Silybin interacting with Cu_4, Ag_4 and Au_4 clusters: Do these constitute antioxidant materials?

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ABSTRACT

We analyze the free radical scavenger capacity of silybin-metal clusters systems by applying Density Functional Theory (DFT) calculations, following two well-known antioxidant mechanisms: the radical adduct formation (RAF) and the hydrogen atom transfer (HAT). As apparent in this investigation, the presence of tetramer clusters increases the antioxidant capacity of silybin and de-protonated silybin. In order to assess further antiradical processes, we also investigated the single electron transfer (SET) process (for the most stable structures derived from RAF and HAT mechanisms). From this study, we concluded that the presence of Cu_4, Ag_4 and Au_4 clusters enhances the free radical scavenging properties of silybin and de-protonated silybin. These metal-organic systems may serve as effective antioxidant materials. These results may provide encouraging guidance for those involved in these experiments and hold promise for future applications.

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1. Introduction

The interaction of metal atoms and clusters to enhance optical, electrical and spectroscopic properties of molecules is an area of active research [1–8]. These coupled systems may serve to produce sensitive biosensors and new antioxidant materials [5]. The presence of metal atoms may have multiple benefits, for example, it is possible to increase the limits for detecting several organic substances that are attached to the metal systems.

Polyphenols comprise a large family of organic molecules that bestow significant beneficial properties [9–14], which correlate strongly with their antioxidant activity. Likewise, they are able to deactivate free radical molecules and retard oxidative stress, using diverse mechanisms [15]. Among polyphenols, silybin (SIL) is a well-documented natural compound that manifests powerful antioxidant activity [16–40]. SIL is the main component of silymarin; a natural mixture that is extracted from the *Silybum marianum* (milk thistle) plant [41].

Previously, we reported that interaction of SIL with Cu, Ag and Au single atoms and tetramer clusters enhances the Raman spectra signal. Our investigation concerning the single electron transfer (SET) process indicates that the capacity to either donate or accept electrons also increases when metal clusters interact with this molecule [5], possibly indicating that these systems are good

antioxidants. In spite of all the studies that focus on the properties of SIL interacting with metal atoms and clusters, only SET has been implemented to analyze the effect of metal clusters on the antioxidant properties of these polyphenol substances. It therefore seems appropriate to study other free radical scavenger mechanisms, to analyze whether these coupled systems constitute good antioxidant materials.

This work aims to study the free radical scavenger capacity of compounds formed from metal clusters and SIL. Under physiological conditions (pH = 7.4) SIL co-exists with the de-protonated [25,28] form ($[SIL_{(-H)}]^{-1}$), thus we also investigate the interaction between metal clusters and ($[SIL_{(-H)}]^{-1}$. Two principal mechanisms for scavenging [42–44] free radicals are studied: the radical addition formation (RAF) and the hydrogen atom transfer (HAT). It is well known that polyphenol molecules such as SIL, mainly use these mechanisms for scavenging free radicals [45]; however as far as we know, there are no investigations concerning SIL attached to metal clusters. Principally, the idea is to analyze whether the presence of metal clusters increases the antioxidant properties of SIL and $[SIL_{(-H)}]^{-1}$. We also investigated the SET mechanism for the most stable structures derived from RAF and HAT, in order to examine further antiradical processes. In the following, it becomes apparent that SIL and $[SIL_{(-H)}]^{-1}$ showed better antioxidant capacity when attached to Cu_4, Ag_4 and Au_4, than they did as bare molecules. These results may serve as an encouraging guide for researchers and may have potential applications.

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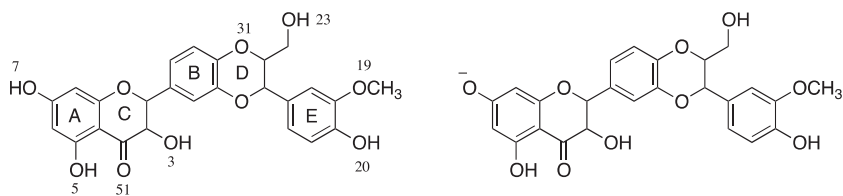 *E-mail address:* mreina.2404@hotmail.com (M. Reina).

Fig. 1. Silybin (SIL) and de-protonated silybin ($[SIL_{(-H)}]^{-1}$).

2. Computational details

Gaussian 09 code [46] was employed to optimize geometries and analyzed the electronic properties of SIL-M_4 and SIL$_{(-H)}$-M_4 (M = Cu, Ag and Au) systems. The interaction with different free radical molecules is also analyzed with the same methodology. Fig. 1 reports a schematic representation of SIL and $[SIL_{(-H)}]^{-1}$ with conventional numbering and labels.

