

5 α -Reductase type 1 inhibition of *Oryza sativa* bran extract prepared by supercritical carbon dioxide fluid

Warintorn Ruksiriwanich^a, Jiradej Manosroi^{a,b}, Masahiko Abe^c, Worapaka Manosroi^d, Aranya Manosroi^{a,b,*}

^a Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

^b Natural Products Research and Development Center (NPRDC), Science and Technology Research Institute (STRI), Chiang Mai University, Chiang Mai 50200, Thailand

^c Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641 Chiba, Japan

^d Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

ARTICLE INFO

Article history:

Received 2 March 2011

Received in revised form 21 July 2011

Accepted 22 July 2011

Keywords:

Antioxidation

5 α -Reductase inhibition

O. sativa crude extract

Supercritical carbon dioxide (scCO₂)

Unsaturated fatty acids

ABSTRACT

The three crude extracts including *Oryza sativa* (bran) from supercritical carbon dioxide fluid (scCO₂) process which gave the highest unsaturated fatty acid contents and biological activities including the antioxidative, tyrosinase inhibition, stimulation index on human normal skin fibroblast were selected from ten edible plants to prepare the semi-purified fractions. Fraction No. 3 of the *O. sativa* bran crude extract gave the highest content of unsaturated fatty acids and 5 α -reductase (type 1) inhibition activity (5AR). Its linoleic acid (LN) and total unsaturated fatty acid (TUC) contents were significantly positive and linear correlated to 5AR on DU-145 cell line (at r of 1.00, $p < 0.01$). Its total phenolic contents and all biological activities also showed positive correlations to 5AR with $r > 0.9$ ($p < 0.05$). This study has demonstrated the potential of fraction No. 3 fractionated from the *O. sativa* bran crude extract prepared by scCO₂ to be developed as anti-androgenic alopecia products.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

In the US, an estimated of 40 million men and 20 million women suffer from baldness and have spent \$1.5 billion annually on hair loss therapies [1]. Dihydrotestosterone (DHT), a potent male hormone, is the cause of genetic male pattern baldness since it has been shown that the DHT levels, numbers of the DHT receptors on the hair follicles and the 5 α -reductase enzyme activity (which converts testosterone to DHT) increase in the balding scalp of androgenic alopecia patients [2–4]. DHT has three times greater affinity for androgen receptors than testosterone, which is the main cause of the androgenic alopecia leading to the miniaturization of hair follicle and hair shedding [5]. The 5 α -reductase inhibitor type 2, finasteride has been approved by the US FDA to use in male pattern baldness, whereas dutasteride (the type 1 and 2 5 α -reductase inhibitor) has been approved for the treatment of symptomatic benign prostatic hyperplasia (BPH), but still hold on phase III for the treatment of male pattern hair loss [6]. The type 1, 5 α -reductase (SRD5A1) related to genetic male pattern baldness, is expressed predominantly in the skin, scalp, sebaceous gland, liver and brain, whereas the type 2, 5 α -reductase (SRD5A2) is found predominantly

in androgen target organs such as prostate, genital skin, and seminal vesicles [7]. Finasteride gives several side effects, such as the decrease of libido, erectile dysfunction, ejaculation disorder and gynecomastia, while dutasteride (5 weeks) has longer half life than finasteride (5–6 h) and is more difficult to reverse the side effects. Although finasteride can block the type 2, 5 α -reductase in androgen target organs more than that in the hair follicle, it has been approved to use in male pattern baldness since it has lower side effects than dutasteride. Both drugs cannot be used in childbearing age females with the pregnancy category X which show the risks of fetal injury or birth defects.

Nowadays, natural extracts from several plants have been used for hair growth promotion such as *Asiasari radix* [8], *Eclipta alba* [9], essential oil of *Chamaecyparis obtuse* [10], *Zizyphus jujube* [11] and *Sophora flavescens* [12]. Most extracts have targeted on the induction of growth factors in hair follicle cells, such as insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF), keratinocyte growth factor (KGF) and epidermal growth factor (EGF), but not on the production of 5 α -reductase enzyme. The unsaturated fatty acids, such as γ -linolenic acid, linoleic acid and oleic acid, have been proved to have anti-hair loss activity by inhibiting 5 α -reductase enzyme in the androgen responsive organs [13]. In fact, several edible plants contain these unsaturated fatty acids in variable amounts, such as *Carthamus tinctorius* L. (safflower), *Helianthus annuus* L. (sunflower), *Linum usitatissimum* L. (flaxseed), *Sorghum bicolor* (L.) Moench (sorghum) and bran of

* Corresponding author at: Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand. Tel.: +66 53 894806; fax: +66 53 894169.

E-mail address: pmpti005@chiangmai.ac.th (A. Manosroi).

Oryza sativa L. (rice). As known, rice bran is discarded or used as livestock feed and oil production. Rice bran oil is edible and has been claimed for improving serum cholesterol levels and lipoprotein profiles similar to other vegetable oils, such as corn and safflower oil [14,15]. Rice bran oil has been extracted from rice bran using scCO₂ by both pilot [16] and lab scale [17]. The health promotion properties of rice bran are from its unsaturated fatty acid contents such as palmitic acid, oleic acid, linoleic acid and γ -linolenic acid, which are known as antioxidants and anti-cancer agents by stimulating the production of the substances which can protect cells from peroxides [18] and anti-hair loss agents [13]. The raw rice bran oil contains both unsaturated and saturated fatty acids, in which palmitic acid is a major acid (C18:0, 12–26%, w/w, typically 18%, w/w). The unsaturated fatty acids are mainly oleic acid (C18:1, 35–46%, w/w, typically 42%, w/w) and linoleic acid (C18:2, 25–38%, w/w, typically 37%, w/w) with the traces of C18:3 acid (0.4–3.8%, w/w) [19,20]. It has been reported that the quality of rice bran oil extracted by SFE was far superior to that produced by hexane extraction, especially at 80 °C [21]. The phenolic compounds and the unsaturated fatty acids containing in *C. tinctorius* have been shown to have antioxidant activity [22] which can recover or slow down the miniaturization of hair follicle. Their redox properties of many bioactive compounds can lead to hair shedding and act as reducing agents, hydrogen donors as well as the singlet oxygen quenchers in the hair follicle [23,24]. Also, there were several anti-hair loss medicinal plant recipes which have been traditionally used by the Chinese and Thai people for over 100 years. But, most of recipes and plants have no scientific evidences on anti-hair loss activity. The relationship between this activity and the antioxidant activity as well as the bioactive compounds in plants still never been explored. Recently, supercritical fluids have been introduced as an alternative one step at low temperature for the preparation of plant extracts. At the critical point, supercritical fluids have the density as liquid, but low viscosity with better flow property as gas. Carbon dioxide is a widely used gas to produce supercritical fluid because of its low critical temperature ($T_c = 31.1$ °C) and pressure ($P_c = 73.8$ bar). It has high solvating power at near critical point. Supercritical carbon dioxide (scCO₂) has been used for the substitution of organic solvent to extract many plant extracts containing thermal sensitive constituents with the advantages of not only being environmental friendly, non-toxic and nonflammable, but also inexpensive [25,26]. It has been used as an alternative to organic solvent for the extraction of many nuts and seeds, such as bran of *O. sativa* (rice) [27], *Arachis hypogaea* L. (peanuts) [28] and *Glycine max* (L.) Merr. (soybean) [29]. Although, the maceration method which is a simply and low cost method, it requires large amount and high purity of the organic solvents which are usually hazardous and flammable solvent wastes and are generally cumbersome [26]. Moreover, it is the non-selective, time consuming and toxic fume emission extraction in comparing to the supercritical carbon dioxide extraction procedure.

This present study has compared the 5 α -reductase type 1 inhibition in DU-145 cell line of the scCO₂ crude extract of *O. sativa* bran, *C. tinctorius* flowers and *S. bicolor* seeds and their semi-purified fractions. The relationship between the 5 α -reductase type 1 inhibition activity and other biological activities as well as the bioactive contents in the crude and semi-purified extracts were evaluated.

2. Materials and methods

2.1. Materials

Gallic acid (99.0%), vitamin C (L-(+)-ascorbic acid, 99.5%), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), EDTA, sulforhodamine B (SRB), dimethyl sulfoxide (DMSO), kojic acid (99.0%), ferrozine,

finasteride (99.5%), Folin–Ciocalteu reagent and ferric chloride (FeCl₂) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mushroom tyrosinase (4187 U/mg) and L-tyrosine were purchased from Fluka (Buchs, Switzerland). Linoleic acid (99.0%), oleic acid (98.5%) and 1,6 diphenyl-1,3,5-hexatriene were from Wako Pure Chemical Industrial Ltd. (Osaka, Japan). γ -Linolenic acid (99.5%) was purchased from Tokyo Chemical Industrial Ltd. (Tokyo, Japan). The standard dutasteride (99.5%) was purchased from Ka-Shing Business Macau Co., Ltd. (Macau, China). Siliga gel 60 was purchased from Merck (Damstadt, Germany). Dulbecco's modified Eagle's culture medium, antibiotics penicillin and streptomycin, fetal bovine serum and trypsin were purchased from HyClone (Logan, UT, USA). All other reagents and solvents were of analytical grade.

2.2. Plant crude extract

2.2.1. Plant sample

Parts of the ten edible plants which have been searched from the literature reviews to contain high amount of unsaturated fatty acids (γ -linolenic acid, linoleic acid and oleic acid) were collected from Chiang Mai Province in Thailand during October to November in 2008 (Table 1). The plant seeds used in this study were packed in vacuum plastic bags and purchased from Thai Cereals World Co., Ltd., Bangkok, Thailand. The % moisture contents in the seeds were in the range of 6–10%. The specimen samples were authenticated by a botanist at the Natural Products Research Development Center (NPRDC), Science and Technology Research Institute (STRI) at Chiang Mai University, Chiang Mai in Thailand.

