

# Antioxidant and Antibacterial Activity of *Consolida orientalis*

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**Abstract**—The antibacterial and antioxidant activity of different extracts from of *Consolida orientalis* was assessed towards selected bacteria as well as in different antioxidant models. The antibacterial screening was carried out by disc diffusion method. Two complementary test system, namely DPPH free radical scavenging and total phenolic compounds, were used for the antioxidant analysis. The extracts from *Consolida orientalis* had interesting activity against *Proteus mirabilis* (13 mm diameter), *Enterobacter cloacae* (14mm diameter), *Klebsiella pneumoniae* (10 mm diameter) and *Staphylococcus aureus* (12mm diameter). The positive control, Valinomycin, Gentamicin and Chloramphenicol had shown zone of inhibition in thicillin resistant all bacterial. IC<sub>50</sub> for DPPH radical – scavenging activity was 478±9.4 μg.mL<sup>-1</sup>. The extract exhibited a good reducing power at that was comparable with ascorbic acid (p<0.05). The extract also showed weak nitric oxide-scavenging activity and Fe<sup>+2</sup> chelating ability. All of extract manifested almost the same pattern of activity as ascorbic acid and BHA at different incubation times (p<0.05).

**Keywords**—*Consolida orientalis*, Antibacterial activity, Antioxidant activity.

## I. INTRODUCTION

THE plant kingdom has been the best source of remedies for curing a variety of disease and pain. This is why medicinal plants have played a key role in the worldwide maintenance of health. Infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant microorganisms have become more widespread in recent years (WHO 1999). Resistance rates among key pathogens continue to grow at an alarming rate in distinct geographic regions worldwide (Bell et al., 1998; Pfaller et al., 1998; Schmitz et al., 1999) and the search for novel antimicrobial agents to combat such pathogens have become crucial for avoiding the threat of post-antibiotic era.

Free radicals cause the oxidation of biomolecules (e.g., protein, amino acids, lipid and DNA) which leads to cell injury and death (McCord, 2000). Moreover, the oxidative stress caused from imbalance between the generation and the neutralization of free radicals by antioxidant mechanism is responsible for many human diseases, including aging, cancer and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's

diseases [19]. Their deteriorative effects can be diminished by natural antioxidants available in foods. Also, oxidative reactions limit the shelf life of fresh and processed food stuffs and are a serious concern in food industry (Sökmen et al., 2004). Synthetic antioxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) are used to decelerate these processes. However, due to their unstable and highly volatile nature, questions about their safety and efficiency have been brought up ever since their first introduction to the food industry [18]. Consequently, the need to identify alternative natural and safe sources of food antioxidant arose and the search for natural antioxidants, especially of plant origin, has notably increased in recent years (López et al., 2007).

*Consolida orientalis* Rech.f. (Ranunculaceae) is a native plant of southern and south-western Europe, central Asia and northern Africa. It acts mostly as a weed of winter crops, especially of winter wheat and less frequently in winter oil seed rape and winter barley. In locations where it is well naturalized and where the share of winter crops are higher the occurrence of *C. orientalis* is more homogenous. This weed species can easily gradate in crop stands when efficient control is absent. Such fields turn to violet-blue, when *C. orientalis* is flowering. The highest occurrence can be found in field margins. This species finds optimal conditions in warmer regions on loamy clay soils. Outside of arable land is only found occasionally and temporarily. On non-arable land and on fallow it disappears in short time. It often grows on soil deposits derived from agricultural Kojour-Nowshar (North of Iran) land where this species occurs. It can also be found in high densities on indigenous fields even if it was not evident, due to effective control.

To the best of our knowledge, there is no report on biological activity of *C. orientalis*. The aim of this study is to determine the antioxidant and antibacterial activities of *C. orientalis* aerial parts extract in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

## II. MATERIAL AND METHOD

### *Plant materials and preparation of freeze-dried extract*

*Consolida orientalis* was collected from the Kojour-Nowshar (North of Iran), Iran, in 2009. The samples were identified by Dr. Bahman Eslami (Assistant Prof. of plant systems, Islamic Azad university of Ghaemshahr, Iran). Voucher specimens are deposited with the faculty of biology herbarium (No 720-722).

