | 43.5554 | 51.3561 | 66.1002 |
| 7                | Endothelial nitric oxide synthase | eNOS | 3NOS | 57.7616 | 72.4500 | 53.7818 |
| 8                | inducible nitric oxide synthase | iNOS | 2NSI | 45.5762 | 74.8195 | 60.7555 |
| Active site directed docking                                 |          |
| 1                | Xanthine oxidase    | XOX    | 3B9J   | 14.3699 | 15.2060 | 15.7181 |
| 2                | Caspase-1           | Casp-1 | 5MMV   | 6.2216  | 11.2864 | 7.5276  |

| Table 2: Results of qualitative and quantitative phytochemical analyses of extracts of A. annua leaf and seed |
|---------------------------------------------------------------|
| Screened phytochemicals | AAL | AAS | MAL | MAS |
|-------------------------|-----|-----|-----|-----|
| Alkaloids (µgQUE/mg extract) | +   | ND  | +   | ND  |
| Flavonoids (µgQUE/mg extract) | 0.033±0.002  | 0.032±0.000  | 0.029±0.000  | 0.026±0.002* |
| Phenols (µgGAE/mg extract) | 0.119±0.010  | 0.109±0.005  | 0.104±0.009  | 0.096±0.002 |
| Saponins | +   | ND  | +   | ND  | +   | ND  |
| Terpenes | +   | ND  | +   | ND  | -   | ND  | +   | ND  |
| Cardiac Glycosides | +   | ND  | +   | ND  | -   | ND  | -   | ND  |
| Balsam | +   | ND  | +   | ND  | +   | ND  | +   | ND  |
| Carbohydrate | +   | ND  | +   | ND  | +   | ND  | +   | ND  |
| Tannins | +   | ND  | +   | ND  | +   | ND  | -   | ND  |
| Resins | +   | ND  | +   | ND  | +   | ND  | -   | ND  |

+ Indicates the presence of the phytochemical; - indicates the absence of the phytochemical; ND-means Not determined; Values are presented in mean ± standard error mean (SEM) of triplicate results (n = 3). Superscript (*) indicates group that is statistically significant as compared to aqueous extract of A. annua seed (AAS). Difference was considered significant at p < 0.05.
(AAS), Methanol extract of A. annua leaf (MAL) and Methanol extract of A. annua seed (MAS). QUE – quercetin equivalent; GAE – gallic acid equivalent; Qtl. = Qualitative.

| Table 3: Bioactive compounds of A. annua and their class of phytochemicals |
|---------------------------------------------------------------|
| **Class of Phytochemicals** | **Sub-class** | **Bioactive compounds of A. annua** |
|----------------------------|---------------|-----------------------------------|
| 1. Polyphenols             |               | 3,4-di-O-caffeoylquinic acid      |
|                            |               | 3,5-di-O-caffeoylquinic acid      |
|                            |               | 4,5-di-O-caffeoylquinic acid      |
|                            |               | 5-O-[(E)-caffeoyl]quinic acid     |
|                            |               | 1,3-di-O-caffeoylquinic acid      |
|                            |               | Methyl-3,4-di-O-caffeoylquinic acid|
|                            |               | Methyl-3,5-di-O-caffeoylquinic acid|
|                            |               | p-hydroxybenzoic acid             |
|                            |               | Salicylic acid                    |
| Cumarins                   |               | Scopoletin                        |
|                            |               | Scoparone                         |
|                            |               | Scopolin                          |
| Flavonols                  |               | Chrysosplenol D                   |
|                            |               | Chrysosplenetin                   |
| Flavonols                  |               | Casticin                          |
|                            |               | Quercetagetin-6,7,4′-trimethyl ether|
|                            |               | Quercetagetin-6,7,3′,4′-tetramethyl ether|
| Flavone                    |               | Artemetin                         |
|                            |               | Arteannuic acid                   |
|                            |               | Arteannuin B                      |
|                            |               | Artemisinin                       |
| 2. Terpenoids              |               | Artemisinic acid                  |
|                            |               | Deoxy-artemisinin                 |
| Sesquiterpenoids           |               | β-sitosterol                      |
|                            |               | Daucosterol                       |
| Triterpenoids (Sterols)    |               | 7,8-dimethylalloxazine            |
|                            |               | Nicotinic acid                    |
| 3. Vitamins                |               | Uracil                            |
|                            |               | Domesticoside                     |
| Riboflavin                 |               | 7,8-dimethylalloxazine            |
| Niacin                     |               | Nicotinic acid                    |
| Nucleotide base            |               | Uracil                            |
| Aryl ketone                |               | Domesticoside                     |