2.2.2. Plant preparation

All plant parts were seeds, except *O. sativa* and *C. tinctorius* were bran and flower, respectively. The bran of *O. sativa* was passed through sieve No. 25 (0.707 mm). Other plant parts were ground into small pieces by a blender (Twist HR 1701, Philips, Indonesia) and passed through sieve No. 20 (0.841 mm). The plant powder was kept in a tight container at 4 °C until use.

2.2.3. Maceration method

Briefly, 200 g of the plant powder were macerated with 1 l of 95% (v/v) ethanol at room temperature (27 ± 2 °C) for 8 h and stirred every 2 h. The extract was filtered through the paper filter Whatman No. 1, connected with a vacuum pump. The residues were re-extracted more by the same process twice. All filtrates were collected, pooled and dried by a rotary evaporator (Rotavapor R210, Buchi, Switzerland) at 40 °C. The crude extracts were kept at –80 °C until use.

2.2.4. Supercritical carbon dioxide fluid extraction

Briefly, 200 g of the plant powder were put in the supercritical carbon dioxide fluid apparatus (scCO₂) (SFE-500MR-2-C50 System, Thar Instruments, Inc., Pittsburgh, USA) together with 25% (w/v) of 95% (v/v) ethanol as a co-solvent, in the chamber at 40 °C and 200 bar [30]. After 2 h, the pressure was released and the extract was collected. The plant extract residues were re-extracted more by the same procedure 3 times. All extracts were collected, pooled, mixed and dried by a rotary evaporator at 40 °C. The crude extracts were kept at –80 °C until use.

2.2.5. Determination of bioactive compounds and biological activities of the crude extract

The resulting extracts were determined for unsaturated fatty acid and total phenolic contents. The phytochemical tests were also investigated. Briefly, 20 mg of the crude extracts were dissolved in 80% (v/v) methanol and used for detecting the presence of alkaloids, anthraquinones, flavonoids, glycosides, carotenoids,

Table 1

Comparison of the percentage yields, unsaturated fatty acid contents and the total phenolic contents of the ten edible plant crude extracts prepared by the two non-heated processes (scCO₂ and ethanolic maceration).

Plants		Unsaturated fatty acid contents (% w/w)								Total phenolic contents (gallic acid equivalent; GAE mg/g of the extract)	
Scientific name	Common name	Percentage yields (%w/w)		γ-linolenic acid		Linoleic acid		Oleic acid		scCO ₂	Maceration
		scCO ₂	Maceration	scCO ₂	Maceration	scCO ₂	Maceration	scCO ₂	Maceration		
<i>Arachis hypogea</i> L.	Peanut	16.94	12.88	–	–	0.17	1.08	0.24	0.72	0.33	0.39
<i>Carthamus tinctorius</i> L.	Safflower	20.23	33.88	9.36	4.45	4.02	2.13	0.65	0.03	1.36	1.44
<i>Glycine max</i> (L.) Merr.	Soybean	5.42	26.28	0.14	0.05	1.08	0.56	0.12	0.08	0.53	0.46
<i>Helianthus annuus</i> L.	Sunflower	14.01	29.93	0.02	0.01	1.97	0.64	0.42	0.82	2.41	1.76
<i>Linum usitatissimum</i> L.	Flax	7.17	12.14	0.28	0.31	0.63	0.77	0.19	0.11	0.43	0.70
<i>Nelumbo nucifera</i> Gaertn.	Lotus	3.34	2.47	0.08	0.11	1.07	2.45	0.19	0.62	0.42	0.58
<i>Oryza sativa</i> L.	Rice	11.21	17.90	5.67	4.41	23.34	20.03	27.28	19.48	0.65	0.51
<i>Sesamum indicum</i> L.	Sesame	12.01	15.88	0.05	0.09	3.96	3.19	2.75	1.89	0.71	0.86
<i>Sorghum bicolor</i> (L.) Moench	Sorghum	2.54	5.12	0.02	0.15	5.56	2.66	1.78	0.51	1.05	1.13
<i>Zea mays</i> L.	Corn	4.55	14.97	0.04	0.05	0.57	1.09	0.2	0.52	0.76	0.96

Note: Values represented mean ($n=3$). “–” represented not found in the sample. Total phenolic contents were presented as gallic acid equivalent (GAE) mg/g of the crude extracts.

tannins and xanthenes according to the standard methods previously described [31–34]. The biological activities [30] including DPPH radical scavenging, lipid peroxidation inhibition, metal ion chelating, tyrosinase inhibition activities and cell proliferation on aged human skin fibroblasts were determined.

2.3. Biological and anti-hair loss activities of the semi-purified fractions from the selected crude extracts

2.3.1. Preparation of the semi-purified fraction containing unsaturated fatty acids from *O. sativa*, *C. tinctorius* and *S. bicolor* crude extracts

The semi-purified fractions of the three selected crude extracts, including *O. sativa*, *C. tinctorius* and *S. bicolor* from the scCO₂ process which gave the highest unsaturated fatty acid contents and antioxidative activities were prepared as previously described with modification [35]. Briefly, 25 g of the crude extracts were loaded on the 750 g of silica gel 60 (Merck, Germany) column (4 cm ϕ \times 100 cm) and eluted with petroleum ether/ethyl acetate (8:1) at the flow rate of 1 ml/min. Each fraction of 100 ml was collected and the solvent was evaporated by a rotary evaporator. The total of 30 fractions were obtained. Each dried fraction was analyzed for unsaturated fatty acid contents by HPLC with the previously described method [30]. The consecutive fractions which contained the same unsaturated fatty acids were pooled and evaporated. There were four dried fractions from each crude plant extracts. The percentages yields, the unsaturated fatty acids and the total phenolic contents of the dried fractions were determined.

2.3.2. Biological activities of the semi-purified fractions

The semi-purified fractions were tested for the DPPH radical scavenging, lipid peroxidation inhibition, metal ion chelating, tyrosinase inhibition and cell proliferation activity on the aged normal human skin fibroblasts with the previously described methods [30].

2.3.3. Cytotoxicity of the semi-purified fractions on DU-145 cell line

2.3.3.1. Cell culture. The human prostate carcinoma cell line (DU-145) was provided by Prof. Dr. Toshihiro Akihisa at the College of Science and Technology, Nihon University in Tokyo, Japan. Cells were cultured under the standard conditions in the complete culture medium containing RPMI medium supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 U/ml) and

streptomycin (100 mg/ml). Cells were incubated in a temperature-controlled and humidified incubator (Shel Lab, model 2123TC, USA) with 5% CO₂ at 37 °C.

2.3.3.2. Cytotoxicity by the SRB assay. The semi-purified fractions were tested for cytotoxicity on DU-145 cells by the SRB assay as previously described [36]. The standard unsaturated fatty acids (γ -linolenic acid, linoleic acid and oleic acid), the standard finasteride and dutasteride at 0.0001–1 mg/ml were used as positive controls. The cells were plated at the density of 1.0×10^4 cells/well in 96-well plates and left overnight for cell attachment on the plate in 5% CO₂ at 37 °C. Cells were then exposed to five serial concentrations of the crude extracts and their semi-purified fractions (0.00001–1 mg/ml) for 24 h. After incubation, the adherent cells were fixed *in situ*, washed and dyed with SRB. The bound dye was solubilized and the absorbance was measured at 540 nm by a microplate reader. The experiments were done in triplicate. The percentages of cell proliferation were calculated according to the following equation: Cell viability (%) = (Absorbance_{sample}/Absorbance_{control}) \times 100. The concentrations of the samples which gave % cell viability of more than 90% were used in the 5 α -reductase inhibition experiment.

2.3.4. Inhibition of 5 α -reductase activity

2.3.4.1. Cultivation of cells. The pellets of human DU-145 cells were plated onto the 6-well plates separately at the density of 2.5×10^5 cells/well, incubated with 10% (v/v) FBS-DMEM medium containing penicillin (100 U/ml) and streptomycin (100 mg/ml) in a 5% CO₂ incubator (Shel Lab, model 2123TC, USA) at 37 °C. Cells were then exposed to the semi-purified fractions, the standard finasteride and dutasteride at the final concentration of 0.1 mg/ml and the standard unsaturated fatty acids at 0.1–0.001 mg/ml for 24 h. The medium were removed, and the cells were washed with PBS, trypsinized with 0.25% trypsin solution for 2 min and suspended in PBS.

2.3.4.2. Total RNA extraction. The total RNA from the cell pellets was extracted by the RNA extraction kit (NucleoSpin[®], Macherey-Nagel, CA, USA) according to the instructions of the manufacturer. The concentration of the total RNA was quantified by Qubit Fluorometer and Quant-iT[™] RNA BR assay kit (Invitrogen, CA, USA). The total RNA solution was kept at –20 °C until used.

2.3.4.3. Reverse transcription-polymerase chain reaction (RT-PCR). The 5 α -reductase type 1 and 2 genes were amplified from the extracted RNA by SuperScrip[™] One-Step RT-PCR with Platinum[®]

Taq kit (Invitrogen, CA, USA) according to the manufacturer's protocol. Briefly, 5 µg of the total RNAs were reverse transcribed with RT/Platinum Taq[®] mix and subjected to PCR cycles with the primers for human 5α-reductase type 1 and 2 (SRD5A1 and 2) as follows: 94 °C for 15 s, 55 °C for 30 s, 72 °C for 45 s for 35 cycles. The human 5α-reductase type 1 primers were designed based on GenBank accession no. NM.001047.2 and NM.000348, respectively, with a forward (5'-CCA TGT TCC TCG TCC ACT AC-3') and reverse (5'-TTC AAC CTC CAT TTC AGC GT-3'), produced 707 bp amplicon and human 5α-reductase type 2 (SRD5A2) forward (5'-GGG TGG TAC ACA GAC ATA CG-3') and reverse (5'-TCA CGA CTA TGA GGA GAG GG-3'), produced 938 bp amplicon [37]. The RT-PCR products were loaded on 1% agarose gel in the 1 × tris-acetate-EDTA (TAE) buffer chamber at 100 V for 30 min. The human 5α-reductase type 1 and 2 dsDNA samples were quantified by the Qubit fluorometer and Quant-iT[™] dsDNA assay kit (Invitrogen, CA, USA).

