Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
1(1), 2012, 12-18

ISSN 2321 – 0958

Short Communication

IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF LORANTHUS ELASTICUS DESV.

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Abstract
Enhancing the shelf life of any product with natural extract from natural source is most desirable. These extracts with known and unknown bioactive compounds can also be exploited to inhibit the oxidation in human system. In the present study, different solvent extracts of *Loranthus elasticus* Desv have been assessed for their antioxidant potentials by five methods selected which represents lipid peroxidation occurring in both foods as well as in biological system. The eextract showed good antioxidant activity except Iron chelating activity; both ethanol and methanol extracts have shown impressing level of the free radical scavenging activity, which were comparable to synthetic antioxidant such as BHA, TBHQ etc. Utilization of the extract may result in formulating better health care products with enhanced shelf life and little or no side effects.

Key words: Antioxidants, *Loranthus elasticus*, DPPH, Metal chelating, Nitric oxide scavenging and 2-deoxy-D-ribose degradation.

Introduction

Oxygen free radicals / reactive oxygen species (ROS) have been a source of the threat in both food systems decreasing the self stability as well as in biological systems in causing chronic diseases. Majority of the present day diseases are reported to be due to the free radicals which are generated continuously during normal metabolic processes, more pronounced in pathological conditions (Mahantesh *et al.*, 2012), which could lead to a shift in the balance between pro-oxidant and antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate due to the increased generation of the free radicals caused by excessive oxidative stress or due to decreased scavenging/quenching in the body caused by depletion of the biological antioxidants (Aruoma 1996; Chandra Mohan

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Received on: December 2012
Accepted after revision: December 2012
Downloaded from: www.johronline.com

et al., 2004). In such a situation supplementing antioxidants through dietary means could prove to be beneficial. Besides medicinal plants, fruits, vegetables and spices; which are a part of our diet also contribute significantly to the dietary antioxidants apart from dietary fibre, trace elements, vitamins which could be exploited for preventing / inhibiting many diseases. On the other hand, the use of synthetic antioxidants namely BHA, BHT, TBHQ, PG etc for food preservation has been restricted since some of them have been found to be toxic. Hence the uses of natural antioxidant from plant sources are being recommended4. There is a practical need for the screening and selection of efficient and stable natural antioxidant as effective alternative in the prevention of food deterioration. It is also shown that natural antioxidants prevent free radical induced diseases and rancidity of food. The present study was undertaken to investigate the antioxidant potentials. In the present study, we have investigated the free radical scavenging activity (inhibition of lipid peroxidation, 1,1 diphenyl 1,2 picrylhydrazyl(DPPH) scavenging, nitric oxide radical scavenging ,, superoxide radical scavenging and inhibiton of hydroxyl radical mediated degradation of deoxyribose (non site specific)). Further more total polyphenols and flavanoids contents were evalvated.

Materials and Methods:

Materials:

Plant materials obtained from Dakshina kannada region of Karnataka. All the chemicals used were procured from Merck, Sigma Aldrich and himedia chemical companies.

Preparation of plant materials:

L.elasticus leaves are separated and shade dried. Shad dried leaves are then pounded. This pounded leaves are used for extraction. Later leaves are taken in the reagent bottles with solvent and agitated or shaked using mechanical shaker for 2 hrs, after that the solvent was added. The process is repeated 5 to 6 times until the solvent colour is retained back. The solvents were removed using the flash evaporator. The crude viscous liquid is obtained. Crude extract obatiened is stored in refrigerator and used as per the need.

Methodology:

DPPH radical scavenging activity:

This method described by Barca .et. al., was used in the assay. DPPH (500µL, 0.5mM in methanol) solution was mixed with different aliquots of sample and volume was made to 3mL using methanol. The mixture was incubated in dark for 45min at room temperature. Absorbance was recorded at 517nm in a UV spectrophotometer. BHA (butyrated hydroxyl anisole) was used as a standard antioxidant compound. A positive control was prepared by mixing 3mL methanol and 0.5mL DPPH solution. Sample blank were prepared with methanol without DPPH solution to eliminate the absorbance of crude extracts. Methanol was used as the blank.

The DPPH radical scavenging activity percentage was calculated by using the formula given below:-

DPPH radical scavenging activity (%) = $[(A_C-A_S)/A_C]$ x100

Where A_C is the absorbance of the positive control solution and A_S is the absorbance of the sample solution.

For this sample solution the IC_{50} value is calculated. IC_{50} value the concentration of the sample required to scavenge 50% of the DPPH free radical in the mixture was calculated using a linear regression equation derived from the graph.

