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*Original paper*

# *In vitro Angiotensin Converting Enzyme Inhibitory and Antioxidant Activities of Some Sulfur Compounds*

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**Abstract** The angiotensin converting enzyme (ACE) catalyzes the conversion of angiotensin I to a key vasoconstrictor angiotensin II in renin-angiotensin system (RAS), thus playing an important role in both regulating blood pressure and maintaining fluid balance through RAS. Free radicals are continuously produced in the biological system. Therefore, organisms need both exogenous and endogenous antioxidants to guard against the damage caused by these free radicals. In this way, they prevent the occurrence of numerous diseases via protecting cellular components and biomolecules. The aim of the current study was to investigate the ACE inhibitory and antioxidant activities of sulfur compounds. The ACE inhibitory and antioxidant activities of all sulfur compounds increased in a concentration-dependent manner. Among these compounds, methionine had the highest ACE inhibitory and antioxidant activityes based on reducing power, DPPH radical scavenging and ORAC methods. Cystine had the highest ABTS radical scavenging, FRAP and nitrite scavenging activities. However, S-benzyl cysteine and S-phenyl cysteine exhibited the lowest ACE inhibitory and antioxidant activities, respectively. These outcomes indicate that sulfur compounds have both ACE inhibitory and antioxidant activities and may serve as gateways for research toward understanding the beneficial pharmacological effects of sulfur compounds against cell damage caused by oxidative stress.

**Keywords** Free radicals; angiotensin converting enzyme; inhibitors; antioxidant activity; sulfur compounds

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# Introduction

The angiotensin converting enzyme (ACE; EC 3.4.15.1) is a zinc metalloprotease of paramount significance in the renin-angiotensin system (RAS). As a result of its peptidyl-dipeptidase activity, ACE hydrolyzes the C-terminal His-Leu dipeptide from angiotensin (Ang)-I to form a key vasoconstrictor octapeptide Ang-II. Thus, it plays an important role in both regulating blood pressure and maintaining fluid/electrolyte/salt balances through RAS in the organism. More so, ACE inhibits a potent vasodilator bradykinin (Tipnis et al., 2000). After discovered as a homologue of human ACE, the ACE2 acts as a carboxypeptidase, cleaves the basic amino acid in C-terminal residue, thereby hydrolyzing Ang-II to Ang (1-7) or produces Ang (1-9) (Simões e Silva et al., 2016; Shukla and Banerjee, 2021). Besides, ACE2, an important regulator of the RAS system, has been proven to have a very crucial role (by acting as a receptor mediating viral entry to the organism) in the pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (Coto et al., 2021; Mascolo et al., 2021; Pagliaro et al., 2022). ACE and ACE2 participate in the synthesis of bioactive peptides of the RAS, thereby are involved in the inflammatory process of conditions such as cardiac hypertrophy, pulmonary hypertension, lung injury, and sepsis (Gaddam et al., 2014). Because of these properties, the main strategy for the prevention of diseases that may result from the alteration of ACE activity may be the search for new molecules for ACE inhibition.

Free radicals are atoms or molecules that have one or more unpaired electrons in their outer orbital electron shell. They are highly unstable, have low activation energy and short life. Because of their high reactivity, excessive formation of free radicals [especially reactive oxygen species (ROS)] disrupts the balance of cellular metabolic processes/reactions, thereby causing inevitable and detrimental effects on important biomolecules such as lipids, proteins, and DNA in cells (Juan et al., 2021). Under normal physiological conditions, there is a balance between ROS that are constantly being formed in cells and antioxidants that neutralize them. Disruption of this balance in favor of ROS leads to oxidative stress, a condition characterized by the accumulation of cellular ROS. Accumulating findings have shown that there is a strong relationship between oxidative stress and many ailments, such as immune deficiency, inflammatory conditions, and several types of cancers, cardiovascular and respiratory diseases (Forman and Zhang, 2021). Antioxidants are chemical compounds not only responsible for preventing oxidative damage, but also detoxifying ROS. They prevent or delay the oxidation of biomolecules by reducing the reactivity of ROS (Sánchez, 2017).

