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INHIBITION OF LOW DENSITY LIPOPROTEIN OXIDATION AND PROTECTION OF DNA CLEAVAGE BY PHENOLIC EXTRACT FRACTION OF *OCIMUM BASILICUM*

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ABSTRACT

The medicinal plant of *Ocimumbasilicum* leave have the valuable plant constituents for the scavenging of free radicals because of their phenolic hydroxyl groups. That the amount of polyphenolic compounds increases, the antioxidant activity also increases. The present study demonstrates that the polyphenolic extract of *Ocimumbasilicum* leave scan protect the body from oxidative stress from ROS, which may be due to the phyto-chemicals in the form of polyphenols that occur in the plant. However, additional studies are necessary to develop a method for the fractionation and identification of polyphenols and to determine the most active antioxidant compounds in the polyphenolic extract free radical scavenging assay were carried out.

KEYWORDS: Antihemolytic, methanolic extract, hLDL, H_2O_2 and NA**.**

INTRODUCTION

Extensive ethno botanical research has focused on the discovery of valuable drugs during the past few decades (Buenz *et al*., 2004). Diverse medicinal plants have been screened and assessed for their ability to agonize free radical-induced oxidative stress (Zhang, 2004). Traditional medicinal plants are often economically favorable, locally available and easily consumable substitutes for commercial medicines. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen

donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin (Gebicka and Banasiak, 2009).

Scope of Antioxidants and DNA protector of Polyphenols

The natural antioxidants from plant sources have attracted much interest due to their safety. Many studies have indicated that there was a high correlation between the antioxidant activity of some plants and their phenolic contents (Velioglu *et al*., 1998). Molecules containing unpaired electrons are known as free radicals that cause tissue collapse by means of DNA, protein, and lipid damage (Guha *et al*., 2009). Free radicals, such as superoxide anion, hydroxyl radicals, and hydrogen peroxide, are known as reactive oxygen species (ROS) (Rajkumar *et al*., 2010).

Oxidation of low density lipoproteins medicinal plants

Oxidation of low density lipoproteins (LDL) has been considered as playing an important role in the initiation and progression of early stage of atherosclerosis and the development of cardiovascular diseases (Heinecke, 1998).

The identification of endothelium-derived relaxing factor as nitric oxide has led to the investigation of the role of this free radical in LDL oxidation. It has been reported that the simultaneous generation of nitric oxide and superoxide causes oxidative modification of LDL, whereas, the release of either free radical alone does not (Jessup *et al*., 1992). Macrophages, stimulated to induce nitric oxide synthase and thus generate nitric oxide, are less able to oxidize LDL than unstimulated cells (Yates *et al*., 1992; Jessup and Dean, 1993).

Hassan *et al.,* (2011) have been investigated the inhibitory effects of *Alliumascalonicom* hydro alcoholic extract (AAE) on LDL oxidation induced by $CuSO_4$ quantitatively *in vitro*. Oxidation of LDL was incubated with CuSO4 and the formation of conjugated dienes and thiobarbituric acid reactive substances (TBARS) was assessed.

MATERIALS AND METHODS

Sterilization of Glassware

Glassware were soaked overnight in cleaning solution and washed thoroughly with running tap water. The glassware and media were sterilized in an autoclave at 15psi for 20 minutes, at120^{o}C.

Preparation of extracts

Organic solvents (methanol) extract of the *Ocimum basilicum* leaf were prepared. The extract obtained was vaccum-dried and used for further test.

Total phenolic content of *Ocimum basilicum* leaf methanol extract

The total phenolic content of *Ocimum basilicum* leaf methanol extracts was determined. The DSM extract (1 mL, 1mg/mL) was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2%Na2CO3, and centrifuged at 13400 X g for 5 min. The absorbance of upper phase was measured using a spectrophotometer (Model UV-1601; Shimadzu, Tokyo, Japan) at 750 nm after 30 min incubation at room temperature. TPC was expressed as a tannic acid equivalent.