2.4. Statistical analysis

The results were presented as the mean of three independent experiments and analyzed by SPSS (version 16.0). ANOVA was used for the analysis of the test results (LSD test) at the significance level of p -value < 0.05. Correlation coefficient (r) was used to determine the relationship between the variables which were calculated using the bivariate correlation statistical function.

3. Results and discussion

3.1. Unsaturated fatty acid and total phenolic contents in the crude extracts

The crude extract from *C. tinctorius* by both ethanolic maceration and scCO₂ showed the highest percentage yields at 33.88 ± 3.67 and $20.23 \pm 1.65\%$ (w/w), respectively (Table 1). Although, the ethanolic maceration of most plants gave higher percentage yields than the scCO₂ technique, it gave lower contents of the main target compounds (unsaturated fatty acids) than that from the scCO₂ technique. The maceration technique gave more impurities than that from the scCO₂ technique. In fact, the polar organic solvents such as methanol and ethanol can also extract many polar compounds such as phenolic compounds, flavonoids and anthraquinone [38], thereby giving the extract with higher impurities and percentage yields. The high lipophilic property and solvating power of the scCO₂ fluid can dissolve the unsaturated fatty acids especially at the pressure of 80–250 bar and the temperature range of 40–80 °C [39]. Thus, the scCO₂ fluid appears to be the best solvent choice to obtain the extract with high content of unsaturated fatty acids. For other methods such as Soxhlet extraction and maceration, they require large amount and high purity of organic solvents (chloroform, hexane or methanol) that are hazardous and can be the flammable solvent wastes and are generally cumbersome [26]. Moreover, the Soxhlet extraction was the non-selective, time consuming and toxic fume emission technique which is harmful to the environments [40]. The crude extract of *O. sativa* by both processes exhibited the highest contents of all unsaturated fatty acids, except γ-linolenic acid in comparing to the crude extracts of *C. tinctorius*. The *O. sativa* crude extract from the scCO₂ technique contained the γ-linolenic acid, linoleic acid and oleic acid contents at 5.67 ± 0.52 , 23.62 ± 2.62 and $27.28 \pm 1.22\%$ (w/w), which were more than those from the maceration method (4.41 ± 0.73 , 20.03 ± 1.89 and $19.48 \pm 1.78\%$, w/w) of 1.29, 1.18 and 1.40 times, respectively. Moreover, by scCO₂ the *O. sativa* crude extract exhibited the highest total unsaturated fatty acids (TUC) at $56.57 \pm 3.73\%$ (w/w) which was higher than those in the *C. tinctorius* ($14.03 \pm 2.17\%$, w/w) and *S. bicolor* ($7.36 \pm 1.18\%$, w/w) crude extract of 4.5 and 8.5 times, respectively.

The crude extracts which gave the highest total phenolic contents (TPC) in the form of gallic acid were observed in *H. annuus* from both by the scCO₂ and the maceration methods at 2.41 ± 0.13 and 1.76 ± 0.07 mg gallic acid equivalent/gram of the crude extract (GAE mg/g), respectively. In fact, various phenolic compounds were found in *H. annuus*, such as 5-O-caffeoylquinic acid, quercetin and kaempferol, respectively [41,42].

3.2. Phytochemicals in the crude extracts

In this study, 95% (w/v) ethanol was used as a co-solvent in the scCO₂ extraction at 25% (w/v) of the liquid CO₂ and as a solvent in the maceration process. Ethanol is a safe solvent and has high polarity than the scCO₂ which can extract polar bioactive compounds from the rice bran such as γ-oryzanol, glycosides and carotenoids. The phytochemical compounds were shown qualitatively and approximated quantitatively in Table 2. Carotenoids which are antioxidants were found in all crude extracts (data not shown). The *C. tinctorius*, *S. bicolor* and *G. max* crude extracts by both processes contained most phytochemicals including alkaloids, carotenoids, glycosides, xanthenes and tannins. Hence, the phytochemical constituents were not affected by the extraction processes, but depended on the types of plants. Xanthone and tannins were found in the crude extracts of *C. tinctorius*, *G. max*, *Nelumbo nucifera* Gaertn., *O. sativa*, *S. bicolor* and *Zea mays* L. As known, natural antioxidants are a broad range of phytochemicals including phenolic, nitrogen and carotenoids [43]. The antioxidative activity of the crude extracts which contained these compounds can be anticipated.

3.3. Biological activities of the crude extracts

The biological activities including DPPH radical scavenging, lipid peroxidation inhibition, metal ion chelation, tyrosinase inhibition activities and stimulation index of the crude extracts of the ten selected edible plants including bran of *O. sativa* prepared by the two non-heated processes were compared (Table 3). Most of plant crude extracts by the scCO₂ showed slightly lower activities than those by the ethanolic maceration technique. This might be from the higher content of hydrophilic substance, phenolic compound as shown in the amount of total phenolic content (TPC).

For DPPH radical scavenging activity, the *C. tinctorius* crude extracts (SC₅₀ value of 1.13 ± 0.02 mg/ml) gave the highest DPPH scavenging activity, which higher than those from the *S. bicolor* and *O. sativa* crude extracts by scCO₂ of about 1.9 and 5.8 times, respectively, but showed lower activity than the standard vitamin C (SC₅₀ value of 0.044 ± 0.004 mg/ml), vitamin E (SC₅₀ value of 0.039 ± 0.002 mg/ml) of about 25 and 29 times, respectively. The highest DPPH scavenging activity of the *C. tinctorius* crude extract may be from the high content of phenolic compounds. The significant correlation between DPPH scavenging activity (A_F) and the total phenolic contents (TPC) in this crude extract ($r = 0.99$, $p < 0.05$) was observed. The chemical constituents in this plant have been reported to be flavonoids [44], lignans [45], triterpene alcohols [46], and polysaccharides [47].

For lipid peroxidation inhibition activity, the three crude extracts by scCO₂ which gave the highest activity were *H. annuus*, *A. hypogaea* and *O. sativa*, respectively. The *O. sativa* crude extract prepared by scCO₂ (IPC₅₀ value of 1.25 ± 0.51 mg/ml) showed high lipid peroxidation inhibition activity but lower than vitamin C (IPC₅₀ value of 0.07 ± 0.04 mg/ml) and vitamin E (IPC₅₀ value of 0.03 ± 0.01 mg/ml) of 17.9 and 41.7 times, respectively. By scCO₂, this crude extract gave higher lipid peroxidation inhibition activity than *C. tinctorius* crude extracts (IPC₅₀ value of 2.27 ± 0.76 mg/ml) and *S. bicolor* (IPC₅₀ value of 4.05 ± 0.69 mg/ml) of about 1.8 and 3.2 times, respectively.

Table 2Comparison of phytochemical compounds of the ten edible plant extracts prepared by the two non-heated methods (scCO₂ and ethanolic maceration).

Scientific name	Common name	Part used	Alkaloid		Antraquinone		Carotenoid		Flavonoid		Glycoside						Xanthone		Tannin		
			S	M	S	M	S	M	S	M	Sucrose		Glucose		Fructose		S	M	S	M	
											S	M	S	M	S	M					
<i>Arachis hypogaea</i> L.	Peanut	Seed	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Carthamus tinctorius</i> L.	Safflower	Flower	+	+	–	–	+	+	–	–	–	–	+	+	+	+	+	+	+	+	+
<i>Glycine max</i> (L.) Merr.	Soybean	Seed	+	+	–	–	+	+	–	–	+	+	–	–	+	+	+	+	+	+	+
<i>Helianthus annuus</i> L.	Sunflower	Seed	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Linum usitatissimum</i> L.	Flax	Seed	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Nelumbo nucifera</i> Gaertn.	Lotus	Seed	–	+	–	–	+	+	–	–	+	+	+	+	–	–	–	–	–	+	+
<i>Oryza sativa</i> L.	Rice	Bran from seed	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	+
<i>Sesamum indicum</i> L.	Sesame	Seed	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Sorghum bicolor</i> (L.) Moench	Sorghum	Seed	+	+	–	–	+	+	–	–	+	+	+	+	–	–	+	+	+	+	+
<i>Zea mays</i> L.	Corn	Seed	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	+	–	–	+

Note: “+” represented presence in the extract. “–” represented absence in the extract. “S” represented supercritical carbon dioxide fluid extraction. “M” represented maceration extraction.

Table 3Comparison of antioxidative, tyrosinase inhibition activities and the stimulation index on human skin fibroblasts (30th passage) of the ten edible plant extracts prepared by the two non-heated methods (scCO₂ and ethanolic maceration).