The fresh of *Consolida orientalis* were washed with distilled water immediately after collection. The collected stems were chopped into small pieces, air dried at room temperature for about 10 days and ground into powder and stored in an airtight container. The resulting stem powder (850g) was extracted in Erlenmeyer flasks with cold MeOH

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for 7 days at room temperature. The whole extract was combined and evaporated to dryness in vacuo to give 45g (yield 5.3 % w/w) of blackish-green colored stem extract which was kept in refrigerator at 4°C.

#### *Antioxidant activity*

##### *DPPH radical-scavenging activity*

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of the free radical scavenging activity of the extracts [6]. Different concentrations of extracts (2 ml, 100, 200, 400, 800 and 1600 µg ml<sup>-1</sup>) were added, at an equal volume, to a ethanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. Vitamine C, BHA and Quercetin were used as standard controls. IC<sub>50</sub> values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals.

##### *Determination of metal chelating activity*

The ability of the *Consolida orientalis* extracts to chelate ferrous ions was estimated in our recently published paper [5]; Nabavi et al. 2009a). Briefly, different concentrations of each extracts (1 ml, 100, 200, 400, 800 and 1600 µg ml<sup>-1</sup>) were added to a solution of 2 mM FeCl<sub>2</sub> (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixtures were then shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solutions was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine- Fe<sup>2+</sup> complex formation was calculated as [(A<sub>0</sub> - A<sub>1</sub>)/A<sub>0</sub>] x 100, where A<sub>0</sub> was the absorbance of the control, and A<sub>1</sub> of the mixture containing the extract or the absorbance of a standard solution. EDTA was used as a standard.

##### *Assay of nitric oxide-scavenging activity*

The procedure is based on the principle that sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using the Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of *Consolida orientalis* extracts dissolved in water (25, 50, 100, 200, 400, 800 and 1600 µg ml<sup>-1</sup>) and incubated at room temperature for 150 min. The same reaction mixture, without the extracts but with an equivalent amount of water, served as control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) were added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as a positive control (Nabavi et al. 2009b; Ebrahimzadeh 2009c).

##### *Scavenging of Hydrogen Peroxide*

The ability of the extracts to scavenge hydrogen peroxide

was determined according to the method of Dehpour et al. [3]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. All the Extracts in distilled water (2 ml, 25, 50, 100, 200, 400, 800 and 1600 µg ml<sup>-1</sup>) were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged [H<sub>2</sub>O<sub>2</sub>] = [(A<sub>0</sub> - A<sub>1</sub>)/A<sub>0</sub>] × 100 where A<sub>0</sub> was the absorbance of the control and A<sub>1</sub> was the absorbance in the presence of the sample of extract and standard.

##### *Reducing power determination*

The reducing powers of *Consolida orientalis* extracts were determined according to the method of Yen and Chen (Nabavi et al. 2008a, Ebrahimzadeh et al. 2010). Different concentrations of extracts in water (2.5 ml; 50, 100, 200, 400 and 800 µg ml<sup>-1</sup>) were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction and was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as a positive control.

##### *Antioxidant activity in a hemoglobin-induced peroxidation of linoleic acid*

The antioxidant activity of extract was determined by a modified photometry assay (Kuo et al., 1999). Reaction mixtures (200 ml) containing 10 ml extract (10 – 400 mg), 1 mmol/l of linoleic acid emulsion, 40 mmol/l of phosphate buffer (pH 6.5) and 0.0016% hemoglobin, were incubated at 37°C for 45 min. After the incubation, 2.5 ml of 0.6% HCl in ethanol was added to stop the lipid peroxidation. The amount of peroxide value was measured in triplicate using the thiocyanate method by reading the absorbance at 480 nm after colouring with 100 ml, 0.02 mol/l of FeCl<sub>2</sub> and 50 ml of ammonium thiocyanate (30 g/100 ml). Vitamin C was used as positive control.