Antihemolytic activity of *acetone extract from the leaves of Ocimum basilicum*

Antihemolytic activity of the flavonoid fraction was assessed as described by with a slight modification. Erythrocytes from male Wistar rat blood were separated by centrifugation and washed with phosphate buffer (pH 7.4), and diluted with phosphate buffered saline to give a 4% suspension. 1 g of extract/ml of saline buffer was added to 2 ml of the erythrocyte suspension and the volume made up to 5 ml with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 ml of H_2O_2 solution in saline buffer added to induce oxidative degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% hemolysis of the blood cells after 240 min. After incubation the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis determined by measuring the absorbance, corresponding to hemoglobin liberation, at 540 nm.

Inhibition activity of human LDL oxidation by *Ocimum basilicum* phenolic extract

Measurement of hLDL oxidation was carried out by using the method described by Hu and Kitts (2000) with slight modifications. To remove ethylene diamine tetra acetic acid (EDTA), hLDL (in PBS, pH 7.4 with 0.01% EDTA) was dialyzed against 10m*M* phosphate buffered saline (PBS) (pH 7.4, 0.15*M*NaCl) at 4°C for 24 h. The EDTA-free HLDL (0.1 mg/mL) was mixed with 10, 25, 50, and 100 ppm levels of extracts (final concentrations), as catechin equivalents, and catechin dissolved in PBS; oxidation was initiated by adding 10m*M* $CuSO_4$ at 37°C for 20h.The mixtures were tested for their content of conjugated dienes (CD)at 234 nm. The percentage inhibition of formation of conjugated dienes was calculated on the basis

of the observed differences in the absorbance values of the oxidized and UN oxidized LDL using appropriate blanks.

Nitric Oxide Radical Scavenging Activity of Poly phenolic Extract

Nitric radical scavenging capacity of polyphenolic extract was measured according to the method described by Olabinri *et al* 2010. 0.1ml of sodium nitroprusside (10mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of ethanolic extract and incubated at room temperature for 150 min. After incubation period, 0.2 mL of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N- (1- Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546nm against blank. All readings were taken in triplicate and Curcumin was used as the standard. The % inhibition was calculated by following equation.

% Nitric oxide radical scavenging capacity= $[(A_0- A_1)/A_0] \times 100$

Where A0 was the absorbance of control and A1 was the absorbance of different solvent extract.

DNA Preparation from Blood

Reagents

Ammonium chloride (NH_4Cl), Chloroform, Ethanol absolute, HCl, Isoamylalcohol, Isopropanol (2-Propanol), Phenol, Potassium carbonate ($KHCO_3$),Proteinase K, Sodium acetate, Sodium chloride, Sodium EDTA (Na_2EDTA),Sodium dodecyl sulfate (SDS 10%), Tris EDTA buffer, pH 8.0.

Procedure

To 10 ml whole blood (EDTA, heparin, citrate) add 30 ml lysis buffer, shakegently, incubate for 30 min on ice, and centrifuge at 1200 rpm for 10min at 4^o C. Remove supernatant (blood waste), add 10 ml lysis buffer, resuspend the pellet, and centrifuge for 10 min at 4°C (1200 rpm). Remove supernatant (It is possible to store the pellet at -80°C. To do so, add 1 ml SE-buffer and resuspend the pellet. Use a cryo-tube and centrifuge at 1200 rpm for 10 min at 4°C.Remove the supernatant and freeze the pellet.) Add 5ml SE-buffer and resuspend the pellet, add 40µl proteinase K (10 mg/ml) and 250µl 20% SDS, shake gently, and incubate overnight at 37°C in a water bath. Add 5 ml SE-buffer and 10 ml phenol, shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C.

Transfer the supernatant into a new tube, add 10 ml phenol/chloroform/isoamylalcohol (25:24:1), shake by hand for 10 min, and centrifuge at 3000 rpm for 5 min at 10°C. Again transfer the supernatant into a new tube, add 10 ml chloroform/isoamylalcohol (24:1), shake by hand for 10 min, and centrifuge at3000 rpm for 5 min at 10°C. Transfer the supernatant into a new tube, add 300µl 3 M sodium acetate (pH 5.2)and 10 ml isopropanol, shake gently until the DNA precipitated, use a glass pipette, make a hook over a bunsen burner, and capture the DNA. Wash the DNA in 70% ethanol and dissolve the DNA in 0.5-1 ml TE-buffer overnight at 4°C on a rotating shaker. (If the DNA is not dissolved leave it longerat 4°C on the rotating shaker). Measure the DNA concentration in a spectrophotometer (Pharmacia,GeneQuant) and run 200 ng on a 1% agarose gel.