Plant scientific name	SC ₅₀ (mg/ml)		IPC ₅₀ (mg/ml)		CC ₅₀ (mg/ml)		IC ₅₀ (mg/ml)		Stimulation index (SI) at 0.1 mg/ml	
	scCO ₂	Maceration	scCO ₂	Maceration	scCO ₂	Maceration	scCO ₂	Maceration	scCO ₂	Maceration
<i>Arachis hypogaea</i> L.	151.23	52.56	1.23	1.44	ND	ND	ND	ND	1.03	0.76
<i>Carthamus tinctorius</i> L.	1.13	1.66	2.27	1.06	3.58	3.26	19.47	6.64	2.60	2.42
<i>Glycine max</i> (L.) Merr.	7.26	5.77	6.66	10.11	93.05	55.35	ND	ND	2.12	1.77
<i>Helianthus annuus</i> L.	6.36	0.25	0.87	1.79	ND	37.33	ND	5.58	1.45	0.86
<i>Linum usitatissimum</i> L.	28.99	27.65	8.99	27.65	ND	ND	ND	ND	1.13	1.49
<i>Nelumbo nucifera</i> Gaertn.	40.69	6.57	3.19	1.79	10.3	5.05	ND	2.6	0.79	0.94
<i>Oryza sativa</i> L.	6.55	6.79	1.25	1.44	68.23	75.04	25.98	48.06	1.10	1.14
<i>Sesamum indicum</i> L.	24.3	8.32	9.3	0.87	ND	ND	ND	19.29	0.85	0.93
<i>Sorghum bicolor</i> (L.) Moench	2.16	3.64	4.05	3.27	36.48	47.82	3.61	17.24	1.22	1.45
<i>Zea mays</i> L.	28.48	22.6	8.48	22.6	7.88	10.41	1.53	3.53	0.73	0.69
Vitamin C	0.044		0.07		NA		0.035		1.20	
Vitamin E	0.039		0.03		NA		NA		NA	
EDTA	NA		NA		0.15		NA		NA	
Kojic acid	NA		NA		NA		0.024		NA	
γ-Linolenic acid	15.49		1.93		1.74		0.77		NA	
Linoleic acid	39.82		1.25		14.79		2.55		NA	
Oleic acid	55.58		3.51		26.34		7.11		NA	

Note: ND represented not detected. NA represented not appreciable. SC₅₀ value (mg/ml) was the concentration of the sample that scavenged 50% of the DPPH radicals. IPC₅₀ value (mg/ml) was the concentration of the sample that inhibited 50% of the lipid peroxidation. CC₅₀ value (mg/ml) was the concentration of the sample that chelated 50% of the metal ion. IC₅₀ value (mg/ml) was the concentration of the sample that inhibited 50% of the tyrosinase enzyme. Stimulation index (SI) was the ratio between the percentages of cell proliferation of the treated sample and the control (no treatment)

For chelating activity, the three crude extracts by scCO₂ which gave the highest activity were *C. tinctorius*, *Z. may* and *S. bicolor*, respectively. The crude extracts from *C. tinctorius* gave the highest chelating activity (CC₅₀ value by scCO₂ of 3.58 ± 0.17 mg/ml), which was higher than *S. bicolor* (CC₅₀ value of 36.48 ± 3.69 mg/ml) and *O. sativa* crude extracts by scCO₂ (CC₅₀ value of 68.23 ± 8.06 mg/ml) of about 10.2 and 19 times, respectively, but indicated lower activity than the standard chelating agent, EDTA (CC₅₀ value of 0.15 ± 0.04 mg/ml) of about 23 times.

For tyrosinase inhibition activity, the three crude extracts by scCO₂ which gave the highest activity were *Z. may*, *S. bicolor* and *C. tinctorius*, respectively. The *S. bicolor* crude extract by scCO₂ showed the high tyrosinase inhibition activity (IC₅₀ value of 3.61 ± 0.82 mg/ml) which was higher than the *C. tinctorius* (IC₅₀ value of 19.47 ± 7.35 mg/ml) and *O. sativa* crude extracts by scCO₂ (IC₅₀ value of 25.98 ± 13.42 mg/ml) of about 5.4 and 7.2 times, respectively, but gave lower activity than the standard vitamin C (IC₅₀ value of 0.035 ± 0.009 mg/ml), kojic acid (IC₅₀ value of 0.024 ± 0.003 mg/ml) of about 103 and 150 times, respectively.

For human skin fibroblast stimulation activity, the *C. tinctorius* crude extract by scCO₂ (SI value of 2.60 ± 0.20) and by maceration (SI value of 2.42 ± 0.67) at 0.1 mg/ml gave the highest stimulation index, which was higher than the standard vitamin C at 0.1 mg/ml (SI value of 1.20 ± 0.08) and *S. bicolor* (SI value of 1.22 ± 0.19 mg/ml) and *O. sativa* crude extracts by scCO₂ (SI value of 1.10 ± 0.12 mg/ml) of 2.2, 2.1 and 2.4 times, respectively.

The scCO₂ crude extracts from the three plants including *C. tinctorius*, *O. sativa* and *S. bicolor* were selected to prepare the semi-purified fractions because of the highest total unsaturated fatty acid contents (TUC) in comparing to other seven edible plants, since the unsaturated fatty acids, γ -linolenic acid, linoleic acid and oleic acid, have been proved to have anti-hair loss activity by inhibiting 5 α -reductase enzyme in the androgen responsive organs [13]. Although extracts from the scCO₂ indicated only slightly lower biological activities than the maceration method, the scCO₂ process gave higher unsaturated fatty acid contents than by the maceration process. Also, the scCO₂ method is not only a non-toxic, non-inflammatory and inexpensive process but also has high solvating power to extract unsaturated fatty acids.

In addition, the crude extract of *C. tinctorius* showed the highest biological activities, followed by *S. bicolor* and *O. sativa*, respectively. The *C. tinctorius* crude extracts from scCO₂ gave the highest DPPH scavenging, chelating and stimulation index, high tyrosinase inhibition activities (in the 3rd rank), moderate lipid peroxidation inhibition (in the 4th rank) and contained the high total unsaturated fatty acids (in the 2nd rank). The crude extract of *O. sativa* which contained the highest total unsaturated fatty acids, showed the moderate biological activities, DPPH scavenging (in the 4th rank), lipid peroxidation inhibition (in the 3rd rank), chelating (in the 4th rank), tyrosinase inhibition activities (in the 4th rank) and stimulation index (in the 6th rank). The *S. bicolor* crude extracts by scCO₂ showed the high DPPH scavenging (in the 2nd rank), chelating (in the 3rd rank), tyrosinase inhibition activities (in the 2nd rank) and moderate lipid peroxidation inhibition (in the 6th rank) and stimulation index (in the 4th rank) and contained high amount of total unsaturated fatty acids (in the 3rd rank).

3.4. Biological activities and 5 α -reductase inhibition of the semi-purified fractions from the three selected plants including *O. sativa* crude extracts

3.4.1. Unsaturated fatty acid and total phenolic contents

Fraction No. 3 of *O. sativa* crude extract showed the highest percentage yields (48.23 ± 3.56%, w/w), followed by fraction No. 1 of *S. bicolor* (43.67 ± 4.48%, w/w) and fraction No. 3 of *C. tinctorius* (37.24 ± 2.72%, w/w) crude extracts. However, fraction No. 3 of the

O. sativa crude extract contained the highest content of unsaturated fatty acids (γ -linolenic acid 7.52 ± 1.12%, w/w; linoleic acid 49.25 ± 3.67%, w/w; oleic acid 42.17 ± 4.12%, w/w), followed by the crude extract of *O. sativa* (γ -linolenic acid 5.67 ± 0.52%, w/w; linoleic acid 23.62 ± 2.62%, w/w; oleic acid 27.28 ± 1.22%, w/w) and the fraction No. 4 of the *O. sativa* crude extract (γ -linolenic acid 9.21 ± 0.78%, w/w; linoleic acid 32.07 ± 1.31%, w/w; oleic acid 13.71 ± 1.34%, w/w), respectively. The higher contents of unsaturated fatty acids were found in fraction No. 3 of *O. sativa* crude extract than in the crude extracts about 2 times because the eluent (petroleum ether/ethyl acetate 8:1) was more non-polar solvent than the salvation system of scCO₂ with 25% (w/v) of ethanol.

As known, the supercritical carbon dioxide fluid was the lipophilic solvent that can also extract the colored substances from the plant parts such as safflower and sorghum. So, the *O. sativa*, *C. tinctorius* and *S. bicolor* crude extract were in green, dark orange and dark yellow appearances which will be undesirable for cosmetic formulation. Therefore, in order to get rid of the colored substances, these crude extract was semi-purified by column chromatography. The semi-purified fraction of *O. sativa* extract fraction 3 (OSF3), *C. tinctorius* extract fraction 3 and *S. bicolor* extract fraction 1 which gave the highest unsaturated fatty acid contents were in pale yellow, pale orange and pale yellow appearances, respectively.

In addition, the crude extract of *C. tinctorius*, fraction No. 2 of the *C. tinctorius* crude extract and the crude extract of *S. bicolor* contained high total phenolic contents at 1.36 ± 0.11, 1.25 ± 0.08 and 1.05 ± 0.09 mg gallic acid equivalent/gram of extract (GAE mg/g), respectively. These crude extracts and fractions appeared to give higher phenolic contents because of the slightly polar property of the extraction condition of 25% (w/v) ethanol in scCO₂.

3.4.2. Biological activities of the semi-purified fractions

3.4.2.1. DPPH radical scavenging assay. The three extracts and fractions which gave the highest scavenging activity were the crude extract of *C. tinctorius*, fraction No. 2 of *C. tinctorius* crude extract and the crude extract of *S. bicolor* with the SC₅₀ values of 1.13 ± 0.12, 1.20 ± 0.25 and 2.16 ± 0.17 mg/ml, respectively (Table 4). However, the *C. tinctorius* crude extract which gave the highest DPPH scavenging activity showed lower activity than the standard vitamin C (SC₅₀ value of 0.04 ± 0.004 mg/ml), vitamin E (SC₅₀ value of 0.04 ± 0.002 mg/ml) of about 28 times, but exhibited higher activity than the standard unsaturated fatty acids, γ -linolenic acid (SC₅₀ value of 15.49 ± 0.02 mg/ml), linoleic acid (SC₅₀ value of 39.82 ± 0.05 mg/ml) and oleic acid (SC₅₀ value of 55.58 ± 0.9 mg/ml) of about 14, 35 and 49 times, respectively. This indicated that the unsaturated fatty acids in the fractions and the extracts may not be the only bioactive compounds which have the effect on this activity, but also other bioactive compounds such as the total phenolic compounds existing in the crude extracts and fractions. The significant positive linear correlation of free radical scavenging activity (A_F) and the total phenolic content (TPC) of the *C. tinctorius* crude extract (A_F and TPC; $r = 1.00$, $p < 0.01$) was observed, while the positive linear correlation between A_F and the total unsaturated fatty acids content (TUC) of the *C. tinctorius* crude extract was 0.85 (A_F and TUC; $r = 0.85$, $p < 0.05$). Thus, the synergistic effects of both total phenolic and unsaturated fatty acid contents had indicated, which may result in the reduction of oxidative stress in the scalp of the alopecia patients [48]. The phenolic compounds may interrupt with the free radical chain and stop further oxidation reaction [49].

