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In vitro angiotensin converting enzyme inhibitory and antioxidant activities of seed extract of *Raphanus sativus* Linn.

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ABSTRACT

The aim of the present study was to investigate the in vitro angiotensin converting enzyme (ACE) inhibitory and antioxidant activities of the aqueous leaf extract and ethanolic seed extract of Raphanus sativus Linn. belonging to the family Brassicaceae. The ability to inhibit ACE was evaluated by quantifying the decrease in N-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine (FAPGG) peak area at 345 nm using reverse phase high performance liquid chromatography (RP-HPLC). FAPGG was used as the substrate in the assay. The antioxidant activity of the extracts was determined by evaluating its hydroxyl radical, superoxide anion radical, nitric oxide radical scavenging abilities, reducing power ability, ferrous chelating ability and total phenolic and flavonoid contents. The ACE inhibitory potency of the seed extract (278.54±1.67µg/ml) was found to be higher than the leaf extract and is compared with the standard drug lisnopril (0.19 ± 0.02 ng/ml). However, both extracts possess significant antioxidant activity when compared with the standard. The results of this study suggest that the ethanolic seed extract of R.sativus has promising bioactive compounds which might have a beneficial effect in the treatment of hypertension and cardiovascular diseases.

Keywords: Angiotensin converting enzyme, *Raphanus sativus* Linn, Cardiovascular diseases, Free radicals.

INTRODUCTION

The renin-angiotensin system participates extensively in the pathophysiology of myocardial infarction, diabetic nephropathy and congestive heart failure. This realization has led to a thorough exploration of the renin-angiotensin system and the development of new ways for inhibiting its actions [1]. Angiotensin converting enzyme (ACE), a crucial enzyme in the regulation of the renin angiotensin system, is a zinc-containing peptidyl dipeptide hydrolase whose active site consists of three parts: a carboxylate binding moiety such as the guanidinium group of arginine, a pocket that accommodates a lipophilic side chain of amino acid residues at C-terminal and a Zn ion. The Zn ion binds to the carbonyl group of the penultimate peptide bond of the substrate, thereby making the carbonyl group polarized and subjecting it to a nucleophilic attack [2].

Currently available ACE inhibitors are synthetic pharmacological drugs and their use in healthy or low-risk populations is not advisable because of their adverse effects like dry cough, skin rashes and angioneuroticedema [3]. Certain compounds derived from plant extracts like hydrolysable tannins [4], phenylpropanes [5], proanthocyanidins [6], flavonoids [7], terpenoids [8] and peptide amino acids [9] have been reported for their in vitro ACE inhibitory activity. These findings opens up the possibility of finding newer plant derived compounds which mimic synthetic ACE inhibitors and provide health benefits without any adverse side effects.

The reactive oxygen species (ROS) include free radicals such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), hydroperoxyl ($HOO^{\cdot}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$) and non-radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) and singlet oxygen ($O_2^{\cdot}$). Similarly, reactive nitrogen species (RNS) include nitric oxide ($NO^{\cdot}$), peroxy nitrite ($ONOO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot}$) and dinitrogen trioxide (N_2O_3). Oxidative stress is a condition in which there is an increased production of oxygen species and diminished levels of antioxidant system resulting in cell damage leading to the pathogenesis of a variety of diseases. Antioxidants prevent the oxidative reactions that occur naturally in tissues by scavenging the free radicals, chelating metal ions and acting as electron donors [10].

The oxidative stress in cardiac and vascular myocytes caused by ROS has been noted to induce cardiovascular tissue injury and to sustain homeostasis of the vascular wall, a balance between the endogenous transmitter's angiotensin II, nitric oxide, and ROS is of great value. It has been clearly noted that hypertension caused by chronically increased levels of angiotensin II is mediated in part by superoxide anions [11]. The cardiovascular diseases caused by increased levels of angiotensin II are found to be mediated by vasoconstriction and thus decreased concentration of vascular nitric oxide seems to promote the angiotensin II dependent cardiovascular diseases [12].

