

Anti-aging Effects of The Extracts from Leaf, Stem, fruit and Seed of Yew (*Taxus cuspidata* Sieb) by Solvent Extraction Method

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Summary

Yew (*Taxus cuspidata* Sieb.) chose that grow as medicine, food, decorative plant in Korea's Kyong-Gi province surroundings. Extracts of yew extracted leaf of 250 g and stems of 300 g with 1,3-butylene glycol (1,3-BG), propylene glycol (PG) and water. As results, external appearance of leaf extract of yew was slightly brown clear extract. The pH was 5.3 ± 0.5 , and specific gravity was 1.012 ± 0.05 , and refractive index was 1.375 ± 0.05 . Also, appearance of stem's extract was slightly brown clear extract, and the pH was 5.4 ± 0.5 , and specific gravity was 1.016 ± 0.05 , and refractive index was 1.358 ± 0.05 . Oil of yew separated from seeds, and extracted polysaccharide high purity from fruits. As a result, specific gravity of oil was 0.987, and obtained 40% of yield. Total polyphenols amount of yew extract is detected 0.563% in leaves, 0.325% in stems, whereas total tannins amount contained 0.054% and 0.037% each in leaves and stems. As effect in cosmetics, the anti-oxidative effect by DPPH method is 75.0% in leaves, and stems was 64.0%. Collagen synthesis rate was shown high activity by 54.16% in stem's extract, 33.18% in leaves' extract. Also, PPE-inhibitory activities were 13.7% and 23.5% each in leaves and stems. Anti-inflammatory effect of yew seed oil displayed superior effect of 41% than control. Polysaccharide's molecular weight that is gotten from fruits was $5\times 10^4 \sim 3\times 10^5$ dalton, and got $20.0\pm 5\%$ of yield.

1. Introduction

Yew (*Taxus cuspidata* Sieb.) that belong to yew tree *Taxales* and belongs to *Taxaceae* be always tree of blue needles-leaves. Fig. 1 showed Korean yew's tree. The height of yew is about 20 m, and bark of a tree is similar with name called yew. Leaf of this plant is some sharp-pointed and the color of face is green, but the color of leaf backside is blue and white. Flowers bloom for March ~ April month and fruit has acquired red testa such as cup [1]~[5]. The major

ingredients of yew have contained alkaloid, taxine, taxinine I~XI, taxane derivatives I~VIII XI [6]~[7], taxinol [8]~[10], taxacin I, II, taxol I ~ III, taxusin [11]~[12] and so on. Leaf that pick in June was contained 0.22% alkaloid, but it was known as that alkaloidal content difference is according to collecting season.

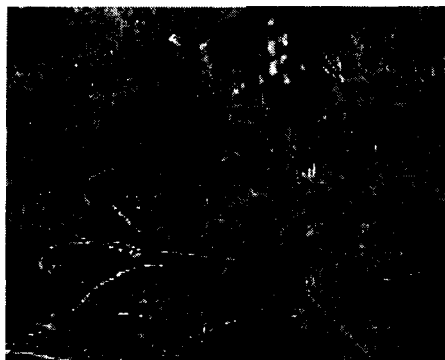


Fig. 1. Yew (*Taxus cuspidate* Sieb)'s tree in Korea.

Flavonoid is contained about 0.1~0.5% of scidopitysin in biflavonoid, and ginkgetin, kayaflavone, sotetsuflavone, and others were contained. As mono-flavonoid, quercetin was contained [13]. By use of these compounds, various effects were expected [14]. Also, diterpene and waxy compound of 0.14% were contained, and material similar to taxusin. As Isotaxiresinol, isolarciresinol and steroid, ponesterone, ecdysterone, taxisterone and others, sorbitol, proanthocyanidin derivatives, sucrose, tannins, and others were contained [15]. Fig.2 showed main ingredients in yew. Effects of yew have various kinds [16]. Wani *et al.* [17] decided structure, and confirmed that is anti-leukemic, anti-tumor agent separating taxol in *Taxus brevifolia*. Liang *et al.* [18] isolated *Taxamairin* I, II of anti-neoplastic diterpene system in *Taxus mairei*, and hepatoma cells' anti-neoplastic activity reported that IC_{50} was 30.21 at 26.78 mg/mL. Miller *et al.* [19] from *Taxus wallicuiana* Zucc to anti-leukemic alkaloids, sorted cephalomannine, and Mirzoev *et al.* [20] had alkaloid taxine's medical efficacy and toxicity test and observed that blood pressure letdown is laziness for 20~30 minute after taking medicine and is in 0.5~1.0 mg. Vohora *et al.* [21] observed effect that biflavonoid of *Taxus baccata* gets in central nervous system, there was genuineness effect, and there was no clause convulsion, anti-inflammatory effect, and was reported special genuineness effect for smooth muscle. Liu *et al.* [22] is using Taiwan yew's heartwood by diabetes treatment in the common people. Also, yew is physiological parameter of heavy metals that is concerned in air open sea, and is used by having bio-indicator, anti-oxidative effect. Thus, it is being a lot of studies about yew in folk medicine or herb medicine. Specially, can seldom seek many studies as skin external application in cosmetics [23], [24].