3.4.2.2. Lipid peroxidation inhibition assay. The three extracts and fractions which gave the highest lipid peroxidation inhibition activity were the *O. sativa* and *C. tinctorius* crude extract and fraction No. 3 of the *O. sativa* crude extract with the IPC₅₀ values of 1.25 ± 0.51, 2.27 ± 0.14 and 3.44 ± 0.23 mg/ml, respectively.

Table 4
Comparison of the percentage yields, unsaturated fatty acid contents, stimulation index on human skin fibroblasts (30th passage) and percentages of cell viability on DU-145 cells of the fractions from *O. sativa*, *C. tinctorius* and *S. bicolor* crude extracts.

Sample	Percentage yield (%w/w)	% Unsaturated fatty acid (% w/w)			TPC (mg GAE/g)	SC ₅₀ (mg/ml)	IPC ₅₀ (mg/ml)	CC ₅₀ (mg/ml)	IC ₅₀ (mg/ml)	SI (at 0.1 mg/ml)	Cell viability (%)
		γ -Linolenic acid	Linoleic acid	Oleic acid							
<i>O. sativa</i> crude extract	–	5.67	23.62	27.28	0.65	6.55	1.25	68.23	25.98	1.10	102.45
<i>O. sativa</i> fraction No. 1	26.44	3.28	16.25	25.32	0.40	7.59	7.92	40.51	39.03	0.99	95.46
<i>O. sativa</i> fraction No. 2	21.85	3.08	24.32	24.31	0.39	7.43	11.09	45.63	32.75	0.98	107.55
<i>O. sativa</i> fraction No. 3	48.23	7.52	49.25	42.17	0.32	10.91	3.44	53.33	11.90	1.01	99.39
<i>O. sativa</i> fraction No. 4	3.38	9.21	32.07	13.71	0.37	8.49	5.09	51.18	22.44	1.03	98.78
<i>C. tinctorius</i> crude extract	–	9.36	4.02	0.65	1.36	1.13	2.27	3.58	19.47	2.60	104.14
<i>C. tinctorius</i> fraction No. 1	31.38	0.00	0.15	0.00	0.69	2.50	7.11	7.98	20.11	1.99	102.14
<i>C. tinctorius</i> fraction No. 2	22.74	0.00	0.32	0.00	1.25	1.20	8.89	6.58	31.27	1.27	97.45
<i>C. tinctorius</i> fraction No. 3	37.24	16.72	15.07	8.40	0.48	5.55	6.29	6.43	19.25	1.59	99.4
<i>C. tinctorius</i> fraction No. 4	8.24	10.80	9.86	3.48	0.36	7.54	5.16	9.56	18.56	1.23	99.81
<i>S. bicolor</i> crude extract	–	0.02	5.56	1.78	1.05	2.16	4.05	36.48	3.61	1.22	111.32
<i>S. bicolor</i> fraction No. 1	43.67	1.22	11.50	3.26	0.47	6.10	3.56	41.03	13.80	0.99	96.58
<i>S. bicolor</i> fraction No. 2	21.23	0.77	9.47	2.74	0.55	5.73	8.33	50.68	13.97	1.12	98.67
<i>S. bicolor</i> fraction No. 3	19.54	0.15	4.54	0.87	0.38	8.58	10.34	43.09	5.44	1.04	102.31
<i>S. bicolor</i> fraction No. 4	14.63	0.11	2.85	0.93	0.37	7.95	9.90	38.78	12.22	1.01	101.78
γ -Linolenic acid	–	–	–	–	–	15.49	1.93	1.74	0.77	0.39	19.67
Linoleic acid	–	–	–	–	–	39.82	1.25	14.79	2.55	0.45	37.52
Oleic acid	–	–	–	–	–	55.58	3.51	26.34	7.11	0.99	101.87
Finasteride	–	–	–	–	–	–	–	–	–	1.01	102.86
Dutasteride	–	–	–	–	–	–	–	–	–	1.02	103.27

Note: NA represented not appreciable. Total phenolic contents were presented as gallic acid equivalent (GAE) mg/g of the sample. SC₅₀ value (mg/ml) was the concentration of the sample that scavenged 50% of the DPPH radicals. IPC₅₀ value (mg/ml) was the concentration of the sample that inhibited 50% of the lipid peroxidation. CC₅₀ value (mg/ml) was the concentration of the sample that chelated 50% of the metal ion. IC₅₀ value (mg/ml) was the concentration of the sample that inhibited 50% of the tyrosinase enzyme. Stimulation index (SI) was the ratio between the percentages of cell proliferation of the treated sample (at 0.1 mg/ml) and the control (no treatment). Cell viability (%) was the percentages of the viable cell absorbance (at 0.1 mg/ml) divided by the control absorbance (no treatment) on DU-145 cell line. Fraction Nos. 1–4 were pooled and evaporated from the consecutive fractions (collected at 1 ml/min with 100 ml in each fraction) which contained the same unsaturated fatty acids to obtain the semi-purified fractions.

The *O. sativa* crude extract gave the highest lipid peroxidation inhibition activity, but lower than the standard vitamin C (IPC₅₀ value of 0.07 ± 0.009 mg/ml), vitamin E (IPC₅₀ value of 0.03 ± 0.005 mg/ml), of about 17.9 and 41.7 times, respectively, while exhibited higher activity than the standard γ -linolenic acid (IPC₅₀ value of 1.93 ± 0.22 mg/ml) and oleic acid (IPC₅₀ value of 3.51 ± 0.29 mg/ml) of about 1.5 and 2.8 times, respectively. The *O. sativa* crude extract and fraction No. 3 of the *O. sativa* crude extract indicated the positive linear correlation between the total phenolic compound (TPC) contents and the lipid peroxidation inhibition activity (A_L) at $r=0.93$ ($p<0.05$), showing that the lipid peroxidation inhibition activity (A_L) might be from the high content of the total phenolic compounds (TPC).

3.4.2.3. Metal ion chelating assay. The three extracts which gave the highest chelating activity were the *C. tinctorius* crude extract, fraction No. 3 of the *C. tinctorius* crude extract and fraction No. 2 of the *C. tinctorius* crude extract with the CC₅₀ values of 3.58 ± 0.24, 6.43 ± 0.45 and 6.58 ± 0.37 mg/ml, respectively. The *C. tinctorius* crude extract which gave the highest chelating activity, showed lower activity than the standard EDTA (CC₅₀ value of 0.15 ± 0.008 mg/ml) and the standard γ -linolenic acid (CC₅₀ value of 1.74 ± 0.32 mg/ml) of about 23.87 and 2.05 times, but exhibited higher activity than the standard linoleic acid (CC₅₀ value of 14.79 ± 1.58 mg/ml) and oleic acid (CC₅₀ value of 26.34 ± 2.93 mg/ml) of about 4.13 and 7.36 times, respectively. Although there was the high correlation coefficient (r) between the total phenolic compound and chelating activity (A_C) of the *C. tinctorius* crude extract at 0.95 ($p<0.05$), the *C. tinctorius* crude extract showed lower chelating activity than the standards. It may not contain the phenolic compounds which had the complex formation property. The previous study has reported that hydroxytyrosol, gallic acid, caffeic acid and chlorogenic acid were the strong chelator, while vanillic acid, syringic acid and ferulic acid cannot chelate the metal ion, showing that not all of the phenolic compounds had complex formation property [50]. However, some chelating activities of these extracts and fractions were observed. In addition, the high Cu level in the occipital region is correlated to the concentrations of free testosterone in the serum which can result in hair loss [51]. Thus, the extracts contained chelators can chelate the excess Cu in the hair follicle and blood, resulting of decreasing hair loss.

3.4.2.4. Tyrosinase inhibition assay. The three extracts which gave the highest tyrosinase inhibition activity were the *S. bicolor* crude extract, fraction No. 3 of the *S. bicolor* crude extract and fraction No. 3 of the *O. sativa* crude extract with the IC₅₀ values of 3.61 ± 0.18, 5.44 ± 1.18 and 11.90 ± 1.47 mg/ml, respectively. The *S. bicolor* crude extract which gave the highest tyrosinase inhibition activity showed lower activity than the standard vitamin C (IC₅₀ value of 0.035 ± 0.009 mg/ml), the standard γ -linolenic acid (IC₅₀ value of 0.77 ± 0.09 mg/ml) and linoleic acid (IC₅₀ value of 2.55 ± 0.09 mg/ml) of about 90, 5 and 1.4 times, respectively.

3.4.2.5. Cell proliferation on the aged normal human skin fibroblasts. The stimulation index (SI) of the crude extracts and their fractions on aged normal human skin fibroblasts (30th passage) was shown in Table 4. At 0.1 mg/ml, all crude extracts and fractions of *O. sativa*, *C. tinctorius* and *S. bicolor*, the standard finasteride and dutasteride and the standard oleic acid showed no cytotoxicity, and at 0.01 mg/ml the standard γ -linolenic acid and linoleic acid also gave no cytotoxicity. But, at 0.1 mg/ml the standard γ -linolenic acid and linoleic acid were toxic to the cells. In fact, linoleic acid has been shown to be toxic to tumor cells with little or no cytotoxic on normal cells at the concentration of more than 0.01 mg/ml [52]. Thus,

these extracts and fractions at 0.1 mg/ml appeared to be safe to be used topically.

3.4.2.6. Cytotoxicity on DU-145 cell line. At 0.1 mg/ml, the crude extracts and fractions of *C. tinctorius*, *O. sativa* and *S. bicolor*, the standard finasteride and dutasteride showed no cytotoxicity on DU-145 cell lines by the SRB assay (Table 4). However, at the concentration of 0.1 mg/ml, the standard γ -linolenic acid and linoleic acid were toxic to the cells. But, γ -linolenic acid at 0.001 mg/ml (105.45 ± 11.52% of the control) and linoleic acid at 0.01 mg/ml (104.43 ± 12.55% of the control) and oleic acid at 0.1 mg/ml (101.87 ± 8.47% of the control) exhibited no toxicity to cells. This indicated that the pure unsaturated fatty acids with higher unsaturation were more toxic than those with lower unsaturation since they can induce apoptosis and also necrosis in the cell [53]. Also, they were more toxic than the crude extracts and semi-purified fractions containing the fatty acids since the phenolic content in the extracts and fractions may have the protective effect against the DNA damage which caused cell death [54]. The crude extracts, their fractions, the standard finasteride, the standard dutasteride and the standard oleic acid at the highest concentration of 0.1 mg/ml and the standard γ -linolenic acid and linoleic acid at the highest concentration of 0.001 and 0.01 mg/ml, respectively, were used in the 5 α -reductase inhibition experiment.