Raphanus sativus Linn. (Brassicaceae), basically known as radish is commonly available throughout the world. Different parts of radish including roots, seeds and leaves are used for medicinal purposes [13]. Literatures suggest that leaves of *R. sativus* possess gut stimulatory [14] and hepatoprotective activities [15] and seeds possess anti-microbial and diuretic activities [16]. However, no report is available in the literature to our knowledge regarding *in vitro* ACE inhibitory and antioxidant activities of the seeds and leaves of *R. sativus*. Hence, the main objective of the present study was to evaluate the ACE inhibitory and antioxidant activities of the seeds and leaves of *R. sativus* L. using various *in vitro* models.

Hence, the main objective of the present study was to evaluate the ACE inhibitory and antioxidant activities of the methanolic seed extract of A. graveolens using in vitro models.

MATERIALS AND METHODS

Plant material

The seeds and leaves of *R. sativus* were collected from Coimbatore district, Tamilnadu, India during the month of June 2010. The plant was identified and authenticated by Mr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference no BSI/SC/5/08-09/Tech-766.

Extraction

The seeds of *R. sativus* L. was washed thoroughly with distilled water and dried in open air at room temperature for 24 h. The seeds were powdered mechanically and sieved through No. 20 mesh sieve. About 500 g of powder was soaked with 2 L of ethanol (95%) for 12 h and then macerated for 4 h at room temperature using a mechanical shaker. The extract was filtered through a muslin cloth and the residue was again soaked with the same volume of 95% ethanol for 12 h and then further extracted for 4 h and filtered. The filtrates were then combined together and concentrated under reduced pressure and concentrated in a rotary evaporator at 40ºC [16].

Around 500 g of *R. sativus* leaves was washed with tap water and then soaked in 2.75 L of distilled water for 3 days. The plant material was filtered through a muslin cloth and the filtrate was collected. This procedure was repeated twice and the collective filtrate was concentrated in a rotary evaporator at 40ºC to yield a thick, viscous extract [17].

Chemicals and instruments

Angiotensin converting enzyme from rabbit lung (EC.3.4.15.1), Furnacryloyl-l-phenylalanylglycylglycine (FAPGG) and Lisinopril were purchased from Sigma Aldrich, USA. 2-deoxy-2-ribose, (2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethane sulphonic acid (HEPES) buffer, pyrocatechol and quercetin were purchased from Himedia Labs., Pvt. Ltd, Mumbai, India. All other drugs and chemicals used in the study were purchased commercially and were of analytical grade. HPLC instrument with L-4000 UV detector, L-6200 Intelligent pump and LiChrosorb RP-18 column (50 × 7 mm) from HITACHI with Data Ace workstation was used for the analytical studies (HITACHI Ltd, Tokyo, Japan).

Phytochemical screening of the extract

Chemical tests were carried out for the methanolic seed extract of *R. sativus* for the presence of phytochemical constituents like phenols, tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides and steroids [18].

Angiotensin converting enzyme inhibition assay

The angiotensin converting enzyme inhibitory activity was carried out by using n-furnacryloyl-l-phenylalanylglycylglycine (FAPGG) as the substrate [19]. The extract and the standard drug Lisinopril (1 mg/ml) were prepared by dissolving in reaction buffer (HEPES 25mM, NaCl 293mM, pH 8.3). The assay mixture (750µl) consisting of 530µl of FAPGG (3mM in reaction buffer) and 200 µl of extract at different concentrations (100-800 µg/ml) was incubated for 3 min at 37°C. The reaction was initiated by adding 20 µl of ACE solution (0.05U/ml) to the test reaction and the samples were incubated for one hour at 37ºC. The reaction was then stopped by adding 80 µl of 5% trifluoroacetic acid solution and samples were centrifuged at 9000 rpm for 5 minutes at room temperature. The enzymatic activity was calculated by quantifying the decrease in FAPGG concentration by recording the decrease in absorbance at 345 nm using RP-18 column (50 mm × 7 mm, 3 µm pore size) with isocratic elution using acetonitrile and 1.1% trifluoroacetic acid in Milli-Q in the ratio of 75:25 v/v; it was filtered through 0.45 µL filter (Sartorius, Germany) and using an ultrasonic bath was degassed before use. The column temperature was ambient and the total running time was 10 min using a flow rate of 1.5 ml/min with retention time of 2.7 min for FAPGG, the injection volume was 20µl and the detection wavelength was 345 nm. Percentage enzyme inhibition was calculated by comparing the enzymatic activity with, and without inhibitor using the following formula,

%ACE inhibition = (1−a) ×100

where a is the activity with inhibitor and activity without inhibitor [2, 20].