Sulfur is an abundant element that plays a crucial role as a component of proteins, vitamins, and other important biomolecules that are essential for life. Sulfhydryl (thiol)-containing amino acids include methionine (Met), cysteine, homocysteine, and taurine (Bin et al., 2017). These are involved in the synthesis of intracellular antioxidants such as glutathione and N-acetyl cysteine (Čolović et al., 2018). On the other hand, some organosulfur compounds such as diallyl sulfide, diallyl disulfide, N-acetyl cysteine, S-allyl cysteine, S-methyl cysteine (SMC), S-ethyl cysteine and S-propyl cysteine have reducing power, metal chelating ability, and superoxide ion scavenging activity (Hsu et al., 2004; Corzo-Martínez et al., 2007; Bayrak and Yanardag, 2021). Antioxidant effects of sulfur compounds have been studied by several researchers (Atmaca, 2004; Battin and Brumaghim, 2008). Sulfur-containing amino acids can be used to reduce cell damage induced by oxidative stress, because of their ROS removing ability (Moskovitz, 2005). Dagsuyu and Yanardag (2021) have revealed that some sulfur compounds have urease and trypsin inhibitory activities. Although vast majority of antioxidants have an active hydroxyl group in their phenolic ring structure which neutralizes free radicals by easily donating hydrogen atoms (Lü et al., 2010), it has been stated that natural or synthetic mixtures consisting of sulfur and nitrogen-containing amino acids, peptides, polypeptides and protein hydrolysates have also antioxidant potential (de Oliveira Filho et al., 2021). Reports show that sulfur-containing amino acids (or their derivatives) with antioxidant activity can be used in the food industry as additives or can be applied as dietary supplements, thereby extending the shelf-life of food because of their free radical scavenging activity (Udenigwe and Aluko, 2011). On the other hand, it has been revealed that cysteine S-conjugates [e.g., S-benzyl cysteine (SBC) and S-phenyl cysteine (SPC)] are intermediary and/or final products in xenobiotic metabolism (Okajima et al., 1984; Hanway et al., 2000). Another cysteine derivative, SMC, has been reported to have chemopreventive effects against hepatocarcino-genesis (Wei et al., 2000), antioxidative and antiinflammatory effects against neurotoxicity (Chen et al., 2007). Senthilkumar et al., (2013) have suggested that SMC shows hypoglycemic and antihyperlipidemic effects in experimental obesity model in rats. Besides, it has been revealed that SMC could be an effective compound against *Cryptosporidium parvum* infection via restoring structural alterations in different tissues of albino mice (Elmahallawy et al., 2020).

Presently, there are only few research articles reporting the *in vitro* ACE inhibitory activities and antioxidant potentials of Met, cystine, SBC, SMC, SPC, and taurine. Thus, the present study was aimed to investigate the *in vitro* ACE inhibitory and antioxidant activities of these sulfur compounds.

# Materials and Methods

### *Chemicals*

Cystine and Met were supplied by Merck Chemical Company (Darmstadt, Germany). SBC, SMC, SPC, taurine, N-[3-(2-Furyl)acryloyl]-Phe-Gly-Gly (FAPGG), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), N,N-dimethyl-4-phenylenediamine (DMPD), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), and fluorescein disodium salt were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

***ACE Inhibitory Activity Assay***

ACE inhibitory activities of sulfur compounds were estimated according to Shalaby et al., (2006). In this assay, lamb kidney homogenate (10 %, weight/volume) was used as a source of ACE. Captopril was used as a standard inhibitor. The percent inhibition of the ACE was calculated using the following equation:

$$\text{ACE inhibitory activity (\%)} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100 \qquad (1)$$

Where: $A_o$ represents the activity of the enzyme without the inhibitor, and $A_1$ is the activity of the enzyme in the presence of the sulfur compounds (or standard inhibitor).

***Reducing Power Assay***

The reducing power of the sulfur compounds was determined using method of Oyaizu (1986). As reference solution, Trolox was used. The intensity of the blue color is directly proportional to the reducing power of the tested sulfur compounds. A high absorbance of the reaction mixture indicates a greater reducing power of the tested sulfur compounds.

***ABTS Radical Scavenging Activity Assay***

ABTS radical scavenging activities of the sulfur compounds and reference antioxidant were assessed by the procedure of Arnao et al., (2001). Trolox was used as a reference antioxidant.

***DPPH Radical Scavenging Activity Assay***

DPPH radical scavenging activities of the sulfur compounds were estimated by the method of Brand-Williams et al., (1995). Trolox was used as a reference antioxidant.

***DMPD Radical Scavenging Activity Assay***

The determination of DMPD radical scavenging activity was performed using method of Fogliano et al., (1999). Ascorbic acid and Trolox were used as the reference antioxidants.