This study uses and extracted 1, 3-BG, PG and water because do stem and leaf of *Taxus cuspidata* Seib that is distributed much in domestic by materials for experiments. These did to

use by cosmetics isolated oil from seeds, and separate polysaccharide powder from fruits. About extract that is gotten from leaf and stem, report result that do polyphenolic compounds and tannin ingredients analyzed quantity. Using this extract, reported result that test elastase inhibitory activity to measure fibroblast proliferation test, vitality of elasticity strengthening about anti-oxidative activity, collagen synthesis effect about vitality of skin. Also, these tested anti-inflammatory effect from oil of seeds, and from fruits result that extract the high purity polysaccharide reported.

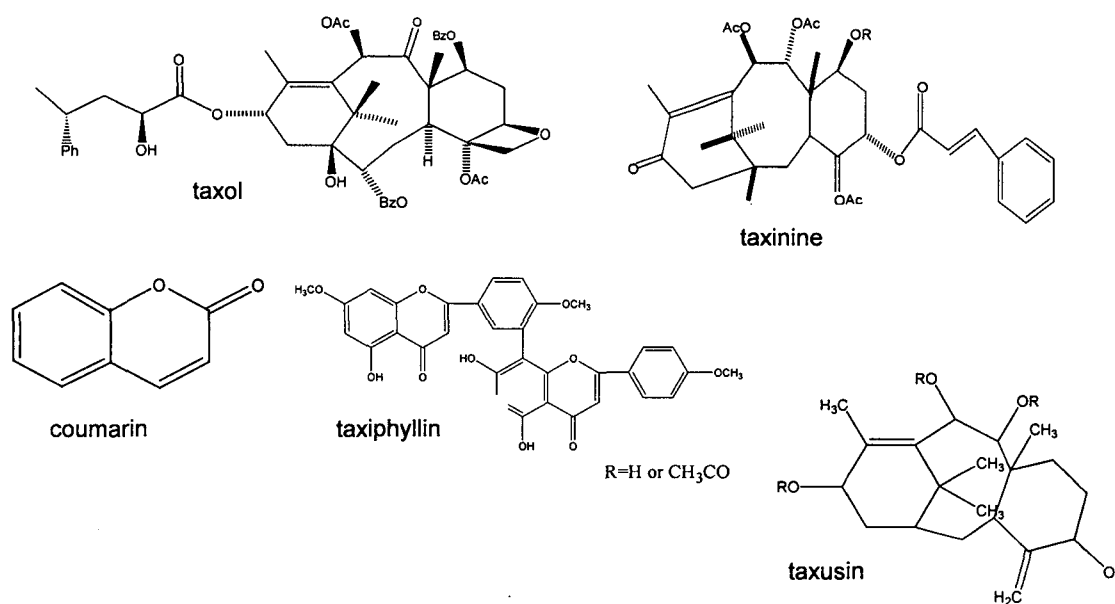


Fig. 2. The molecular structure of major ingredients in Yew's tree.

2. Experimental

2.1. Materials

It chose Yew (*Taxus cuspidata* Sieb) that is growing by medical application, food and decorative plant around Kyong-Gi province in October from September 2000, and used in an experiment picking leaves and stems. To use leaf and stem's extract of yew by cosmetics, used solvent using 1,3-BG (Dow chem., USA), PG (Dow chem. USA) and D.I. water as solvent. To measure anti-oxidative activity, used reagent that is 1,1-diphenyl-2-picryl-hydrazyl (DPPH). To measure all contents of polyphenol, used Folin-Ciocalteu's phenol reagent (Sigma Co.) and gallic acid by standard reagent, and used tannic acid (Aldrich Co.) to measure total tannin contents. Measurement of effectiveness measured causing normally person's desmocyte (normal human fibroblast) to measure collagen synthesis rate (collagen proliferation), and experimented using porcine pancreatic elastase (PPE) to test the inhibitory activity of elastase. It tested using N-succinyl-(alanin)-3-p-nitroanilide (Sigma Co.) that PPE is synthesis stroma. Other reagents used as it is without special refining by foods and cosmetics.