Antioxidant activity

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extract is determined by its ability to scavenge the hydroxyl radicals produced by the EDTA-Fe^{3+}-H_2O_2-ascorbic acid system by the Fenton reaction [21]. The reaction mixture consists a final volume of 1.0 ml which contains 100 µl of 2-deoxy2-ribose (28 mM) in phosphate buffer solution (20 mM, pH 7.4), 500 µl of the extract at various concentrations (5-80 µg/ml) in buffer solution, 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1v/v), 100 µl of H_2O_2 (1.0 mM) and 100 µl of ascorbic acid (1.0 mM). Test samples were incubated at 37°C for 1 h. The free radical damage on the substrate, deoxyribose was assessed with the thiobarbituric acid test. The positive control used for this assay was quercetin (5-80 µg/ml). The percentage inhibition of the extract and standard was calculated [22].

Superoxide radical scavenging activity

Superoxide radical generation using the phenazine methosulfate(PMS)-b-nicotinamide adenine dinucleotide (NADH) system. The superoxide radicals are generated in a PMS-NADH system by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 78 mM NADH, 50 mM NBT, 10 mM PMS and samples to be tested at different concentrations (25-400 µg/ml). The color reaction between superoxide radicals and NBT was detected at 560 nm using a UV-spectrophotometer and the percentage inhibition calculated. Ascorbic acid was used as positive control [23].

Nitric oxide radical scavenging activity

Various concentrations of the extracts (50-800 µg/ml) and sodium nitroprusside (5mM) in phosphate buffer saline (0.025 M, pH 7.4) in a total volume of 3 ml was incubated at room temperature for a period of 150 min. After which, 0.5 ml of the incubated solution and 0.5 ml Griess' reagent (1% sulphanilamide, 2% O-Phosphoric acid and 0.1% naphthyethylene diamine dihydrochloride) were added togather and allowed to react for 30 min. Control samples without the test compounds but with equal volume of buffer was prepared in a similar manner as done for the test. The absorbance of the chromophore formed during diazotisation of nitrite with sulphanilamide and successive coupling with naphthyethylene diamine dihydrochloride was measured at 546 nm. The experiment was carried out using curcumin (50-800 µg/ml) as positive control [24]. The percentage inhibition of the extract and standard was calculated.

Ferrous chelating ability

In the Fe^{2+} chelating assay, Fe^{2+} level in the assay mixture is determined by measuring the formation of the Fe^{2+}-ferrozine complex. The reaction mixture containing various concentrations (50-800 µg/ml) of the extract was added to 2mM ferric chloride (0.1 ml) and 5mM ferrozine (0.2 ml) to begin the reaction and the resulting mixture was shaken and left to stand for 10 min at 25º C. The absorbance of the assay solution was measured at 562nm. The experiment was carried out using ascorbic acid (50-800µg/ml) as positive control. The percentage chelating effect of ferrozine-Fe^{2+} complex formation was calculated [25].

Reducing power ability

Reducing power ability was measured by mixing 1 ml extract of various concentrations (50-800 µg/ml) prepared with distilled water to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 30 min. Next an aliquot of 2.5 ml of trichloroacetic acid (10%) was added to the mixture and centrifuged for 10 min at 3000 rpm, 2.5 ml from the upper part was diluted with 2.5 ml water and shaken with 0.5 ml fresh 0.1% ferric chloride. The absorbance was measured at 700 nm. The reference solution was prepared as above, but contained water instead of the samples. Increased absorbance of the reaction mixture indicates increased reducing power. Butylated hydroxyltoluene (BHT) was used as positive control [26].

Estimation of total phenolic content

Total phenolics present in the extract can be estimated using folin-ciocalteu reagent and pyrocatechol as the standard. An aliquot of 1 ml of extract solution in a test tube was added to 0.2 ml of Folin-Ciocalteu reagent (1:2 in distilled water) and, after 20 min, 2 ml of purified water and 1 ml of sodium carbonate (15%) was added. After 30 min, the absorbance was measured at 765 nm. The total phenolic compounds present in the extract were determined as µg pyrocatechol equivalent (PCE) with the use of the standard pyrocatechol graph [27].