##### *Assay for antibacterial activity of plant extract*

Antibacterial activity of plant extract was determined by disc diffusion method as described by Bauer et al. Three Gram (-) bacteria (*Proteus mirabilis* PTCC(1076); *Enterobacter cloacae* PTCC(1003), and *Klebsiella pneumonia* PTCC(1290) and one Gram (+) bacteria (*Staphylococcus aureus* PTCC(1112) )were used for the present study. All the test bacteria were collected from Pastor Institute of Iran. Dried filter paper discs (4mm in diameter) impregnated in known

amount of test substances (500  $\mu\text{g}/\text{discs}$ ) were placed on Mueller-Hinton agar medium uniformly seeded with the test organisms. Valinomycin, Gentamicin and Chloramphenicol discs (30  $\mu\text{g}/\text{disc}$ ) soaked in respective solvent were used as positive control. These plates were then kept at low temperature (4°C) for two to four hours to allow maximum diffusion of compound. The diffusion occurred according to the physical law that controls the diffusion of molecules through agar gel.

The plates were then incubated at 37°C for 24 hours to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called "Zone of Inhibition". The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

#### Statistical analysis

Experimental results are expressed as means  $\pm$  SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ( $p < 0.05$ ) and the means separated by Duncan's multiple range tests. The EC<sub>50</sub> values were calculated from linear regression analysis.

### III. RESULTS

Determination of total phenolic compounds and flavonoid content. The total phenolic content was  $72.12 \pm 1.20$  mg gallic acid equivalent g<sup>-1</sup> extract by reference to standard curve ( $y = 0.0054x + 0.0628$ ,  $r^2 = 0.987$ ). The total flavonoid content was  $15.2 \pm 0.4$  mg quercetin equivalent g<sup>-1</sup> extract, by reference to standard curve ( $y = 0.0063x$ ,  $r^2 = 0.999$ ).

#### Antioxidant activities

IC<sub>50</sub> for DPPH radical-scavenging activity was  $478 \pm 9.4$  mg ml<sup>-1</sup>. The IC<sub>50</sub> values for ascorbic acid, quercetin and BHA were  $1.26 \pm 0.04$ ,  $1.32 \pm 0.07$  and  $13.49 \pm 0.09$  mg ml<sup>-1</sup>, respectively. Figure 1 shows dose-response curves for the reducing powers of extract. It was found that the reducing powers of extract increased with increasing concentrations. The extract exhibited a fairly good reducing power at 25 and 800  $\mu\text{g ml}^{-1}$ . The extract also showed good nitric oxide scavenging activity between 0.1 and 1.6 mg ml<sup>-1</sup>. The % inhibition was increased with increasing concentration of extract. IC<sub>50</sub> was  $1104.6 \pm 28.9$  mg ml<sup>-1</sup> for *C.orientalis* and  $17.01 \pm 0.4$  mg ml<sup>-1</sup> for quercetin. The absorbance of Fe<sup>2+</sup>-ferrozine complex was decreased dose-dependently, that is, the activity was increased on increasing concentration from 0.2 to 1.6 mg ml<sup>-1</sup>. Extract showed moderate Fe<sup>2+</sup> chelating ability. IC<sub>50</sub> was  $909.3 \pm 21.7$  mg ml<sup>-1</sup>. EDTA showed very strong activity (IC<sub>50</sub> = 18 mg ml<sup>-1</sup>). The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner. IC<sub>50</sub> for H<sub>2</sub>O<sub>2</sub> scavenging activity was  $964.4 \pm 13.16$  mg ml<sup>-1</sup>. The IC<sub>50</sub> values for ascorbic acid and BHA were  $21.4 \pm 1.1$  and  $52.0 \pm 2.3$  mg ml<sup>-1</sup>, respectively. Tested extract showed good activity in hemoglobin-induced

linoleic acid system. There was significant difference between extract and control ( $p < 0.05$ ) (Figure 2). In addition, extract showed good inhibition capacity on the hemoglobin-catalyzed peroxidation of linoleic acid.