N/B: This classification is based on the reviews by Matsui, et al. [43], Ferreira, et al. [32] & Brown [17]

| Table 4: Docking score of compounds of Artemisia annua plant against antioxidant protein targets (kcal/mol) |
|---------------------------------------------------------------|
| **Compounds** | **PubChem CID** | **SOD** | **CAT** | **GSH-Px** |
|----------------|-----------------|---------|---------|------------|
| 1 Trehalose (standard ligand) | 7427 | -5.50 | - | - |
| 2 Metformin (standard ligand) | 4091 | - | -5.40 | - |
| 3 Vitamin E (standard ligand) | 14985 | - | - | -5.80 |
| 4 3,4-di-O-caffeoylquinic acid | 5281780 | -5.90 | -8.40 | -6.50 |
| 5 3,5-di-O-caffeoylquinic acid | 6474310 | -7.20 | -7.70 | -7.10 |
| 6 4,5-di-O-caffeoylquinic acid | 6474309 | -6.40 | -9.40 | -6.80 |
| 7 Domesticoside | 75072039 | -6.10 | -6.50 | -5.80 |
| 8 Arteannuic acid | 578305 | -5.30 | -6.60 | -6.70 |
| 9 Arteannuin B | 6543478 | -5.80 | -6.90 | -6.50 |
| 10 Artemisinin | 68827 | -6.00 | -7.90 | -6.60 |
| 11 Artemisinic acid | 10922465 | -5.50 | -7.40 | -6.80 |
| 12 Artemetin | 5320351 | -5.90 | -6.80 | -6.60 |
| 13 β-sitosterol | 222284 | -6.30 | -9.30 | -7.00 |
| 14 5-O-[E]-caffeoylquinic acid | 25244622 | -6.70 | -7.00 | -6.40 |
| 15 1,3-di-O-caffeoylquinic acid | 6474640 | -6.40 | -8.70 | -7.20 |
| #   | Compounds                                      | PubChem CID | iNOS | eNOS | Casp-1 | XOX | NOX |
|-----|-----------------------------------------------|-------------|------|------|--------|-----|-----|
| 16  | Chrysosplenol D                               | 5280699     | -6.10| -6.80| -6.90  |     |     |
| 17  | Chrysosplenetin                               | 5281608     | -6.20| -7.60| -6.80  |     |     |
| 18  | Casticin                                      | 5315263     | -5.90| -7.10| -6.60  |     |     |
| 19  | Deoxy-artemisinin                            | 12814879    | -6.00| -7.00| -6.40  |     |     |
| 20  | Daucosterol                                   | 5742590     | -6.50| -8.60| -7.00  |     |     |
| 21  | 7,8-dimethylalloxazine                       | 5326566     | -6.50| -7.60| -6.70  |     |     |
| 22  | Methyl-3,4-di-O-caffeoylquinic acid           | 5319160     | -7.30| -7.90| -6.50  |     |     |
| 23  | Methyl-3,5-di-O-caffeoylquinic acid           | 5319161     | -6.60| -7.90| -7.20  |     |     |
| 24  | Nicotinic acid                                | 938         | -4.50| -4.80| -4.50  |     |     |
| 25  | p-hydroxybenzoic acid                        | 135         | -4.50| -6.50| -4.70  |     |     |
| 26  | Quercetagetin-6,7,4'-trimethyl ether          | 44259869    | -6.10| -7.70| -6.60  |     |     |
| 27  | Quercetagetin-6,7,3',4'-tetramethyl ether     | 14376220    | -6.20| -6.60| -6.70  |     |     |
| 28  | Salicylic acid                                | 338         | -4.40| -6.50| -5.00  |     |     |
| 29  | Scopoletin                                    | 5280460     | -5.30| -7.00| -5.80  |     |     |
| 30  | Scoparone                                     | 8417        | -5.30| -6.00| -5.80  |     |     |
| 31  | Scopolin                                      | 439514      | -6.20| -6.80| -6.40  |     |     |
| 32  | Uracil                                        | 1174        | -4.40| -5.30| -4.00  |     |     |