Estimation of total flavonoid content

Total flavonoids present in the extract can be determined by treating it with aluminium nitrate and then comparing it to that of the standard quercetin. One mg of the extract was first added to 1ml of ethanol (80%) and from which 0.5 ml was added to the test tubes containing 0.1 ml of aluminium nitrate (10%), 0.1 ml of 1 M potassium acetate and 4.3 ml of ethanol (80%). After a period of 40 min the absorbance of the supernatant solution was measured at 415 nm using UV spectrophotometer [28].

Calculation of 50% inhibitory concentration (IC_{50})

The concentration (µg/mL) of the extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition (I%) was calculated using the formula:

$$I\% = A_c - A_t / A_c \times 100$$

where A_c is the absorbance of the control and A_t is the absorbance of the sample.

Statistical analysis

All the experiments were carried out in triplicate and results expressed as mean ± SEM. Significant differences among means of samples were evaluated by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Phytochemical screening of the extract

Phytochemical screening showed the presence of tannins, phenolics, flavonoids glycosides and steroids in the extract.

Angiotensin converting enzyme assay

The ACE inhibitory activity of *R. sativus* seed and leaf extracts were represented as percentage ACE inhibition. Among the extracts assayed, the seed extract demonstrated ACE inhibitory activity at a concentration of 800 µg/ml, showing an inhibition greater than 50%. The seed extract showed an IC_{50} value of 278.54±1.67 µg/ml at a concentration below 800 µg/ml, whereas the leaf extract did not show prominent ACE inhibitory activity at the same concentration. These results were compared with that of the standard drug lisnopril, which showed 81.98 ± 0.56% inhibition at a concentration of 0.8 ng/ml with an IC_{50} value of 0.19±0.02ng/ml. Both the extracts inhibited ACE in a concentration-dependent manner and the inhibition by the seed extract was moderate when compared to the standard drug, lisnopril (Table 1). However, at higher doses of the extract, ACE would be significantly inhibited. Flavonoids are a group of polyphenolics compounds, which have been reported to possess ACE inhibitory activity [7]. Thus, the presence of phenolic and flavonoid content in the extracts would have contributed towards ACE inhibition.

Table 1. ACE inhibitory activity of extracts of *R. sativus*

Plant extracts	Concentration µg/ml	% Inhibition	IC_{50} µg/ml
RSEE	100 200 400 800	41.02 ± 0.50 47.31 ± 0.73 55.28 ± 0.66 62.20 ± 0.72	278.54 ± 1.67
RLAE	100 200 400 800	05.05 ± 0.25 4.21 ± 0.40 21.46 ± 0.56 22.06 ± 0.47	-
Standard Lisnopril (ng/ml)	0.1 0.2 0.4 0.8	43.12 ± 0.23 50.38 ± 0.32 77.92 ± 0.45 81.98 ± 0.56	0.19 ± 0.02

RSEE- Raphanus sativus seed ethanolic extract
RLAE- Raphanus sativus leaf aqueous extract
IC_{50}- concentration needed to obtain 50% inhibition of ACE activity
All values determined were mean ±SEM; n= 3

Antioxidant assay

Superoxide radical scavenging activity

The superoxide anion radical scavenging ability of the extracts were determined by the PMS-NADH system, where it generates superoxide radicals which reduce the NBT to form a chromophore (diformazan) that absorbs at 560 nm. Determination of the resulting absorbance provides a measure of the level to which the extract was able to inhibit NBT reduction by the superoxide radical [29]. The extracts showed significant ($P<0.01$) superoxide scavenging activity. The scavenging activity of the seed extract (IC_{50} 239.99 ± 0.32µg/ml) was higher than that of the leaf extract (IC_{50} 345.45 ± 0.46µg/ml) when compared with standard ascorbic acid (IC_{50} 61.71 ± 0.85µg/ml). Thus, both the extracts exhibited superoxide anion scavenging activity in a concentration dependent manner (Table 2).