Total Polyphenols:

Total polyphenol content of the extract was determined using Folin - Ciocalteu reagent (FC reagent) by the method of Singleton and Rossi, 1965. Briefly, sample in the different concentration was mixed with distilled water to make up final volume (3mL). Then 0.5mL FC reagent was mixed and incubated for 10min at room temperature. After

incubation 2mL of 7% sodium carbonate was added and boiled the content in the water bath for 1minute. After cooling, absorbance was measured at 750nm. Gallic acid was used as a standard compound and amount of the total polyphenols content was calculated using the standard graph.

Nitric oxide scavenging assay

Nitric oxide radical inhibition was estimated by the use of Griess Illosvory reaction. In this investigation, Griess Illosvory reagent was generally modified by using Napthyl ethylene diamine dihydrochloride (0.1%w/v) instead of the use of 1-napthylamine (5%)(Sreejayan and Rao 2007). The reaction mixture (3ml) containing 2ml of 10 mM sodium nitroprusside, 0.5ml saline phosphate buffer and 0.5ml of standard solution or aqueous and ethanolic extract of (500 -1000µg/ml) were incubated at 25°C for 150min. After incubation, 0.5ml of the reaction mixture was mixed with 1ml Sulfanilic acid reagent (0.33% in 20%glacial acetic acid) and allowed to stand for 5min for the completion of the reaction of diazotization. After that further 1ml of the Napthyl ethylene diamine dihydrochloride was added, mixed and was allowed to stand for 30min at 25°C. The concentration of nitrite was assayed at 546nm and was calculated with the Control absorbance of the standard nitrite solution (without extracts or standards, but the same condition should be followed).Here the blank is taken as the buffer and make up solvents and the Ascorbic acid and Quercetin (10 -50 µg/ml) was taken as standard. Results are provided in (.The percentage inhibition was calculated using the formula:

% Scavenging Activity = A control - A test or A Std/ A control * 100

Non-site specific Hydroxyl radical mediate 2-deoxy-D-ribose degradation.

The assay was performed as reported by Halliwell etal, with modifications. The reaction mixture contained 100µl of 28mM 2-deoxy-D-ribose dissolved in phosphate buffer (P^H = 7.4). 500µl solution of various concentration of the 70% Methanolic extract , 200µl of 200µM $FeCl_3$ and 1.04mM EDTA (1:1V/V), 100µl of H_2O_2 (1.0mM) and 100µl Ascorbic acid (1.0mM). Aften an incubation period of 60min at 37 c the extent of deoxy ribose degradation was measured by the TBA reaction. 1ml of

TBA (1% in 50mM NaOH) and 1ml of TCA were added to the reaction mixture, tubes were heated at 100ºc for 20min. after cooling the absorbance was read at 532nm against a blank (containing only buffer and deoxy ribose).

% Inhibition = $[(A_0-A_1)/A_0]\times1000$

Where, A_0 is the absorbance of control and A_1 is the absorbance of the sample.

All the values expressed are the mean values carried out in triplicates. BHA was used as a positive control.

Total Flavonoids:

Total flavonoids assay is to find out the amount of flavonoid present in the sample. In this assay quercetin (0.1 mg/ml) is taken as the standard compound. Different aliquots of standard were taken in different test tube, and $AlCl_3$ (1 g/10ml) 100µl, $KOOCCH_3$ (0.98 g/10ml) 100µl is added, then the volume of the solution is made up to 3 ml with double distilled water. The test tube are then incubated for 30 minutes. After 30 minutes of incubation absorbance is taken at 416 nm. Similarly it is repeated for the sample. Methanol is taken as the blank. The presence of total flavonoids is calculated from the standard graph

Superoxide anion scavenging activity:

Measurement of superoxide scavenging activity of the loranthus elasticus extracts was based on the methof reported by Liu et al. With modifications. Hte superoxide is generated in 3 ml of tris HCl (16 mM pH 8.0) containg 1 ml of NBT (50 µM), 1 ml of NADH (78µM) solution and sample solution of different concentration. The reaction started by adding 1 ml of PMS solution (10µM) to the mixture. The reaction mixture was incubated at 25° C for 5 min and the

absorbance at 560nm was measured against the blank sample. L- ascorbic acid was used as a control. BHA was used as the positive control.

% inhibition = [(A control- A sample) / A control) * 100)] .

