In vitro screening of antioxidant and anti-inflammatory capacities of plant extract *Curculigo orchioides* Gaertn

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Abstract

Curculigo orchioides Gaertn is a medicinal herb belonging to Amaryllidaceae family and is recognized as a potent source of biologically active compounds with many biological properties. The rhizome of the plant was used to prepare aqueous extracts in order to screen antioxidant and anti-inflammatory capacity. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were evaluated to determine the phytochemical composition of the extracts. Antioxidant activity was screened using different assays, DPPH, total antioxidant activity, inhibition of protein oxidation, lipid peroxidation and deoxyribose oxidation and ferric ion reducing power. Anti-inflammatory activity was screened using human red blood cell membrane stability test and inhibition of protein denaturation assays. The TPC and TFC were 101.2±1.3 mg GA/g (Gallic Acid /g) and 178.4±2.4 mg EGCG/g (epigallocatechin gallate/g) respectively. The values of EC_{50} for DPPH, inhibition of deoxyribose oxidation, inhibition of lipid peroxidation, inhibition of protein oxidation and inhibition of BSA denaturation assays were 25.6±1.6, 10.1±1.0, 29.0±0.7, 82.6±3.9 and 32.9±3.5 µg/mL respectively. Further, RCO extract showed less ferric ion reducing ability. CO demonstrated comparable values for EC_{50} with Diclofenac sodium standard on membrane stabilizing activity (49.7±1.4 and 49.7±1.4 µg/mL respectively). Result of the study suggests that CO exerts antioxidant as well as anti-inflammatory activities by stabilizing biological membranes.

Keywords: *Curculigo orchioides*, protein oxidation, lipid peroxidation, deoxyribose oxidation, human red blood cell membrane stability

Introduction

The therapeutic potential of plants has been well explored over years in traditional practices including Chinese traditional, Ayurveda, and Unani medicine[1]. An antioxidant is a substance that delays or inhibits oxidative damage to a target molecule[2]. The main characteristic of an antioxidant is its ability to trap free radicals. Natural antioxidants in plants are chemical constituents that occur in all parts of the plant, very effective to prevent cellular oxidative stress. Polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) are the major natural antioxidants compounds present in plants [3,4]. Oxidative stress is caused by excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) results in cellular damage leading to degenerative diseases such as cancer, cardiovascular diseases and inflammatory disorders.

Plant origins with anti-inflammatory activities are considered to be an important source for the development of new therapeutic agents. Chronic inflammatory diseases are still one of the main health problems of the world's population[5].

Although several modern drugs are used to treat these types of disorders, however, prolonged use may cause several adverse side effects.

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Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. Several plants are being used in traditional medical systems for treating these disorders which are inflammatory like rheumatism and arthritis. Flavonoid, polyphenols, proanthocyanidin, alkaloid, terpenoid and steroid compounds in plants are usually responsible for the anti-inflammatory activities [6].

Curculigo orchioides Gaertn belongs to Amaryllidaceae family and are distributed in Asia, Europe and North Africa[7]. In Sri Lanka, it is commonly known as *Heen Binthal*. *Curculigo* plants are perennial herbs, often with tuberous rhizomes 7. The rhizomes of this plant possess medicinal properties which can be used to treat skin diseases, asthma, bronchitis and jaundice etc[8]. In addition to that, the rhizome also possesses immune simulative[9] hepatoprotective, anticancer and antioxidant properties[10]. Water extracts of *C. orchioides* are used for many herbal preparations in Sri Lanka. However, there is no compressive scientific report available in Sri Lanka. Thus, the present study has been directed to investigate the antioxidant and anti-inflammatory activity of *C. orchioides* water extract.

Materials and Methods

Preparation of extract

The plant was obtained from "*Weda waththa*" (6.801746, 79.977027) located in Maththegoda of Colombo district area from August to September 2021, in Sri Lanka. The plant was identified by a senior lecturer, at the Institute of indigenous medicine, University of Colombo. Rhizome of the plant was used for the study. Rhizome was washed with tap water followed by distilled water and de-ionized water and dried in a freeze drier to avoid oxidation of endogenous substances. Rhizome was cut into small pieces and ground to a fine powder using a clean kitchen blender. Fine powder biomass, 60 g was boiled with 1920 mL of deionized water until the total volume was reduced to 240 mL (1/8th of the original volume) using a glass beaker. The plant extract was filtered through a cotton wool plug followed by filter paper (Whatman No.1). The filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was freeze-dried. The freeze-dried sample was weighed and stored at -20^0 C in sterile glass tubes until further use.

