

## Effect of anti-inflammation extracts from Korean traditional medicinal herb

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### Abstract

Five Mix Plant Extracts according to different extraction solvents were assessed for its cell viability and anti-inflammatory activity by *in vitro* methods. The single plant extract was extracted with 70% ethanol(EtOH) and the mix plants(*C.kousa*, *R.multiflora*, *T.nucifera*, *M.basjoo* and *S.glabra*) were extracted with EtOH 30%, 70%; Butylene Glycol(BG) 30%, 70%; Propylene Glycol(PG) 30%, 70%; Distilled Water(D.W). Cell viability was measured using the Micro culture tetrazolium (MTT) assay method and Human fibroblast cells, CCD 1102 KERTr were used. The plant extracts with the maximum concentration that none toxic to the cells were evaluated for anti-inflammatory activity. Anti-inflammatory activity was evaluated using lipoxigenase inhibition assay method. A dose response curve was plotted to determine the IC50 values. Results showed that, at the 5 kinds of single plant extracts by 70% EtOH extraction solvent, it showed the IC50 was 280ug/ml of S1, 370ug/ml of S2, 380ug/ml of S3, 170ug/ml of S4 and 190ug/ml of S5. At the mix plant extracts by 7 kinds of extraction solvents (70%, 30% EtOH; 70%, 30% BG; 70%, 30% PG; D.W), it showed the IC50 was 140ug/ml of M E70, 140ug/ml of M E30, 120ug/ml of M BG70, 110ug/ml of M BG30, 120ug/ml of M PG70, 136ug/ml of M PG30 and 120ug/ml of M D.W. From the results, it is concluded that when these five plants mixed before extraction, it will extract more active ingredients with anti-inflammatory effects. Further study we will analyzing plants effective single compound using high performance liquid chromatography (HPLC) profiling and progressing the experiments *in vivo*.

**Keywords:** *Cornus kousa* Buerger et. Hance, *Rosa multiflora* Thunberg, *Torreya nucifera* Siebold et. Zucc, *Musa basjoo* Siebold, *Sarcandra glabra* Nakai and anti-inflammatory.

### 1. Introduction

As the largest organ in the body, the skin provides a barrier against UV radiation, chemicals, microbes and physical pollutants. Challenges of this nature can contribute to skin ageing and inflammation.<sup>1,2</sup> Although anti-inflammatory drugs are used extensively, prolonged consumption of these medications is usually coupled with numerous side effects.<sup>3,4</sup> And many herbal extracts and natural products prevent or reduce oxidative stress in *in vitro* models.<sup>5</sup> Therefore, it is necessary to explore the natural products with anti-inflammatory activity.

The five plant materials *Cornus kousa* Buerger et. Hance(*C.kousa*), *Rosa multiflora* Thunberg(*R.multiflora*), *Torreya nucifera* Siebold et. Zucc(*T.nucifera*), *Musa basjoo* Siebold(*M.basjoo*) and *Sarcandra glabra* Nakai(*S.glabra*) was used in this study. These five plants were widely distributed at South Korea and many effects were known from Korean traditional medicinal herb. *C.kousa* is a tree distributed in the mountains of Korea, China and Japan. Some chemical constituents have been reported from the leaves of *Cornus kousa* such as isoquercitrin, gallic acid, tannin, phenolics and flavonoids.<sup>6</sup> And the fruits of this plant have been used in Korean traditional medicine as a hemostatic agent and for the treatment of diarrhea.<sup>7</sup> *R.multiflora* is a species of rose native to eastern Asia, in China, Japan and Korea. Roses contain different types of secondary metabolites, such as phenolic acid, flavonoids, fragrant components, carotenoid pigments, folate and condensed tannins.<sup>8,10,11</sup> As an herbal remedy, rose various parts are used for the treatment of various diseases including catamenia disorder, trauma, cold, flu and inflammation.<sup>12,13</sup> It is also concerned in hemostasia and the control of pain and diarrhea.<sup>14</sup> Recent studies have shown that rose extracts also possess high antioxidative capacity in all tested assays as well as antimutagenic effects. *T.nucifera* is a slow-growing, coniferous tree from the pine family native to southern Japan and to South Korea's Jeju Island. The fruit of the plant was used to cure vermicide<sup>15,16</sup> and the oil from the seed is edible. The oil has many health benefits— applied on the skin it can heal inflammation, treat acne and boils.<sup>17</sup> *Musa* is a genus over 50 of tropical monocot tree-like plants, important for food, fiber, and ornamentals. *M. basjoo* is a species belonging to the genus *Musa*. The plant of fruits leaves and stems have numerous traditional medicinal uses, including treating dysentery, diarrhea, and digestive disorders. And in traditional Chinese medicine, the stem, flower, leaves and rhizome of *Musa basjoo* are considered useful for clearing heat-toxins, quenching thirst and disinhibiting urine.<sup>18</sup> *S. glabra*