***Ferric Reducing Antioxidant Power (FRAP) Assay***

The FRAP assay was carried out according to Benzie and Strain (1996). Reference solutions of $FeSO_4.7H_2O$ were employed to obtain calibration curve. Ascorbic acid and Trolox were used as the positive control. The results were expressed as μM $Fe^{2+}$ per 100 mL sample.

***Metal Chelating Activity Assay***

The metal chelating activities of the sulfur compounds were determined using the method of Decker and Welch (1990). EDTA was used as a reference metal chelator. A low absorbance indicates a higher chelating activity of the tested sulfur compounds.

***Nitrite Scavenging Activity Assay***

Nitrite scavenging activities of the sulfur compounds and quercetin (as a reference antioxidant) were carried out according to Liu et al., (2011).

***Oxygen Radical Absorbance Capacity (ORAC) Assay***

To measure ORAC of the sulfur compounds and Trolox (as a reference antioxidant), a modified method of Huang et al., (2002) was employed. The fluorescence was measured at 37° C every min for 35 min at 485 nm as the excitation wavelength and 528 nm for the emission wavelength. The following formula was used to calculate ORAC values, and the results were given as % inhibition value.

$$\text{ORAC value (\%)} = \frac{[(I_2 - I_1)]}{[(I_0 - I_1)]} \times 100 \qquad (2)$$

$I_0$ is initial fluorescence intensity value of fluorescein
$I_1$ is fluorescence intensity value of fluorescein remaining intact during incubation medium in the absence of the sulfur compounds or standard
$I_2$ is fluorescence intensity value of fluorescein remaining in the incubation medium in the presence of the sulfur compounds or standard

For antioxidant activities (ABTS, DPPH, DMPD, metal chelating, and nitrite scavenging activities) of the sulfur compounds (or standards) were calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{[(A_0 - A_1)]}{A_0} \times 100 \qquad (3)$$

$A_0$ is the absorbance of the control, $A_1$ is the absorbance of the sulfur compounds

For ACE inhibitory and antioxidant activities, the sulfur compounds (or standard) concentration providing 50 % inhibitions ($IC_{50}$) were calculated by the regression equations (by plotting concentration of sulfur compounds versus percentage inhibition). A low $IC_{50}$ indicates a higher inhibitory potential and antioxidant activity of the tested compounds.

# Results and discussion

Free radicals are extremely reactive and unstable atoms, molecules or molecular fragments. They have one or more unpaired electrons that can easily interact with many other biomolecules (e.g., nucleic acids, proteins, and lipids) in the physiological conditions (Kumar et al., 2021). Besides, they can quickly propagate radical chain reactions, which have harmful effects on cells. The continuous formation and elimination of free radicals (i.e., ROS) in living cells are precisely kept under control by a phenominum called redox-balance (Pizzino et al., 2017; Ramana et al., 2018).

The disruption of this redox balance in favor of ROS causes oxidative stress, which in turn promotes the development of a wide variety of disorders that include cancer, diabetes, aging, Alzheimer's, and Parkinson's diseases (Suleman et al., 2019; Forman and Zhang, 2021).

The sulfur atom is present in all living cells, and is also essential for life. Besides, it is a structural component of some amino acids, proteins, and many other biomolecules that have important biological functions. Met, cysteine, cystine, homocysteine, N-acetyl cysteine, and taurine are well known sulfur-containing amino acids. Among them, Met and cysteine are designated as proteinogenic amino acids, whereas the others are called non-proteinogenic amino acids. Moreover, they are involved in many biochemical processes such as methylation reaction in the form of S-adenosylmethionine and biosynthesis of one of the most important intracellular antioxidants known as glutathione (Čolović et al., 2018).

The present study investigated the ACE inhibitory activity as well as antioxidant potential of Met, cystine, SBC, SMC, SPC, and taurine based on electron and hydrogen atom transfer methods. The outcomes were compared with activities of some natural and synthetic antioxidant compounds.

