ejpmr, 2017,4(2), 170-174

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIOXIDANT ACTIVITY OF *CARPESIUMROSEULATUM*ON THE ETHANOL EXTRACTION

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Article Received on 02/12/2016 **Article Revised on 22/12/2016** **Article Accepted on 12/01/2017**

ABSTRACT

The objectives of this study were to determine antioxidant activities and reducing power activity of ethanol extracts of *Carpesium roseulatum* (Composite) leaves, stems, and roots. The antioxidant activity of the *C. roseulatum* extracts was measured on the basis of the scavenging activity of the hydroxyl radical (-OH), 1, 1- diphenyl 2-picrylhyorazyl (DPPH), and ferric reducing antioxidant power (FRAP). These results show that *C. roseulatum* has some phytochemical constituents which may be active against the free radicals (OH and DPPH) and reducing power activity. OH scavenging activity of *C. roseulatum* was evaluated at 22.2-31.1 µg/ml at 0.1 mg/ml and the highest as found to be 67.4% at 8.0 mg/ml. DPPH scavenging activity of *C. roseulatum* was evaluated at 17.9-45.1 µg/ml at 0.1 mg/ml and the highest as found to be 71.1% at 8.0 mg/ml. Reducing power activity of *C. roseulatum* was evaluated at 26.8-38.1 µg/ml at 0.1 mg/ml and the highest as found to be 64.2% at 8.0 mg/ml.

KEYWORDS: Antioxidant activities, hydroxyl radical (OH), 1, 1- diphenyl 2-picrylhyorazyl (DPPH), ferric reducing antioxidant power (FRAP), *Carpesium roseulatum*.

INTRODUCTION

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals”. Oxidative stress occurs when reactive oxygen/nitrogen species overwhelm the antioxidant defense system. This is observed as a change in the organism’s redox status that favors a disproportionate increase in reactive species or a decrease in the antioxidant defense.[1] This may lead to damage, disease and severe disorders. Oxidative damage to DNA, proteins and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases; most notably heart disease and cancer.[2]

About 5% or more the inhaled oxygen is converted to reactive oxygen species such as O^{2-}, H_2O_2, OH by universal reduction of O_2.[3] In nature there are a wide variety of naturally occurring antioxidants which are different in their composition, physical and chemical properties, mechanisms and site of action.[4] Their compounds in food play an important role as a health protecting factor. This free radical is stable at room temperature and is reduced in the presence of an antioxidant molecule.

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs.[5] In both cases, there is a preference for antioxidants from natural rather than from synthetic sources.[6] There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants.

The hydroxyl radical ($OH^{\bullet}$) is the most potent yet short-lived of the reactive oxygen species (ROS) radicals. This radical acts positively in reproduction, germination, and growth,[8] whilst also playing a part in cell death.[9] The hydroxyl radical constitutes the chemically most reactive species of `activated oxygen' formed by successive monovalent reduction of dioxygen (O_2) in cell metabolism, and is primarily responsible for the cytotoxic effects of oxygen in plants, animals and micro-organisms, living in an oxygenic atmosphere.

DPPH is a common abbreviation for the organic chemical compound 1, 1- diphenyl 2-picrylhyorazyl or 2,2-diphenyl-1-picrylhydrazyl. The 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is a well-known radical and a trap (scavenger) for other radicals.[10] Because DPPH can be kept indefinitely with little decomposition and because it neither dimerizes nor reacts with oxygen, it proved to be quite useful in a variety of investigations,

such as polymerization inhibition or radical chemistry,[11] the determination of antioxidant properties of amines, phenols or natural compounds (vitamins, plant extracts, medicinal drugs) and for inhibiting homolytic reactions.[12]

The ferric reducing/antioxidant power (FRAP) assay is a developed, direct test of total antioxidant power.[13] The FRAP assay is robust, sensitive, simple, and speedy and facilitates experimental and clinical studies investigating the relationship among antioxidant status, dietary habits, and risk of disease.

Carpesium is a genus of flowering plants in the aster family, Compositae or Asteraceae. They are distributed in Europe and Asia andmost occur in China, Korea, and Japan. Several species are endemic to their country. *Carpesium roseulatum* Miquel (Compositae) is a small species native to southeastern Asia. The species is a low-growing perennial plant that only reaches a height of 10cm, but spreads to form clumps 30cm or more wide. The plant is gathered from the wild for local use as a food and medicine. This plant can be used in treating life threatening diseases like cancer.[14]
There were no report on antioxidant activity of OH, DPPH, and reducing power on *C. roseulatum* yet. The antioxidant potential was evaluated in relation to the scavenging of stable nitrogen-centered radicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical ($OH^{\bullet}$). The reducing power of antioxidants was also evaluated by ferric reducing antioxidant power (FRAP) assay as well as anti-bleaching of ß-carotene activity.

The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants for OH and DPPH and to examine whether the herbal medicine (*C. roseulatum*) having significant reducing power activity.