***Invitro* DNA cleavage protector activity of *Ocimum basilicum* phenolic extract**

The experiments were performed in a volume of 20 ml containing 33mmol/Lin bp (7.56 nmol/L) of pBR322 plasmid DNA in 5mmol/L phosphate buffer contained 10 mmol/L NaCl, pH 7.4, in the presence of different concentrations(200-400 mmol/L) of catechin, naringin, and rutin. Immediately prior to irradiating the samples with UV light, H_2O_2 was added to mM concentration of 2.5mmol/L. The reaction volumes were held in caps of polyethylene micro-centrifuge tubes, which were placed directly on the surface of a trans illuminator (8000 mW/cm) at300 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 4.5 ml of a mixture containing 0.25% bromophenol blue, 0.25% xylenecyanol FF, and 30% glycerol was added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris borate buffer (45mmol/L Tris-borate, 1mmol/L reactive substances (TBARS) were determined as described by Stocks (Stocks and Dormandy, 1971). EDTA).Untreated pBR322 plasmid was included as a control in each run of gel electrophoresis, which was carried out at 1.5 V/cm for 15 h. Gel was stained in ethidium bromide (1 mg/ml; 30 min) and photographed on Polaroid-Type 667positive land control.

RESULTS AND DISCUSSION

Total phenolic content of leaf extract of *Ocimumbasilicum*

In this context, the preliminary experiments revealed that 80% methanol was the best solvent for the extraction of phenolics from *Ocimum basilicum* at 60 °C for 60 min since it afforded a maximum yield of phenolics. The yields leaf of *Ocimum basilicum* extracts ranged from 29 % (w/w). Therefore, the total phenolic contents were reported as catechin equivalents (Table-

1). The polyphenol antioxidant activity is due to the chemical structure and ability to donate/accept electrons, thereby delocalizing the unpaired electron within the aromatic structure (Ross and Kasum, 2009).

Table 1: Yield and phenolic content leaf of *Ocimumbasilicum*.

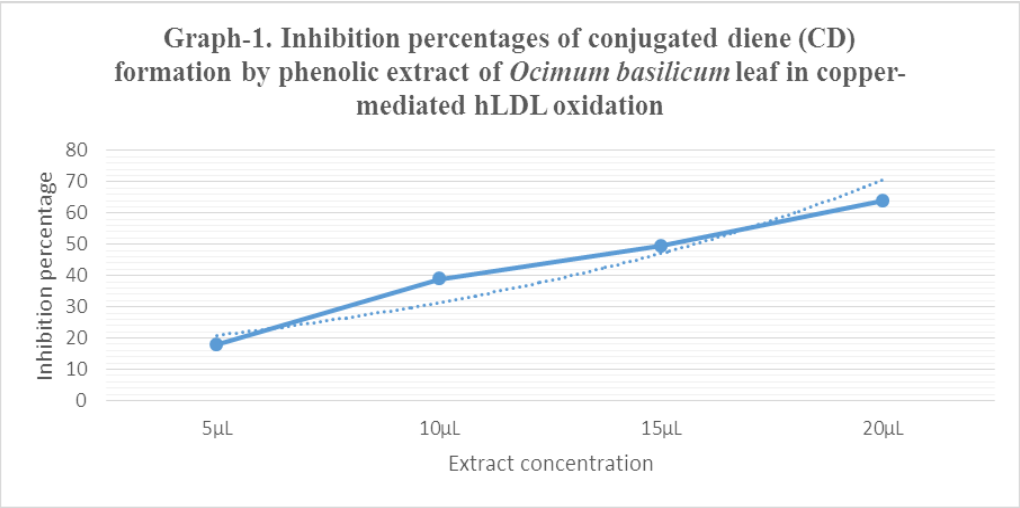
Sample	Yield of extract (g/100 g of defatted CONTENT)	Total phenolic content (mg catechin equivalents per gram methanol extract)
leaf phenolic exctract of *Ocimumbasilicum*	29.1±1.5[a]	96.2±1.3[b]

[a] Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.

