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Full Length Research Paper

Cytotoxic effect of nanoparticles synthesized from *Salvia officinalis* L. and *Ricinus communis* aqueous extracts against vero cell line and evaluation of their antioxidant activities

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The development of safe carriers for the use of plant extracts in industrial and health fields constitute a matter of serious concern. The development of plant derived nanoparticles may help to overcome such barriers. However, the major concern is still the safety of these carriers. The present study describes the synthesis of nanoparticles derived from *Salvia officinalis* L. and *Ricinus communis* and the evaluation of their cytotoxic and antioxidant effects. It is shown in this study that the aqueous extracts of *S. officinalis* L. and *R. communis* have the potentials to reduce silver nitrate ions to silver nanoparticles. The biosynthesized nanoparticles were analyzed by spectrophotometer and granulometric tests. The biological activities of these extracts and nanoparticles were carried out. *S. officinalis* L. leaf extract had the strongest antioxidant activity, followed by *R. communis* leaf and fruit extracts. Altogether, the synthesized nanoparticles were safe and may serve as antioxidant products in many fields.

Key words: Plant material, biosynthesis of nanoparticle, cytotoxicity, cell culture, free radicals.

INTRODUCTION

Salvia officinalis (common sage) is one of the most known and used aromatic and medicinal plant, to which several ethnopharmaceutical properties are attributed. *S. officinalis* is a popular herb commonly used as a culinary spice for flavouring and seasoning that has also been used for centuries in folk medicine, for the treatment of a variety of aliments. Due to the increasing scientific evidence of the health benefits of natural antioxidants, *S. officinalis* turned into a good candidate as a source of those compounds, for the cosmetic and pharmaceutical industries. The antioxidant properties of sage were extensively studied using *in vitro* studies. Sage alcoholic extracts revealed strong antioxidant activity by increasing the stability of food oils (Miura et al., 2002; Zainuddin et al., 2002; Ozcan, 2003; Jaswir et al., 2005), contributing to its application in food industry. The antioxidant activity of sage extracts were also shown using other methods, such as by the accelerated autoxidation of methyl linoleate (Cuvelier et al., 1994, 1996), by the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Lamaison et al., 1991) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphoric acid (ABTS) free radicals (Shan et al., 2005), as well as by the oxygen

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radical absorbance capacity (ORAC assay) (Zheng and Wang, 2001).

Ricinus communis is a soft wooden small tree, wide spread throughout tropics and warm temperatures regions of the world (Parekh and Chanda, 2007). In the Indian system of medicine, the leaf, root and seed oil of this plant have been used for the treatment of the inflammation and liver disorders, and as hypoglycemic laxative (Dhar et al., 1968; Capasso et al., 1994); because of the side-effects of synthetic antioxidants such as butylated hydroxytoluene (BHT) used as food preservative on cancer risk, the interest on antioxidants of natural sources has increased considerably in recent years (Deans and Simpson, 2000).

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. Spices used in different types of food to improve flavours, since ancient times, are well known for their antioxidant properties (Madsen and Bertelsen, 1995). In various studies, sage has been shown to be the most potent natural antioxidants of the common spices (Herrmann, 1993).

Free radicals are molecules/atoms with unpaired electrons. Radicals are produced in the cells as by-products of normal oxidation. Most of the radicals are reactive oxygen species (ROS), formed during normal cell aerobic respiration (Gutteridge and Halliwell, 2000).

ROS are oxygen derived chemically reactive molecules (Fridovich, 1999; Betteridge, 2000; Halliwell, 1996, 1999). Free radicals and ROS react with several biomolecules and begin a chain reaction. These reactions only stop when the free radicals are eliminated, the generated free radical reacts with another free radical, or when it reacts with a chain breaking or primary antioxidant. Antioxidant phytochemicals in foods, especially in vegetables, fruits, and grains are found to have human disease prevention abilities, and may improve food quality (Yu et al., 2002). There is a continuous search for foods rich in antioxidants. Every year, numerous papers are being published on this area.

Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries (Halliwell et al., 1996). Natural antioxidants exhibit a wide range of pharmacological activities, and have been shown to have anticancer, anti-inflammatory and anti-aging properties (Matés et al., 1999; Noguchi and Niki, 2000; Mayne, 2003; Pinnell, 2003).