is an herb native to Southeast Asia. It is also known as "9-knotted flower" and "bone-knitted lotus"; a semi-bush plant affiliated to the chestnut magnolia, and is one of rare Chinese herbal medicines. It is widely used in Traditional Chinese Medicinal (TCM) as antipyretic, detoxicate, antitumor, anti-inflammatory, and anti-infectious drug.<sup>19</sup>

In the present study, we explored the anti-inflammatory activity of mixed plant extracts, and tested there cell cytotoxic effect using human fibroblasts. The aims of this study were to develop a cosmetic composition containing mix plant extracts which has inflammation inhibition effective at the minimal concentration.

## 2. Materials and Methods

### 2.1 Preparation of the extracts

In this study, the extraction solvent used during sampling were selection with distilled water(D.W), ethanol, butylene glycol(BG) and propylene glycol(PG) that were used widely at developing functional cosmetics. The sampling methods and soluble solid content of the plant extracts in this study are shown in Table 1.

#### 2.1.1 Single plant extract

All plants used in this study were washed, dried and then 100g of each shade dried powder was extracted with 1L 70 % (v/v) ethanol at room temperature for 14 days. After 14 days, a percolation was done and the extract solution was sterilised by a 0.3  $\mu$ m filter.

#### 2.1.2 Mix plants extract

Mix plants (20g each of the plants, total 100g) were extracted with 1L ethanol(EtOH) 30%, 70%; Butylene Glycol(BG) 30%, 70%; Propylene Glycol(PG) 30%, 70%; Distilled Water(D.W) at room temperature for 14 days. After 14 days, a percolation was done and the extract solution was sterilized by a 0.3  $\mu$ m filter.

**Table 1. Sampling methods and soluble solid content of the plant extracts.**

Number	Extract Component	Extraction Solvent	Filtrate Yield	Soluble Solid Content
S 1	<i>C.kousa</i> <sup>1</sup>	70 % EtOH	893 g	1.36 %
S 2	<i>R.multiflora</i> <sup>2</sup>		889 g	0.84 %
S 3	<i>T.nucifera</i> <sup>3</sup>		876 g	1.26 %
S 4	<i>M.basjoo</i> <sup>4</sup>		864 g	1.19 %
S 5	<i>S.glabra</i> <sup>n</sup>		881 g	1.22 %
M E70	<i>Mix Extract</i>	70 % EtOH	885 g	1.18 %
M E30		30 % EtOH	825 g	1.06 %
M BG70		70 % BG	864 g	1.18 %
M BG30		30% BG	875 g	1.56 %
M PG70		70 % PG	836 g	1.17 %
M PG 30		30 % PG	848 g	7.88 %
M D.W		D.W	872 g	0.92 %

*C.kousa*<sup>1</sup>: *Cornus kousa* Buerger et Hance, *R.multiflora*<sup>2</sup>: *Rosa multiflora* Thunberg, *T.nucifera*<sup>3</sup>: *Torreya nucifera* Siebold et Zucc, *M.basjoo*<sup>4</sup> : *Musa basjoo* Siebold, *S.glabra*<sup>n</sup>: *Sarcandra glabra* Nakai, EtOH: ethanol, BG: butylene glycol, PG: propylene glycol, D.W: distilled water