[b] Means in each column sharing the same letter are not significantly (P = 0.05) different from other.

Effect on hLDL oxidation of phenolic fraction from the leaf of *Ocimumbasilicum*

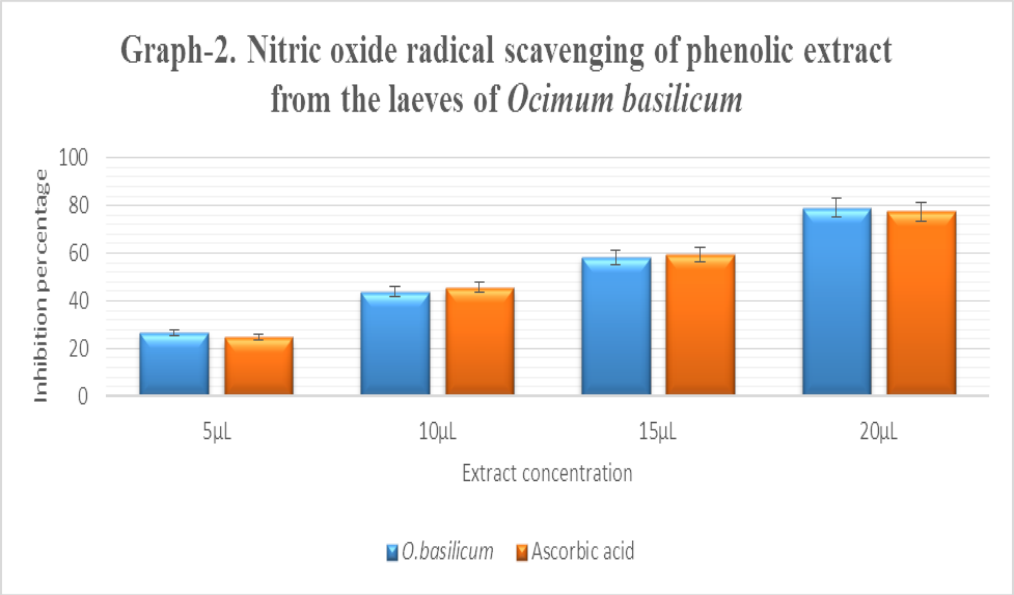
In this study, EDTA-free hLDL was used to initiate the oxidation process in the presence of different concentrations of phenolic extract of *Ocimum basilicum* (5 to 20µl/ml). Phenolic extract of *Ocimum basilicum* showed almost 100% inhibition of oxidation at 40 µl/ml concentration ("Fig-1"). Phenolic extract of *Ocimum basilicum* showed a significantly (P < 0.05) higher activity than catechine against LDL oxidation, at both 40 µl/ml levels. This behavior may be attributed to the synergistic effect of mixtures of anti-oxidative compounds present in bean extracts. In present study was indicated that *Caralluma attenuate* phenolic extract containing more phenolic hydroxyl groups showed relative higher anti-Ox-LDL activities ("fig. 1"). The phenolic rich fraction 20 µl/ml showed the highest anti-Cu2+-induced-Ox-LDL activity with the inhibitory percentage of 70.56±7.03%.



"Fig. 1"Effect on hLDL oxidation of phenolic fraction from the leaf of *Ocimumbasilicum*