3.4.2.7. The 5 α -reductase type 1 inhibition assay. The type 1, 5 α -reductase, is the main cause of hair loss, predominated in human scalp skin especially in dermal papilla [55]. In several studies, the DU-145 human androgen insensitive prostate adenocarcinoma cell line which has the type 1, 5 α -reductase has been used for the 5 α -reductase type 1 inhibition assay [56,57]. The fraction No. 3 of the *O. sativa* crude extract at 0.1 mg/ml showed the highest 5 α -reductase type 1 inhibition activity on DU-145 cell line at 93.33 ± 10.93% of the control which was higher than the standard finasteride (57.07 ± 6.52%) and dutasteride (76.56 ± 4.76%) of about 1.64 and 1.22 times, respectively (Fig. 1). The percentages inhibition on 5 α -reductase (type 1) of γ -linolenic acid (0.001 mg/ml) and oleic acid (0.1 mg/ml) were only at 38.72 ± 5.36 and 35.69 ± 5.01%, respectively, which was lower than fraction No. 3 of the *O. sativa* crude extract at about 2.41 and 2.61 times, respectively. However, the standard linoleic acid (0.01 mg/ml) showed similar inhibition activity (94.17 ± 8.27%) to the *O. sativa* fraction No. 3. Moreover, the inhibition activity of the standard linoleic acid was also dose-dependent which was in the same trend as fraction No. 3 of the *O. sativa* crude extract. When linoleic acid was increased from 0.0001, 0.001 and 0.01 mg/ml, it gave an increasing inhibition at 51.23 ± 5.38, 61.58 ± 7.30 and 94.17 ± 8.27%, respectively ($y=4047x+54.01$, $r^2=0.977$). Thus, linoleic acid appeared to play the main role for the inhibition of the 5 α -reductase inhibition activity. Besides linoleic acid, other bioactives existing in the fractions, such as ferulic acid, vanillic acid, γ -oryzanol and phytic acid [58] in the fraction No. 3 of the *O. sativa* crude extract may be synergistic for this activity. For fraction No. 3 of the *O. sativa* crude extract, the significant positive linear correlation between the total unsaturated fatty acids and 5 α -reductase inhibition activity (TUC and 5AR; $r=1.00$, $p<0.01$) and linoleic acid contents and 5AR (LN and 5AR; $r=1.00$, $p<0.01$) were observed. Moreover, the 5 α -reductase inhibition activity of the fraction No. 3 of the *O. sativa* crude extract also correlated with the antioxidative activities including, free radical scavenging activity (A_F and 5AR; $r=0.98$, $p<0.05$), lipid peroxidation inhibition activity (A_L and 5AR; $r=0.95$, $p<0.05$) and chelating activity (A_C and 5AR; $r=0.89$, $p<0.05$) and its phenolic contents (TPC and 5AR; $r=0.97$, $p<0.05$). This indicated that the 5 α -reductase inhibition activity of the fraction No. 3 of the *O. sativa* crude extract was related to antioxidant activities and the phenolic contents. For DPPH radical scavenging activity, inadequate

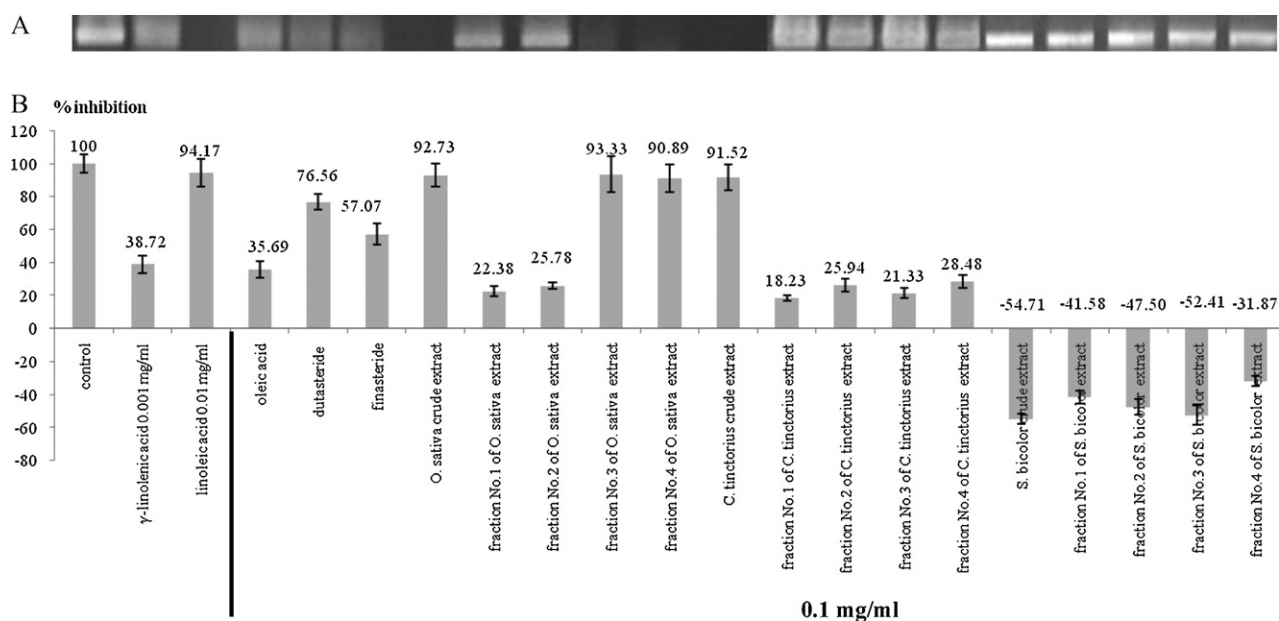


Fig. 1. The 5α-reductase (type 1) inhibition on DU-145 cells at 0.1 mg/ml of *O. sativa*, *C. tinctorius* and *S. bicolor* crude extracts and their fractions in comparing to the standards finasteride (0.1 mg/ml), dutasteride (0.1 mg/ml), standard unsaturated fatty acids, γ-linolenic acid (0.001 mg/ml), linoleic acid (0.01 mg/ml) and oleic acid (0.1 mg/ml). (A) agarose gel electrophoresis of dsDNA of 5α-reductase (type 1) enzyme after the inhibition of the samples and (B) the percentages of 5α-reductase (type 1) inhibition. %Inhibition = [(control – sample)/control] × 100.

Table 5

The correlation matrix (Pearson's correlation coefficients) of the unsaturated fatty acid contents, total phenolic contents, stimulation index on human skin fibroblasts (passage 30th) and the 5α-reductase inhibition activity of *O. sativa* crude extract, fraction No. 3 of *O. sativa* crude extract and *C. tinctorius* crude extract (at 95% confidence interval).

Extract/fraction	Variable	GLA	LN	OL	TUC	TPC	A _F	A _L	A _C	A _T	SI	5AR
<i>O. sativa</i> crude extract	GLA	–										
	LN	0.88	–									
	OL	0.82	0.82	–								
	TUC	0.78	0.98	0.91	–							
	TPC	0.94	0.93	0.93	0.95	–						
	A _F	0.91	0.89	0.91	0.91	0.96	–					
	A _L	0.91	0.87	0.89	0.92	0.93	0.84	–				
	A _C	0.93	0.88	0.91	0.89	0.95	0.78	0.88	–			
	A _T	0.90	0.91	0.89	0.88	0.92	0.83	0.89	0.79	–		
	SI	0.91	0.87	0.93	0.85	0.82	0.91	0.93	0.91	0.90	–	
	5AR	0.95	0.99 [*]	0.97	0.99 [*]	0.95	0.95	0.93	0.91	0.89	0.91	–
Fraction No. 3 of <i>O. sativa</i> crude extract	GLA	–										
	LN	0.87	–									
	OL	0.82	0.82	–								
	TUC	0.76	0.99 [*]	0.91	–							
	TPC	0.89	0.93	0.93	0.95	–						
	A _F	0.93	0.89	0.91	0.91	0.97	–					
	A _L	0.94	0.87	0.89	0.92	0.93	0.84	–				
	A _C	0.89	0.88	0.91	0.89	0.95	0.78	0.88	–			
	A _T	0.90	0.91	0.89	0.88	0.92	0.83	0.89	0.79	–		
	SI	0.90	0.87	0.93	0.85	0.82	0.91	0.93	0.91	0.90	–	
	5AR	0.96	1.00 ^{**}	0.95	1.00 ^{**}	0.97	0.98	0.95	0.90	0.93	0.93	–
<i>C. tinctorius</i> crude extract	GLA	–										
	LN	0.84	–									
	OL	0.79	0.80	–								
	TUC	0.89	0.85	0.76	–							
	TPC	0.87	0.82	0.73	0.85	–						
	A _F	0.78	0.83	0.89	0.85	1.00 ^{**}	–					
	A _L	0.87	0.78	0.71	0.84	0.98	0.84	–				
	A _C	0.67	0.74	0.87	0.73	0.95	0.67	0.78	–			
	A _T	0.80	0.71	0.89	0.88	0.88	0.83	0.89	0.79	–		
	SI	0.88	0.85	0.88	0.89	0.91	0.94	0.93	0.89	0.79	–	
	5AR	0.89	0.95	0.90	0.93	0.98	0.91	0.90	0.83	0.89	0.92	–

Note: GLA: γ-linolenic acid content; LN: linoleic acid content, OL: linoleic acid content, TUC: total unsaturated fatty acid content, TPC: total phenolic content, A_F: free radical scavenging activity, A_L: lipid peroxidation inhibition activity, A_C: chelating activity, A_T: tyrosinase inhibition activity, SI: stimulation index, 5AR: 5α-reductase inhibition activity.

