EVALUATION OF ESTROGEN-LIKE EFFECT OF 13 HERBAL MEDICINES FOR ANTI-SKIN-AGEING PRODUCT

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Abstract: The objective of present study was to search for the appropriate herbal extracts by comparatively analysis for their biological activities. Thirteen herbal extracts with potential estrogenic activity were investigated by E-screen assay. The extracts having a promising activity were further evaluated in vitro for their anti-oxidation, anti-protein glycation, and cytotoxicity activities. Results showed 10 extracts had estrogenic properties namely *Pueraria candollei* var. *mirifica, Linum usitatissimum, Glycine max, Curcuma aeruginosa, Cissus quadrangularis, Tadehagi godefroyanum, Curcuma comosa, Butea superba, Trigonella foenum-graecum,* and *Punica granatum. P. candollei* var. *mirifica* exhibited the highest estrogenic relative potency but its potency was less than 17β-estradiol. The proliferative activity of those extracts could be completely inhibited by addition of an estrogen receptor antagonist. The extract of *T. foenum-graecum* exhibited the strongest cytotoxicity on mouse fibroblast cells (cell viability <80% at 100 μg/ml) while growth promoting effect could be observed for *P. candollei* var. *mirifica, C. aeruginosa, C. quadrangularis,* and *C. comosa.* Pre-treatment of those 10 extracts to the mouse fibroblast cells prior to addition of H_2O_2 reduced the apoptotic cells as well as increased the percentage of cell survival. However, all herbal extracts showed low anti-oxidative and anti-nonenzymatic protein glycation activities. Therefore, 9 extracts, except for *T. foenum-graecum,* were selected as the potentially active ingredients for the treatment of skin-ageing in postmenopausal women.

Introduction

One of important problems associated with skin ageing in postmenopausal women is deficiency of endogenous estrogen level in blood circulation [1]. According to this theory, estrogen replacement therapy is presented as new approach to reverse features of skin ageing. Estrogens could improve several features of skin ageing [2-3]. However, the serious adverse side effect caused by continuous reliance of estrogens should be caution in cosmetic products since they may sometimes associated with risk of stimulation in the growth of estrogen-dependent tumors in certain groups of consumers. Additionally, some women have unusual vaginal bleeding or a history of blood clots as well as consumers who have liver disease should avoid using these drugs [4].

To date, phytoestrogens are found beneficial to skin ageing in postmenopausal women [5]. These efficacies are through the exertion of estrogen-mimicking effect via the structural similarity to estrogens. However, most studies have determined the estrogenic activity of individual pure compounds or herb species [6-8]. Comparative investigation of estrogenic activity of most herbs containing phytoestrogens have not been elucidated in order to select the more appropriate one to be used as compositions in anti-skin-ageing products.

The present study was aimed to search for the appropriate herbal extracts by comparative analysis for their biological activities. The herbal extract with good estrogen-like effect and low degree of cytotoxicity would be further used as active ingredient in anti-skin-ageing preparation. To achieve this aspect, crude ethanolic extract of 13 herbal medicines was investigated for their biological activities. The criteria for selecting the suitable extracts were good estrogen-like effect, low cytotoxicity, evident data reported, and information from indigenous practioners. Moreover, their other biological activities associated with anti-ageing effect, such as protective effect against oxidative stress, anti-oxidation, and anti-nonenzymatic protein glycation were also compared.

Materials and Methods

Chemicals: The following chemicals were used in this study: 17β-estradiol (E2), ICI 182780, ascorbic acid, trolox, quercetin (Sigma-Aldrich Inc., St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum albumin (Gibco, Invitrogen Corporation, NY, USA). All chemicals were of analytical grade or higher.

Herbal extraction: Thirteen herbal medicines were identified. The herbal powders were extracted by maceration in ethyl alcohol. The macerated mixtures were pooled and solvents were removed under vacuum of a rotary evaporator at 45±1 °C (BÜCHI, Flawil, Switzerland). The resulting extracts were kept at -20°C until use.

E-screen assay: Estrogenic/anti-estrogenic activity of herbal extracts was carried out in estrogen receptor positive human mammary adenocarcinoma (MCF-7) cells using MTT assay [9]. The solution of 0.1 nM E2

was used as a positive control and 0.1% dimethyl sulfoxide as a negative control.