The activity of the ACE enzyme is very crucial because of its important role in both regulating blood pressure and maintaining fluid/electrolyte/salt balance in the organism through RAS (Fagyas et al., 2014). An increase in the activities of ACE is associated with cardiovascular and renal disorders (e.g., high blood pressure, heart failure, acute kidney injury, diabetes-mediated kidney disease). Because of being a key factor of experimental and clinical approaches in the treatment of the aforementioned diseases, inhibition of the ACE is at the center of research and paramount for the discovery of new and safe molecules with inhibitory properties (Giani et al., 2021). The inhibition effects of sulfur compounds as well as that of captopril against ACE activity are summarized in Table 1. The results depict $IC_{50}$ values of ACE inhibitory activities of the sulfur compounds. $IC_{50}$ values were calculated by plotting the inhibition percentage values as function of concentrations. According to the results, all the sulfur compounds and captopril had an $IC_{50}$ values in the range of 0.11-389.07 µM. Considering the high inhibitory activities (associated with the lower $IC_{50}$ values) these compounds demonstrated lower inhibitory activity against ACE in comparison to captopril ($IC_{50}$ values of 0.11±0.02 µM). On the other hand, it was observed that Met had approximately two times more inhibitory effect than that of SMC. In addition, Met was observed to have the best ACE inhibitory activity among the sulfur compounds. The ACE inhibitory activity of sulfur compounds and the standard decreased in the order of: captopril>Met>SMC>taurine>cystine> SBC>SPC (Table 1). The various inhibitors may bind to the amino acids such as aspartate, histidine, phenylalanine, and serine at the binding pocket in the active center of the enzyme, thereby giving rise to inhibition of the ACE (Masuyer et al., 2012). Bioactive sulfur compounds in *Asparagus officinalis* have been revealed to possess anti-ACE activity (Nakabayashi et al., 2015). Sulfur-containing N-mercaptoalkanoyl amino acids had been reported to be potent ACE inhibitors whose SMC and SEC derivatives had the same $IC_{50}$ value for SMC and lower $IC_{50}$ value for SEC when compared to captopril (Komori et al., 1987). In the current study, SMC and other compounds showed weaker ACE inhibitory activities than that of captopril. This may be due to lack of sulfhydryl group in the substances we used in the current study or these substances may not fully interact with the amino acids in the active site of the enzyme.

**Table 1.** Inhibitory activities of the sulfur compounds on ACE.

| Compounds / Standard | Concentrations (µM) | $IC_{50}$ (µM)* |
|---|---|---|
| **Cystine** | 0.1<br>1<br>10 | 90.97 ± 9.25 |
| **Met** | 0.01<br>0.1<br>0.5 | 1.46 ± 0.16 |
| **SBC** | 1<br>10<br>100 | 274.18 ± 38.74 |
| **SMC** | 0.5<br>1<br>2 | 2.46 ± 0.17 |
| **SPC** | 0.1<br>1<br>100 | 389.07 ± 98.03 |
| **Taurine** | 1<br>10<br>20 | 83.53 ± 14.69 |
| **Captopril** | 0.01<br>0.05<br>0.1 | 0.11 ± 0.02 |

*Mean ± SD of triplicate values.

ACE: Angiotensin converting enzyme; Met: Methionine; SBC: S-benzyl cysteine; SMC: S-methyl cysteine; SPC: S-phenyl cysteine.

The reducing power of molecules is related to the ability of a test sample to donate an electron or hydrogen atom to ferric iron (Shen et al., 2019). In the present study, the reducing power of the all compounds were estimated based on reducing $Fe^{3+}$ to $Fe^{2+}$. FRAP method measures the antioxidant capacities of compounds with redox potential to form the $Fe^{2+}$-TPTZ complex, by giving an electron to the $Fe^{3+}$-TPTZ complex in an acidic pH (Magalhães et al., 2008). The findings in Table 2 show reducing power and the FRAP values of all tested compounds. It was observed that the reducing power of the sulfur compounds and standard antioxidant increased with concentration. Cystine and Met had the highest reducing power, whereas taurine had the lowest reducing power value. On the other hand, the reducing power of 500 µM Trolox was almost 5.5 times greater than that of Met (Table 2). The fact that the sulfur compounds have lower reducing power values than Trolox

is an indication of their lesser ability to reduce $Fe^{3+}$. In the current study, the FRAP values of all sulfur compounds exhibited low FRAP activities than that of standard antioxidant. Cystine was found to have the highest FRAP values. On the other hand, aromatic amino acids have been reported to have very good antioxidant potential, especially non-radical single electron transfer-based experimental systems such as FRAP (Munteanu and Apetrei, 2021). Theoretically, any compound whose redox potential is less than that of Fe(III)/Fe(II) pair can reduce Fe(III) to Fe(II) (Amin et al., 2013). On the contrary, FRAP method is reported to insufficiently measure the antioxidant capacities of compounds possessing free thiol groups such as glutathione (Gulcin, 2020). The reason for having the moderate FRAP values of the sulfur compounds in our study may be due to the presence of an aryl group with an electron delocalized system or an alkyl group covalently bonded to the sulfur atom (Guidea et al., 2020). However, FRAP value of cystine at 500 µM was nearly four-fold less than that of Trolox (Table 2). The FRAP values of all tested compounds in the present study were much lower than that of the standard antioxidant. FRAP values decreased in the order of: ascorbic acid>Trolox>cystine>SBC>SPC>SMC> taurine>Met (Table 2).