Inhibition of Lipid Peroxidation in chicken Liver Homogenate

The inhibition of lipid peroxidation of rat liver homogenate was assayed according to the method described by Ng et al. (*26*) with some modifications. Liver tissues obtained from normal Wister rats were homogenized with a Polytron homogenzier in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 10% (w/v) homogenate. The homogenate was centrifuged at 3000*g* for 10 min, and an aliquot (0.5 mL) of supernatant was mixed with the extracts of various concentrations, followed by the addition of 0.1 mL of 10 *í*M FeSO4 and 0.1 mL ,0.1 mM L-ascorbic acid. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by adding 0.5 mL of trichloroacetic acid followed by 0.38 mL of thiobarbituric acid and heating at 100 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the absorbance of the supernatant containing the thiobarbituric acid-reactive substances (TBARS) was measured at 532 nm in a spectrophotometer. BHA was used as control. The percent inhibition was calculated using the following formula:

% inhibition = [(A - A 1) / A control) * 100)]

A is the absorbance of the control, and *A*1 is the absorbance of the test Sample

Result and Discussion:

Total polyphenol content was assessed by Folin - Ciocalteu method. The assay shows the presence of good amount of polyphenol. Gallic acid curve is used as the standard curve. The flavanoids content was assessed through aluminium chloride method. The values of the L.elasticus were range between 10 to 100 µg.

In addition the damaging action of free radicals in the strongest among free radicals. amount of flavanoids present is calculated using Quercetin standard curve, ethanolic and methanolic extracts shows the presence of good amount of polyphenols and flavanoids content per gram extract (Table 1 and 2). The free radical scavenging activity of L.elasticus was assessed by DPPH assay (Table 3). All the extract of different solvent shown significant inhibitory activity against the DPPH radical. Radical inhibition was observed varies with different extract used. DPPH is the stable free radical used to evaluate the antioxidant activity of plant andmicrobial extracts. In the current study DPPH radical scavenging activity was found in all the extracts of L elasticus, which is compared to activity of TBHQ and BHT. The radical scavenging activity varies with test sample and similar to those of the reference sample and their IC 50 (the concentration required to inhibit radical formation by 50%) ranged from 10 to 100 µg. This indicates that the L elasticus extract are good source of natural antioxidant. In addition, the ability to scavenge the DPPH radical is related to lipid peroxidation of liver homogenate of chicken. All the extract shows the inhibition. But the ethanolic and methanolic extract has shown good inhibition activity compared o other extracts. Thus indicateds that there is higher inhibition activity of lipid peroxidation in L.elasticus.

Superoxide and hydroxyl radical are two most representative free radicals. In cellular oxidation reactions, superoxide are normally formed first and their effects can be magnified because they produce other kinds of cell damaging free radicals and oxidizing agents. Reaction between PMS and NADH produces superoxide radicals, which reduces NBT to yield formazan derivative. The L.elasticus extract done dependently inhibited the NBT reduction. The IC 50 In biochemical system superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can substantially generate extremely reactive hydroxyl

radicals in presence of some transition metals such as iron and copper. They also have ability to attack DNA and cause stand scission. Activity was found to be good with respect to inhibition of hydroxyl radical (Table 4 and 5).

The result from deoxyribose degradation might reveal some of the antioxidant mechanism of the extract in non site specific assay. EDTA forms complex with iron (Fe^{+++}) and hydroxyl radicals are generated solution. Thus inhibition of the degradation of deoxyribose; both methanol and ethanolic extract shown good inhibitory activity than other extracts, when compared with standard (Table 6).

Inhibition of NO radicals is another important activity because it also a important source for inflammation. Both methanol and ethanol have shown good inhibition of NO radical. Which mean it may even act as an anti-inflammatory drug (Table 7).

Aknowledgement:

Author acknowledges to UGC for funding and St.Philomena's college Mysore for support

Table 1: Polyphenols content of *L elasticus*

Different extracts	mg/g extract
Ethanolic	315
Methanolic	221
Hexane	53
Ether	89
Ethyle acetate	125

Table 2: Flavonoids content of *L elasticus*

Different extracts	mg/g extrat
Ethanolic	145
Methanolic	113
Hexane	23
Ether	36
Ethyle acetate	54

Table 3: DPPH scavenging activity of *L elasticus*

Different extracts	IC_{50} (µg)
Ethanolic	39
Methanolic	40
Hexane	50
Ethyl acetate	40
Ether	56
TBHQ	30
BHT	38

Table 4: Lipid peroxidation inhibition activity of *L elasticus*

Different extracts	IC_{50} (µg)
Ethanolic	25
Methanolic	23

Hexane	ND
Ethyl acetate	ND
Qucertine	ND
Vitamin C	ND

Table 5: Superoxide scavenging activity of *L elasticus*

Different extracts	IC_{50} (µg)
Ethanolic	42
Methanolic	35
Hexane	ND
Ethyl acetate	ND
Qucertine	30
Vitamin C	ND

Table 6: Hydroxyl scavenging activity of *L elasticus*

Different extracts	IC_{50} (µg)
Ethanolic	12
Methanolic	10
Hexane	ND
Ethyl acetate	ND
Qucertine	10
Vitamin C	ND

Table 7: NO scavenging activity of *L elasticus*

Different extracts	IC_{50} (µg)
Ethanolic	100
Methanolic	120
Hexane	ND
Ethyl acetate	ND
Qucertine	20
Vitamin C	35

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