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Proteome analysis for inhibitory elastin degradation in human dermal fibroblast by the extract of *Coffea canephora*

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ABSTRACT

The extract of *Coffeea canephora* inhibited the degradation of elastin in human dermal fibroblast. The extract prepared with hot water after de-oiling with supercritical carbon dioxide was dosed to fibroblast and proteome analysis using 2-D was made to observe the expression of anti-aging related proteins. By the proteome expression profiling, down-regulation of 4 proteins including CELA2B, CELA3A, CELA3B and MMP3 resulted that the deoiled Robusta extract inhibits the degradation of elastin and dermal extracellular matrix. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Coffeea canephora;
Skin anti-aging;
Elastin degradation;
human dermal fibroblast.

INTRODUCTION

Coffee canephora, Robusta coffee, accounts for one third of coffee production, is reported to produce a bitter brew, with a musty flavor and relatively less body; therefore, it is frequently included in espresso blends or typically employed as instant coffee or fast food. Researchers have been studying how the flavor and taste of coffee are influenced by roasting and extraction conditions. Also, the profile of antioxidants is significantly influenced by the type of coffee as well as processing[1, 2].

The roasting of coffee beans dramatically increases their total antioxidant activity. A roasting time of 10 minutes (medium-dark roast) was found to produce coffee with optimal oxygen scavenging and chain breaking activities in vitro[3]. The total antioxidant activities of different plant phenol-containing beverages have been compared and it has been shown that coffee has significantly more total antioxidant activity than either cocoa, green tea, black tea or herbal tea[4]. It can be concluded that coffee possesses greater in-vitro antioxidant activity than other beverages, due in part to intrinsic compounds such as chlorogenic acid, in part to compounds formed during roasting such as melanoidins and in part to as yet unidentified compounds.

It has been shown that plant phenols have strong antioxidant activity in vitro[5]. As a result it has been hypothesised that plant phenols might protect cellular DNA, lipids and proteins from free radical- mediated damage in vivo[6]. Therefore, coffee extracts are expected to have positive effects on human skin such as

skin whitening or anti-aging. However, until now there have been fewer researches about cosmeceutical properties of coffee. From this point, it is completely interesting for doing research about these cosmeceutical properties of coffee.

This study was carried out to evaluate the anti-aging efficacy of Vietnamese Robusta extract. Vietnamese Robusta green beans were roasted at 230 °C for 15 minutes and deoiled by supercritical carbon dioxide. *In vitro* test on human fibroblast cells were made with the hot water extract. First, MTT assay was conducted to identify the toxicity of the coffee extract. After that, proteome analysis using 2-D was conducted to observe the changes of proteins that are involved in skin aging process, especially skin anti-wrinkle process.

EXPERIMENTAL

Materials

Normal human dermal fibroblast cells (NF) were maintained in DMEM containing 10% fetal bovine serum (FBS) and 1% antibiotic (AA). Experimental cells were prepared at passage 7th and at density of 3×10^4 cells/ml. Cells were incubated at 37 °C in 5% CO_2. Deoiled Vietnamese Robusta Extracts: green Vietnamese Robusta beans were roasted at at 230 °C for 15 minutes and deoiled by Supercritical CO^2 extractor. The deoiled coffee powder was then extracted by hot DW at the ratio 1:10 in about 3h. The clear liquids were collected for further experiments.

MTT assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is used to determine cytotoxicity of coffee extracts for the viability and growth of human fibroblast cells. 100 µl of experimental cell solution was plated into each well of 96-well culture plate and incubated for 24 h in 5% CO^2 incubator. After treatment of cells for 48 h by coffee extracts at 1; 5; 10; 50 and 100 mg/L in DMEM containing 1% AA, experimental media are removed and the cells are incubated with 50 µl basal medium containing 2.0 mg/ml MTT in CO^2 incubator at 37 °C for 3 h. The medium is aspirated, and the formazan product is solubilized with 200 ul dimethyl sulfoxide (DMSO) every well. Absorbance at 595 nm was measured for each well using iMark™ microplate absorbance reader (Bio-Rad).