**Table 2.** Reducing power and FRAP values of the sulfur compounds.

| Compounds / Standards | Concentrations (µM)* | Reducing Power (Absorbance)* | Concentrations (µM)* | Ferric Reducing Antioxidant Power ($Fe^{2+}$ µM)* |
|---|---|---|---|---|
| **Cystine** | 100<br>250<br>500 | 0.035±0.009<br>0.049±0.006<br>0.061±0.006 | 300<br>400<br>500 | 35.40±3.92<br>46.23±1.42<br>57.81±0.71 |
| **Met** | 100<br>250<br>500 | 0.026±0.002<br>0.070±0.003<br>0.125±0.003 | 1250<br>1500<br>2000 | 1.66±0.36<br>2.92±0.01<br>18.53±2.85 |
| **SBC** | 100<br>250<br>500 | 0.015±0.001<br>0.023±0.012<br>0.026±0.014 | 1250<br>1500<br>2000 | 44.72±4.45<br>51.26±8.43<br>75.69±1.95 |
| **SMC** | 100<br>250<br>500 | 0.014±0.001<br>0.026±0.013<br>0.032±0.018 | 1250<br>1500<br>2000 | 25.08±2.85<br>32.88±1.07<br>53.03±1.07 |
| **SPC** | 100<br>250<br>500 | 0.051±0.011<br>0.068±0.012<br>0.094±0.013 | 1000<br>1250<br>1500 | 23.57±2.82<br>33.89±2.04<br>43.71±5.84 |
| **Taurine** | 100<br>250<br>500 | 0.005±0.005<br>0.008±0.004<br>0.013±0.008 | 1250<br>1500<br>2000 | 4.94±0.01<br>16.01±1.42<br>21.80±0.36 |
| **Trolox** | 100<br>250<br>500 | 0.367±0.002<br>0.489±0.001<br>0.671±0.006 | 250<br>500<br>1000 | 130.83±2.85<br>211.15±5.34<br>242.88±0.36 |
| **Ascorbic acid** | - | - | 250<br>500<br>1000 | 107.92±0.36<br>194.28±1.42<br>246.15±2.14 |

*Mean ± SD of triplicate values.

Met: Methionine; SBC: S-benzyl cysteine; SMC: S-methyl cysteine; SPC: S-phenyl cysteine.

The ABTS radical scavenging activity method is frequently used to determine the antioxidant activities of both lipophilic and hydrophilic compounds based on electron and/or hydrogen atom transfer. Radical scavenging capability of complex mixtures and individual compounds is inversely proportional to the discolorizing of ABTS and DPPH radicals (Gulcin, 2020; Muntenau and Apetrei, 2021). The outcomes of ABTS and DPPH radical scavenging activities of the sulfur compounds and standard antioxidant are presented in Table 3. Standard antioxidant was better ABTS radical scavenger than the sulfur compounds. The $IC_{50}$ values of the sulfur compounds were found to be high and ranged from 2029.67 to 10350.25 µM. The present findings suggest that ABTS radical scavenging activity can be employed in a wide pH range for both hydrophilic and hydrophobic molecules (Osman et al., 2006). Cystine (2029.67±106.54 µM) was found to have the lowest $IC_{50}$ value among sulfur compounds (Table 3). For all the tested sulfur compounds, an increase in concentration resulted in elevation of the DPPH radical scavenging potential. Trolox had the strongest antiradical activity with $IC_{50}$ of 27.28±0.76 µM compared to the sulfur

compounds. The antiradical power of the sulfur compounds are ordered as: Met>cystine>taurine>SMC>SPC>SBC (Table 3). Ripoll et al. found that taurine mildly scavenged DPPH radicals whereas it did not exhibit ABTS scavenging effect (Ripoll et al., 2012). More so, the findings of Kim et al., revealed that Met and taurine have no ABTS and DPPH radical scavenging activities (Kim et al., 2020). Similarly, Heng et al., (2020) revealed that Met did not exhibit DPPH radical scavenging activity at room temperature, but had a valuable antioxidant activity based on the oxidative stability index test at a higher temperature. The outcomes of the current study are not in line with the findings of the aforementioned researchers. In addition, the present findings show that sulfur compounds do not completely bleach ABTS radical cations. This may be due to the lack of hydrogen atoms that can be transferred to the ABTS radical cation in the side chains of these compounds. A recent study revealed that organic oligosulfides had not shown DPPH scavenging activity. Like in our study, this was associated with the fact that the organic oligosulfides are not strongly involved in hydrogen atom transfer to the DPPH radical (Osipova et al., 2021).