Nitric oxide radical scavenging assay

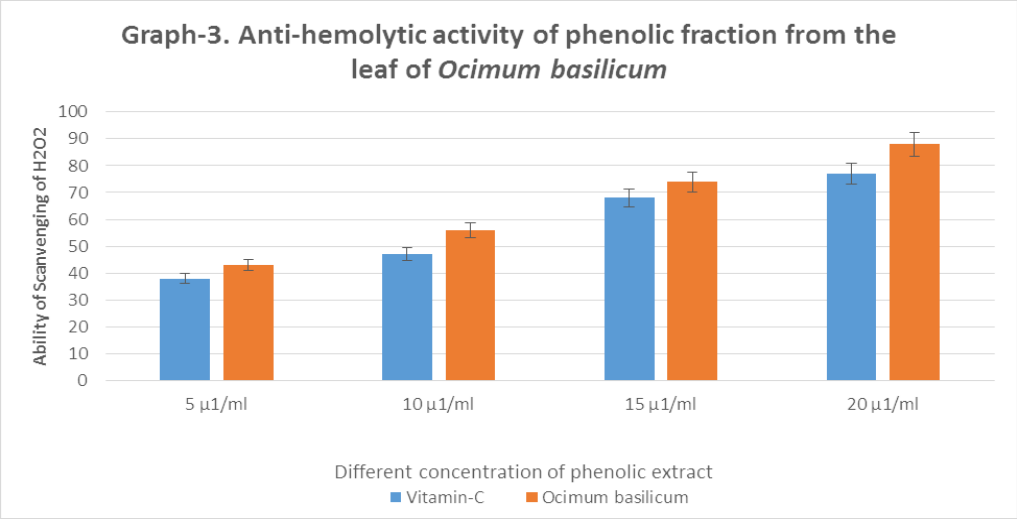
In the present study, the nitric oxide radical quenching activity of the phenolic extract of *Ocimumbasilicum* was detected and compared with the standard ascorbic acid. The phenolic extract of *Ocimumbasilicum* exhibited the maximum inhibition of 68% at a concentration of 20µg/ml, in a concentration-dependent manner when compared to ascorbic acid that showed lowest activity against nitric oxide ("Fig. 2").



"Fig. 2"Nitric oxide radical scavenging of phenolic extract from *Ocimum basilicum* leaves.

Anti-hemolytic activity of phenolic fraction from the leaf of *Ocimumbasilicum*

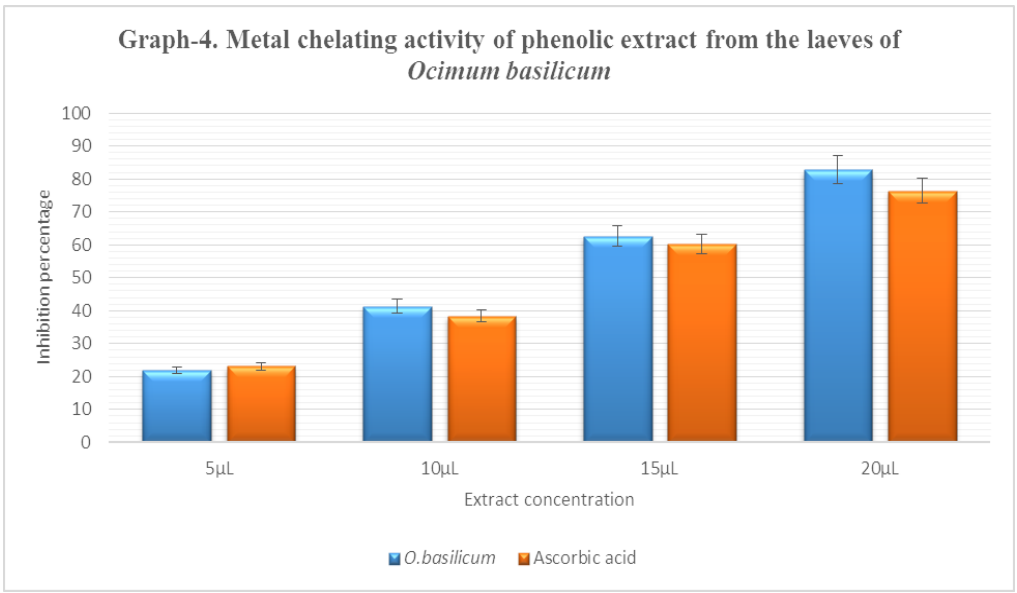
The phenolic fraction of *Ocimum basilicum* showed higher anti hemolytic activity with the increase in concentration. The anti-hemolytic activity of the acetone extract was in dose dependent manner exhibiting higher activity in 20µg/ml concentration. The membrane damage induced by the H_2O_2 in the erythrocytes was inhibited by the phenolic fraction of *Ocimum basilicum* ("Fig. 3").