2.2 Manufacturing methods of yew's extract

2.2.1. Extraction methods of leaves and stems of yew

To extract the leaf and stem of yew into cosmetics, it used by sample that dry in cool shade during 3 days after soak for 10 minutes in hot water picking leaves, stems of *Taxus cuspidata* Sieb. Extracting method of yew appeared to Fig.3. First, put in beaker of 1 L cutting leaves of 250 g to 1~3 mm size, and 200 g 1,3-BG, 200 g PG and 450 g D.I. water, and mixed by agitator. Mixed enough for 72 hours by 15 rpm in $40\pm 5^{\circ}\text{C}$ using agitator so that included active components come out well within yew's leaf. This removes the residual substance filtrating by 100mesh, and removed the fine residual substance by 400mesh again and got leaf extract of yew.

Stem of yew extracted 300 g stem by method such as equal solvent. To extract leaf of yew and stem into cosmetics, used by sample that dry in cool shade during 3 days after soak for 10 minutes in hot water picking leaf, stem of *Taxus cuspidata* Sieb. Also, extract of stem extracted by method such as equal solvent.

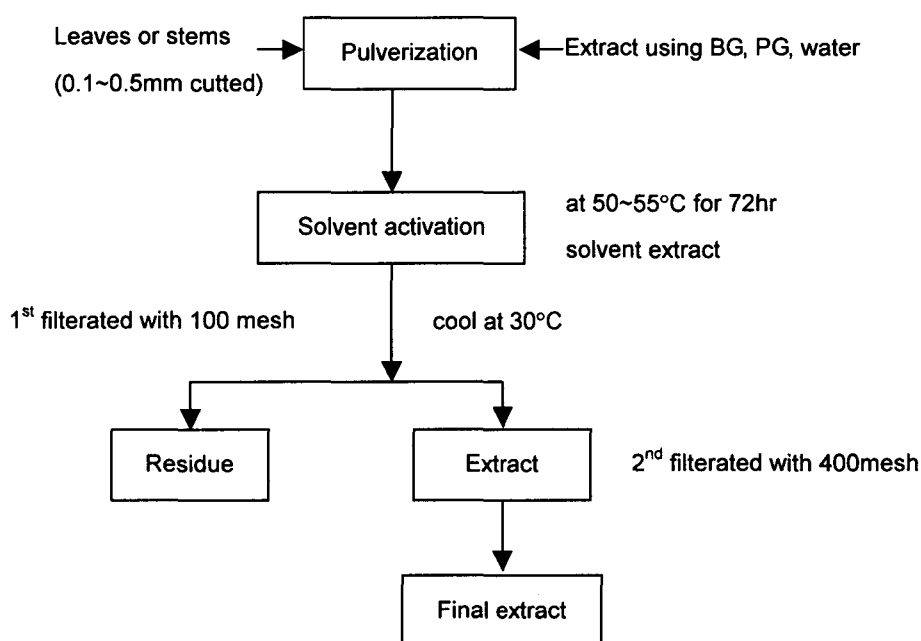


Fig. 3. Extract of yew's leaf and stems with polyols.

2.2.2. Extraction method of oil from seed

To extract oil from yew's seed, must cultivate them after dried seeds of *Taxus cuspidata* Sieb. Then, put this to hexane and agitated by agitator. These agitated enough for 48 hours by 15 rpm in $20\pm 5^{\circ}\text{C}$ so that oil's active components that is contained in seeds may come out well.

This removes the residual substance filtrating by 200mesh, and the fine residual substance by 400mesh. Using distillatory apparatus, this sample made the seed oil of yew removing organic solvent.

2.2.3. Extracting method of polysaccharide

Extracting method of polysaccharide is as following. First, after pick fruit of 1.0 kg and separate fruits and seeds, agitated for 2 hours by 700~1,000 rpm using agitator putting 500 g of D.I. water. And then, this sample filtrates it by 50mesh and removed shells of fruits. To separate polysaccharide, adding alcohol of the regular content, and uses 5×10^5 daltons' filtrating apparatus and make from powder because make this do freeze drying and extracted pure natural polysaccharide powder.

