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

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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 (Fe^{3+}) to ferrous (Fe^{2+}) 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.

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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 *SOD*s to (a less reactive species) H_2O_2. H_2O_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 chemo-protection 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^{o}C and air-dried. Dried extracts were labeled *AAL* (aqueous leaf extract of *A. annua*) and *AAS* (aqueous seed

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^{o} 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 Bonferioni 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-caffeoylquinic acid, 5-O-[(E)-caffeoyl]quinic acid, Daucosterol, 7,8-dimethylalloxazine, Methyl-3,4-di-O-caffeoylquinic acid and Methyl-3,5-di-O-caffeoylquinic 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-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, Artemisinin, β-sitosterol, 1,3-di-O-caffeoylquinic acid and Daucosterol were higher than that of standard ligand (Metformin (-5.40 kcal/mol)) with 4,5-di-O-caffeoylquinic being the highest (-9.40 kcal/mol) and closely followed by β-sitosterol (-9.30 kcal/mol) against *CAT*. Similarly, 3,5-di-O-caffeoylquinic acid, β-sitosterol, 1,3-di-O-caffeoylquinic acid, Daucosterol and Methyl-3,5-di-O-caffeoylquinic 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-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic 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-caffeoylquinic 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	Dimension		
	#	Name	Abbrev	ID	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	42.7357	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*	
	Qlt.	Quantitative	Qlt.	Quantitative	Qlt.	Quantitative	Qlt.	Quantitative
Alkaloids	+	ND	+	ND	+	ND	-	ND
Flavonoids (µgQUE/mg extract)	+	0.033±0.002	+	0.032±0.000	+	0.029±0.000	+	0.026±0.002[a]
Phenols (µgGAE/mg extract)	+	0.119±0.010	+	0.109±0.005	+	0.104±0.009	+	0.096±0.002
Saponins	+	ND	+	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 ([a]) indicates group that is statistically significant as compared to aqueous extract of *A. annua* seed (*AAS*). Difference was considered significant at $p < 0.05$ Aqueous extract of *A. annua* leaf (*AAL*), aqueous extract of *A. annua* seed

(*AAS*), Methanol extract of *A. annua* leaf (*MAL*) and Methanol extract of *A. annua* seed (*MAS*). QUE – quercetin equivalent; GAE – gallic acid equivalent; Qlt. = 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	Phenolic acid (Chlorogenic acid)	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 Casticin Quercetagetin-6,7,4′-trimethyl ether Quercetagetin-6,7,3′,4′-tetramethyl ether
	Flavone	Artemetin
2. Terpenoids	Sesquiterpenoids	Arteannuic acid Arteannuin B Artemisinin Artemisinic acid Deoxy-artemisinin
	Triterpenoids (Sterols)	β-sitosterol Daucosterol
3. Vitamins	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)-caffeoyl]quinic acid	25244622	-6.70	-7.00	-6.40
15	1,3-di-O-caffeoylquinic acid	6474640	-6.40	-8.70	-7.20

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)