Initial geometries were fully optimized at M06/LANL2DZ level of theory in gas phase [47–54]. All coupled systems were optimized as singlets or doublets. In order to verify optimized minima, we performed harmonic analysis and we identified local minima (zero imaginary frequencies). To study the RAF mechanism, several initial geometries were analyzed. The ·OOH was bonded to every carbon atom forming double bonds, and also to the metal atoms of the clusters. The RAF mechanism was studied according to the following reaction schemes. M represents Cu, Ag or Au in all cases:

For SIL

$$SIL\text{-}M_4 + {}^{\cdot}OOH \rightarrow [SIL\text{-}M_4\text{-}OOH]^{\cdot}$$
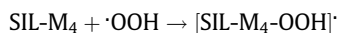
$${}^{\cdot}G_{RAF} = G(SIL\text{-}M_4\text{-}OOH^{\cdot})\text{-}G(SIL\text{-}M_4) + G({}^{\cdot}OOH)$$
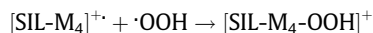
$$[SIL\text{-}M_4]^{+\cdot} + {}^{\cdot}OOH \rightarrow [SIL\text{-}M_4\text{-}OOH]^{+}$$

$${}^{\cdot}G_{RAF} = G([SIL\text{-}M_4\text{-}OOH]^{+}\text{-}G([SIL\text{-}M_4]^{+\cdot}) + G({}^{\cdot}OOH)$$
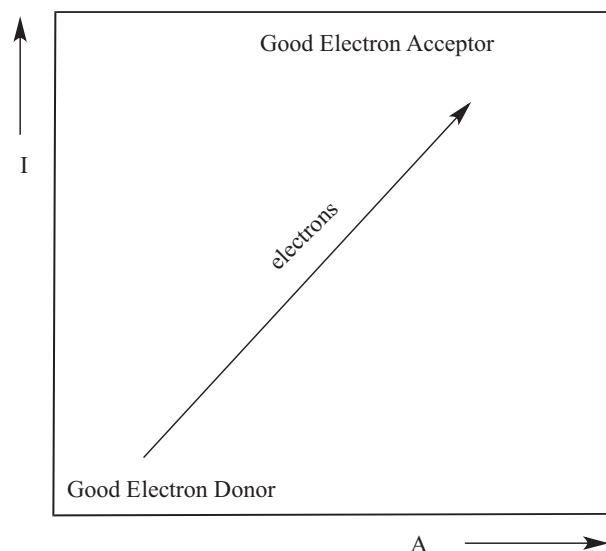
Fig. 2. Full Electron Donor-Acceptor Map (FEDAM).

For $[SIL_{(-H)}]^{-1}$

$$[SIL_{(-H)}\text{-}M_4]^{-1} + {}^{\cdot}OOH \rightarrow [SIL_{(-H)}\text{-}M_4\text{-}OOH]^{-1\cdot}$$
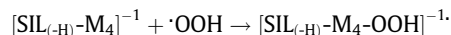
$${}^{\cdot}G_{RAF} = G([SIL_{(-H)}\text{-}M_4\text{-}OOH]^{-1\cdot})\text{-}G([SIL_{(-H)}\text{-}M_4]^{-1}) + G({}^{\cdot}OOH)$$
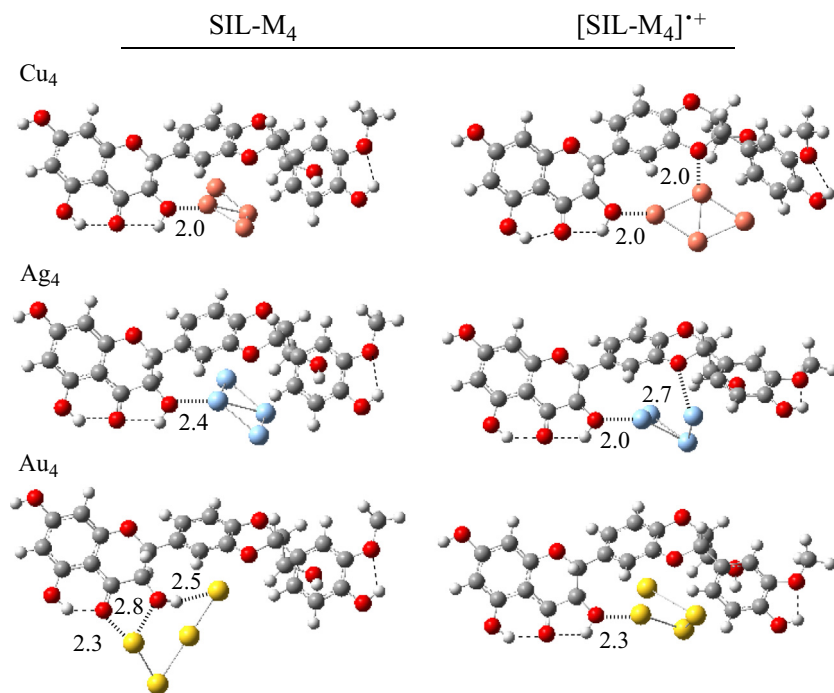
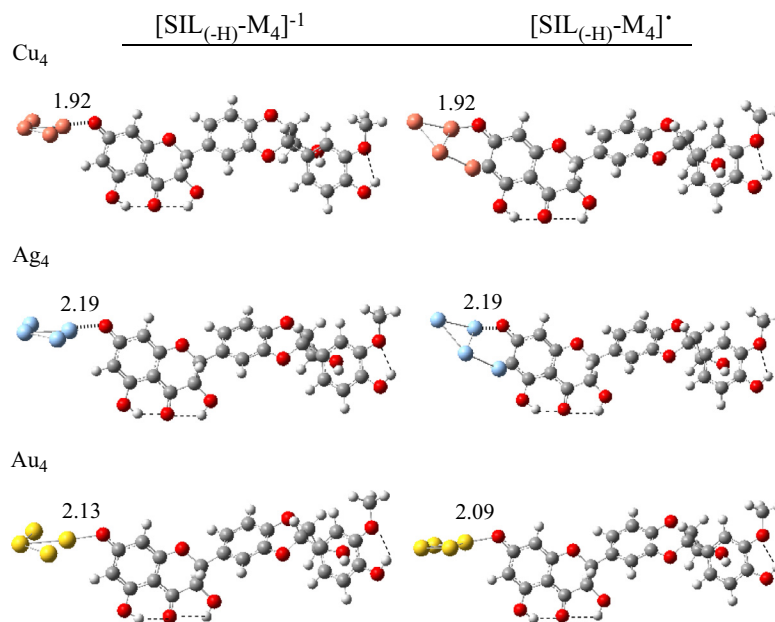Fig. 3. Most stable structures of SIL-M_4 and $[SIL\text{-}M_4]^{\cdot+}$ (M = Cu, Ag or Au).