Protein extraction

Experimental NF cells were plated into 100mm-petri dish. After 24 h being incubated at 370C and 5% CO2, the cells were treated by Robusta extract at the non-toxic concentration and incubated 48 h more. Cells were collected by Trypsin EDTA solution and washed by 10-time diluted PBS. Proteins were extracted from the cell pellet by adding 100 ul Lysis buffer (7 M Urea, 2 M Thiourea, 4% CHAPS, 1% DTT, 2% Carrier ampholyte, 4% PIC, 0.002% Bromo Phenol Blue), sonicating in 1min and incubating at 300C in 5 h, centrifuging at 13400 rpm in 1 min and finally collecting the clear solution. The protein concentration was determined by Bradford method.

Proteome analysis: Two-dimensional electrophoresis

Cells washed in PBS were isolated via centrifugation. Proteins were extracted by cell lysis. The lysis buffer solution was prepared with 7 M urea, 2 M thiourea, 4% CHAPS, 1% DTT, 2% Carrier ampholyte, 4% PIC, and 0.002% BPB. Cells in lysis buffer were sonicated for 1 min and maintained for 30 min at room temperature. The solution was centrifuged for 5 min at 4000×g. The supernatant was collected and preserved at -20°C. The amount of total protein extracted was measured via the modified Bradford method[7]. Bovine γ-globulin was utilized as the standard protein.

Immobiline Dry strips (13cm, pI 3-10L, GE Healthcare, USA) were used with an IPGphore fixed-length strip holder. The strip was rehydrated for 12 h with rehydration solution and the sample proteome was injected simultaneously. The rehydration solution was prepared with 7 M urea, 2 M Thiourea, 2% CHAPS, 1% DTT, 2% Carrier ampholyte, 10% glycerol, 0.002% BPB. Isoelectric focusing was conducted in IPGphore (Amersham Bioscience, Sweden). After 8 hours of rehydration, stepwise focusing was performed for 60 min at 500 V, 60 min at 2000 V, and then increased to 8000 V and maintained until no current change was observed[8]. Focused IPG strips were equilibrated for 15 min in a solution (7 M urea, 2 M Thiourea, 2% SDS, 50 mM Tris-HCl, 30% glycerol and 1% DTT),

and then for an additional 15 min in the same solution containing 2.5% iodoacetamide rather than DTT[9]. After equilibration, the second dimension was run on 11.5% polyacrylamide homogenous gels (T-13%, C-2.5%, 18×24 cm)[10]. The gels were stained with silver nitrate[11]. The stained gel was scanned and the protein spot images were analyzed using 2D Elite (Amersham Biosciences, Sweden) image analysis software. The MALDI-tof MS analysis was made for some protein spots to make the proof identification.

RESULTS AND DISCUSSION

Toxicity of vietnamese robusta extracts on human fibroblast

Cell viability of fibroblast cells depended on treating Robusta extract concentration as shown in Figure 1. At treating extract concentrations from 0 to 10 mg/L, the cells were still alive. At 50 and 100 mg/L of deoiled Robusta extract, about 20% and 35% of cells were killed respectively. Therefore, 10 mg/L of deoiled VN Robusta extract was chosen to treat the in 2-D experiments because of its no toxicity.