Removal of polyphenols

Removal of polyphenols was carried out using Polyvinylpolypyrrolidone (PVVP) column according to the method described by Ranatunge *et al* 2017[11]. The antioxidant activity of the polyphenol-free water extract was determined using a DPPH assay.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content was estimated by the Folin – Ciocalteu method[12] and the total flavonoid content (TFC) was determined using the aluminum chloride (AlCl3) method described by Zhishen et al., 1999[13]. TPC and TFC were expressed as gallic acid equivalents (mg Gallic Acid /g) and epigallocatechin gallate (EGCG) equivalents per gram of dried sample respectively.

DPPH radical scavenging activity assay

The free radical scavenging activity of the plant extract was measured by 2,2,0- diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described by Blois1958[14] with modifications. The capacity to scavenge the DPPH radical was calculated using the following equation:

% DPPH scavenging effect = (AC – AS)/ AC ×100

Where, AC and AS are the absorbance of the control and sample respectively.

EC_{50} value was calculated using a graph constructed with different concentrations vs % DPPH scavenging effect.

Total antioxidant capacity

The total antioxidant activity of the plant extract was evaluated by the phosphomolybdenum reduction assay method according to the procedure described by Prieto *et al* 1999[15]. Ascorbic acid was used as the positive standard. The total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per gram of dried sample.

Ferric reducing power assay

The reducing power was evaluated by determining the ability to convert ferric ions to ferrous ions by plant extract as previously described with slight modifications[16]. In briefly different concentrations (7.81-250 µg/mL) of CO (100 µL) was mixed with phosphate buffer (0.2 M, pH 6.6, 250 µL) and potassium ferricyanide (1% w/v, 250 µL). The reaction mixture was incubated at 50º C for 20 minutes and trichloroacetic acid (10% w/v, 250 µL) was added and samples were centrifuged at 6500 rpm for 10 minutes. The supernatant was mixed with deionized water and ferric chloride (0.1% w/v) at a ratio of 1:1:2 respectively. L- Ascorbic acid was used as the positive control. Absorbance was measured at 700 nm wavelength.

Inhibition of protein oxidation

The effect of CO on protein oxidation was carried out according to the slightly modified method of Wang and co-workers 2006[17]. Bovine serum albumin (BSA) was oxidized by a Fenton-type reaction. Different concentrations of CO (0.5 mL) were mixed with reaction mixture (1.5 mL), containing potassium phosphate buffer (20 mM, pH 7.4, 300 µL), BSA (4 mg mL^{-1}), $FeSO_4$ (2 mM, 300 µL), H_2O_2 (30%, 400 µL) and was incubated for 30 min at 37º C. For the determination of protein carbonyl content in the samples, 1.0 mL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl was added to the reaction mixture. Samples were incubated for 30 min at room temperature. Then, 1.0 mL of cold TCA (10%, w/v) was added to the mixture and centrifuged at 3000 rpm for 10 min. The protein pellet was washed three times with 2.0 mL of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1.0 mL of guanidine hydrochloride (6 M, pH 2.3). L- Ascorbic acid was used as the positive control. The absorbances of the samples were measured at 370 nm wavelength. The percentage inhibition of protein oxidation was calculated by the following equation.

% inhibition = (AC – AS)/AC× 100

Where, AC and AS are the absorbance of the control and sample respectively.

EC_{50} value was calculated using a graph constructed with different concentrations vs % inhibition.