Table 2. Antioxidant activity of the extracts of *R. sativus*

Antioxidant Activity	IC_{50} µg/ml		
	Standard	RSEE	RLAE
Superoxide radical scavenging assay	61.71 ± 0.85	239.99 ± 0.32	345.45 ± 0.46
Hydroxyl radical scavenging assay	16.90 ± 0.18	30.27 ± 0.14	57.77 ± 0.75
Ferrous chelating ability	89.28 ± 0.14	665.66 ± 0.46	271.42 ± 0.43
Nitric oxide radical scavenging assay	173.42 ± 0.15	193.93 ± 0.79	363.63 ± 0.64

RSEE- Raphanus sativus seed ethanolic extract
RLAE- Raphanus sativus leaf aqueous extract
All values determined were mean ±SEM; n= 3

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by assessing the inhibition of deoxyribose degradation by hydroxyl radicals produced by Fenton reaction. Both the extracts and the standard (quercetin) inhibited the formation of hydroxyl radicals. The scavenging activity of the *R. sativus* seed extract was higher (IC_{50} 30.27 ± 0.14µg/ml) than that of the leaf extract (IC_{50} 57.77 ± 0.75µg/ml) and is compared with the standard quercetin (IC_{50} 16.90 ± 0.18µg/ml) (Table 2). The hydroxyl radical is an exceedingly reactive free radical which has been implicated as an extremely damaging species of ROS in free radical pathology, capable of damaging almost all molecules present in living cells. It can join nucleotides in DNA as well as cause strand breakage which ultimately lead to carcinogenesis, mutagenesis and cytotoxicity. The hydroxyl radical scavenging ability of an extract gives a direct relation to its antioxidant activity. The ability of the extracts to inhibit hydroxyl radical mediated deoxyribose damage was determined by means of the Fe^{2+}-dependent deoxyribose assay. Malondialdehyde, a degradation product of deoxyribose produces a pink chromogen with thiobarbituric acid [23]. Both the plant extracts scavenged the hydroxyl radicals present in the reaction mixture and thus the degradation of deoxyribose was prevented.

Nitric oxide radical scavenging assay

The extracts of *R. sativus* effectively reduced the generation of nitric oxide from sodium nitroprusside. The seed extract showed strong nitric oxide scavenging activity (IC_{50} 193.93±0.79 µg/ml) and that of standard curcumin was 171.42 ± 0.15 µg/ml. The leaf extract (IC_{50} 363.63 ± 0.64µg/ml) also showed good scavenging ability (Table 2). Nitric oxide (NO) is a potent pleiotropic agent involved in various physiological processes like smooth muscle relaxation, inhibition of platelet aggregation and regulation of cell mediated toxicity. However, overproduction of NO manifest in various pathological conditions basically by formation of peroxynitrites. In the nitric oxide scavenging assay, sodium nitroprusside reacts with oxygen to produce nitrite ions that is determined by using Griess

reagent [30]. The extracts were found to decrease the quantity of nitrite ions which can be attributed to the presence of phytochemical constituents.

Ferrous chelating ability

Addition of the extracts of *R. sativus* interferes with the ferrous-ferrozine complex and the red colour of the complex decreased with the increasing concentration of the extracts. Thus metal chelating ability of the extracts was denoted by the extent of ferrous-ion-ferrozine complex formation [29]. Both the extracts captured ferrous ions before ferrozine and thus have ferrous chelating ability. The absorbance of the extracts decreased with increasing concentration (50 to 800 µg). Among the extracts tested, the leaf showed higher chelating activity (IC_{50} 271.42 ± 0.43 µg/ml) than the seed extract (IC_{50} 665.66 ± 0.46 µg/ml). The IC_{50} of the standard, ascorbic acid was found to be 89.28 ± 0.14 µg/ml (Table 2). It was reported that the chelating agents that can form σ bond with a metal, are most efficient as secondary antioxidants because they decrease the redox potential and thus steady the oxidized form of the metal ion. Our results show that the extracts have good ability for iron binding, signifying its antioxidant activity.