**Table 3.** ABTS and DPPH radical scavenging activities of the sulfur compounds.

| Compounds / Standard | ABTS Radical Scavenging Activity | | DPPH Radical Scavenging Activity | |
|---|---|---|---|---|
| | Concentration (µM) | $IC_{50}$ (µM)* | Concentration (µM) | $IC_{50}$ (µM)* |
| **Cystine** | 250<br>350<br>500 | 2029.67±106.54 | 100<br>200<br>300 | 495.51±31.85 |
| **Met** | 1500<br>2000<br>3000 | 5325.61±229.40 | 100<br>200<br>300 | 318.43±17.31 |
| **SBC** | 1500<br>2000<br>3000 | 8099.15±726.43 | 500<br>1000<br>2000 | 5516.57±263.37 |
| **SMC** | 1500<br>2000<br>3000 | 6337.68±75.21 | 500<br>1000<br>2000 | 2959.77±25.47 |
| **SPC** | 1500<br>2000<br>3000 | 5199.90±25.67 | 500<br>1000<br>2000 | 3512.74±200.51 |
| **Taurine** | 1500<br>2000<br>3000 | 10350.25±569.48 | 100<br>200<br>300 | 756.11±1.75 |
| **Trolox** | 250<br>300<br>500 | 515.41±2.02 | 10<br>25<br>50 | 27.28±0.76 |

*Mean ± SD of triplicate values.

Met: Methionine; SBC: S-benzyl cysteine; SMC: S-methyl cysteine; SPC: S-phenyl cysteine.

DMPD radical scavenging activity method can be used for both hydrophilic and lipophilic molecules. When the $DMPD^{\bullet+}$ cation radical abstracts hydrogen atoms from surrounding molecules, it turns into a purple-colored product that is proportional to the antioxidant capacity of the test molecule (Gulcin et al., 2020). Metal chelators may act as important secondary antioxidants, due to their ability to lessen the redox potentials and stabilizations of the oxidized form of the transition metals such as Fe(II) (Končić et al., 2011). The results in Table 4 shows $IC_{50}$ values of DMPD radical scavenging and metal chelating activities of the sulfur compounds and standard antioxidants and chelator (EDTA). According to DMPD radical scavenging activities, $IC_{50}$ values of 8.12-9.82 µM were recorded for the sulfur compounds, while $IC_{50}$ values of 0.73±0.01 µM and 0.88±0.01 µM were found for ascorbic acid and Trolox, respectively. The lowest $IC_{50}$ value was exhibited by SPC (8.12±0.02 µM) (Table 4). The lower $IC_{50}$ values of standard antioxidants for DMPD radical scavenging activity compared to the sulfur compounds are in line with the outcomes of both ABTS and DPPH radical scavenging activities. In DMPD experimental system, the dependence on the DMPD radical scavenging activity on the sulfur atoms in the structure of the compounds could not be fully established. Therefore, the standard antioxidants had better scavenging effects in comparison to the sulfur compounds, with ascorbic acid having a better antiradical effect than Trolox. Similar findings were reported by Schlesier et al., (2002). It was observed that ascorbic acid scavenged DMPD radical in a much shorter time than Trolox. Like ABTS, DMPD radical has a positively charged chromophore molecule that reacts with antioxidant in reaction media (Ahmed et al., 2020) in a process that can be explained by the different kinetic