Inhibition of lipid peroxidation

The inhibition of lipid peroxidation was carried out according to the slightly modified method of Dhar et al. 2013[18]. Lipid peroxides formed in the egg yolk was used as the lipid-rich source. Briefly, fresh egg yolk emulsion was diluted to 10% v/v with 1.15% w/v KCl. Egg yolk emulsion (50 µL), different concentrations (7.81-1000 µg/mL) of CO, aqueous trichloroacetic acid (20%, 150µL) and 0.67% w/v thiobarbituric acid (150µL) were added respectively. The reaction mixture was then vortexed thoroughly and incubated at 95° C in the water bath for 1 hour. The mixture was cooled and centrifuged at 3000 rpm for 10 min. L- Ascorbic acid was used as the positive control. The absorbance of the upper layer was measured at 532 nm wavelength and percentage inhibition was calculated with the following formula.

% Inhibition = (AC- AS) /AC X 100

Where, AC and AS are the absorbance of the control and sample respectively.

EC_{50} value was calculated using a graph constructed with different concentrations vs % inhibition.

Inhibition of deoxyribose oxidation

The inhibition of deoxyribose oxidation was measured according to the modified method of Halliwell 1987[19]. Gallic acid was used as the positive control. The absorbance was measured at 532 nm wavelength. Percentage inhibition of deoxyribose oxidation was calculated with the following formula.

% inhibition= (AC –AS)/AC) X 100

Where, AC and AS are the absorbance of the control and sample respectively.

EC_{50} value was calculated using a graph constructed with different concentrations vs % inhibition.

Assessment of in vitro anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of plant extract was studied by using inhibition of albumin denaturation according to the method published by Leelaprakash and Mohan Dass 2011[20] with minor modifications. Different concentrations (7.81-1000 µg/mL) of CO

(0.05 mL) mixed with 0.45 mL bovine albumin (5%). pH of the reaction mixture was adjusted to 6.3 using 1N HCl. The resulting mixture was incubated at 37º C for 20 min and then heated to 51º C for 20 min. After cooling the mixture, the turbidity was measured at 660 nm wavelength. Diclofenac sodium was used as a positive control. The percentage inhibition of protein denaturation was calculated as follows.

% inhibition = (AC –AS) / AC X 100

Where, AC and AS are the absorbance of the control and sample respectively.

EC_{50} value was calculated using a graph constructed with different concentrations vs % inhibition.

Human red blood cell membrane stability assay

The human red blood cell (HRBC) membrane stabilization method was carried out according to the method described by Stability and Azam 2013[21]. The blood sample was collected from a health volunteer. The collected blood was mixed with sterilized alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water). The blood sample was centrifuged at 3000 rpm and packed cells were washed with isosaline and a 10% (V/V) suspension. Different concentrations (7.81-1000 µg/mL) of CO (l mL) mixed with phosphate buffer (1mL), hyposaline (2 mL) and HRBC suspension (0.5 mL). Diclofenac sodium was used as a positive control and instead of hyposaline 2 mL water was used as the negative control. The hemoglobin content in the supernatant was calculated using a spectrophotometer at 560 nm wavelength. The result was estimated by the following equations.

% Hemolysis = ODS/ODCX 100

Where, ODS and ODC are the optical density of the sample and control respectively.

The percentage of membrane protection was calculated by the following equation

% Membrane protection=100- the percentage of hemolysis

EC_{50} value was calculated using a graph constructed with different concentrations vs % Membrane protection.

Statistical analysis

All the results were expressed as the mean ± standard deviation (Mean ± SD) of at least three independent experiments. Calibration curves were considered linear if $R^2 > 0.99$. The EC50 values were calculated from linear dose-response curves where $R^2 > 0.95$. A student t-test was carried out for the statistical calculations using Microsoft Excel (2010).

Results

Extraction yield, polyphenolic and flavonoid content of *C. orchioides* extract is mentioned in Table 01. The extraction yield of the CO was 10.5 ±0.5%.

Table 01: Extraction yield, polyphenolic and flavonoid content of *C. orchioides* extract.

Sample/ Control	Extraction yield %	Total polyphenolic content (TPC) (mg GAE/g)	Total Flavonoid content (TFC)(mg EGCG/g)
CO	10.5 ±0.5 (n=2)	101.2±1.3 (n=6)	178.4±2.4 (n=6)

Figure 01 shows the comparison of the total antioxidant capacity of CO with ascorbic acid. Total antioxidant capacity increases with the concentration and is comparable with ascorbic acid at a lower concentration (< 25 µg/mL).