$[SIL_{(-H)}-M_4]^{-1}$ $[SIL_{(-H)}-M_4]^{\bullet}$

Cu$_4$

Ag$_4$

Au$_4$

Fig. 4. Most stable structures of $[SIL_{(-H)}-M_4]^{-1}$ and $[SIL_{(-H)} - M_4]^{\bullet}$ (M = Cu, Ag or Au).

ΔE (kcal/mol)	[SIL-Cu$_4$-OOH]•
34.4	
0.0	

ΔE (kcal/mol)	[SIL-Ag$_4$-OOH]•
18.5	
0.0	

ΔE (kcal/mol)	[SIL-Au$_4$-OOH]•
5.6	
0.0	
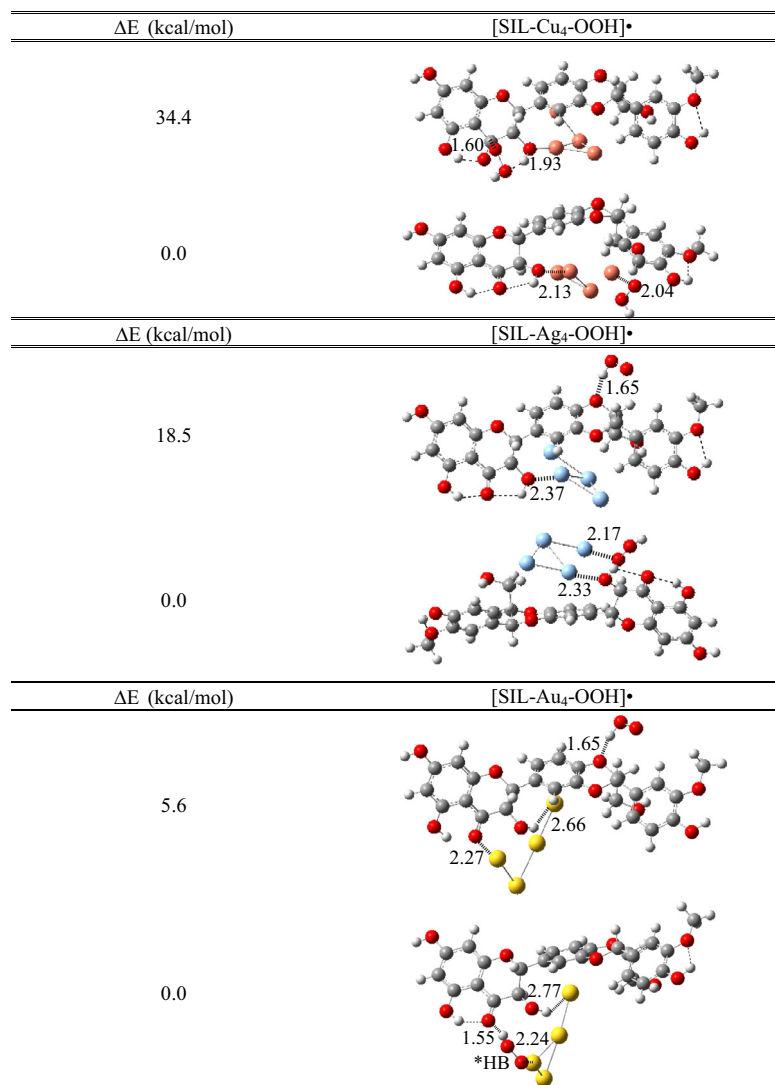
Fig. 5. Most stable structures of $[SIL-M_4-OOH]^{\bullet}$ (M = Cu, Ag or Au).

$[SIL_{(-H)}-M_4]^{·} + ^{·}OOH \rightarrow [SIL_{(-H)}-M_4-OOH]$
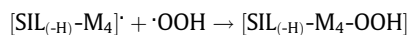
$^{·}G_{RAF} = G([SIL_{(-H)}-M_4-OOH])-G([SIL_{(-H)}-M_4]^{·}) + G(OOH)$

The HAT mechanism is analyzed in terms of the Gibbs free energy (ΔG_{HAT}) obtained as follows for SIL and for $[SIL_{(-H)}]^{-1}$:

$SIL-M_4 + ^{·}R \rightarrow \{[SIL-M_4](-H)\}^{·} + RH$

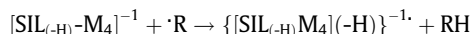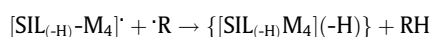$^{·}G_{HAT} = G(\{[SIL -M_4](-H)\}^{·}) + G(RH)-G(SIL-M_4)-G(^{·}R)$