"Fig. 3" Anti-hemolytic activity of phenolic extract from *Ocimum basilicum* leaves.

Metal chelating activity of phenolic extract of *O.basilum*

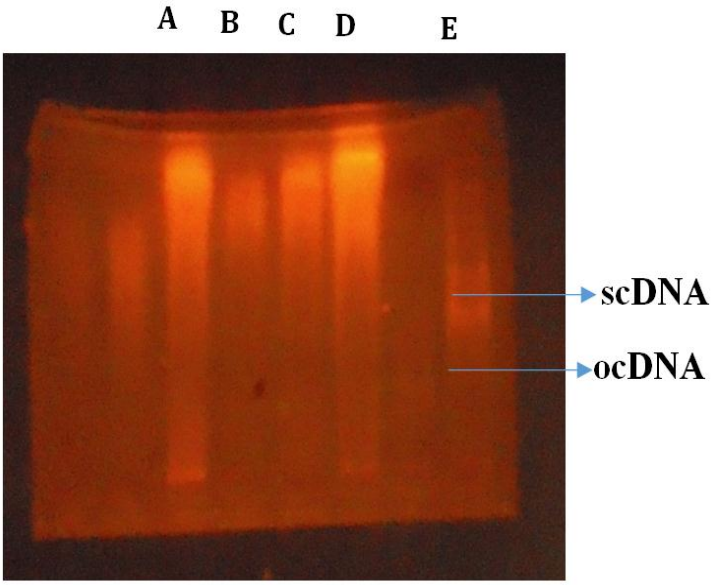
The metal chelating activity of phenolic extract of *O.basilum* shown as in ("Fig. 4"). The phenolic extract of *O.basilum* was assessed for their ability to compete with ferrozine for ferrous iron in the solution. In this assay, the leaf extract of *O.basilum* interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are capable of capturing ferrous iron before ferrozine. The phenolic extract of *O.basilum* reduced the red color complex immediately and showed the highest chelating activity.



"Fig. 4"Metal chelating activity of phenolic extract from *Ocimum basilicum* leaves.

Effects of *O.basilicum* Phenolic extract on the protection of super coiled DNA against OH• generated by the photolysis of H_2O_2

The electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (2.5mmol/L) in the absence or presence of the *O.basilicum* phenolic extract. DNA derived from pBR322 plasmid showed two bands on agarose gel electrophoresis(lane 1), the faster-moving band corresponding to the native form of super coiled circular DNA (sc DNA) and the slower-moving band being the open circular form(oc DNA). The UV irradiation of DNA in the presence of H_2O_2 (lane 2) caused the cleavage of scDNA to give ocDNA and the linear form (linDNA), indicating that**.** OH generated by UV-photolysis of H_2O_2 produced DNA strand scission. The presence of all the phenolic under investigation suppressed the formation of linDNA ("Fig. 5").



"Fig. 5"Protection of supercoiled DNA against OH• generated by the photolysis of H_2O_2 by *Ocimumbasilicum* phenolic extract.

A-Cleavage DNA;

B- pBR322 DNA;

C- Treated H_2O_2 with Phenolic fraction

D- Treated UV Phenolic fraction;

E- Treated H_2O Phenolic fraction

CONCLUSION

Polyphenols are valuable plant constituents for the scavenging of free radicals because of their phenolic hydroxyl groups. This, together with the obtained results, suggests that as the amount of polyphenolic com-pounds increases, the antioxidant activity also increases. In

conclusion, the present study demonstrates that the polyphenolic extract of *Ocimumbasilicum* leave scan protect the body from oxidative stress from ROS, which may bedue to the phyto-chemicals in the form of polyphenols that occur in the plant. These may be used in nutraceuticals and the food industry. However, additional studies are necessary to develop a method for the fractionation and identification of polyphenols and to determine the most active antioxidant compounds in the polyphenolic extract..

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