### 2.2 Cell viability assay

Cell viability was measured using the Microculture tetrazolium (MTT) assay method.<sup>20</sup> Human fibroblast cells, CCD 1102 KERTr were purchased at the American Type Culture Collection (ATCC). CCD 1102 KERTr cells were grown in DMEM (Dubelcco's Modified Eagle Medium, BRL, USA) containing 10% fetal bovine serum (FBS). Upon reaching 90% confluence, the cells were trypsinized and  $1 \times 10^4$  cells/ml were seeded in 24 well-plates and incubated at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. After 24h, the cells were washed with warmed phosphate buffered saline for two washes before the culture medium was replaced. The extract samples were diluted with the cell growth medium until 100 mg/ml, 200 mg/ml concentration and added 20ul to each well. The plate returned to the incubator (37°C, 5% CO<sub>2</sub>) for 24h. After incubation, cytopathic effect (CPE) was observed and 200  $\mu$ l MTT (3 - (4,5-Dimethylthiazol-2-yl) -2) solution was added to each well and reacted for 4 hours in the incubator(37°C, 5% CO<sub>2</sub>). After the reaction, removed the MTT solution and added the dimethyl sulfoxide(DMSO) 1 ml to each well. And the absorbance at 570 nm was measured with the Micro plate Spectrophotometer (Biotech, Eon, USA). The extract sample solvents as a negative control and cell proliferation effect from the difference in absorbance were compared.

### 2.3 Anti-inflammatory activity

Anti-inflammatory activity was measured using the Lipoxygenase Inhibition assay method.<sup>9</sup> It was studied using linoleic acid as substrate and lipoxidase as enzyme. Test solution was dissolved in 0.5ml of 0.1 M sodium phosphate buffer (PBS, pH 7.0) and added 0.5 ml of lipoxidase enzyme solution (60,000units/ml). Test samples 50ul, 100ul were dissolved in test solution and incubated for 10 min at 25°C. After incubated, 1.0 ml of linoleic acid solution (1mM) was added, mixed well and absorbance was measured at 234nm using the Micro plate Spectrophotometer (Biotech, Eon, USA). Test solution was used instead of test sample as the control, and lipoxidase enzyme solution was used instead of PBS buffer as the blank.

The percentage inhibition for both of these assays is calculated by:

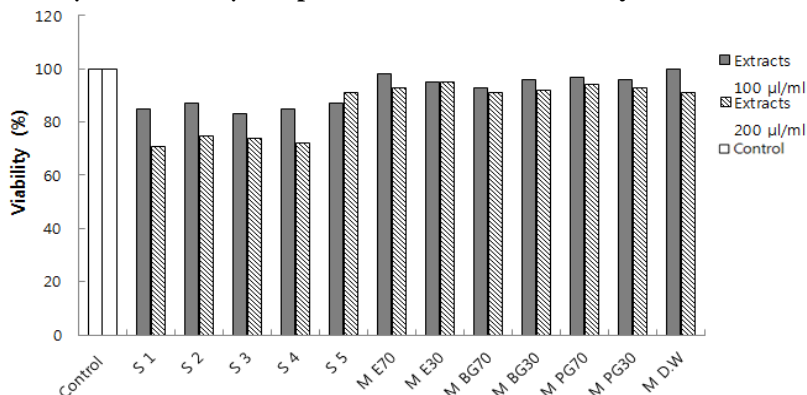
$$\text{Enzyme inhibition activity (\%)} = 1 - [(\text{Absorbance of blank}) / (\text{Absorbance of control})] \times 100$$

A dose response curve was plotted to determine the IC<sub>50</sub> values. IC<sub>50</sub> is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