Numerous vegetables, crops, spices and medicinal herbs have been tested, in an effort to identify new and potentially useful antioxidants (Vinson et al., 1998; Ganthavorn and Hughes, 1997; Jitoe et al., 1992; Zheng and Wang, 2001). More recently, it has become evident that phenolic natural products may reduce oxidative stress by indirect antioxidant action. For example, various flavonoids, which are found naturally in fruits, vegetables and some beverages, have been demonstrated to exert antioxidant effects through a number of different mechanisms (Nijveldt et al., 2001).

This report deals with the antioxidant activity of some plant extracts and their derived nanoparticles, and the evaluation of their cytotoxic potential against vero cell line.

MATERIALS AND METHODS

Biosynthesis of nanoparticles

One Milliliter aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of plant extracts was added to 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions, and kept at room temperature for 5 h. The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer Evolution EV 60 (Thermo Fisher Scientific, France). Particles size measurements of the powder samples were carried out with a Beckman-Coulter LS 230 laser granulometer in the 0.1 to 10000 nm range. The nanosize range was determined for the colloidal suspensions, using a Zetasizer nano system from Malvern instruments.

Determination of antioxidant activity of aqueous extract of the *Salvia officinalis* L. and *Ricinus communis* DPPH• radical scavenging assay

Radical scavenging activity of plant extracts against stable 2,2-diphenyl-2 picrylhydrazyl - (DPPH• hydrate, Sigma-Aldrich, Tunisia) was determined spectrophotometrically. When DPPH• reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light-yellow) were measured at 517 nm on a UV/visible light spectrophotometer.

Radical scavenging activity of extracts was measured by a modified method of Koleva et al. (2002) adapted for microplate analysis. Extract solutions were prepared by dissolving 1 mg of dry sample in 1 ml of methanol. Four concentrations of two-fold dilutions were prepared for each sample (1 mg/ml, 500 µg/ml, 250 µg/ml, and 125 µg/ml). The solution of DPPH• in ethanol (20 mg/L) was prepared daily, before UV measurements. For each well, 66.5 µl of each sample was added to 133.5 µl DPPH• solution. As negative control, 200 µl DPPH• in ethanol was used as negative control, and 200 µl of ethanol was used as a blank. The experiment was carried out in triplicate. The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Radical scavenging activity was calculated by the following formula:

$$\text{Activity (\%)} = 100 - [(x - b) \times 100 / (y - b)]$$

Where, x = absorbance of the sample in the presence of DPPH; y = absorbance of negative control and; b = absorbance of blank sample.

Cytotoxicity assay

Cell culture

The vero cell line was derived from the kidney of a normal, adult, African green monkey (Cercopithecus) in 1962, by Yasumura and Kawakita at the Chiba University in Japan. This cell line has been used extensively for virus replication studies and plaque assays.