$[SIL-M_4]^{+·} + ^{·}R \rightarrow \{[SIL-M_4](-H)\}^{+} + RH$

$^{·}G_{HAT} = G(\{[SIL-M_4](-H)\}^{+}) + G(RH)-G([SIL-M_4]^{+·}) + G(^{·}R)$

$[SIL_{(-H)}-M_4]^{-1} + ^{·}R \rightarrow \{[SIL_{(-H)}M_4](-H)\}^{-1·} + RH$
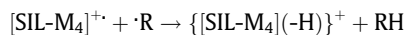
$^{·}G_{HAT} = G(\{[SIL_{(-H)}M_4](-H)\}^{-1·}) + G(RH)-G([SIL_{(-Hn)}-M_4]^{-1}) + G(^{·}R)$

$[SIL_{(-H)}-M_4]^{·} + ^{·}R \rightarrow \{[SIL_{(-H)}M_4](-H)\} + RH$

$^{·}G_{HAT} = G(\{[SIL_{(-H)}M_4](-H)\}) + G(RH)-G([SIL_{(-H)}-M_4]^{·})-G(^{·}R)$

M is Cu, Ag or Au, $\{[SIL-M_4](-H)\}^{·}$ and $\{[SIL-M_4](-H)\}^{+}$ are the dehydrogenated systems from SIL, $\{[SIL_{(-H)}-M_4](-H)\}^{-1·}$ and $\{[SIL_{(-H)}-M_4](-H)\}$ are the dehydrogenated systems from $[SIL_{(-H)}]^{-1}$. $R^{·}$ is the free radical (.OH, .OOH, NO_2 and $C_6H_5O.$) and RH is the free radical bonded to one hydrogen atom (H_2O, H_2O_2, HNO_2 and C_6H_5OH).

The products of the most exergonic reactions coming from both mechanisms were selected to analyze the SET mechanism *i.e.* the capability to either donate or accept an electron. To investigate the SET mechanism, the vertical ionization energy (*I*) and the vertical electron affinity (*A*) were obtained from single point calculations of cationic and anionic molecules, using the optimized structure of the neutrals at M06/6-311+g(d,p)/LanL2DZ level of theory. Water was included to simulate polar environment using the SMD continuum model [55]. FEDAM (Full Electron Donor-Acceptor Map) is a previously reported visual tool [56,57]. In this map (see Fig. 2) *I* and *A* are plotted and allow us to classify substances as either donors or acceptors of electrons. Electrons will be transferred from molecules located down to the left of the map (good electron donors) to those molecules that are up to the right (good electron acceptors).

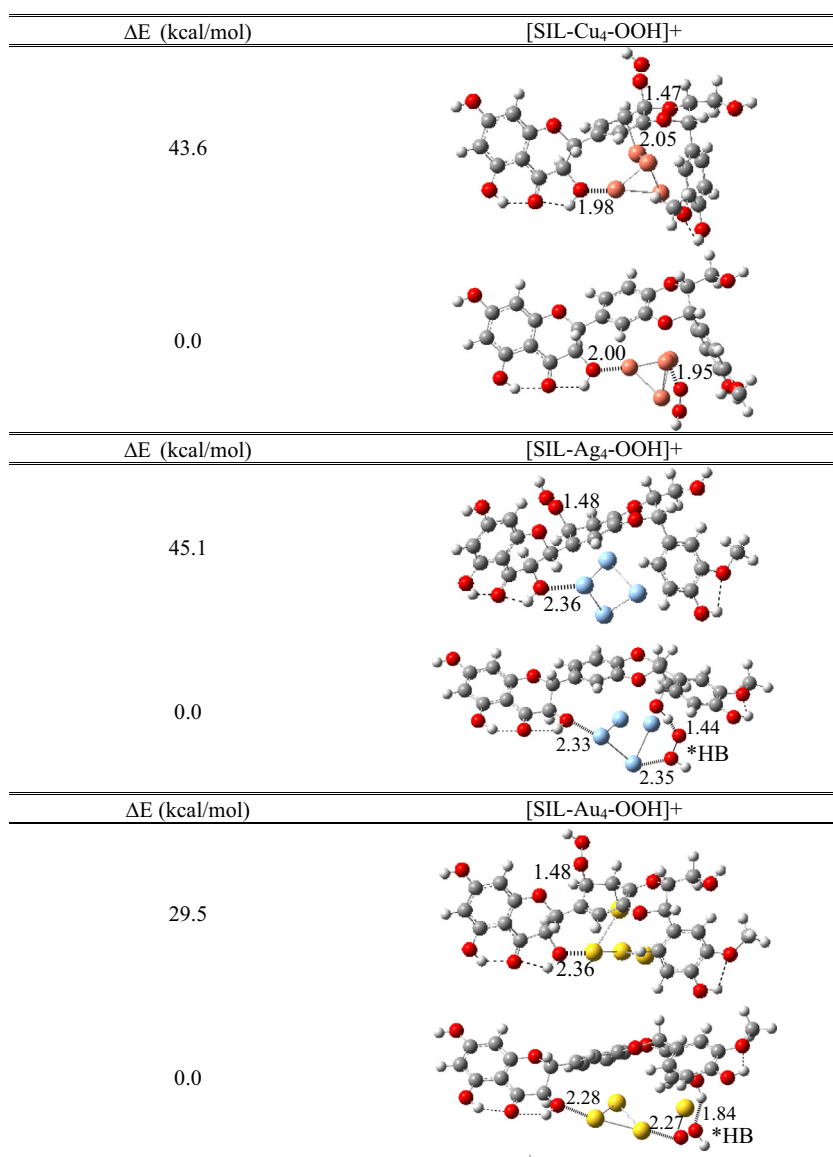| ΔE (kcal/mol)	[SIL-Cu₄-OOH]+
43.6	
0.0	
ΔE (kcal/mol)	[SIL-Ag₄-OOH]+
45.1	
0.0	
ΔE (kcal/mol)	[SIL-Au₄-OOH]+
29.5	
0.0	

Fig. 6. Most stable structures of [SIL-M₄-OOH]⁺ (M = Cu, Ag or Au).