**Table 5:** Docking score of compounds of *Artemisia annua* plant against oxidative stress protein targets (kcal/mol)
Table 6: Active site directed docking score of compounds of Artemisia annua plant against antioxidant protein targets (kcal/mol)

| #  | Compounds                               | PubChem CID | SOD   | CAT   | GSH-Px |
|----|-----------------------------------------|-------------|-------|-------|--------|
| 1  | Trehalose (standard ligand)              | 7427        | 43.30 | -     | -      |
| 2  | Metformin (standard ligand)              | 4091        | -     | 4.80  | -      |
| 3  | Vitamin E (standard ligand)              | 14985       | -     | -     | -      |
| 4  | 3,4-di-O-caffeoylquinic acid             | 5281780     | 90.60 | 76.70 | -      |
| 5  | 3,5-di-O-caffeoylquinic acid             | 6474310     | 92.50 | 94.90 | -      |
| 6  | 4,5-di-O-caffeoylquinic acid             | 6474309     | 93.60 | 92.00 | -      |
| 7  | Domesticoside                           | 75072039    | 56.60 | 31.80 | -      |
| 8  | Arteannuin acid                         | 578305      | 50.80 | 19.80 | -      |
| 9  | Arteanuin B                             | 6543478     | 51.90 | 33.70 | -      |
| 10 | Artemisinin                             | 68827       | 75.00 | 58.10 | -      |
| 11 | Artemisinic acid                        | 10922465    | 42.20 | 21.60 | -      |
| 12 | Artemetin                               | 5320351     | 88.70 | 54.70 | -      |
| 13 | β-sitosterol                            | 222284      | 122.30| 85.60 | -      |
| 14 | 5-O-[(E)-caffeoyl]quinic acid           | 25244622    | 55.60 | 38.60 | -      |
| 15 | 1,3-di-O-caffeoylquinic acid            | 6474640     | 96.10 | 86.70 | -      |
| 16 | Chrysosplenol D                         | 5280699     | 89.50 | 52.80 | -      |
| 17 | Chrysosplenetin                         | 5281608     | 87.40 | 51.70 | -      |
| 18 | Casticine                               | 5315263     | 85.10 | 63.60 | -      |
| 19 | Deoxy-artemisinin                       | 12814879    | 64.40 | 39.90 | -      |
| 20 | Daucosterol                             | 5742590     | 154.60| 143.40| -      |
| 21 | 7,8-dimethylalloxazine                  | 5326566     | 56.30 | 31.80 | -      |
| 22 | Methyl-3,4-di-O-caffeoylquinic acid      | 5319160     | 94.90 | 88.40 | -      |
| 23 | Methyl-3,5-di-O-caffeoylquinic acid      | 5319161     | 97.10 | 101.10| -      |
| 24 | Nicotinic acid                          | 938         | 3.90  | 0.80  | 49.10 |
| 25 | p-hydroxybenzoic acid                   | 135         | 6.80  | 0.00  | 83.50 |
| 26 | Quercetagentin-6,7,4'-trimethyl ether    | 44259869    | 83.20 | 45.50 | -      |
| 27 | Quercetagentin-6,7,3',4'-tetramethyl ether| 14376220   | 83.70 | 53.80 | -      |
| 28 | Salicylic acid                          | 338         | 5.90  | 0.20  | 61.60 |
| 29 | Scopoletin                              | 5280460     | 20.60 | 7.00  | -      |
| 30 | Scoparone                               | 8417        | 25.90 | 14.60 | -      |
| 31 | Scopolin                                | 439514      | 74.60 | 42.70 | -      |
| 32 | Uracil                                  | 1174        | 1.00  | -2.00 | 29.10 |