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

antioxidant protection or excess production of reactive oxygen species (ROS) creates a condition known as oxidative stress, which can cause hair loss [59,60]. In fact, the relationship between the free radical scavenging activity and the anti-androgenic alopecia activity can be anticipated, since there is a balance between the oxidative damage and antioxidant protection in normal aerobic cells. This study has shown the correlation of A_F and the 5α -reductase inhibition activity (5AR) of the *C. tinctorius* crude extract (A_F and 5AR; $r=0.91$, $p<0.05$) (Table 5). For lipid peroxidation activity, lipid peroxidation can be caused by free radicals which can initiate the free radical chain reaction on the cell membrane in hair dermal papilla. Thus, these extracts and fractions may inhibit free radicals which initiated lipid peroxidation reaction in cell membrane involved hair dermal papilla, resulting in cell damage and hair shedding [61]. Moreover, the metal ion chelating activity may be able to reduce the excess of Cu level in the occipital area and also in the blood circulation of the androgenic alopecia patient [51]. The moderate tyrosinase inhibition activity of these fractions and extracts might be useful for the 5α -reductase inhibition activity by binding with the enzyme that can have the synergistic effect with other compounds.

The *O. sativa* and *C. tinctorius* crude extracts and fraction No. 4 of the *O. sativa* crude extract also exhibited marked 5AR inhibition activity at 92.73 ± 6.91 , 91.52 ± 7.69 and $90.89 \pm 8.5\%$ of the control, respectively, while the *S. bicolor* crude extract and their fractions showed the negative result which stimulated the production of 5α -reductase enzyme. Similarly, the fractions of *C. tinctorius* crude extract exhibited low 5AR inhibition activity. The significant positive linear correlation between TUC and 5AR ($r=0.99$, $p<0.05$) and LN and 5AR ($r=0.99$, $p<0.05$) of the *O. sativa* crude extracts were shown. The *C. tinctorius* crude extracts showed positive correlation between TUC and 5AR and between LN and 5AR at linear relationship of $r=0.93$ and 0.95 , $p<0.05$, respectively. The unsaturated fatty acids may have less effect on the 5α -reductase inhibition activity than TPC with the correlation at (TPC and 5AR, $r=0.98$, $p<0.05$) in the *C. tinctorius* crude extracts.

4. Conclusion

The crude extracts from the three edible plants, including *O. sativa*, *C. tinctorius* and *S. bicolor* prepared by $scCO_2$ method which showed high unsaturated fatty acids contents and anti-oxidative activities, including the DPPH radical scavenging, lipid peroxidation inhibition, metal ion chelating, tyrosinase inhibition activities, stimulation index on human normal skin fibroblast, were selected from ten edible plants to prepare the semi-purified fractions because of not only the non-toxic, non-inflammatory and inexpensive but also the high solvating power of the $scCO_2$ extraction. The synergistic effect of the unsaturated fatty acids and the phenolic compounds containing in these plants were responsible for these biological activities. Fraction No. 3 of *O. sativa* crude extract, the *O. sativa* and *C. tinctorius* crude extracts demonstrated high antioxidant activities (A_F , A_L and A_C) and had high total phenolic contents which showed positive linear correlations to the 5α -reductase inhibition activity (5AR) with $r>0.95$ ($p<0.05$) that can reinforce the healthy tissue around the hair follicles leading to the reduction of the shedding hair before the telogen phase.

Moreover, fraction No. 3 of the *O. sativa* crude extract gave the highest content of unsaturated fatty acids (γ -linolenic acid $7.52 \pm 1.12\%$, w/w; linoleic acid $49.25 \pm 3.67\%$, w/w; oleic acid $42.17 \pm 4.12\%$, w/w), high anti-oxidative activities with high stimulation index on human normal skin fibroblast (stimulation index = 1.01 ± 0.34) and the highest 5α -reductase (type 1) inhibition ($93.33 \pm 10.93\%$ of control) on DU-145 prostate cancer cell line. The linoleic acid content (LN) and the total unsaturated fatty acid

content (TUC) in fraction No. 3 of the *O. sativa* crude extract were significantly related to the 5α -reductase inhibition activity (5AR) (LN and 5AR; $r=1.00$, $p<0.01$) and (TUC and 5AR; $r=1.00$, $p<0.01$). In addition, the positive correlation between 5α -reductase inhibition and antioxidant activity of fraction No. 3 of the *O. sativa* crude extract, free radical scavenging activity (A_F and 5AR; $r=0.98$, $p<0.05$), lipid peroxidation inhibition activity (A_L and 5AR; $r=0.95$, $p<0.05$) and chelating activity (A_C and 5AR; $r=0.89$, $p<0.05$) were observed. Therefore, the genetic anti-hair loss activity of the crude extracts and fractions prepared by $scCO_2$ from these three plants, especially fraction No. 3 of the *O. sativa* crude extract can be anticipated since they inhibited the 5α -reductase mRNA production which can lead to the formation of 5α -reductase type 1 protein, the major cause of hair loss.

Acknowledgements

This work was supported by the Thailand Research Fund (TRF) under the Royal Golden Jubilee – PhD (RGJ – PhD) program in Thailand, Natural Products Research and Development Center (NPRDC), Science and Technology Research Institute (STRI), Chiang Mai University, Thailand.

References

- [1] R. Irving, Baldness cure firm heads for AIM, in: The Times, Times Newspapers Ltd., England, 2007.
- [2] N.A. Hibberts, A.E. Howell, V.A. Randall, Balding hair follicle dermal papilla cells contain higher levels of androgen receptors than those from non-balding scalp, *Journal of Endocrinology* 156 (1998) 59–65.
- [3] M.E. Sawaya, V.H. Price, Different levels of 5 alpha-reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenetic alopecia, *Journal of Investigative Dermatology* 109 (1997) 296–300.
- [4] R.M. Trüeb, Molecular mechanisms of androgenetic alopecia, *Experimental Gerontology* 37 (2002) 981–990.
- [5] G. Teutsch, F. Goubet, T. Battmann, A. Bonfils, F. Bouchoux, E. Cerede, D. Gofflo, M. Gaillard-Kelly, D. Philibert, Non-steroidal antiandrogens: synthesis and biological profile of high-affinity ligands for the androgen receptor, *The Journal of Steroid Biochemistry and Molecular Biology* 48 (1994) 111–119.
- [6] H.C. Eun, O.S. Kwon, J.H. Yeon, H.S. Shin, B.Y. Kim, B.I. Ro, H.K. Cho, W.Y. Sim, B.L. Lew, W.-S. Lee, H.Y. Park, S.P. Hong, J.H. Ji, Efficacy, safety, and tolerability of dutasteride 0.5 mg once daily in male patients with male pattern hair loss: a randomized, double-blind, placebo-controlled, phase III study, *Journal of the American Academy of Dermatology* 63 (2010) 252–258.
- [7] M.A. Titus, M.J. Schell, F.B. Lih, K.B. Tomer, J.L. Mohler, Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer, *Clinical Cancer Research* 11 (2005) 4653–4657.
- [8] S.-S. Rho, S.-J. Park, S.-L. Hwang, M.-H. Lee, C.D. Kim, I.-H. Lee, S.-Y. Chang, M.-J. Rang, The hair growth promoting effect of *Asiasari radix* extract and its molecular regulation, *Journal of Dermatological Science* 38 (2005) 89–97.
- [9] K. Datta, A.T. Singh, A. Mukherjee, B. Bhat, B. Ramesh, A.C. Burman, *Eclipta alba* extract with potential for hair growth promoting activity, *Journal of Ethnopharmacology* 124 (2009) 450–456.
- [10] G.-S. Lee, E.-J. Hong, K.-S. Gwak, M.-J. Park, K.-C. Choi, I.-G. Choi, J.-W. Jang, E.-B. Jeung, The essential oils of *Chamaecyparis obtusa* promote hair growth through the induction of vascular endothelial growth factor gene, *Fitoterapia* 81 (2010) 17–24.
- [11] J.I. Yoon, S.M. Al-Reza, S.C. Kang, Hair growth promoting effect of *Zizyphus jujuba* essential oil, *Food and Chemical Toxicology* 48 (2010) 1350–1354.
- [12] S.-S. Roh, C.D. Kim, M.-H. Lee, S.-L. Hwang, M.-J. Rang, Y.-K. Yoon, The hair growth promoting effect of *Sophora flavescens* extract and its molecular regulation, *Journal of Dermatological Science* 30 (2002) 43–49.
- [13] T. Liang, S. Liao, Inhibition of steroid 5 alpha-reductase by specific aliphatic unsaturated fatty acids, *Biochemical Journal* 285 (1992) 557–562.
- [14] M.M. Most, R. Tully, S. Morales, M. Lefevre, Rice bran oil, not fiber, lowers cholesterol in humans, *American Journal of Clinical Nutrition* 81 (2005) 64–68.
- [15] R. Tahira, R. Ata-ur, A.B. Muhammad, Characterization of rice bran oil, *Journal of Agricultural Research* 45 (2007) 225–230.
- [16] Z. Shen, M.V. Palmer, S.S.T. Ting, R.J. Fairclough, Pilot scale extraction and fractionation of rice bran oil using supercritical carbon dioxide, *Journal of Agricultural Food Chemistry* 45 (1997) 4540–4544.
- [17] X. Chen, D. Ahn, Antioxidant activities of six natural phenolics against lipid oxidation induced by Fe^{2+} or ultraviolet light, *Journal of the American Oil Chemist's Society* 75 (1998) 1717–1721.
- [18] H.B. MacDonald, Conjugated linoleic acid and disease prevention: a review of current knowledge, *Journal of the American College of Nutrition* 19 (2000) 111s–118s.