3. Results and discussion

3.1. Geometry optimization

The most stable structures of SIL-M$_4$ and [SIL-M$_4$]$^{+}$ were reported previously [5]. They are presented in Fig. 3 for easy reference. In all the structures, metal clusters are located near to C and B rings. As expected, distances are shorter for Cu than for Ag and Au clusters; and for charged systems, distances are shorter than for neutral ones.

Fig. 4 reports the most stable optimized geometries for [SIL$_{(-H)}$-M$_4$]$^{-1}$ and [SIL$_{(-H)}$-M$_4$]$^{\cdot}$. The acid proton in SIL corresponds to OH(7) (see Fig. 1). Therefore in this position, the most stable structures correspond to the interaction between de-protonated SIL and metal clusters. Other geometries were previously reported as less stable [5]. The structures of Fig. 3 and 4 are the reactants for the RAF and the HAT mechanisms.

3.2. Radical adduct formation (RAF)

Two initial structures were used to analyze the interaction of these systems with $\cdot$OOH: bonded to all C—C double bonds of the organic molecule or bonded directly to the metal clusters. Figs. 5–8 present the most stable optimized structures of compounds coming from the RAF mechanism.

Figs. 5 and 6 present [SIL-M$_4$-OOH]$^{\cdot}$ and [SIL-M$_4$-OOH]$^{+}$ systems, respectively. Energy differences compared to the most stable structures are reported. The energy difference is larger for the cationic systems than for the neutrals. It becomes clear that in all cases, the most stable geometries occur when the $\cdot$OOH interacts directly with the metal clusters. In some of the structures ([SIL-Au$_4$-OOH]$^{\cdot}$, [SIL-Ag$_4$-OOH]$^{\cdot}$ and [SIL-Au$_4$-OOH]$^{\cdot}$ hydrogen bonds are also presented (indicated in the figures, *HB).

Figs. 7 and 8 include the most stable optimized structures for de-protonated silybin interacting with the clusters; i.e. [SIL$_{(-H)}$-M$_4$-OOH]$^{\cdot-1}$ and [SIL$_{(-H)}$-M$_4$-OOH], respectively. Similarly, in these systems the most stable structures are those with the $\cdot$OOH bonds to the metal clusters. In all [SIL$_{(-H)}$-M$_4$-OOH]$^{\cdot-1}$ systems (Fig. 7), the most stable structures present hydrogen bonds (indicated as *HB). For the [SIL$_{(-H)}$-M$_4$-OOH], the most stable structures are comparable, regardless of which metal forms the cluster (Fig. 8, structures located at 0.0). Metal clusters interact with O(7) of the organic molecule and $\cdot$OOH. In all cases, the $\cdot$OOH is located between two metal atoms.

ΔE (kcal/mol)	[SIL$_{(-H)}$-Cu$_4$-OOH]$^{\bullet-1}$
23.6	
0.0	
ΔE (kcal/mol)	[SIL$_{(-H)}$-Ag$_4$-OOH]$^{\bullet-1}$
9.4	
0.0	
ΔE (kcal/mol)	[SIL$_{(-H)}$-Au$_4$-OOH]$^{\bullet-1}$
10.1	
0.0	
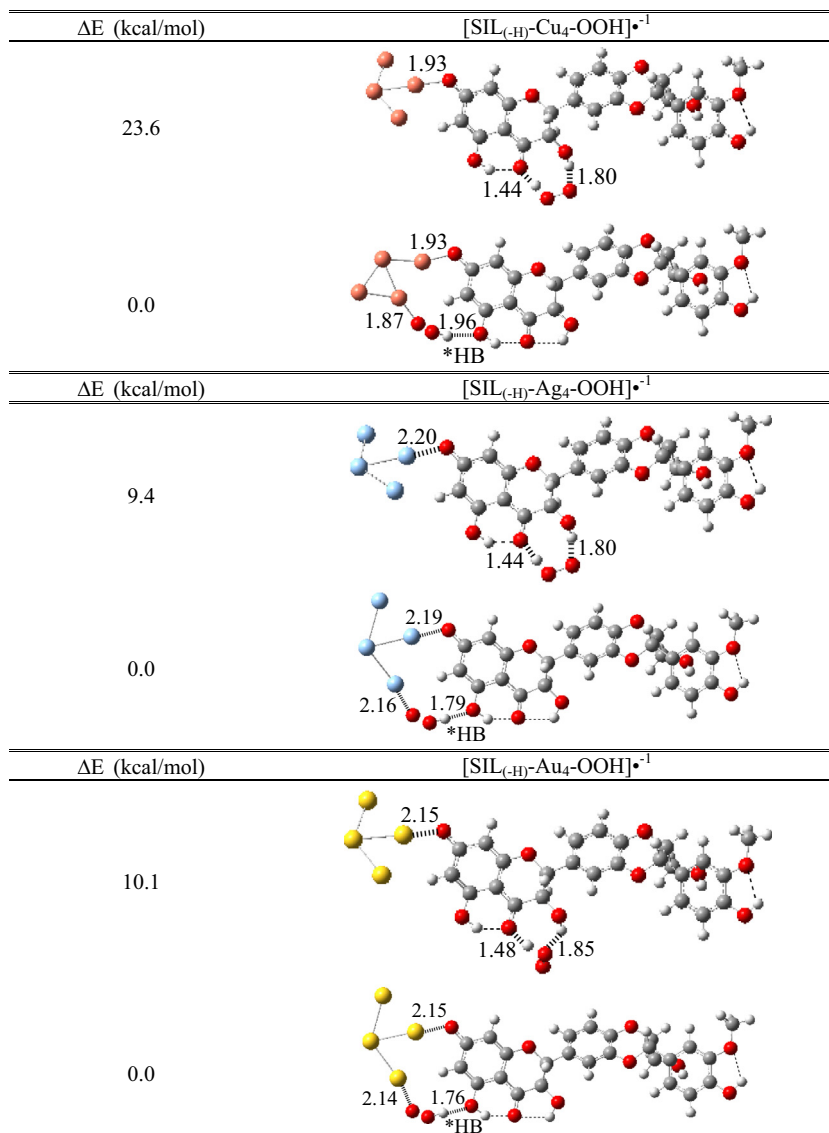
Fig. 7. Most stable structures of [SIL$_{(-H)}$-M$_4$-OOH]$^{\cdot-1}$ (M = Cu, Ag or Au).