Table 7: Active site directed docking score of compounds of Artemisia annua plant against xanthine oxidase and caspase-1 (kcal/mol)

| #  | Compounds                               | PubChem CID | XOX   | Casp-1 |
|----|-----------------------------------------|-------------|-------|--------|
| 1  | 6-mecaptopurine (standard ligand)        | 667490      | -4.6  | -      |
| 2  | Allopurinol (standard ligand)            | 135401907   | -5.7  | -      |
| 3  | Ayclovir (standard ligand)               | 1353985213  | -6.4  | -      |
| 4  | Fenbufen (standard ligand)               | 3335        | -     | -6.3   |
| 5  | Indoprofen (standard ligand)             | 3718        | -     | -6.8   |
| 6  | Aspirin (standard ligand)                | 2244        | -     | -5.0   |
| 7  | 3,4-di-O-caffeoylquinic acid             | 5281780     | 3.10  | 99.00  |
| 8  | 3,5-di-O-caffeoylquinic acid             | 6474310     | 4.70  | 68.80  |
| 9  | 4,5-di-O-caffeoylquinic acid             | 6474309     | 2.50  | 79.60  |
| 10 | Domesticoside                           | 75072039    | -2.60 | 12.70  |
| 11 | Arteannuin acid                         | 578305      | -5.30 | 10.80  |
| 12 | Arteanuin B                             | 6543478     | -5.40 | 40.50  |
| 13 | Artemisinin                             | 68827       | -0.80 | 33.80  |
| 14 | Artemisinic acid                        | 10922465    | -5.30 | 17.60  |
| 15 | Artemisin B                             | 5320351     | -5.50 | 98.90  |
| 16 | β-sitosterol                            | 222284      | 0.70  | 80.80  |
| 17 | 5-O-[(E)-caffeoyl]quinic acid            | 25244622    | -6.10 | 25.60  |
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| No. | Compound                              | CAS    | AAE | pEC50 |
|-----|---------------------------------------|--------|-----|-------|
| 18  | 1,3-di-O-caffeoylquinic acid          | 6474640| 0.20| 91.90 |
| 19  | Chrysoplenol D                        | 5280699| -5.60| 35.00 |
| 20  | Chrysoplenetin                        | 5281608| -5.50| 78.00 |
| 21  | Casticin                              | 5315263| -5.60| -5.80 |
| 22  | Deoxy-artemisinin                     | 12814879| -2.70| -6.20 |
| 23  | Daucosterol                           | 5742590| 5.70 | -7.00 |
| 24  | 7,8-dimethylalloxazine                | 5326566| -8.10| -6.90 |
| 25  | Methyl-3,4-di-O-caffeoylquinic acid   | 5319160| 0.90 | -6.50 |
| 26  | Methyl-3,5-di-O-caffeoylquinic acid   | 5319161| 2.50 | -6.40 |
| 27  | Nicotinic acid                        | 938    | -5.90| -4.00 |
| 28  | p-hydroxybenzoic acid                 | 135    | -6.70| -4.50 |
| 29  | Quercetagettin-6,7,4′-trimethyl ether | 44259869| -6.70| -5.90 |
| 30  | Quercetagettin-6,7,3′,4′-tetramethyl ether | 14376220| -4.50| -6.00 |
| 31  | Salicylic acid                        | 338    | -6.70| -5.50 |
| 32  | Scopoletin                            | 5280460| -7.90| -5.40 |
| 33  | Scoparone                             | 8417   | -6.10| -5.20 |
| 34  | Scopolin                              | 439514 | -4.50| -6.10 |
| 35  | Uracil                                | 1174   | -5.10| -4.10 |

**Figure 1:** Iron reducing power of aqueous and methanol extracts of *Artemisia annua* leaf and seed

*Values are mean ± SEM of triplicate results (n = 3). (*) indicates group that is statistically significant as compared to MAS at 27.28μg/ml. Differences are considered significant at p < 0.05 with Two-way ANOVA and the Bonferroni post-hoc t – test correction factor. (AAE-Ascorbic acid Equivalent)*

**Figure 2:** DPPH scavenging activities of aqueous and methanol extracts of *Artemisia annua* leaf and seed
Values are mean ± SEM of triplicate results (n = 3). (*) indicates difference in the DPPH scavenging activity between 83.3μg/ml and 166.7μg/ml. (a) indicates group that is statistically significant as compared to AAL at 83.3μg/ml and the superscripts (a, b) indicate groups that are statistically significant as compared to AAS and MAS respectively at 83.3μg/ml. Differences are considered significant at p < 0.05 with Two-way ANOVA with the Bonferoni post-hoc t–test correction factor.