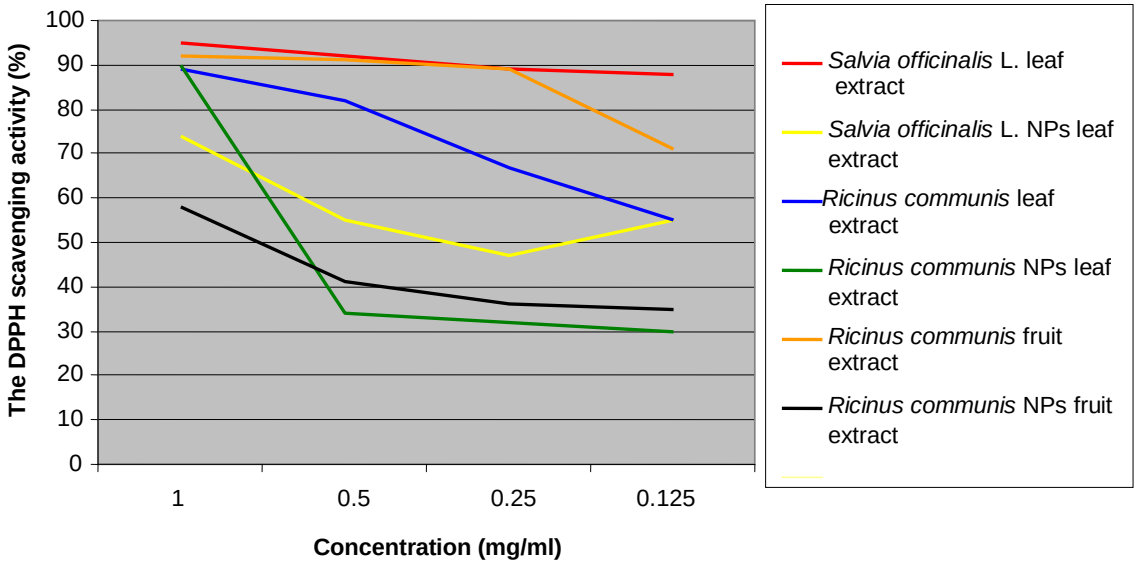

Figure 1. DPPH scavenging activity (%) of different plant extracts and nanoparticles (NPs) at different concentrations. Each extract is shown by different colour.

The vero cell line was maintained in RPMI 1640 supplemented with fetal bovine serum (10% v/v), L-glutamin (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). Cells were incubated at 37°C, in a 5% CO_2 humidified atmosphere.

Cytotoxicity assay

In this assay, aqueous extract and nanoparticles (NPs) were tested individually. The evaluation was based on the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), by the mitochondrial dehydrogenase of viable cells to give a blue formazan product which can be measured spectrophotometrically. The MTT colorimetric assay was performed in 96-well plates. Cells were seeded in 96-well plates at a concentration of 5×10^4 cells/well and incubated for 24 h at 37°C, in a 5% CO_2 humidified atmosphere. Microscopic examination ensured that stable normal cell layers were maintained in each well throughout every experiment. After treatment with various concentration of the test compound (78, 156, 312.5, 625, 1250, 2500, 5000, and 10000 µg/ml), the cells were incubated for an additional 48 h at 37°C. The cells were examined daily under a phase-contrast microscope to determine the minimum concentration of compound that induced alterations in cell morphology. After that, the medium was removed and cells in each well were incubated with 100 µl of MTT solution (5 mg/ml) for 4 h, at 37°C. MTT solution was then discarded and 50 µl dimethyl sulfoxide (DMSO) was added to dissolve insoluble formazan crystal, and the plates were incubated at 37°C for 30 min. Optical density (OD) was measured at 540 nm using a standard microplate reader (BIO-TEK® ELx800™ Universal Microplate Reader, USA). Data were obtained from triplicate wells. Cell viability was expressed with respect to the absorbance of the control wells (untreated cells), which were considered as 100% of absorbance. The percentage of cytotoxicity was calculated as $[(A - B) / A] \times 100$, where, A and B are the OD_{540} of untreated and of treated cells, respectively. The 50% cytotoxic concentration (CC_{50}) was defined as the compound's concentration (µg/ml) required for the reduction of cell viability by 50%, which were calculated by regression analysis.

RESULTS AND DISCUSSION

In vitro antioxidant activity

DPPH is a free radical stable at room temperature, which produces a violet solution in ethanol. In the presence of anti-oxidant compounds, the DPPH is reduced, producing a non-color ethanol solution. We found that the *S. officinalis* L. leaf extract had the strongest antioxidant properties (Figure 1) followed by *R. communis* leaf and fruit extracts, which had important antioxidant properties, with the IC_{50} of 172 and 1.6 µg/ml, respectively (Table 1). The *R. communis* fruit extract had higher antioxidant activity than *R. communis* leaf extract; this good activity may be due to the higher content of phenolic compounds. Falleh et al. (2008) pointed out the correlation coefficient between phenolics (total polyphenol, flavonoids, and condensed tannins) and IC_{50} values of the DPPH, indicating that polyphenolics may play an important role in free-radical scavenging. In general, the antiradical and antioxidant activities of the plant extracts are ascribed to the phenolic contents (Lu and Foo, 2001; Miliauskas et al., 2004; Chidambara-Murthy et al., 2002). The antioxidant activities of plant extracts were significantly decreased with the biosynthesized nanoparticles. The antioxidant potential of nanoparticles derived from *S.officinalis* L. leaf extract, *R. communis* leaf and fruit extracts decreased, with 351, 518, and 745 µg/ml, respectively (Table 1). The reducing ability of a compound depends on the presence of reductants, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.

In terms of limitations, it is well known that colorimetric

Table 1. Percentage of antioxidant activity and IC_{50} of plant extracts and nanoparticles (NPs).