ΔE (kcal/mol)	[SIL(-H)-Cu₄-OOH]
66.9	
0.0	

ΔE (kcal/mol)	[SIL(-H)-Ag₄-OOH]
51.5	
0.0	

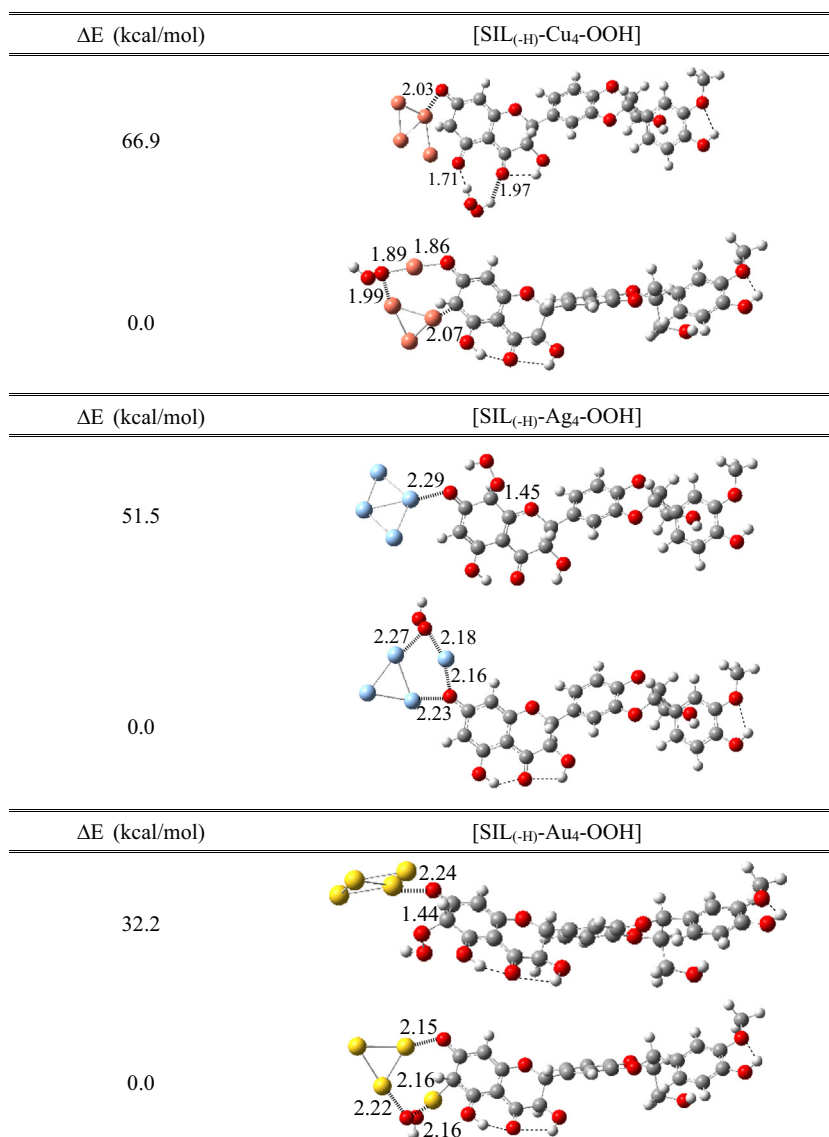| ΔE (kcal/mol)	[SIL(-H)-Au₄-OOH]
32.2	
0.0	

Fig. 8. Most stable structures of [SIL(-H)–M₄-OOH] (M = Cu, Ag or Au).

Table 1
Gibbs free energies (ΔG_{RAF}) considering the reactions of the RAF mechanism.