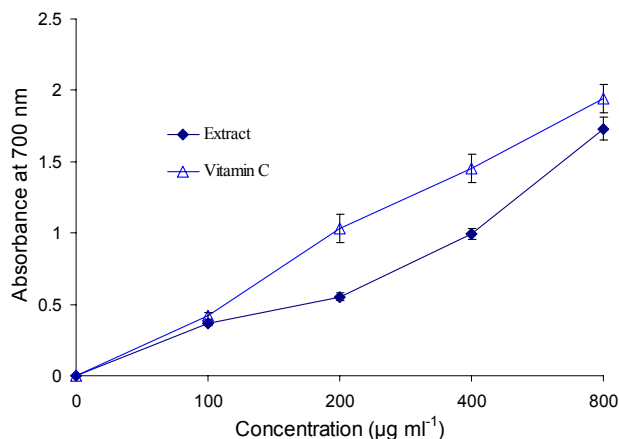


Fig. 1. Reducing power of *Consolidia orientalis*. Vitamin C was used as control.

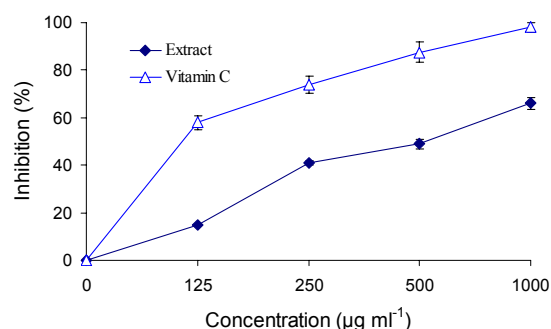


Fig. 2. Effect of *C.orientalis* extract on hemoglobincatalyzed peroxidation of linoleic acid. Vitamin C was used as control.

#### Assay for antibacterial activity

Crude extract of *C.orientalis* (500  $\mu\text{g}/\text{disc}$ ) showed moderate activity against all three Gram (-) bacteria and Gram (+) bacteria. The zone of inhibition against *Proteus mirabilis*, *Enterobacter cloacae*, *Klebsiella pneumonia* and *Staphylococcus aureus* was 13, 14, 10 and 12 mm diameter, respectively. On the other hand, standard antibiotic Valinomycin, Gentamicin and Chloramphenicol showed significant antibacterial activity against all tested Gram (+) and Gram (-) bacteria (Table 1).

### IV. DISCUSSION

Total phenol compound, as determined by the Folin Ciocalteu method, was reported as gallic acid equivalents and total flavonoid content was reported as the quercetin equivalent/g of extract powder by AlCl<sub>3</sub> colorimetric method. This plant showed high total phenol and flavonoid contents.

TABLE I ANTIBACTERIAL ACTIVITY OF *C. ORIENTALIS* METHANOL EXTRACT EXPRESSED AS MINIMUM INHIBITORY CONCENTRATIONS (MICs) IN G/ML

	<i>C.orientalis</i> methanol extract (500 µg/disc) (mm diameter)	Gentamicine (30 µg/disc) (mm diameter)	Valinomycine (30 µg/disc) (mm diameter)	Chloramphenicol (30 µg/disc) (mm diameter)
<i>Proteus mirabilis</i> PTCC(1076)	13	17	13	31
<i>Enterobacter cloacae</i> PTCC(1003)	14	26	20	25
<i>Klebsiella pneumonia</i> PTCC(1290)	10	20	12	22
<i>Staphylococcus aureus</i> PTCC(1112)	12	16	15	25

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [21]. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases [13]. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [14]. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [1]. It was found that the radical-scavenging activity of the extracts increased with increasing concentration. IC<sub>50</sub> for DPPH radical-scavenging activity was  $380 \pm 12 \text{ mg ml}^{-1}$ . The high total phenol and flavonoid contents of this plant may lead to its good DPPH-scavenging activity. Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important mechanism of phenolic antioxidant action [23]. In this assay, the presence of reductants (antioxidants) in the samples would result in the reducing of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by donating an electron. Amount of  $\text{Fe}^{2+}$  complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 1 shows the dose- response curves for the reducing powers of the extracts from *C.orientalis*. The extracts exhibited a fairly weak reducing power at 25 and 800  $\mu\text{g ml}^{-1}$  which was not comparable with Vit C ( $p < 0.001$ ). It was evident that *C.orientalis* did not show reductive potential and could not serve as electron donors for terminating the radical chain reaction.