#	Compounds	PubChem CID	iNOS	eNOS	Casp-1	XOX	NOX
1	ADMA (standard ligand)	123831	-5.80	-6.40	-	-	-
2	Indoprofen (standard ligand)	3718	-	-	-6.80	-	-
3	6-mercaptopurine (standard ligand)	667490	-	-	-	-4.50	-
4	VAS2870 (standard ligand)	4058452	-	-	-	-	-7.50
5	3,4-di-O-caffeoylquinic acid	5281780	-9.30	-9.00	-6.80	-7.20	-7.80
6	3,5-di-O-caffeoylquinic acid	6474310	-9.10	-9.40	-7.40	-7.40	-8.30
7	4,5-di-O-caffeoylquinic acid	6474309	-8.90	-9.40	-6.80	-7.30	-7.30
8	Domesticoside	75072039	-6.90	-6.90	-5.80	-6.70	-6.40
9	Arteannuic acid	578305	-7.40	-7.10	-5.70	-6.20	-6.40
10	Arteannuin B	6543478	-7.50	-7.20	-6.00	-7.20	-6.30
11	Artemisinin	68827	-8.40	-8.50	-6.50	-6.80	-7.40
12	Artemisinic acid	10922465	-6.70	-6.80	-5.40	-6.80	-6.70
13	Artemetin	5320351	-7.50	-6.80	-6.00	-7.00	-6.30
14	β-sitosterol	222284	-8.50	-6.80	-6.80	-7.80	-6.00
15	5-O-[(E)-caffeoyl]quinic acid	25244622	-8.10	-6.80	-6.40	-7.10	-6.50
16	1,3-di-O-caffeoylquinic acid	6474640	-9.60	-6.80	-6.90	-6.70	-6.20
17	Chrysosplenol D	5280699	-7.50	-6.80	-5.90	-7.10	-6.40
18	Chrysosplenetin	5281608	-7.60	-6.80	-6.10	-7.10	-6.50
19	Casticin	5315263	-7.30	-6.80	-5.80	-7.00	-6.30
20	Deoxy-artemisinin	12814879	-7.80	-6.80	-6.20	-6.80	-7.30
21	Daucosterol	5742590	-8.80	-6.80	-7.00	-8.10	-7.60
22	7,8-dimethylalloxazine	5326566	-8.50	-6.80	-6.90	-7.50	-7.60
23	Methyl-3,4-di-O-caffeoylquinic acid	5319160	-9.20	-6.80	-6.50	-7.40	-7.30
24	Methyl-3,5-di-O-caffeoylquinic acid	5319161	-9.10	-6.80	-6.40	-7.00	-7.90
25	Nicotinic acid	938	-5.30	-6.80	-4.00	-4.60	-4.70
26	p-hydroxybenzoic acid	135	-6.10	-6.80	-4.50	-4.90	-5.00
27	Quercetagetin-6,7,4′-trimethyl ether	44259869	-7.90	-6.80	-5.90	-7.30	-6.50
28	Quercetagetin-6,7,3′,4′-tetramethyl ether	14376220	-8.00	-6.80	-6.00	-6.60	-6.30
29	Salicylic acid	338	-6.20	-6.80	-5.50	-4.90	-5.40
30	Scopoletin	5280460	-6.70	-6.80	-5.40	-5.90	-5.70
31	Scoparone	8417	-7.00	-6.80	-5.20	-6.00	-5.60
32	Scopolin	439514	-8.20	-6.80	-6.10	-7.50	-6.70
33	Uracil	1174	-5.10	-6.80	-4.10	-4.30	-4.70

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	Arteannuic acid	578305	50.80	19.80	-
9	Arteannuin 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	Casticin	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	Quercetagetin-6,7,4′-trimethyl ether	44259869	83.20	45.50	-
27	Quercetagetin-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	Acyclovir (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	Arteannuic acid	578305	-5.30	10.80
12	Arteannuin B	6543478	-5.40	40.50
13	Artemisinin	68827	-0.80	33.80
14	Artemisinic acid	10922465	-5.30	17.60
15	Artemetin	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

18	1,3-di-O-caffeoylquinic acid	6474640	0.20	91.90
19	Chrysosplenol D	5280699	-5.60	35.00
20	Chrysosplenetin	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	Quercetagetin-6,7,4′-trimethyl ether	44259869	-6.70	-5.90
30	Quercetagetin-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