Cytotoxicity study: Toxicity of herbal extracts was investigated in mouse fibroblast cells using MTT assay [10]. Doxorubicin HCl was used as a positive control. The cell proliferative/anti-proliferative effects were expressed as percentage of cell survival.

Protective effect against H_2O_2-induced oxidative stress: Protective effect of selected phytoestrogenic extracts on the H_2O_2-induced oxidative stress was evaluated in mouse fibroblast cells using MTT assay [11]. The cells were treated with various concentrations of those extracts for 2 hours. After that, H_2O_2 (1000 μM) was added and incubated for an additional 24 hours.

Antioxidative capacity: Antioxidative capacity of herbal extracts was determined using ferric reducing antioxidant power (FRAP) [12] and $ABTS^{\bullet+}$ free radical decolorization assays [13].

Antinonenzymatic protein glycation: Effect of herbal extracts on inhibition effect of protein glycation was slightly modified from method previously described [14]. The mixture of bovine serum albumin, glucose and herbal extract was incubated at 55°C for 40 hours. The amount of glycated protein was read at the excitation and emission wavelength of 330 nm and 440 nm. Results were expressed as percent inhibition of advanced glycation end products. Quercetin was served as positive control.

Statistical analysis: Data of experimental investigation were analyzed by one-way analysis of variance. In all cases, a minimal level of significance was set at $p<0.05$ using SPSS software version 15 for Windows (SPSS Inc., Chicago, USA).

Results and Discussion

Estrogen-like activity of 13 herbal extracts was examined in MCF-7 cells and the results were shown in Figure 1. Ten extracts exhibited growth-promoting effect in MCF-7 cells. The extract of *P. candollei* var. *mirifica* gave the highest level in growth promoting activity. It significantly stimulated cell proliferation at concentrations of 0.1 – 50 μg/ml ($p<0.05$) whereas higher concentration (100 μg/ml) suppressed the growth of such cells. The maximal proliferative effect of this extract was achieved at 50 μg/ml which higher than effect displayed by 0.1 nM E2. The extract of *P. granatum* pericarp exhibited the lowest estrogen-like effect. Overall, the estrogenic activity of all test extracts could be classified into 3 groups as follows: (i) strong estrogenicity (*P. candollei* var. *mirifica, L. utitatissimum, G. max*), (ii) moderate estrogenicity (*C. aeruginosa, C. quadrangularis, T. godefroyanum, C. curcuma, B. superba*), and (iii) weak estrogenicity (*T. foenum-graecum, P. granatum*). The remaining 3 extracts namely *T. cruciatum, C. argentatum* and *E. hirta* were excluded from this study because they did not exert either proliferative or anti-proliferative effect in the cells. These findings were in agreement with other reports that low concentrations of phytoestrogenic extracts stimulated the growth of MCF-7 cells but at high concentrations they inhibited the growth of the cells [15]. This is because most phytoestrogens strongly bind to ERβ than to ERα This property can cause either agonistic or antagonistic effect depended on target of organ. The difference in estrogenic activities of these extracts could be caused from their phytoestrogenic contents and chemical structures.

To evaluate whether possible mechanism of action of 10 extracts on the growth of MCF-7 cells, co-treatment of the cells with each herbal extract plus estrogen receptor antagonist (10 nM ICI 182780) was examined. In the absence of this inhibitor, all extracts significantly stimulated the growth of MCF-7 cells in a dose-dependent manner at all test concentrations (data not shown). Neither the cell treated with E2 nor herbal extracts in combination with ICI 182780 had any effect on the growth of the cells. These co-treatments led to decrease in value of relative proliferative effect by almost 50% of E2 did ($p<0.05$). The values obtained were not significantly different compared to effect of untreated cells. These results indicated that the phytoestogenic substances in the extracts exerted their estrogenic activities through estrogen receptor pathway in such cells.