Extract	Concentration (mg/ml ± SD)				IC_{50} (µg/ml)
	1	0.5	0.25	0.125	
Salvia officinalis L. (A)	95±2	92±1	89±1	88±1	ND
Salvia officinalis L. NPs (A)	74±0.5	55±1	47±1.5	55±1	351
Ricinus communis (A)	89±1	82±1.7	67±2	55±0.5	172
Ricinus communis NPs (A)	90±2	34±3	32±2	30±1	518
Ricinus communis (B)	92±2	91±0.5	89±1	71±1	1.6
Ricinus communis NPs (B)	58±2	41±1	36±1	35±1	745

ND, Not determined; (A), leaf extract; (B), fruit extract.

Table 2. CC_{50} and viability test of plant extracts and nanoparticles (NPs) against vero cells.

Extract	Concentration (µg/ml)								CC_{50} (mg/ml)
	78	156	312	625	1250	2500	5000	10000	
S. officinalis L. (A)	73	73	73	73	69	69	69	62	22.7
S. officinalis L. NPs (A)	89	89	82	69	69	69	69	56	11.7
R. communis (A)	97	76	74	72	70	69	6	64	16.5
R. communis NPs (A)	92	87	87	75	73	70	65	50	10.0
R. communis (B)	87	76	75	70	68	66	64	60	14.0
R. communis NPs (B)	74	74	74	73	73	73	73	55	15.0

(A) Leaf extract; (B) fruit extract.

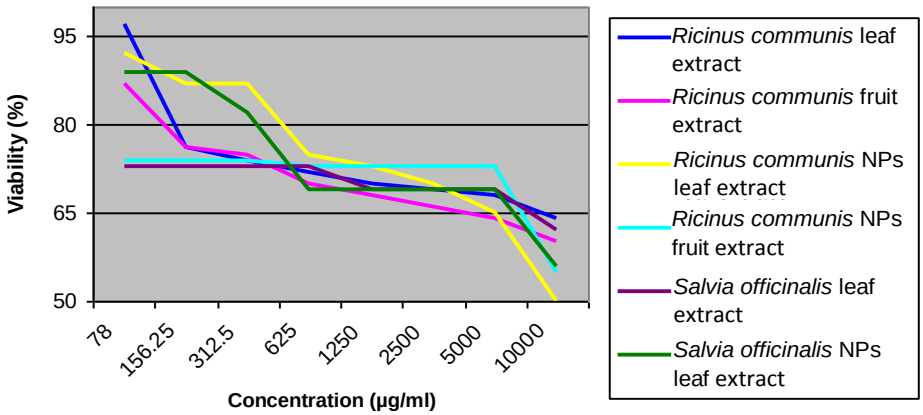

Figure 2. Cell viability of plant extracts and nanoparticles (NPs) at different concentrations.

assays to determine biological activity of extracts can be interfered with by the extract colour, and may represent assay artefact. To overcome this problem in the present study, it was necessary to test the extracts by calorimetric methods prior to study their cytotoxic effects, and the obtained optical density of these extracts was served as blank.

Cytotoxicity test

The MTT assay indicated that the cell treatment with different plant extracts and nanoparticles reduced the cell growth of vero cell line in a concentration-dependent manner. The CC_{50} values for vero cell line were 22.7, 11.7, 16.5, 10.0, 14.0, and 15.0 mg/ml for *S. officinalis* L. leaf extract, *S. officinalis* L. NPs leaf extract, *R. communis* leaf extract, *R. communis* NPs leaf extract, and *R. communis* fruit extract, respectively (Table 2 and Figure 2).

In this study, the nanoparticles were tested against a single transformed cell line. The results do not imply the activity of the extracts in other cell lines (primary or

transformed), nor the anti-proliferative actions *in vivo*. However, the non cytotoxic effect of these nanoparticles constituted an important finding that need to be considered for their future use.

Conclusion

As far as we know, this is the first report describing the used of *S. officinalis* L. and *R. communis* for nanoparticles synthesis. This present study reveals that these plant aqueous extracts can be used for the biosynthesis of silver nanoparticles. The antioxidant activity of nanoparticles was less than that for plant aqueous extracts; however they did not show potent cytotoxic effect. Altogether, these results enhance the use of these nanoparticles in many fields.

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