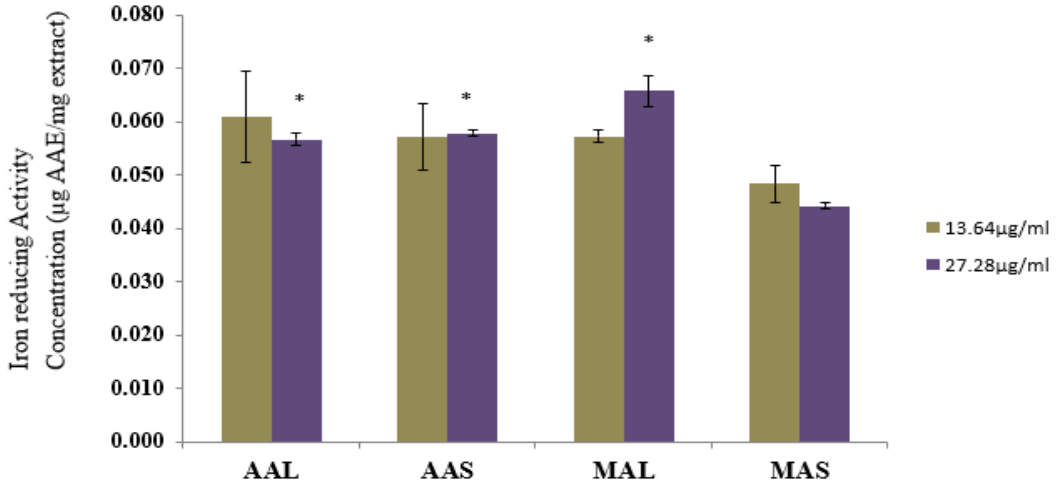

Figure 1: Iron reducing power of aqueous and methanol extracts of *Artemisia annua* leaf and seed
Values are mean ± SEM of triplicate results (n = 3). (*) indicates group that is statistically significant as compared to MAS at 27.28µg/ml. Differences are considered significant at p < 0.05 with Two-way ANOVA and the Bonferioni post-hoc t – test correction factor. (AAE-Ascorbic acid Equivalent)

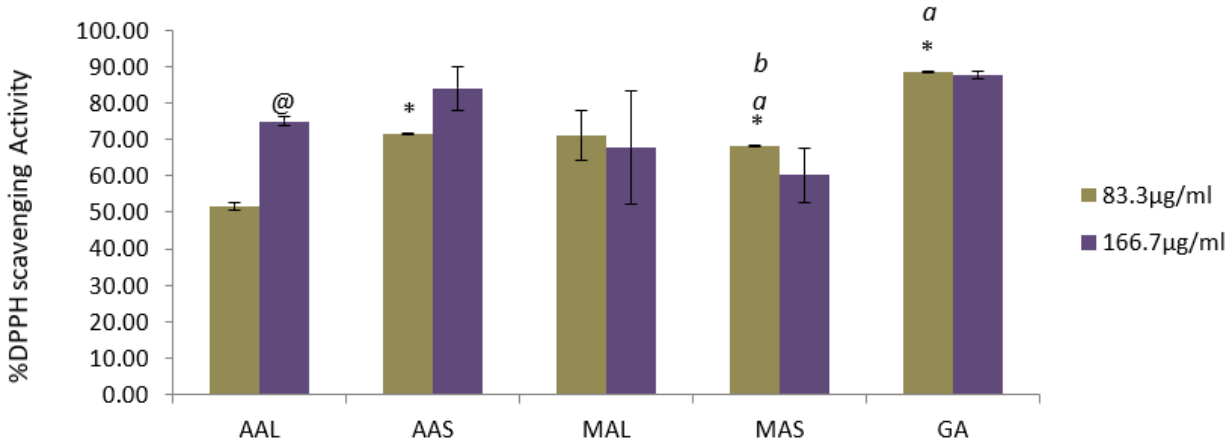

Figure 2: DPPH scavenging activities of aqueous and methanol extracts of *Artemisia annua* leaf and seed

Values are mean ± SEM of triplicate results (n = 3). ($^{@}$) indicates difference in the DPPH scavenging activity between 83.3µg/ml and 166.7µg/ml. (*) 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 Bonferioni post-hoc t – test correction factor

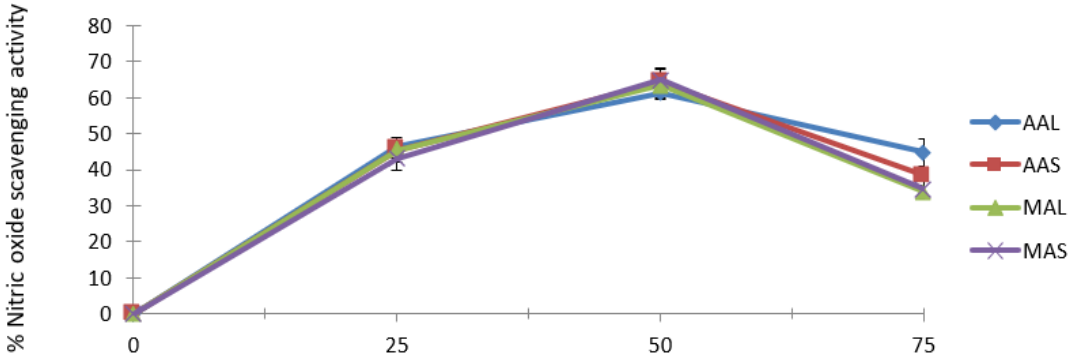

Figure 3: Nitric oxide scavenging activities of aqueous and methanol extracts of *Artemisia annua* leaf and seed *There is no significant difference in the nitric oxide radical scavenging ability of extracts of the different solvent system used.*

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 (-8.10 kcal/mol), Scopoletin (-7.90 kca/mol) were among the five (5) topmost compounds in their binding affinity to the active site of *XOX* than acyclovir (-6.40 kcal/mol), a known xanthine oxidase inhibitor. Similarly, 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), daucosteroal (-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 (-8.10 kcal/mol) and scopoletin (-7.90) against *XOX* are shown in Figure 5.