Cytotoxicity of 10 herbal extracts was investigated in mouse fibroblast cells using MTT assay. Figure 2 illustrates the cytotoxicity of those extracts in term of cell viability to untreated cells. Cell viability of doxirubin HCl (20 μg/ml, positive control) was about 70% for all experiments. Among 10 extracts tested, 8 extracts did not shown detectable cytotoxicity over the concentration tested. However, cytotoxic effect was observed when the cells were treated with high concentrations of *C. aeruginosa* extract (50 and 100 μg/ml) and *T. foenum-graecum* (25, 50, and 100 μg/ml). High concentration of *P. candollei* var. *mirifica* (25 μg/ml or higher) could significantly stimulate the growth of the cells as compared to untreated cells with cell viability of 104.84±1.84% ($p<0.05$), 109.34±0.38% ($p<0.05$), and 111.80±1.34% ($p<0.01$) for 25, 50, and 100 μg/ml. *C. quadrangularis* extract promoted the growth of cells to 113.33±5.75% and 116.01±3.62% of control at 50 and 100 μg/ml, respectively. *C. comosa* extract at concentrations between 10 – 50 μg/ml stimulated cell growths while no effect was observed at 100 μg/ml. At low concentration (0.1 and 1 μg/ml), *C. aeruginosa* extract promoted cell growth. Four extracts of *L. usitatissimum* seeds, *G. max* seeds, *T. godefroyanum* roots, and *P. granatum* pericarp showed no effect on the growth or death of the cells ($p>0.05$). It is well documented that the safe herbal extracts for dermal use should have the viability of the cells higher than 80% after exposure to 100 μg/ml extract [16]. Therefore, 9 extracts represented as promising candidates for cosmetic use, except for *T. foenum-graecum.*

The protective effect of 10 herbal extracts against oxidative stress induced by H_2O_2 was investigated in mouse fibroblast cells. Results showed that cell

survival was slightly increased in a dose dependent manner (data not shown). No significant increase in cell viability was observed for cells pre-treated with 1 μg/ml of all herbal extracts ($p>0.05$). However, viabilities of cells pre-treated with 10 μg/ml *L. utitatissimum* and *T. foenum-graecum* increased to 70.73±1.82 and 72.56±2.51% of control. The highest level for cell viabilities was observed when cells were pretreated with 100 μg/ml herbal extracts. These results suggested that 10 herbal extracts could protect cell death under oxidative stress. The mechanism of those extract might partly associate with their anti-oxidative activities. Previous reports confirmed these findings that phytoestrogens or phytoestrogenic extracts could protect cells from oxidative-induced cell death [11,17]

The anti-oxidation and anti-glycation properties of 10 extracts were also investigated. As expected, all extracts showed high IC_{50} values for both FRAP, $ABTS^{\bullet+}$, and glucose-bovine serum albumin glycation assays (data not shown). This was because they were crude extracts which contain a mixture of both active and non-active compounds. Moreover, the presence of low content of total phenolics and flavonoids also contributed to weak activity for such properties. However, the weak properties of such extracts might play synergistic of action together with strong estrogenicity when incorporation them into cosmetic preparation.

Conclusions

This work demonstrated that 10 extracts exhibited estrogen-like effect in MCF-7 cells. These included *P. candollei* var. *mirifica*, *L. usitatissimum*, *G. max*, *C. aeruginosa*, *C. quadrangularis*, *T. godefroyanum*, *C. comosa*, *B. superba*, *T. foenum-graecum*, and *P. granatum*. Nine herbal extracts, except for *T. foenum-graecum*, were promising candidates as ingredients for anti-skin-ageing in postmenopausal women.

References

[1] Wolff E, Pal L, Altun T, et al. Skin wrinkles and rigidity in early postmenopausal women very by race/ethnicity: baseline characteristics of the skin ancillary study of the KEEPS trial. Fertil Steril 2011; 95(2): 658-622.e3.

[2] Hall G, Phillips TJ. Estrogen and skin: the effects of estrogen, menopause, and hormone replacement therapy on the skin. J Am Acad Dermatol 2005; 53(4): 555-568.

[3] Sauerbronn AVD, Fonseca AM, Bagnoli VR, et al. The effects of systemic hormonal replacement therapy on the skin of postmenopausal women. Int J Gynaecol Obstet 2000; 68: 35-41.

[4] Spencer AL, Bonnema R, McNamara M. Helping women choose appropriate hormonal contraception: update on risk, benefits, and indications. Am J Med 2009; 122(6) 497-506.