### 3. Results

#### 3.1 Cell viability

**Fig. 1. Effect of 100µl/ml and 200µl/ml plant extracts on cell viability in CCD 1102 KERTr cells.**

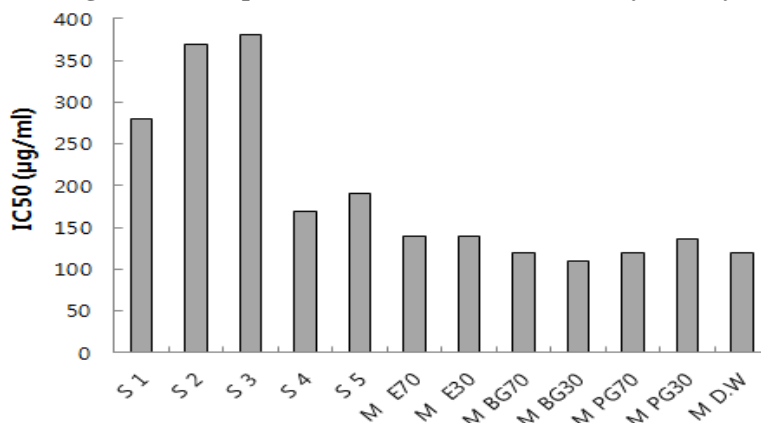


Quantitative analysis of the enzyme activity by MTT assay. The vehicle alone without extract served as a control.

The cell viability effect of the 100ul/ml and 200ul/ml plant extracts was evaluated by MTT reduction assay and the results are tabulated in Fig.1. Human fibroblast cell, CCD 1102 KERTr cell viability of mix plant extracts according to extraction solvents was measured. At the 5 kinds of single plant extracts by 70% EtOH extraction solvent, the percentage of cell viability were showed more than 83% at 100ul/ml, 71% at 200ul/ml. At the 7 kinds of mix plant extracts by extraction solvents (70%, 30% EtOH; 70%, 30% BG; 70%, 30% PG; D.W), the percentage of cell viability were showed more than 93% at 100ul/ml, 91% at 200ul/ml.

#### 3.2 Anti-inflammatory activity

**Fig. 2. Effect of plant extracts at anti-inflammatory activity.**



Quantitative analysis of the enzyme activity by Lipoxygenase Inhibition assay.

Linolenic acid as a substrate for the lipoxygenase reaction by the peroxide results of examination is shown in Fig.2. And anti-inflammatory effects of mix plant extracts according to extraction solvents are measured by the IC<sub>50</sub>. At the 5 kinds of single plant extracts by 70% EtOH extraction solvent, it showed the IC<sub>50</sub> was 280ug/ml of S1, 370ug/ml of S2, 380ug/ml of S3, 170ug/ml of S4 and 190ug/ml of S5.

Amongthem, the maximum inhibition with low IC<sub>50</sub> value of 170ug/ml at S4 and the minimum inhibition with high IC<sub>50</sub> value of 380ug/ml at S3. At the mix plant extracts by 7 kinds of extraction solvents (70%, 30% EtOH; 70%, 30% BG; 70%, 30% PG; D.W), it showed the IC<sub>50</sub> was 140ug/ml of M E70, 140ug/ml of M E30, 120ug/ml of M BG70, 110ug/ml of M BG30, 120ug/ml of M PG70, 136ug/ml of M PG30 and 120ug/ml of M D.W. Among them, the maximum inhibition with low IC<sub>50</sub> value of 110ug/ml at M BG30 and the minimum inhibition with high IC<sub>50</sub> value of 140ug/ml at M E.

#### 4. Discussion

In our study, the percentage of viability was ranged between 98% (at concentration 100ul/ml) and 71% (at concentration 200ul/ml), plant extracts of cell viability was appeared to be very low. In particular, mix plant extracts (M E70 ~ M DW) is higher than single plant extract (S1 ~ S5) in the whole cell viability was confirmed. After experiments, the plant extracts with the maximum concentration that none toxic to the CCD 1102 KERTr cells were evaluated for anti-inflammatory activity. While in the single plant extracts extracted with the same solvent, the IC50 value between the plant extracts is very large and showed little anti-inflammatory effect. On the other hand, at the mix plant extracts, although plant extracts extracted from the different ratios of solvents, but the IC50 value between the plant extracts is very small and showed very high anti-inflammatory effect. These showed when these five plants mixed before extraction; it will extract more active ingredients with anti-inflammatory effects. Further study was done analyzing plants effective single compound using high performance liquid chromatography (HPLC) profiling. And at the mix plant extracts, all the plant extracts according the seven different extraction solvent at low concentrations had excellent anti-inflammatory effect. So father study we will be conducted repeated tests to find the optimal ratio of solvents. In this study, we only tested the anti-inflammatory activity *in vitro*, but in future study, we will progressing the experiments *in vivo*

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