Conventional H^+ bond, Van der waals' force, Pi-akyl 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 scopoletin (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.

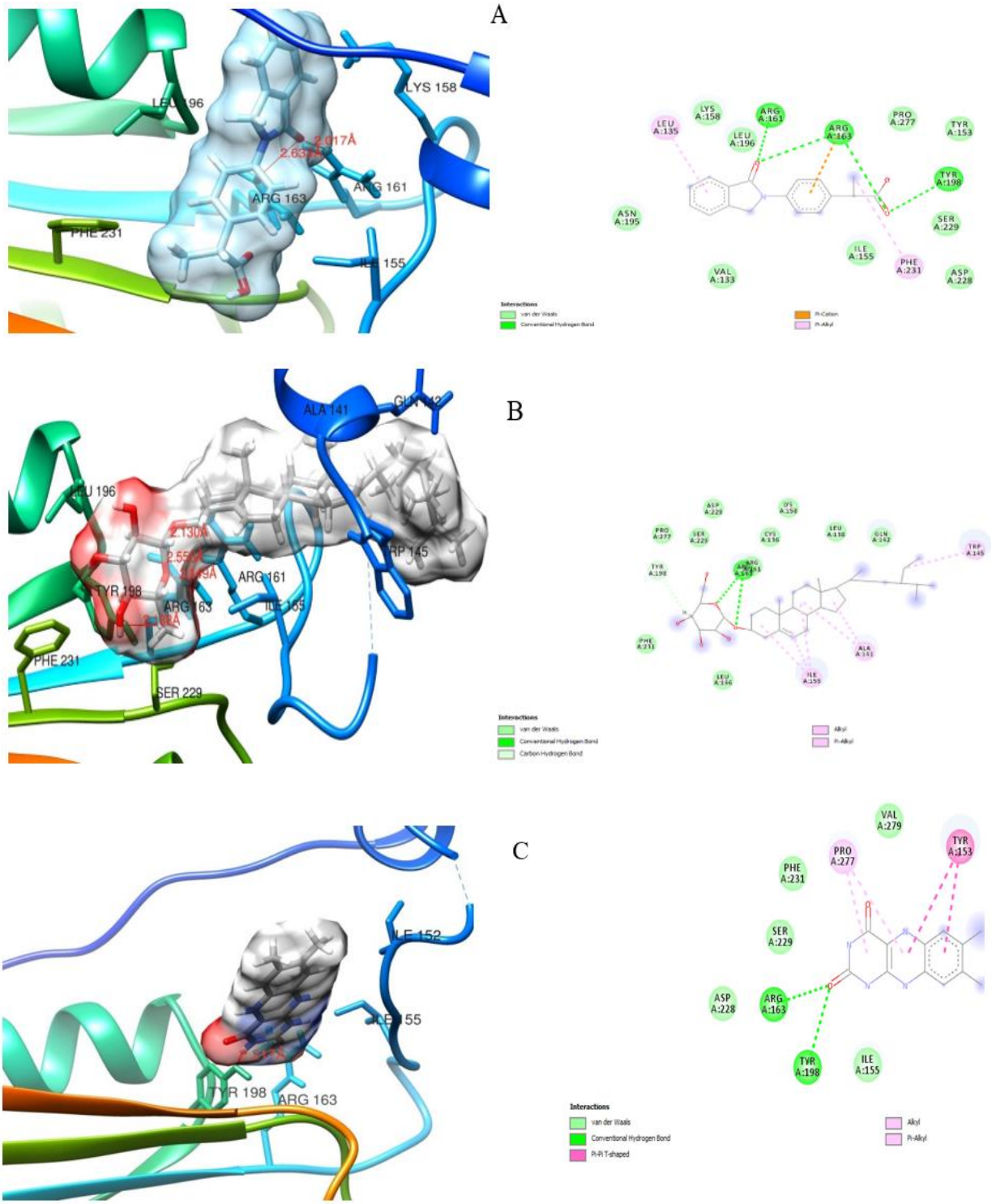

Figure 4: 3D (left) and 2D (right) interactions of indoprofen (A), daucosterol (B) and 7, 8 – dimethylalloxazine (C) with caspase-1