Reducing power ability

The reducing power ability of the plant extracts were determined by measuring the transformation of Fe^{3+} ions to Fe^{2+} ions. Table 3 shows the reductive capabilities of seed and leaf extract of *R. sativus* and the standard, BHT. The reducing power increased significantly with increasing concentration of the extracts. The seed extract of *R. sativus* showed higher reducing ability (absorbance 0.3654 ± 0.0037) than the leaf extract (0.3137 ± 0.0072). However, the activity was less than the standard, BHT (absorbance 1.0653 ± 0.024). The reducing power ability of a compound usually depends on the existence of reductones, which mainly act by breaking the free radical chain reaction by donating a proton [31]. The antioxidant principles present in the plant extracts could have caused the reduction of ferric complex into its ferrous form, thus accounting for the reducing power ability.

Table 3. Reducing power ability of extracts of *R. sativus*

Plant extracts	Concentration µg/ml	Absorbance nm
RLAE	50	0.0619 ± 0.0073
	100	0.1245 ± 0.0042
	200	0.1802 ± 0.0019
	400	0.2524 ± 0.0170
	800	0.3137 ± 0.0072
RSSE	50	0.0846 ± 0.0018
	100	0.1392 ± 0.0019
	200	0.1890 ± 0.0071
	400	0.2658 ± 0.0035
	800	0.3654 ± 0.0037
Standard BHT	50	0.6842 ± 0.013
	100	0.7933 ± 0.005
	200	0.8865 ± 0.005
	400	0.9536 ± 0.014
	800	1.0653 ± 0.024

RSEE- Raphanus sativus seed ethanolic extract
RLAE- Raphanus sativus leaf aqueous extract
BHT- Butylated hydroxyl toluene
All values determined were mean ±SEM; n= 3

Total phenolic content

Estimation of total phenolic content using Folin-Ciocalteu reagent is an easy, suitable and reproducible method employed regularly in studying phenolic antioxidants. It is calculated from the standard pyrocatechol graph and expressed as µg pyrocatechol equivalents (PCE). The seed and leaf extract contains 16.79 ± 0.012 µg PCE/mg and 10.19 ± 0.083 µg PCE/mg respectively. Phenolics are secondary metabolites found in great abundance in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient free radical scavengers and metal chelators [32]. It has been reported that the antioxidant activity of phenols is principally due to their redox potential, hydrogen donating and singlet oxygen quenching ability.

Total flavonoid content

The total flavonoid content in the extracts was determined as µg quercetin equivalent (QE) using the standard quercetin graph. The total flavonoid content in the seed and leaf extract was found to be 0.72 ± 0.012 µg QE/mg and 1.44 ± 0.021 µg QE/mg. Flavonoids are water soluble pigments present in the cytosol or stored in the vacuole of the plant cell and represent the largest group of compounds in plants. They exhibit numerous biological effects like

antihepatotoxic, anti-ulcer, antiviral, anti-inflammatory, anticancer and anti-allergic activities. They interact with the cardiovascular system in several ways like, reducing reactive oxygen species, increasing nitric oxide concentration and also by inhibiting ACE mainly by binding to the Zn^{2+} at the active site. Flavonoids due to the presence of their phenolic hydroxyl groups are highly capable of scavenging ROS and are known to be potent antioxidants [33].

CONCLUSION

Currently available ACE inhibitors are synthetic pharmacological drugs; their use in healthy or low-risk populations is not advisable due to their adverse effects. Certain compounds derived from plant extracts like hydrolysable tannins [4], phenylpropanes [5], proanthocyanidins [6] flavonoids [7], xanthones, fatty acids, terpenoids [8], alkaloids [34] oligosaccharides and peptide amino acids [9] have been reported for their *in vitro* ACE inhibitory activity. These findings opens up the possibility of finding newer plant derived compounds which mimic synthetic ACE inhibitors and provide health benefits without any adverse effects.

The present study suggests that the seed extract of *Raphanus sativus* possess angiotensin converting enzyme inhibitory and antioxidant activities that might be helpful in treating hypertension and preventing or slowing the progress of cardiovascular disorders and free radical related disorders. Further investigations on the isolation of active compounds present in the extract and *in vivo* studies are necessary to identify a potential chemical entity for clinical use in the treatment of hypertension and other related cardiovascular disorders.

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