Reaction	ΔG_{RAF} (kcal/mol)
SIL + ·OOH → [SIL-OOH]·	−2.8
SIL-Cu₄ + ·OOH → [SIL-Cu₄-OOH]·	−45.4
SIL-Ag₄ + ·OOH → [SIL-Ag₄-OOH]·	−23.0
SIL-Au₄ + ·OOH → [SIL-Au₄-OOH]·	−3.8
[SIL-Cu₄]⁺· + ·OOH → [SIL-Cu₄-OOH]⁺	−75.5
[SIL-Ag₄]⁺· + ·OOH → [SIL-Ag₄-OOH]⁺	−52.0
[SIL-Au₄]⁺· + ·OOH → [SIL-Au₄-OOH]⁺	−38.5
[SIL(-H)]⁻¹ + ·OOH → [SIL(-H)-OOH]·⁻¹	−14.5
[SIL(-H)-Cu₄]⁻¹ + ·OOH → [SIL(-H)-Cu₄-OOH]·⁻¹	−33.0
[SIL(-H)-Ag₄]⁻¹ + ·OOH → [SIL(-H)-Ag₄-OOH]·⁻¹	−19.6
[SIL(-H)-Au₄]⁻¹ + ·OOH → [SIL(-H)-Au₄-OOH]·⁻¹	−13.3
[SIL(-H)-Cu₄]· + ·OOH → [SIL(-H)-Cu₄-OOH]	−65.8
[SIL(-H)-Ag₄]· + ·OOH → [SIL(-H)-Ag₄-OOH]	−43.8
[SIL(-H)-Au₄]· + ·OOH → [SIL(-H)-Au₄-OOH]	−48.4

Table 1 presents Gibbs free energies for the radical adduct formation of the most stable structures. The values for the most exergonic reactions of SIL and [SIL(-H)]⁻¹ without the metal clusters are

also presented for the purpose of comparison. Negative values indicate that the formation reaction is exergonic. Out of all systems that included metal clusters, Gibbs free energies are more negative than values for SIL and [SIL(-H)]⁻¹. The presence of metal clusters may serve to promote an interesting synergic effect, which may be involved in the free radical scavenging enhancement properties of SIL. All these systems can be considered as antioxidant materials, as they exhibit exergonic capacity to stabilize ·OOH. The ability of SIL and [SIL(-H)]⁻¹ to scavenge ·OOH through the RAF mechanism is greatly enhanced in the presence of the metal clusters. The best metal for enhancing the capacity to capture ·OOH is Cu (greater negative values; approximately 20 kcal/mol).

3.3. Hydrogen atom transfer (HAT)

Hydrogen atom transfer is one of the main free radical scavenging mechanisms applied by polyphenols [42–44]. In order to investigate the HAT mechanism, hydrogen atoms from all the OH groups were removed one by one. Only the most stable de-hydrogenated systems are considered in what follows. The HAT mechanism was studied according to the corresponding equations. R· is the free

Table 2
Gibbs free energy values (ΔG_{HAT}) considering the reactions of the HAT mechanism.

Reaction	R· =	ΔG_{HAT} (kcal/mol)			
		·OH	·OOH	NO$_2$	C$_6$H$_5$O·
SIL + R· → SIL(-H)· + RH		−24.0	3.8	8.3	2.7
SIL-Cu$_4$ + R· → {[SIL-Cu$_4$](-H)}· + RH		−43.3	−15.5	−11.0	−16.6
SIL-Ag$_4$ + R· → {[SIL-Ag$_4$](-H)}· + RH		−40.3	−12.5	−8.0	−13.6
SIL-Au$_4$ + R· → {[SIL-Au$_4$](-H)}· + RH		−23.6	4.2	8.7	3.1
[SIL-Cu$_4$]·$^+$ + R· → {[SIL-Cu$_4$](-H)}$^+$ + RH		−61.5	−33.7	−29.2	−34.8
[SIL-Ag$_4$]·$^+$ + R· → {[SIL-Ag$_4$](-H)}$^+$ + RH		−61.4	−33.6	−29.1	−34.7
[SIL-Au$_4$]·$^+$ + R· → {[SIL-Au$_4$](-H)}$^+$ + RH		−41.9	−14.1	−9.6	−15.2
[SIL$_{(-H)}$]$^{-1}$ + R· → {[SIL$_{(-H)}$](-H)}·$^{-1}$ + RH		−27.3	0.5	5.0	−0.6
[SIL$_{(-H)}$-Cu$_4$]$^{-1}$ + R· → {[SIL$_{(-H)}$-Cu$_4$](-H)}$^{-1}$· + RH		−28.6	−0.8	3.7	−1.9
[SIL$_{(-H)}$-Ag$_4$]$^{-1}$ + R· → {[SIL$_{(-H)}$-Ag$_4$](-H)}$^{-1}$· + RH		−26.8	1.0	5.5	−0.1
[SIL$_{(-H)}$-Au$_4$]$^{-1}$ + R· → {[SIL$_{(-H)}$-Au$_4$](-H)}$^{-1}$· + RH		−25.0	2.8	7.3	1.7
[SIL$_{(-H)}$-Cu$_4$]· + R· → {[SIL$_{(-H)}$-Cu$_4$](-H)} + RH		−32.8	−5.0	−0.5	−6.1
[SIL$_{(-H)}$-Ag$_4$]· + R· → {[SIL$_{(-H)}$-Ag$_4$](-H)} + RH		−26.8	1.0	5.5	−0.1
[SIL$_{(-H)}$-Au$_4$]· + R· → {[SIL$_{(-H)}$-Au$_4$](-H)} + RH		−25.6	2.2	6.7	1.1
	RH =	H$_2$O	H$_2$O$_2$	HNO$_2$	C$_6$H$_5$OH

radical (·OH, ·OOH, NO$_2$ and C$_6$H$_5$O·) and RH is the free radical bonded to one hydrogen atom (H$_2$O, H$_2$O$_2$, HNO$_2$ and C$_6$H$_5$OH).