[5] Accorsi-Neto A, Haider M, Simões M, et al. Effects of isoflavones on the skin of postmenopausal women: pirot study. Clinic (Sao Paulo) 2009; 64(6): 505-510.

[6] Hu C, Liu H, Du J, et al. Estrogenic activities of extract of Chinese licorice (*Glycerhiza uralensis*) root in MCF-7 breast cancer cells. J Steroid Biochem Mol Biol 2009; 113(3-5): 209-216.

[7] Yang S, Zhou Q, Yang X. Caspase-3 status is a determinant of the differential responses to genistein between MDA-MB-231 and MCF-7 breast cancer cells. Biochim Biophys Acta 2007; 1773(6): 903-911.

[8] Cherdshewasart W, Sriwatcharakul S. Matabolic activation promotes estrogenic activity of the phytoestrogen-rich plant. Maturitas 2008; 59(2): 128-136.

[9] Zhao Q, Li B, Weber N, et al. Estrogen-like effects of ethanol extracts from several Chinese legumes on MCF-7 cell. Eur Food Res Technol 2005; 221: 828-833.

[10] Talebi S, Afshari JT, Rakhshandeh H, et al. In vitro antiproliferative effect of fresh red garlic on human transitional cell carcinoma (TCC-5637 cell line) Int J Agri Biol 2006; 8(5): 609-614.

[11] Jitsanong T, Khanobdee K, Piyachaturawat P, et al. Diarylheptanoid 7-(3,4 dihydroxyphenyl)-5-hydroxy-1-phenyl-1(E)-1-heptene from *Curcuma comosa* Roxb. Protects retinal pigment epithelial cells against oxidative stress-induced cell death. Toxicol in Vitro 2011; 25(1): 167-176.

[12] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239(1): 70-76.

[13] Re R, Pellegrint N, Proteggente A, et al. Antioxidant activity applying an improved ABTS radical cation declorization assay. Free Radic Biol Med 1999; 26(9-10) 1231-1237.

[14] Wang J, Sun B, Cao Y, et al. Protein glycation inhibition activity of wheat bran feruloyl oligosaccharides. Food Chem 2009; 112(2): 350-353.

[15] Sebastian KS, Thampan RV. Differential effects of soybean and fenugreek extracts on the growth of MCF-7 cells. Chem Biol Interact 2007; 170(2): 135-143.

[16] Wang KH, Lin RD, Hsu FL, et al. Cosmetic applications of selected traditional Chinese herbal medicines. J Ethopharmacol 2006; 106: 353-359.

[17] Yu W, Fu YC, Zhou XH, et al. Effects of resveratrol on H_2O_2-induced apoptosis and expression of SIRTs in H9c2 Cells. J Cell Biochem 2009; 107(3): 741-747.