All experiments were repeated independently at least three times, and data (n=3 for each concentration) are expressed as the mean ± SD; **p <.05 and ***p<.001 or ****p< .0001 in comparison to the positive control (Ascorbic acid); paired t test.

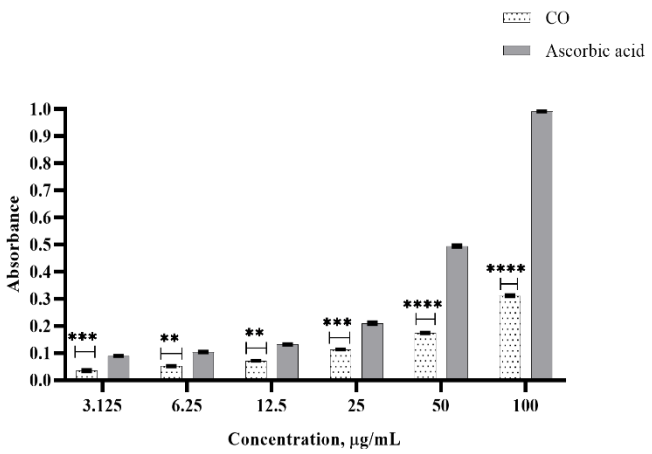

Fig. 01: Total antioxidant capacity of CO water extract

EC_{50} values for inhibition of lipid peroxidation, inhibition of protein oxidation, and inhibition of deoxyribose oxidation is mentioned in Table 02.

Table 02: EC_{50} values for inhibition of lipid peroxidation, inhibition of protein oxidation, and inhibition of deoxyribose oxidation.

Sample/ Control	EC_{50}(µg/mL)		
	Inhibition lipid peroxidation	Inhibition protein oxidation	Inhibition deoxyribose oxidation
CO	29.0±0.7*	82.6±3.9*	10.1±1.0
Ascorbic acid	58.4 ± 2.2	51.2±0.1	NA
Gallic acid	NA	NA	8.7±0.6

NA- Not Applicable
(*P<0.05 when compared with positive control)

Table 02 shows the inhibition of lipid peroxidation of the CO water extract showed significantly higher activity compared with the standard ascorbic acid (P<0.05). However, CO exhibited moderate inhibition of protein oxidation compared with ascorbic acid (P<0.05). There was no significant difference observed between CO and gallic acid in protective activity against the oxidation of deoxyribose.

The effect of polyphenols on DPPH radical scavenging activities shows in Table 03.

Table 03: The effect of polyphenols on DPPH radical scavenging

Sample/ Control	EC_{50}(µg/mL)	
	Before removal of polyphenols (PP)	After removal of polyphenol
CO	25.6±1.6 *** (n=6)	704.1±4.6 (n=3)
Gallic acid	4.5±0.2 (n=3)	NA

EC_{50} values confirm that polyphenol present in the CO extract contributes significantly to scavenging free radicals (Table 03). (***P<0.001 between PP and PP free extracts)

Anti-inflammatory potential of plant extract (Human red blood cell membrane stability and inhibition of BSA denaturation assay) is shown in Table 04.

Table 04: Anti-inflammatory potential of plant extract (Human red blood cell membrane stability and inhibition of BSA denaturation assay)

Sample/ Control	EC_{50}(µg/mL)	
	Human Red blood cell membrane stability	Inhibition of BSA denaturation
RCO	49.7±1.4	32.9±3.5
Diclofenac Sodium	47.8±2.1	23.8±3.6

This was only preliminary testing and higher red blood cell membrane protection was observed in CO compare with the anti-inflammatory drug diclofenac Sodium (p>0.05). Further inhibition of BSA denaturation confirms that higher anti-inflammatory properties of CO compared with Diclofenac sodium (p >0.05).

Figure 02 shows the comparison of the reducing potential of CO with ascorbic acid. Ascorbic acid was used as a positive control and CO showed poor reducing potential when compared with the ascorbic acid. The reducing potential of CO slowly increases with the concentration.