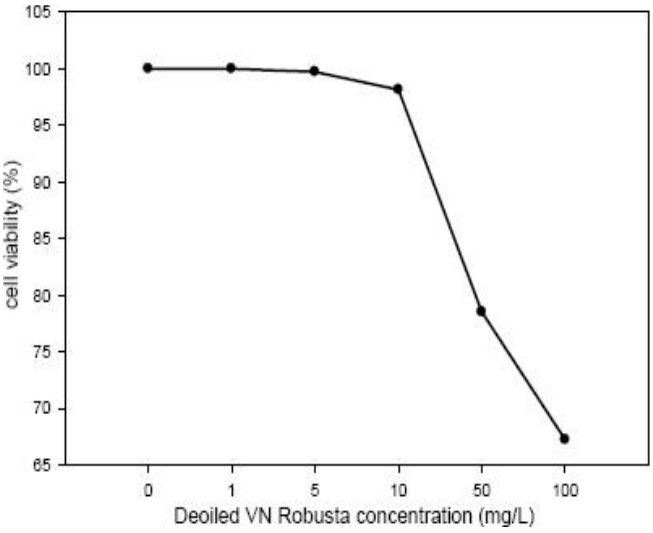
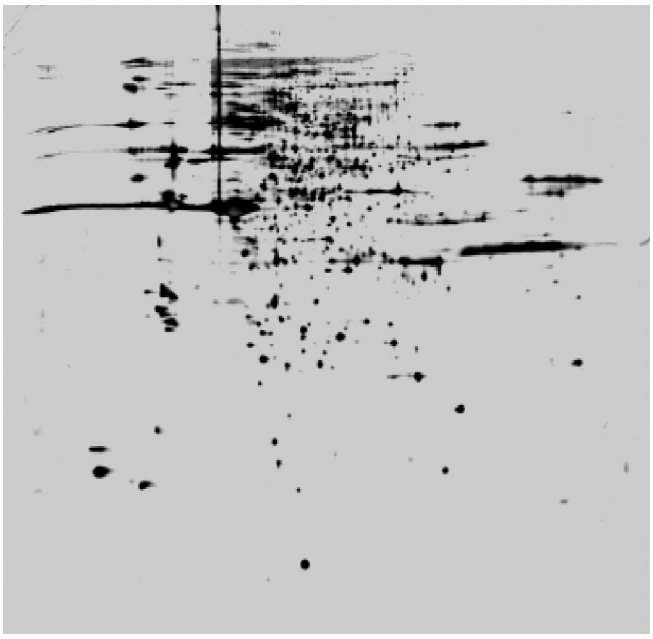


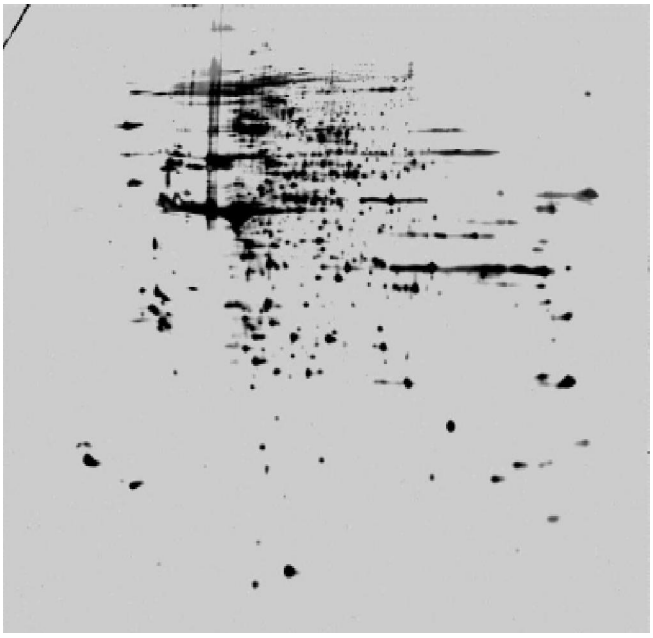
Figure 1 : Cell viability (%) of human fibroblast treated by deoiled VN Robusta extract.

Effects of coffea canephora deoiled by the supercritical fluid on elastin degradation in human dermal fibroblast

Changes of anti-aging related proteins were observed by comparing the control 2-D gel and sample 2-D gel which was treated by 10 mg/L of deoiled Vietnamese Robusta extract as shown in Figure 2. On control gel and sample gel, there were 717 and 612 detected spots respectively. Among them, 447 spots were matched in which 160 spots were up-regulated and 287 spots were down-regulated. Base on isoelectric point (pI) and molecular weight (MW), 6 cellular senescence



(a)



(b)

Figure 2 : 2D-PAGE images of proteins extracted from human fibroblast treated by 10 mg/L of deoiled Robusta extract. (a) Control, (b) deoiled Robusta extract.