MATERIALS AND METHODS

Sample extract

Whole plant of *C. roseulatum* in full bloom was collected in the August and September at Mt. Giri forest area, the South Korea. The plants of *C. roseulatum* divided into three parts: leaves, stems, and roots. Each sample (100 g) of *C. roseulatum* was ground with pestles and liquid nitrogen at –70℃ and homogenized prior to beginning extraction experiments. The samples were blended with 50% ethanol, and then an aliquot of the mixture (100 µL, 200 mg sample / ml 50% ethanol) was further mixed with 100 mM Tris-HCl buffer (400 µL, pH 7.4).The mixture was further stirred with a magnetic bar at 65°C for 2 hours. The sample was treated with ultrasound at room temperature for a given duration. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature and left in the dark at room temperature for 20 min. Extracted sample was filtered. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

Hydroxyl radical assay

The scavenging activity for hydroxyl radicals was measured with fenton reaction. Reaction mixture contained 60 µL of 1.0 mM $FeCl_2$, 90 µL of 1mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2, and 1.0 mL of extract at various concentrations (0.1, 1.0, 2.0, 4.0, and 8.0 mg/ml). Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with UV visible spectrometer (Shimadzu, UV-1800, Japan).

DPPH free radical

The antioxidant activity of the *C. roseulatum* extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams et al.[15] with slight modifications. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol.[16] DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time. 1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of the previous plant extracts of various concentrations (0.1, 1.0, 2.0, 4.0, and 8.0 mg/ml). DPPH was added to the solutions prepared with plant extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 µL of ethanol DPPH solution (final concentration 300 µM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using theUVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 517 nm. Absorbance changes are measured at 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (1.0 µg/ml) was used as reference standard (positive control). The inhibition % was calculated using the following formula.

Ferric reducing antioxidant power (FRAP) Assay
FRAP assay was determined according to the method described by Nenadis et al.[17] This assay is based on the reducing power of antioxidants in which a potential antioxidant reduces the oxidized ferric ions to produce ferrous ions, which form a blue colored complex with tripyridyl triazine. The FRAP reagent was freshly prepared by mixing 2,4,6-tripyridyl triazine (10 mM, 1 mL) and ferric chloride (20 mM, 1mL) in 0.25 M acetate buffer (10 mL, pH 3.6). Plant extract sample (50 µL) was added to the FRAP reagent (3 mL) and the absorbance was measured at 593 nm after 8 min incubation at room

temperature. In this assay the yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each compound. A calibration curve of ascorbic acid was established, the antioxidant capacity of the plant extracts was then expressed as mM ascorbic acid equivalent/g dry extract. All measurements were carried out in triplicates.

Statistical analysis

All the analysis were carried out in triplicate and the results were expressed as the mean ±SD. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0). The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

Inhibition % = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

IC_{50} is defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction of a maximum scavenging capacity. To determine the IC_{50} value of the active component, the technique using 96-well microplates was employed.[18] Regression analysis by a dose response curve was plotted to determine the IC_{50}values.

RESULTS

Table 1 was shown the antioxidant activities for OH radical of the *C. roseulatum.* Various concentrations of leaf extracts were higher than those of stem and root extracts. The rates of antioxidant activities of the ethanol extracts for *C. roseulatum* were dependent on concentrations. OH scavenging activity of leaves of *C. roseulatum* was evaluated at 0.1 mg/ml was 41.6%, that of stems was 29.5% at same concentration, and root was 27.3%. OH scavenging activity of leaves of *C. roseulatum* was evaluated at 8.0 mg/ml was 67.4%, that of stems was 53.5% at same concentration, and root was 50.8%. The all values of OH scavenging activity of leaves were higher than those of stems and roots. However, the all groups for stems and roots did not show a statistically significant difference ($p < 0.05$).

DPPH scavenging activity of leaves of *C. roseulatum* was evaluated at 0.1 mg/ml was 45.1% and that of stems was 33.6% at same concentration, and that of root was 32.7% (Table 2). It is also observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in the assay mixture. DPPH scavenging activity of leaves of *C. roseulatum* was evaluated at 8.0 mg/ml was 71.1% and that of stems was 60.3% at same concentration, and that of root was 57.2%. The high antioxidant activity for DPPH found on leaf extracts. The all values of DPPH scavenging activity of leaves were higher than those of stems and roots. However, the all groups for stems and roots did not show a statistically significant difference ($p < 0.05$).

The results of the Ferric Reducing Power Assay test of the alcoholic and aqueous extracts of *C. roseulatum* in comparison with the standard (ascorbic acid) at 700 nm were shown in Table 3. The rates of reducing power activity of the ethanol extracts were also dependent on concentrations. The reducing power activity of leaves of *C. roseulatum* was evaluated at 0.1 mg/ml was 34.5% and that of stems was 24.7% at same concentration, and root was 19.1%. Reducing power activity of leaves of *C. roseulatum* was evaluated at 8.0 mg/ml was 64.2% and that of stems was 49.4% at same concentration, and root was 42.5%.