- [19] M.M. Chakrabarty, Rice bran – a new source for edible and industrial oil, in: R.E. David (Ed.), *The World Conference on Edible Fats and Oils Processing – Basic Principles and Modern Practices*, Maastricht, The Netherlands, 1989.
- [20] R.N. Sayre, R.M. Saunders, *Lipid Technologies and Applications: Rice Bran and Rice Bran Oil*, Marcel Dekker, New York, 1990.
- [21] M.S. Kuk, M.K. Dowd, Supercritical CO₂ extraction of rice bran, *Journal of the American Oil Chemists Society* 75 (1998) 623–628.
- [22] M. Hiramatsu, M. Komatsu, Y. Xu, Y. Kasahara, *In vitro* and *in vivo* study of antioxidant action in food plant (*Carthamus tinctorius*), *Pathophysiology* 5 (1998), 79–79(71).
- [23] A.B. Caragay, Cancer-preventive foods and ingredients, *Food Technology* 56 (1992) 65–68.
- [24] C.A. Rice-Evans, N.T. Miller, G. Paganga, Antioxidant properties of phenolic compounds, *Trends in Plant Science* 4 (1997) 304–309.
- [25] A. Manosroi, R. Chutoprapat, M. Abe, J. Manosroi, Characteristics of niosomes prepared by supercritical carbon dioxide (scCO₂) fluid, *International Journal of Pharmaceutics* 352 (2008) 248–255.
- [26] F. Sahena, I.S.M. Zaidul, S. Jinap, A.A. Karim, K.A. Abbas, N.A.N. Norulaini, A.K.M. Omar, Application of supercritical CO₂ in lipid extraction – a review, *Journal of Food Engineering* 95 (2009) 240–253.
- [27] A. García, A. de Lucas, J. Rincón, A. Alvarez, I. Gracia, M. García, Supercritical carbon dioxide extraction of fatty and waxy material from rice bran, *Journal of the American Oil Chemists' Society* 73 (1996) 1127–1131.
- [28] J.W. Goodrum, M.B. Kilgo, Peanut oil extraction with SCCO₂: solubility and kinetic functions, *Transactions of the American Society of Agricultural Engineers* 30 (1987) 1865–1868.
- [29] J.P. Friedrich, G.R. List, Characterization of soybean oil extracted by supercritical carbon dioxide and hexane, *Journal of Agricultural and Food Chemistry* 30 (2002) 192–193.
- [30] A. Manosroi, W. Ruksiriwanich, M. Abe, H. Sakai, W. Manosroi, J. Manosroi, Biological activities of the rice bran extract and physical characteristics of its entrapment in niosomes by supercritical carbon dioxide fluid, *The Journal of Supercritical Fluids* 54 (2010) 137–144.
- [31] S.T. Allen, *Chemical Analysis of Ecological Material*, Blackwell Scientific Publication, New York, 1974.
- [32] J.R. Harbone, *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*, Charpan & Hall, London, 1976.
- [33] F. VanMiddlesworth, R.J.P. Cannell, *Dereplication and Partial Identification of Natural Products, Methods in Biotechnology: Natural Product Isolation*, Humana Press, New Jersey, 1998.
- [34] K.M. Yoo, C.H. Lee, H. Lee, B. Moon, C.Y. Lee, Relative antioxidant and cytoprotective activities of common herbs, *Food Chemistry* 106 (2008) 929–936.
- [35] A. Chatterjee, P.K. Dutta, R. Chowdhury, Effect of fatty acids and cholesterol present in bile on expression of virulence factors and motility of *Vibrio cholerae*, *Infection and Immunity* 75 (2007) 1946–1953.
- [36] K.T. Papazisis, G.D. Geromichalos, K.A. Dimitriadis, A.H. Kortsaris, Optimization of the sulforhodamine B colorimetric assay, *Journal of Immunological Methods* 208 (1997) 151–158.
- [37] J.M. Torres, E. Ortega, Quantitation of mRNA levels of steroid 5[alpha]-reductase isozymes: a novel method that combines quantitative RT-PCR and capillary electrophoresis, *The International Journal of Biochemistry & Cell Biology* 36 (2004) 78–88.
- [38] X. Lou, H.G. Janssen, C.A. Cramers, Effects of modifier addition and temperature variation in SFE of polymeric materials, *Journal of Chromatographic Science* 34 (1996) 282–290.
- [39] J. Chrastil, Solubility of solids and liquids in supercritical gases, *The Journal of Physical Chemistry* 86 (1982) 3016–3021.
- [40] Y. Naudé, W.H.J. de Beer, S. Jooste, L. van der Merwe, S.J. van Rensburg, Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of DDT, DDD and DDE in sediment, *Water SA* 24 (1998) 205–214.
- [41] Y. Jin, Y.-s. Xiao, F.-f. Zhang, X.-y. Xue, Q. Xu, X.M. Liang, Systematic screening and characterization of flavonoid glycosides in *Carthamus tinctorius* L. by liquid chromatography/UV diode-array detection/electrospray ionization tandem mass spectrometry, *Journal of Pharmaceutical and Biomedical Analysis* 46 (2008) 418–430.
- [42] G.M. Weisz, D.R. Kammerer, R. Carle, Identification and quantification of phenolic compounds from sunflower (*Helianthus annuus* L.) kernels and shells by HPLC-DAD/ESI-MSn, *Food Chemistry* 115 (2009) 758–765.
- [43] Y.S. Velioglu, G. Mazza, Y.L. Gao, B.D. Oomah, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, *Journal of Agricultural and Food Chemistry* 46 (1998) 4113–4117.
- [44] K. Kazuma, T. Takahashi, K. Sato, H. Takeuchi, T.T.O. Matsumoto, T. Okumo, Quinochalcones and flavonoids from fresh florets in different cultivars of *Carthamus tinctorius* L., *Bioscience Biotechnology & Biochemistry* 64 (2000) 1588–1599.
- [45] R. Palter, R.E. Lundin, W.F. Haddon, A cathartic lignan glycoside isolated from *Carthamus tinctorius*, *Phytochemistry* 11 (1972) 2871–2874.
- [46] T. Akihisa, K. Yasukawa, H. Oinuma, Y. Kasahara, S. Yamanouchi, M. Takido, K. Kumaki, T. Tamura, Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects, *Phytochemistry* 43 (1996) 1255–1260.
- [47] T. Hirokawa, S. Hirokawa, N. Yamauchi, T. Kataoka, J.-T. Woo, K. Nagai, Immunomodulating activities of polysaccharide fractions from dried safflower petals, *Cytotechnology* 25 (1997) 205–211.
- [48] M. Naziroglu, I. Kokcam, Antioxidants and lipid peroxidation status in the blood of patients with alopecia, *Cell Biochemistry and Function* 18 (2000) 169–173.
- [49] E.R. Sherwin, Oxidation and antioxidants in fat and oil processing, *Journal of the American Oil Chemist's Society* 55 (1978) 809–841.
- [50] M. Andjelkovic, J. Van Camp, B. De Meulenaer, G. Depaemelaere, C. Socaciu, M. Verloo, R. Verhe, Iron-chelation properties of phenolic acids bearing catechol and galloyl groups, *Food Chemistry* 98 (2006) 23–31.
- [51] M.G. Skalnaya, V.P. Tkachev, Trace elements content and hormonal profiles in women with androgenetic alopecia, *Journal of Trace Elements in Medicine and Biology* 25 (2011) S50–S53.
- [52] X. Lu, H. Yu, Q. Ma, S. Shen, U. Das, Linoleic acid suppresses colorectal cancer cell growth by inducing oxidant stress and mitochondrial dysfunction, *Lipids in Health and Disease* 9 (2010) 106.
- [53] M.F. Cury-Boaventura, C. Pompeia, R. Curi, Comparative toxicity of oleic acid and linoleic acid on Raji cells, *Nutrition* 21 (2005) 395–405.
- [54] W. Greenrod, M. Fenech, The principal phenolic and alcoholic components of wine protect human lymphocytes against hydrogen peroxide- and ionizing radiation-induced DNA damage *in vitro*, *Mutagenesis* 18 (2003) 119–126.
- [55] D. Thiboutot, G. Harris, V. Iles, G. Cimis, K. Gilliland, S. Hagari, Activity of the type 1, 5 alpha reductase exhibits regional differences in isolated sebaceous glands and whole skin, *Journal of Investigative Dermatology* 105 (1995) 209–214.
- [56] S. Délos, C. Iehlé, P.-M. Martin, J.-P. Raynaud, Inhibition of the activity of [5]basic' 5[alpha]-reductase (type 1) detected in DU 145 cells and expressed in insect cells, *The Journal of Steroid Biochemistry and Molecular Biology* 48 (1994) 347–352.
- [57] V.D. Handratta, T.S. Vasaitis, V.C. Njar, L.K. Gediya, R. Kataria, P. Chopra, D.R.F. Newman Jr., Z. Guo, Y. Qiu, A.M. Brodie, Novel C-17-heteroaryl steroidal CYP17 inhibitors/antiandrogens: synthesis, *in vitro* biological activity, pharmacokinetics, and antitumor activity in the LAPC4 human prostate cancer xenograft model, *Journal of Medicinal Chemistry* 48 (2005) 2972–2984.
- [58] P.K. Das, A. Chaudhuri, T.N.B. Kaimal, U.T. Bhalerao, Isolation of gamma-oryzanol through calcium ion induced precipitation of anionic micellar aggregates, *Journal of Agricultural and Food Chemistry* 46 (1998) 3073–3080.
- [59] W. Löntz, A. Sirsjö, W. Liu, M. Lindberg, O. Rollman, H. Törmä, Increased mRNA expression of manganese superoxide dismutase in psoriasis skin lesions and in cultured human keratinocytes exposed to IL-1[beta] and TNF-[alpha], *Free Radical Biology and Medicine* 18 (1995) 349–355.
- [60] Y. Miyachi, Photoaging from an oxidative standpoint, *Journal of Dermatological Science* 9 (1995) 79–86.
- [61] U.N. Das, Tumorcidal action of cis-unsaturated fatty acids and its relationship to free radicals and lipid peroxidation, *Cancer Letters* 56 (1991) 235–243.