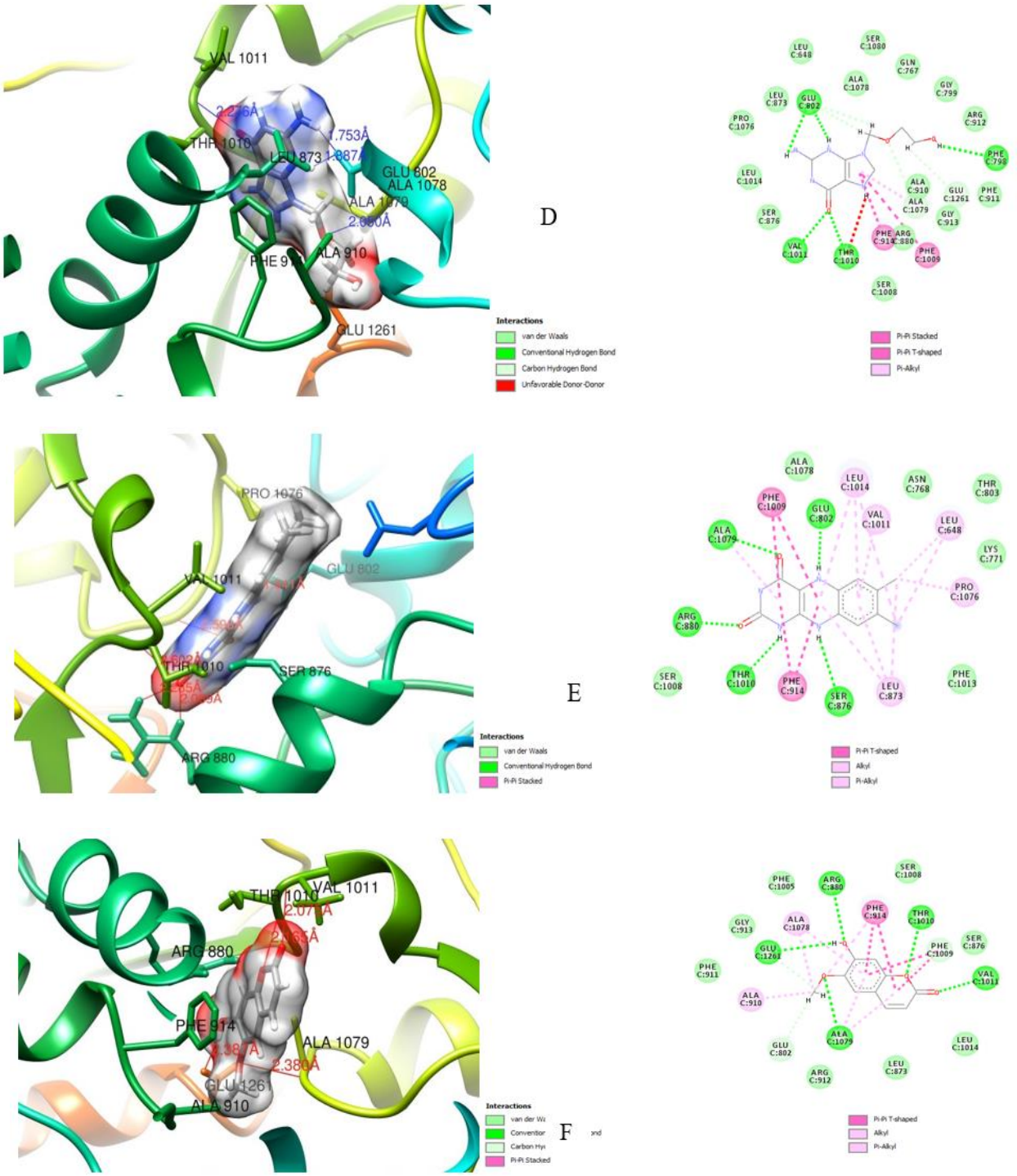

Figure 5: 3D (left) and 2D (right) interactions of acyclovir (D), 7, 8 – dimethylalloxazine (E) and scopuletin (F) with xanthine oxidase

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.

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