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TABLE 1 : List of identified proteins related to cellular senescence of NF treated by deoiled Robusta coffee extract

Protein name	NCBI accession No.	Theoretical		Absolute spot volume		Relative spot volume (%)	
		pI	MW (kDa)	Control	Sample	Control	Sample
CELA2B	P08218	6.8	28.809	487268	476823	100	97.86
CELA3A	P09093	6.3	29.488	230524	200008	100	86.76
CELA3B	P08861	5.85	29.263	72469	33412	100	46.11
MMP3	P08254	5.77	53.977	256916	130247	100	50.70
CDKN2A	P42771	5.52	16.532	150205	43375	100	28.88
IL-1α	P01583	5.04	30.606	160551	135212	100	84.22

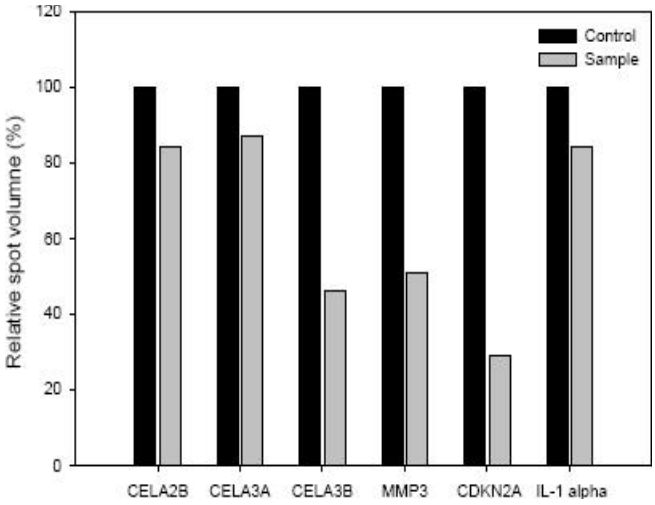

Figure 3 : Comparison of identified cellular senescence related protein spot volume from NF treated by deoiled Robusta extract.

related proteins were identified as shown in TABLE 1. Comparison of identified 6 cellular senescence related protein spot volume from fibroblast treated by deoiled Robusta extract to non-treated control was made as shown in Figure 3.

In collagen synthesis, there was no related protein which was identified. However, three proteins in elastase family were identified – CELA2B, CELA3A and CELA3B. Elastase is an enzyme family which breaks down elastin, an elastic fiber that, together with collagen, determines the mechanical properties of connective tissue[13-15]. The three elastase amounts were all down-regulated. This meant the elastin degradation occurred in NF cells treated by deoiled Robusta extract less than in the cells without treating. Therefore skin treated by deoiled Robusta extract could have higher chance to return to its original position when it is poked or pinched.

Furthermore, the expression of MMP3, a degrading enzyme, decreased about 50%. A negative growth regulator inhibiting cyclin dependent protein kinases - CDKN2A was significantly down-regulated. IL-1alpha protein, which involves in the inflammatory response and stimulates the release of prostaglandin and collagenase, is reported to increase expression in senescent fibroblasts[12] but showed a decrease of nearly 20% in fibroblast treated by deoiled Robusta extract.

From the results of this study, deoiled Robusta extract inhibits the degradation of elastin and dermal extracellular matrix with the down-regulation of 4 proteins including CELA2B, CELA3A, CELA3B and MMP3. Also the two proteins, which are reported to increase expression in senescent fibroblast, were down-regulated in human dermal fibroblast treated by deoiled Robusta extract. Further studies to identify components involved in this promotion would be anticipated to develop the possible anti-aging agent.

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