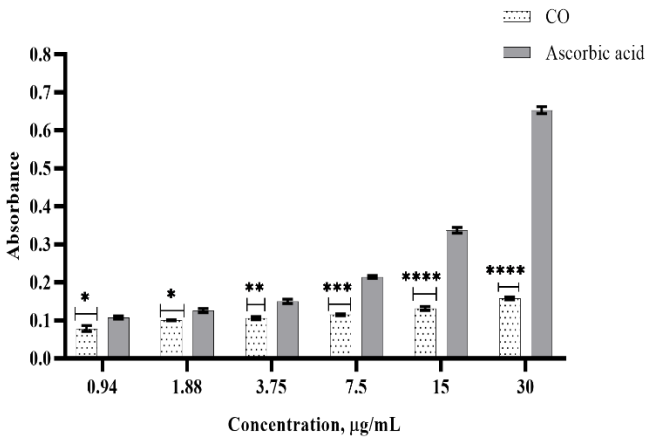

Fig. 02: Ferric iron-reducing potential of CO extract and positive control

All experiments were repeated independently at least three times, and data (n=3 for each concentration) are expressed as the mean ± SD; *p < .01 **p < .05 and ***p < .001 or ****p < .0001 in comparison to the Positive control (Ascorbic acid) ; paired t test.

Discussion

Among the medicinal plants of the genus Curculigo, *C. orchioides* is the most commonly used herbal medicine in Sri Lanka. The rhizome of the *C. orchioides* is used in traditional and folk medicine to treat diseases such as urinary and skin diseases, asthma, bronchitis, and jaundice etc[8]. The water extract which is prepared according to Sri Lanka traditional medicine was used in the present study to explore the antioxidant activity of the water extract and polyphenol free extract of *C. orchioides.*

Antioxidants are substance which is capable of reducing or preventing cellular damage[2]. The main characteristic of an antioxidant is its ability to trap free radicals. Polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamin E and C) are the major natural antioxidants present in plants[3,4]. Herbal plants are considered good antioxidants. Therefore, different *in vitro* antioxidant assays were carried out to screen the antioxidant capacity of the CO based on different mechanisms involved. Total phenolic content and total flavonoid content were used to quantify the phenolic compounds of the extracts. Free radical scavenging capacity was screened using a DPPH radical scavenging assay, inhibition of lipid peroxidation, inhibition of protein oxidation and inhibition of deoxyribose oxidation. Reducing power assay and total antioxidant capacity were used to measure the ability of the antioxidant to produce a reduction of metal ions.

Plant polyphenols are secondary metabolites; frequently involve as defensive agents against abiotic and biotic stress conditions. They also play numerous roles in biological systems such as antioxidants, antimicrobial and anticancer agents[22]. The TPC of CO was 101.2±1.3 mg GA/g (Gallic Acid /g) (Table 01). The study of Hejazi et al., 2018 showed that the TPC of water extract is 83.95±0.02 mg GA/g [23]. The review of Wang *et al* 2021[8] is revealed that phenolic compounds are the main metabolites present in the plants of the Genus Curculigo. Nie *et al.,* 2013 has reported that total of 31 phenols and phenolic glycosides have been isolated from the Curculigo species[7]. Further, it describes that these compounds are characterized mainly as benzyl benzoate glucosides, followed by phenol glycosides and simple phenol[7]. In the case of total phenol content determination, the results strongly suggest that phenolics are important components of the CO. Previous studies report that the antioxidant activity of plant extract is mainly associated with the presence of phenolic compounds [24], which may exert antioxidant effects as free radical scavengers, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators[25]. Qiong *et al*., 2005 also reported that phenolic compounds are major contributors to the antioxidant activity of *C. orchioides*[26]. There is a highly positive relationship between total phenols and antioxidant activity [27].

Flavonoids are another subset of polyphenols. They involve in regulatory mechanisms in cell proliferation and differentiation to protect eukaryotic cells from oxidative stress by regulating the activity of different protein kinases[28]. The antioxidant properties of flavonoids are due to their ability to scavenge free radicals and also serve to chelate metals. According to the present study, the higher contents of flavonoids (178.4±2.4 mg EGCG/g, Table 01) in CO explain its higher radical scavenging activity.