Figure 3: Nitric oxide scavenging activities of aqueous and methanol extracts of Artemisia annua leaf and seed. There is no significant difference in the nitric oxide radical scavenging ability of extracts of the different solvent system used.

Molecular docking interactions (site directed docking). The active sites of targets were accessed as deposited in the Computed Atlas of Surface Topography of protein (CASTp) database [40] to assess the binding interactions of ligands and the amino acid side chains positioned at the active site of these proteins. The results of our study revealed that the bioactive compounds of A. annua and the standard ligands had a positive change in Gibb’s energy (ΔG) (kcal/mol) in the docking interaction with the antioxidant target proteins, except for uracil (-2.00 kcal/mol) in the docking against CAT (Table 6). Following the fact that the more negative the binding energy, the better the protein-ligand association and the stability of complex [41], our high docking scores (− ΔG kcal/mol) in the non-site specific docking against these antioxidant proteins (Table 5) suggest that these ligands are not inhibitors of SOD, CAT and GSH-Px.

Protein active site directed docking against oxidative stress targets (Casp-1 and XOX) revealed that 7,8-dimethylalloxazine (-6.90 kcal/mol) and Daocosterol (-7.00 kcal/mol) had higher docking scores than indoprofen (-6.80 kcal/mol) against casp-1 (Table 7). The molecular (3D and 2D) interactions of indoprofen (-6.80 kcal/mol), daucosterol (-7.00 kcal/mol) and 7, 8-dimethylalloxazine (-6.90 kcal/mol) against Casp-1 are expressed in Figure 4, and those of acyclovir (-6.40 kcal/mol), 7, 8-dimethylalloxazine (-6.90 kcal/mol) and scoleotin (-7.90) against XOX are shown in Figure 5.

Conventional H⁺ bond, Van der waals’ force, Pi-alkyl bond, Pi-carbon and Carbon hydrogen bonds were among the several bond interactions involved in the ligand-target binding. The conventional H⁺ bond is said to be stable and a reliable bond in this interaction [42]. In Figure 4, indoprofen (A), daucosterol (B), and 7, 8-dimethylalloxazine (C) interacted with conventional H⁺ bonds to at least two amino acids at the active site of Casp-1 (ARG 161, ARG 163 and TYR 198) with several other amino acids in which they share other bond types. The interaction with xanthine oxidase (Figure 5) reveals that acyclovir (D), 7, 8-dimethylalloxazine (E) and scoleotin (F),
bond with at least five conventional H⁺ bonds with the amino acids, GLU 802, ALA 1079, THR 1010, VAL 1011 and ARG 880. Acyclovir, however, had a weaker binding (van der waals’ force with ALA 1079 and ARG 880.

Figure 4: 3D (left) and 2D (right) interactions of indoprofen (A), daucosterol (B) and 7, 8 – dimethylalloxazine (C) with caspase-1
These interactions (Figure 4 & 5) suggest therefore a stable binding of these bioactive compounds of *A. annua* to these targets, and daucosterol, 7,8-dimethylalloxazine, scopoletin may be good substitutes for the existing target inhibitors that are known with adverse effects.
Conclusion. Plant extracts of Artemisia annua leaf and seed from Gangnim showed reasonable antioxidant activity. Literature reports that antioxidants reduce oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer. The antioxidant assay, molecular docking scores and molecular interactions of compounds of A. annua with respective protein targets used is suggestive of A. annua plant having the prospect to prevent oxidative stress and cure pathological conditions that may arise due to oxidative stress.

Acknowledgment. We are grateful to Prof. John C. Aguyi of Africa Center of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria for generously donating the Artemisia annua leaf and seed used for this study, and Mr. Adeboyega E. Abayomi, a postgraduate student of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Jos, for lending his computational biology skill for this study.

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