Table 2 reports ΔG_{HAT} for all systems under study. Negative ΔG_{HAT} values indicate that formation reactions are exergonic. Generally, the presence of Cu, Ag and Au tetramer clusters increases the exergonicity of the HAT mechanism compared to SIL and [SIL$_{(-H)}$]$^{-1}$. HAT reactions of the systems that have metal clusters attached to SIL are more exergonic than those with metal clusters attached to [SIL$_{(-H)}$]$^{-1}$ (greater ΔG_{HAT} negative values were found). For SIL-M$_4$ and [SIL-M$_4$]·$^+$ systems, only the reaction with SIL-Au$_4$ with some of the free radicals is endergonic, so that these system is not capable of scavenge the four free radicals studied here.

For [SIL$_{(-H)}$-M$_4$]$^{-1}$ and [SIL$_{(-H)}$-M$_4$]· systems, only the reactions with [SIL$_{(-H)}$-Cu$_4$]· system and four free radicals are exergonic. These results suggest that HAT mechanism would be predominant under acidic conditions, if SIL is not deprotonated. For systems with Cu, antioxidant capacity is better (more exergonic reactions) than for systems with Ag and Au. In particular SIL-Cu$_4$ and [SIL-Cu$_4$]·$^+$ are optimum, as the reaction with four free radicals is exergonic.

3.4. Single electron transfer (SET)

SET mechanism for the most stable structures derived from RAF and HAT is investigated in order to examine further antiradical processes. Figs. 9 and 10 present the FEDAM for the most stable systems. We are considering the products of the reaction that are generated by means of RAF (Fig. 9) and HAT (Fig. 10) mechanisms.

Fig. 9 shows that all systems containing metal cluster are better electron donors and worse electron acceptors than [SIL-OOH]· and [SIL$_{(-H)}$-OOH]$^{-1}$. The only exception is [SIL-Au$_4$-OOH]· which is the best electron acceptor among the compounds studied. It is clear that metal clusters increase the antioxidant properties. Fig. 10 presents the FEDAM for systems constituting products of HAT mechanism that present exergonic reactions. In this Figure, it is generally apparent that systems containing metal clusters are better antioxidant molecules than silybin dehydrogenated molecules [SIL(-H)· and {[SIL$_{(-H)}$](-H)}$^{-1}$] as they have lower I values. {[SIL-Au$_4$](-H)}$^+$ represents the exception.

In this regard, products from RAF and HAT structures may present interesting antiradical properties in terms of their capacity to either donate or accept electrons. The presence of copper tends to
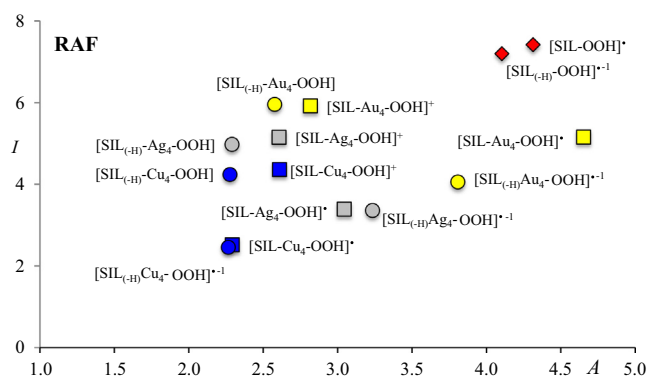

Fig. 9. FEDAM for the most stable products of the reaction according to the RAF mechanism.
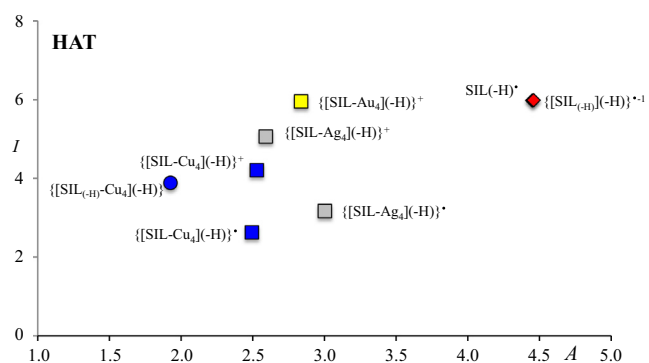

Fig. 10. FEDAM for the most stable products of the reaction according to the HAT mechanism.

increase electron donor capacity and the presence of gold augments electron acceptor ability.

4. Concluding remarks

The presence of tetramer clusters significantly enhances the free radical scavenging properties of SIL and [SIL$_{(-H)}$]$^{-1}$, when applying RAF and HAT mechanisms. In particular, copper systems showed the best free radical scavenging properties.

The FEDAM for the most stable structures derived from RAF and HAT reactions indicates that the presence of tetramer metal clusters increases the capability to donate an electron. The presence of copper generally increases electron donor capacity and the presence of gold augments electron acceptor ability.

These metal-organic coupled systems may represent promising antiradical materials. These results may provide encouraging guidance for those involved in these experiments and hold promise for future applications.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.comptc.2017.03.034.

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