properties of both compounds (Fogliano et al., 1999). As for metal chelating activities, it was found that SPC ($IC_{50}$ value of 42.83±0.41 µM) had the highest metal chelating activity. $IC_{50}$ values of standard compound and other sulfur compounds are as follows: 60.00±6.47 µM for EDTA, 74.87±8.15 µM for SBC, 105.03±4.02 µM for cystine, 153.11±0.59 µM for Met, 154.18±2.00 µM for SMC, and 181.54±7.37 µM for taurine (Table 4). The high metal chelating activities of SPC and SBC is likely related to the presence of the aromatic group in their structure (Carrasco-Castilla et al., 2012). Owing to the resonance structures of aromatic amino acids, they are suggested to act as effective radical scavengers, and can easily neutralize free radicals by donating protons (Rajapakse et al., 2005). Kim et al., (2020) reported that sulfur amino acids (such as Met, cysteine and taurine) failed to chelate $Fe^{2+}$ ions but could chelate both $Cu^{2+}$ and $Zn^{2+}$. This may vary depending on the different number of metal binding sites of the chelator (for example, hexadentate as in EDTA) and the affinity of the chelator to the metal. In addition, dietary supplements containing cysteine and Met have been shown to reduce oxidative stress in animals by acting as metal chelators (Patra et al., 2001; Nandi et al., 2005; Martínez et al., 2017).

**Table 4.** DMPD radical scavenging and metal chelating activities of the sulfur compounds.

| Compounds / Standards | DMPD Radical Scavenging Activity | | Metal Chelating Activity | |
|---|---|---|---|---|
| | Concentration (µM) | $IC_{50}$ (µM)* | Concentration (µM) | $IC_{50}$ (µM)* |
| **Cystine** | 10<br>100<br>200 | 9.82±0.01 | 25<br>50<br>100 | 105.73±4.02 |
| **Met** | 10<br>100<br>1000 | 8.75±0.03 | 50<br>100<br>150 | 153.11±0.59 |
| **SBC** | 10<br>100<br>1000 | 9.66±0.04 | 25<br>50<br>100 | 74.87±8.15 |
| **SMC** | 10<br>100<br>1000 | 9.56±0.13 | 50<br>100<br>150 | 154.18±2.00 |
| **SPC** | 10<br>100<br>1000 | 8.12±0.02 | 25<br>50<br>100 | 42.83±0.41 |
| **Taurine** | 10<br>100<br>1000 | 9.32±0.05 | 50<br>100<br>150 | 181.54±7.37 |
| **Ascorbic Acid** | 1<br>100<br>500 | 0.73±0.01 | - | - |
| **Trolox** | 1<br>100<br>500 | 0.88±0.01 | - | - |
| **EDTA** | - | - | 50<br>100<br>150 | 60.00±6.47 |

*Mean ± SD of triplicate values.

EDTA: Ethylenediaminetetraacetic acid; Met: Methionine; SBC: S-benzyl cysteine; SMC: S-methyl cysteine; SPC: S-phenyl cysteine.

Nitrite (or nitrate), found in residual pesticides, protein-rich foods, and cosmetics/medicines, can react with secondary amine groups to form S-nitroso compounds such as nitrosamines. Nitrosamines are then converted to alkane-linked DNA, proteins and nitrogenous intracellular components that may increase the risk of cancer (Zhan et al., 2016). Also, they are important molecules that can precipitate methemoglobinemia (Choi et al., 2008). Therefore, the nitrite scavenging activity method is widely used to investigate the antioxidant potential of both natural and synthetic compounds. The ORAC method is an accepted standard method used in nutraceutical, pharmaceutical and food industry to assess the antioxidant capacity (Gorinstein et al., 2009). Moreover, it is widely employed in other sectors assessing antioxidant power and oxidative stress. $IC_{50}$ values of the nitrite scavenging activities and ORAC values of the sulfur compounds and standard antioxidant are depicted in Table 5. Nitrite scavenging activity ($IC_{50}$ value of 2185.27±76.50 µM) of quercetin was found to be lower than that of the sulfur

compounds. Alongside this, the $IC_{50}$ value of cystine (812.78±41.74 μM) was lower than that of other sulfur compounds. The nitrite scavenging activity of the sulfur compounds are ordered as follows: cystine>SMC>Met> SPC>taurine>SBC>quercetin (Table 5). The present findings indicate that sulfur compounds are potentially powerful nitrite scavengers. In contrast, a report by Vriesman et al., (1997) demonstrated that some sulfur compounds having disulfide group (-S-S-) and S-methyl group (e.g., oxidized glutathione and S-methyl glutathione) do not exhibit nitrite scavenging activity at physiological pH. Thus, the *in vitro* nitrite scavenging activity exhibited by all sulfur compounds in the present study may be linked to the acidic reaction medium used. More so, the dependence of capability of nitrite scavenging on the sulfur atoms in the structure of the compounds may be due to different reaction kinetics. As of the ORAC values, in the present study showed that Trolox with an $IC_{50}$ value of 32.77±0.47 μM was a more effective scavenger of peroxyl radicals when compared to sulfur compounds. Met ($IC_{50}$ value of 220.19±13.53 μM) had the best antioxidant activity as compared to other compounds (Table 5). The ORAC values of all tested compunds are ordered as follows: Trolox>Met> taurine>SMC>SPC>cystine>SBC. It has been revealed that biologically active sulfur compounds (cystine, taurine, and Met, etc.) had close ORAC values by using both spectrophotometric and voltammetric methods and Met had the lowest whereas cystine had the highest ORAC values (Dorozhko and Korotkova, 2011). These findings were not in harmony with our results. In an inhibitory diagrams study of the ORAC values of 10 amino acids and some other natural components it was suggested that cystine had higher ORAC values than Met (Nakajima et al., 2016). In another study involving a combination of tripeptides and 20 amino acids, Met was shown to have the highest ORAC value after tryptophan and tyrosine (Ohashi et al., 2015).