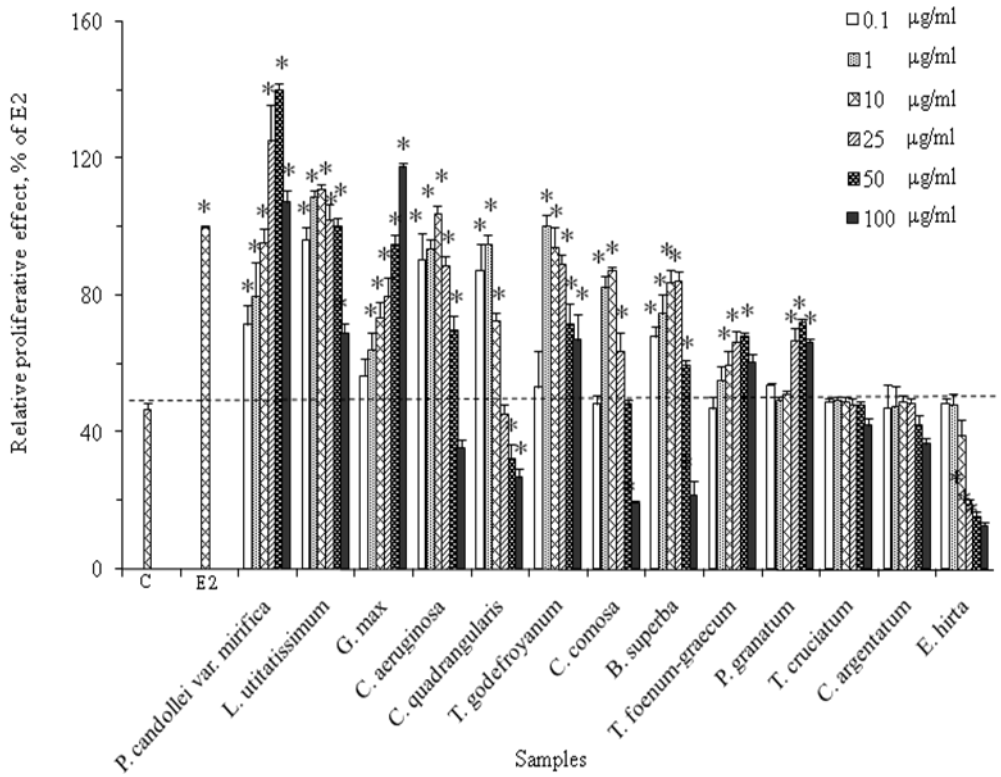

Figure 1. Effect of types and concentrations of 13 herbal extracts on the proliferation of MCF-7 cells (∗p<0.05 versus the control group).The results are expressed as relative proliferative effect of E2 (0.1 nM, 100%).

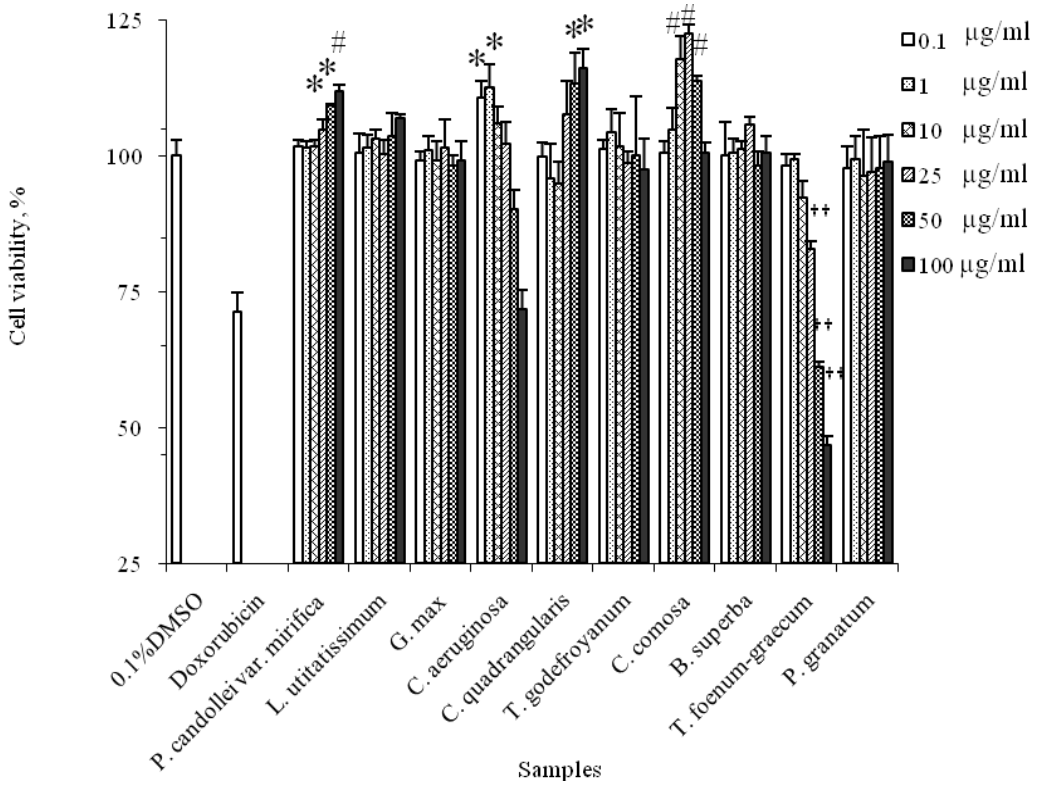

Figure 2. Effect of types and concentrations of 10 herbal extracts on viability of mouse fibroblast cells (∗p<0.05, #p<0.01, and ††p<0.001 versus untreated cells).