DPPH is the most extensively used assay to determine the antiradical and/or antioxidant power of biological extracts and/or purified compounds. The violet colour of the DPPH solution converts into the yellow colour due to the presence of a substance that can donate hydrogen depending on the antioxidant activity. It is a relatively stable free radical that, upon reduction by an antioxidant loses its absorption (517 nm) [29]. The present study showed that the CO contains a potent scavenger of DPPH free radical and has an EC_{50} value of 25.6±1.6 µg/mL (Table 03). Previous studies also showed a moderate DPPH radical scavenging capacity of *C. curculigo*[23]. The methanol extract of *C. orchioides* rhizomes was found to be moderately effective in scavenging DPPH radicals [30]. The study of Hejazi et al., 2018 revealed that the DPPH radical scavenging capacity of aqueous extract of *C. orchioides* was EC_{50} 104.8±0.6 [23]. Literature indicates that variation of plant secondary metabolites occur due to geographic location and connected environmental factors like temperature, rainfall, soil type, and composition [31]. The higher DPPH radical scavenging capacity of the plant extract may be due to the presence of the polyphenol. Therefore, experiment was carried out to investigate the polyphenol contribution for DPPH radical scavenging capacity. The polyphenol of the CO was removed using polyphenol adsorbant PVPP. PVPP is a highly cross-linked polymer that has a high affinity toward polyphenols [32,33].

Interestingly CO showed poor DPPH radical scavenging capacity after the removal of polyphenol (Table 03). The EC_{50} ratios with the absence and presence of polyphenols are over 100-fold extracts. These results indicate that polyphenols are the main factor that contributed to DPPH radical scavenging activity and the involvement of non-polyphenols in the antioxidant activity is negligible. Total antioxidant activity is another standard assay used to evaluate the antioxidant potential of plant extract. The total antioxidant capacity assay is based on the reduction of molybdenum (VI) to molybdenum (V) which forms a green chromophore with phosphate in the acidic medium[34]. CO exhibits moderate total antioxidant capacity. The evidence further confirms the CO was with higher antioxidant potential (Figure 02).

Lipid peroxidation is the process where the reaction between unsaturated lipids and reactive oxygen species[35]. Malondialdehyde (MDA) is one of the final yields of polyunsaturated acids peroxidation in the cells[36]. MDA level is commonly recognized as a marker of oxidative stress and overproduction of MDA due to an increase of free radicals [37]. The thiobarbituric acid (TBA) is a common method is used to determine the degree of malondialdehyde (MDA) compound in biological solution[38].

The mechanism of the assay is MDA reacts with TBA and produces a pink colour which read at 532 nm. A higher reduction of lipid peroxidation is observed in CO extract (EC_{50}, 29.0±0.7 µg/mL) compare with the positive control (Ascorbic acid EC_{50} 58.4±2.2 µg/mL) (Table 02). Molecules present in the CO extract may have a high contribution to the inhibition of lipid peroxides. Results suggest that plant extracts are capable of reducing cell membrane damage by scavenging lipid peroxides. The previous study of Bafna and Mishra, 2005 showed that methanol extract exhibit potent inhibition of lipid peroxidation induced by Iron/ADP/Ascorbate complex in rat liver homogenate, EC_{50} value was 94.7µg/mL [30].

Reactive species also damage proteins due to oxidation of the protein. Superoxide ($O2•–$) hydroxyl (•OH), peroxyl(RO_2•), alkoxyl (RO•), hydroperoxyl (HO_2•), and non-radical species such as hydrogenperoxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen (O_2), and

peroxy- nitrite ($ONOO^-$) are the ROS leading to protein oxidation [39].

The direct attack of oxidants damages the backbone of a protein to cause fragmentation and conformational changes in the secondary and tertiary structure of the protein [40]. The generation of carbonyls is the most common damage for oxidized proteins[40]. DNPH derivation method has been developed as a convenient and regular method to quantify the levels of protein oxidation in the food system [41]. In this method, DNPH reacts with the carbonyl groups of proteins to generate hydrazones and the absorbance is read at 370 nm wavelength[42]. In the present study, inhibition of protein oxidation by CO was tested. The results revealed that CO contains the moderate potential to prevent protein oxidation ($EC_{50,}$ 82.6±3.9 µg/mL, Table 02). Ascorbic acid (Positive control) was EC_{50} 51.2±0.1 µg/mL. Therefore, CO is highly applicable for a disease that arises due to increased levels of protein carbonyls such as neurodegenerative diseases (amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, and Huntington's diseases), cataractogenesis, systemic amyloidosis, muscular dystrophy, progeria, Werner's syndrome, rheumatoid arthritis, and respiratory distress syndrome [43].