**Table 5.** Nitrite scavenging scavenging activities and ORAC values of the sulfur compounds.

| **Compounds / Standards** | **Nitrite Scavenging Activity** | | **Oxygen Radical Absorbance Capacity** | |
|---|---|---|---|---|
| | **Concentration (μM)** | **$IC_{50}$ (μM)*** | **Concentration (μM)** | **$IC_{50}$ (μM)*** |
| **Cystine** | 100<br>300<br>500 | 812.78±41.74 | 250<br>500<br>1000 | 680.14±5.42 |
| **Met** | 1000<br>1500<br>2000 | 896.27±33.30 | 250<br>500<br>1000 | 222.19±13.53 |
| **SBC** | 1000<br>1500<br>2000 | 2161.96±28.65 | 250<br>500<br>1000 | 807.93±23.92 |
| **SMC** | 1000<br>1500<br>2000 | 846.34±61.45 | 250<br>500<br>1000 | 470.46±11.73 |
| **SPC** | 1000<br>1500<br>2000 | 1094.62±19.80 | 250<br>500<br>1000 | 637.31±12.00 |
| **Taurine** | 1000<br>1500<br>2000 | 1486.81±11.05 | 250<br>500<br>1000 | 430.23±9.77 |
| **Quercetin** | 1000<br>1500<br>2000 | 2185.27±76.50 | - | - |
| **Trolox** | - | - | 25<br>37.50<br>50 | 32.77±0.47 |

*Mean ± SD of triplicate values.

Met: Methionine; SBC: S-benzyl cysteine; SMC: S-methyl cysteine; SPC: S-phenyl cysteine.

# Conclusions

In the current study, *in vitro* ACE inhibitory and antioxidant activities of the several sulfur compounds were determined. The outcomes show that the ACE inhibitory and antioxidant activities of all the sulfur compounds increased with an increase in concentration. Among the sulfur compounds, Met was found to have the highest ACE inhibitory activity, as well as the highest reducing power, DPPH radical scavenging, and the ORAC effect. Cystine had the highest ABTS radical

scavenging, FRAP, and nitrite scavenging activity. While SPC was the sulfur compound with the lowest ACE inhibitory activity, SBC had the lowest antioxidant activity. Our findings indicate that sulfur compounds have moderate *in vitro* ACE inhibitory activity and antioxidant effect. On the basis of present outcomes, consumption of sulfur compounds might be beneficial for not only the regulation of inflammatory processes (e.g., cardiac hypertrophy, pulmonary hypertension, lung injury, and sepsis) but also for the prevention of some diseases caused by oxidative stress resulting from the detrimental effects of ROS. We suggest further research (*in vivo*) should be conducted so as to understand and unravel the biological activities of these compounds.

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## Author Contributions

Conception/Design of Study-B.B.B. and R.Y.; Data Acquisition-S.Y., B.B.B.; Data Analysis/Interpretation-S.Y., B.B.B. and R.Y.; Drafting Manuscript-S.Y., B.B.B. and R.Y.; Critical Revision of Manuscript-B.B.B. and R.Y.; Final Approval and Accountability-B.B.B. and R.Y.; Technical or Material Support-S.Y., B.B.B. and R.Y.; Supervision-R.Y.

## Data Availability

All data generated or analyzed during this study are included in this published article.

## Declarations

## Ethics Approval

This article does not contain any studies with human participants or animal subjects performed by any of the authors.

## Informed Consent

All authors declare that the current paper has not been under review by other journals, besides approving its submission on Applied Biochemistry and Biotechnology.

## Conflict of Interest

The authors have no conflict of interest to declare.

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