Table 1: The hydroxyl radical (OH) by *Carpesium roseulatum* at different concentrations

Concentration (mg/ml)	Leaf	Stem	Root
0.1	41.60±0.67	29.45±1.48	27.28±1.21
0.5	46.85±1.45	33.65±1.29	30.81±2.12
1.0	54.80±2.83	38.77±2.45	36.98±1.61
2.0	57.60±2.26	43.81±1.49	42.14±0.22
4.0	60.24±2.71	50.49±2.14	48.47±1.40
8.0	63.37±2.51	53.48±2.06	50.82±1.26
t-test			

Data represent the mean ± SD from three replicates.

Table 2: The free radical scavenging (DPPH) effects by *Carpesium roseulatum* at different concentrations

Concentration (mg/ml)	Leaf	Stem	Root
0.1	45.08±2.90	33.57±0.83	32.67±1.43
0.5	49.13±2.42	36.02±1.32	36.23±1.04
1.0	56.33±1.14	41.81±0.37	41.31±1.27
2.0	60.19±1.06	48.52±1.19	46.09±0.39
4.0	65.14±0.78	54.19±1.38	53.15±0.33
8.0	71.14±0.90	60.33±0.67	57.20±0.75
t-test			

Data represent the mean ± SD from three replicates.

Table 3: The reducing power effects by *Carpesium roseulatum* at different concentrations

Concentration (mg/ml)	Leaf	Stem	Root
0.1	34.58±1.59	24.69±0.56	19.14±0.78
0.5	39.24±0.96	27.95±1.24	23.42±1.71
1.0	44.86±1.42	34.20±1.56	28.17±2.26
2.0	51.87±3.89	41.13±1.26	35.11±1.85
4.0	56.29±2.30	46.44±1.53	39.42±0.83
8.0	64.21±1.74	49.35±1.88	42.45±1.41
t-test			

Data represent the mean ± SD from three replicates.

Figure 1 was shown the comparative data of OH, DPPH radical scavenging activity and reducing power as determined by the IC_{50} values of the different vegetable tissues. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} value is inversely related to the antioxidant activity of crude extracts. The OH activity of leaves (IC_{50} = 22.15 µg/ml) was at the same levels as that of L-ascorbic acid and IC_{50} of stem was 31.06 µg/ml, and that of root was 22.55 µg/ml. The DPPH activity of leaves (IC_{50} = 17.89 µg/ml) was at the same levels as that of L-ascorbic acid and IC_{50} of stem was 45.12 µg/ml, and that of root was 31.75 μg/ml. The reducing power activity of leaves (IC_{50} = 26.77 µg/ml) was at the same levels as that of L-ascorbic acid and $IC_{50}$of stem was 38.30 µg/ml, and that of root was 30.19 µg/ml.

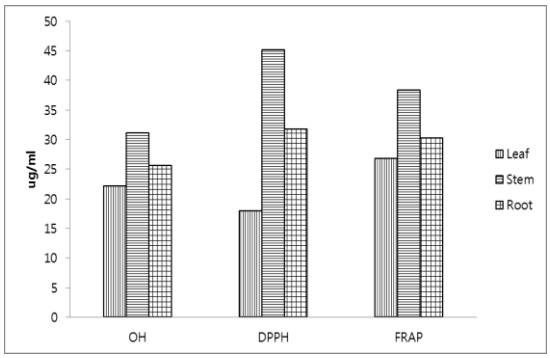

Figure 1: Inhibitory effects {IC_{50} (mg/ml)} on OH, DPPH, and FRAP by *Carpesium roseulatum* on 1.0 M.

DISCUSSION

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage *in vivo*.[19] In this study, hydroxyl radical scavenging activity of *C. roseulatum* was 67.4% (Table 1). In *Hydrocotyle sibthorpioides*, the hydroxyl radical scavenging activity observed was 78.6% at the concentration of 8.0 mg/ml.[20] In *Bauhinia vahlii*, the hydroxyl radical scavenging activity observed was in the range of 29.3–84.4% at the concentration of 80 µg/ml.[21] At 500 µg/ml of *Leucas linifolia*, hydroxyl radical scavenging showed 78% inhibition.[22]

The DPPH radical had been used widely in the model systems to investigate the scavenging activities of several natural compounds such as phenolic compounds, anthocyanins, or crude extracts of plants. In this study, DPPH values of *C. roseulatum* were slightly high (71.1%) (Table 2). At 500 µg/ml of *Leucas linifolia*, DPPH scavenging showed 63% inhibition with an IC50 value of 175 µg/ml).[22] At 8.0 mg/ml of *Hydrocotyle sibthorpioides*, DPPH showed 86.0% inhibition.[20]

The extract of *C. roseulatum* was 64.2% inhibitory effects on the activation of FRAP (Table 1). *Glycyrrhiza glabra* was 191.91% and 294.69 % in case of alcoholic and aqueous extracts respectively at 800 µg/ml concentration.[23]

Higher level of antioxidant activity is observed in the ethanol extract of *C. roseulatum* leaves when compared with other cited species. Thus, these extracts can be considered as new sources of natural antioxidants.

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