DNA damage is one of the major effects of ROS [44]. DNA is the cell's genetic material and OH- radicals react with all purine and pyrimidine bases also the deoxyribose backbone results in changes in the encoded proteins, which may lead to malfunctions or complete inactivation of the encoded proteins. Further, changes in the nucleotides of one strand can result in mismatches with the nucleotides in the other strand, yielding subsequent mutations[45]. Accordingly, inhibition of DNA oxidation power of RCO was evaluated in the present study. The hydroxyl radical formed in the reaction between iron (III)-EDTA and H_2O_2 in the presence of ascorbic acid. The attacked pentose sugar 2-deoxyribose is on heating with thiobarbituric acid at low pH, yielding a pink chromogen that can be measured by its absorbance at 532 nm wavelength[46]. Interestingly higher inhibition of deoxyribose oxidation was observed in RCO ($EC_{50,}$ 10.1±1.0 µg/mL, Table 02) compared with the positive control (Ascorbic acid, $EC_{50,}$ 8.7±0.6 µg/mL).

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity[47]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants [48]. Present study CO showed less reducing power potential compared with ascorbic acid (Figure 02). The antioxidant potential of plants is directly linked to their anticancer potential [49].

Inflammation is a complex process and it is a protective reaction to tissue damage caused by physical injury and harmful chemicals[50]. The most commonly used drug for the management of inflammatory conditions is the non-steroidal anti-inflammatory drugs (NSAIDs). But there are many various adverse effects, especially gastric irritation, leading to the formation of gastric ulcers are associated with the above synthetic drugs [51]. Secondary metabolite of plant acts as a potent anti-inflammatory drug with the minimum side effect. Inhibit the function of cycloxygenase (COX) enzyme that is responsible for conversion of arachidonic acid to prostaglandin (PG is the major function of the anti-inflammatory agents[21]. The anti-inflammatory capacity of plant extract can be evaluated *in vitro* and *in vivo*. Red blood cell membrane stability and inhibition of protein denaturation are *in vitro* methods frequently used for screening the anti-inflammatory activity. Since the erythrocyte membrane is analogous to the. lysosomal membrane, the prevention of hypotonicity-induced HRBC membrane lysis has been used as a measure for estimating the anti-inflammatory property of extracts [52, 53].

Stabilization of the lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extracellular release[53]. The lysosomal enzymes released during inflammation

produce various disorders. Therefore non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [54]. Interestingly RCO water extract showed high red blood cell membrane stability (EC_{50} 49.7±1.4 µg/mL, Table 04) compare with the commercially available non-steroid drug Diclofenac sodium (EC_{50}47.8±2.1 µg/mL). The study of Asif and kumara 2016 revealed that root tubers methanolic extract obtained from the plant *Curculigo orchioides* at a dose of 200 mg/kg and 400 mg/kg have significant anti-inflammatory activity[55].

Application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heats are the factors that cause protein denaturation[56]. Denaturation of proteins is a well-documented cause of inflammation. Accordingly, the ability of plant extract to inhibit protein denaturation was studied as part of the investigation of the anti-inflammatory activity. It was effective in inhibiting heat-induced albumin denaturation ($EC_{50,}$ 32.9±3.5 µg/mL, Table 04) compare with the positive control (Diclofenac sodium, EC_{50} 23.8±3.6 µg/mL).

Conclusion

The results of this study demonstrate the antioxidant and anti-inflammatory capacity of the rhizome CO. The finding explains that bioactive molecules present in water extract act as potential antioxidant agents with different mechanisms. The present study confirms that polyphenol contributes to the antioxidant potential. Furthermore, water extract exerts high in vitro anti-inflammatory capacity. Hence, the present study indicates that bioactive molecules present in the plant extract can be used as a prototype for the development of new drugs and/or as a source of antioxidants and anti-inflammatory pharmaceutical raw material.

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Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

The author reports no conflicts of interest in this work.

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