The extracts also showed weak nitric oxide scavenging activity between 0.1 and 800  $\text{mg ml}^{-1}$ . The procedure is based on the principle that sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. The % inhibition increased with an increasing

concentration of the extract. IC<sub>50</sub> was  $1104 \pm 28.9$  for *C.orientalis* vs.  $17.01 \pm 0.03 \text{ mg ml}^{-1}$  for quercetin. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [17]. The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health. Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications in humans and thereby improves quality of life and overall survival of some diseases such as Thalassemia major [10]. In addition, brain iron dysregulation and its association with amyloid precursor protein plaque formation are implicated in Alzheimer's disease (AD) pathology and so iron chelation could be considered a rational therapeutic strategy for AD [20]. Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts in oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton chemistry [11]. These processes can be delayed by iron chelation and deactivation. The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease [7],[19]. Because  $\text{Fe}^{2+}$  also has been shown to cause the production of ox radicals and lipid per oxidation, minimizing  $\text{Fe}^{2+}$  concentration in Fenton reactions affords protection against oxidative damage. The chelating of ferrous ions by the extract was estimated by the method of Dinis et al., [5, 6, 7], [18]. Ferrozine can quantitatively form complexes with  $\text{Fe}^{2+}$ . In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. In this assay, both extract and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The absorbance of  $\text{Fe}^{2+}$  ferrozine complex decreased dose-dependently, i.e. the activity increased with the increasing concentration from 0.2 to 3.2  $\text{mg ml}^{-1}$ . Metal chelating capacity was significant since the extract reduced the concentration of the catalyzing transition metal in lipid peroxidation [4]. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [9]. *C.orientalis* extract showed good  $\text{Fe}^{2+}$  chelating ability. IC<sub>50</sub> was  $909.3 \pm 21.7 \text{ mg ml}^{-1}$ . Figure 2 shows the time-course plots for the antioxidative activity of the *F. assafoetida* extract using the FTC method. Membrane lipids are rich in unsaturated fatty acids which are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation [24]. The inhibition of lipid peroxidation by antioxidants may be due to their free radical scavenging

activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical [18]. Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. The tested extracts exhibited very low antioxidant activity. There were significant differences between extracts and controls ( $p \leq 0.01$ ).

The antimicrobial activities of various plants have been reported by many Researchers (Cowan, 1999; Dewanjee, 2008). As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide range of microorganism. In the present study a variety of gram positive and gram negative strains were selected for screening antimicrobial effects of ethanolic and methanolic extract of *C.orientalis*. The result of this study showed that the methanolic extract of *C.orientalis* exhibited varied range of antimicrobial activity against the tested organism including gram positive and gram negative bacteria, which is comparable to standard antibiotic effect. The *C.orientalis* extracts exhibited the greatest antimicrobial activities (as determined by the diameters of the inhibition zones towards the most susceptible bacteria like *Proteus mirabilis*, *Enterobacter cloacae*, *Klebsiella pneumonia* and *Staphylococcus aureus*. In some cases methanolic extract showed stronger effect on Gram(+) and Gram(-) bacteria.

#### V. CONCLUSION

In summary, pharmacological evaluation of *C.orientalis* extract reveals some interesting activities like antioxidant and antibacterial activities of this plant. Since, crude methanol extract of *C.orientalis* showed antioxidant and antibacterial effect, we assume that different active secondary metabolites are present in its extracts and perhaps some of these compounds may function in a synergistic manner. However, further studies are necessary to elucidate the mechanism lying with this effect. This report may serve as a *footstep* regarding the biological and pharmacological activities of *C.orientalis*.

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