2.3. Determinations of polyphenols and tannins

After content measurement of phenolic compounds [25], [26] puts extraction liquid 1 mL in 60 mL distilled water according to Folin-Ciocalteu law [27], controlled by total volume 100 mL with distilled water after put 5 mL of Folin-Ciocalteu reagent, and puts and mix 15 mL of Na_2CO_3 solution after 30 seconds. After station for 2 hours at normal temperature, yielded amount of polyphenolic compounds using standard curve that is made measuring absorbance in 670 nm and does gallic acid by standard solution. Also, determination of tannin measured absorbance in 540 nm because uses spectrophotometer, and did tannic acid's amount by tannin's content after react for 10 minutes 0.1 M FeCl_3 and 0.008 M $\text{K}_3\text{Fe}(\text{CN})_6$ putting each 3 mL to extract of yew. Also, measured absorbance in 540 nm because fixed quantity of tannin of extraction liquid uses spectrophotometer after react for 10 minutes 0.1 M FeCl_3 and 0.008 M $\text{K}_3\text{Fe}(\text{CN})_6$ after impose each 3 mL to taxus extract according to method of Price etc., and did amount of tannic acid by content of tannin.

2.4. Free radical scavenging effect

Scavenging effect against free radical was measured by the Fugita et al., using moderately stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The sample solution (2 mL) was added to 2 mL of 60 μM DPPH ethanolic solution and kept at room temperature for 30 min. The absorbance was measured at 516 nm [28]. The radical elimination rate appeared in (1) expression below.

$$\text{DPPH radical scavenging activity (\%)} = 100 - \frac{\text{OD}_{\text{exp}} - \text{OD}_{\text{control}}}{\text{OD}_{\text{standard}}} \times 100 \text{ ----- (1)}$$

2.5. The effect of collagen biosynthesis

This is test that can measure wrinkle improvement effect of increasing collagen synthesis in fibroblast. Method of experiment [29] treated 5 mM or 10 mM samples after cultivating (normal human fibroblast) for 24 hours which segregate in newborn skin of number that is equal to plate that have 6 wells. After cultivating this cells for 48 hours, quantity of collagen that desmocyte secretes synthesis newly measures color reaction of dyeing reagent that attach specially to [Gly-X-Y]_n triple helical sequence structure that is discovered differentially in collagen using Sircol™ collagen assay kit by absorbance (OD₅₄₀: From 540 nm to optical density) value using spectrophotometer. According to this calculated amount of collagen that is integrated, and corrected quantity of the collagen by cellular number or protein amount.

2.6. Elastase inhibitive activity of anti-ageing

The elastase activity was assayed spectrophotometrically by a modified method of Bieth *et al.* [30] using [N-Succ-(Ala)3-p-nitroaniline at 400 nm for 10 min at 37°C. The reaction mixture (final volum 2.5 mL) contained: 200 mM different concentrations (10 nM – 5 mM) of the title compounds dissolved in DMSO or buffer. Each inhibitor was preincubated for 15 min at 37°C and the reaction was started by addition of substrate. Blanks contained no enzyme. The percentage of inhibition (mean of 5 determination) was calculated as follows:

$$\text{PPE-inhibitory activity (\%)} = (1 - B/A) \times 100 \text{ ----- (2)}$$

A: OD_{400nm} enzyme activity without inhibitor

B: OD_{400nm} enzyme activity in inhibitor presence

3. Results and Discussions

3.1. Leaf, stem, oil, polysaccharide extract of yew

There are various methods that extract several herb medicine ingredients, but this study does by solvent and extracted polyol to use properly in cosmetics industry [30]. Extract of cosmetics can have efficacy by few amount, but must observe in use content because side effect or skin irritation is worried in case of use high concentration. By above experimental method, extracted leaf of yew and stem, and displayed this result to Table I. As see in Table I, appearance of leaf extract of yew was brown clear liquid. The pH was 5.3±0.5. Specific gravity was 1.012±0.05. The refractive index was 1.375 (20°C). Also, appearance of stem extract was brown clear liquid. The pH was 5.4±0.5, specific gravity was 1.016±0.05, and the refractive index was 1.358 (20°C). From seeds of yew, result extract taxus seed oil, received yield rate of about 40%, the specific gravity was 0.897. Polysaccharide could be assumed by about 1.0x10⁴ ~ 5x10⁵ dalton, and

received yield rate of 10~30%.

Table I. Specifications for the Extracts of *Taxus Cuspidata* Sieb

	Extract of leaf	Extract of stem	Taxus seed oil
Appearance	Light yellowish liquid	Light yellowish liquid	Slightly dark brown
PH	5.3±0.5	5.4±0.5	-
Gravity	1.012±0.05	1.012±0.05	0.922±0.05
Refractive index	1.397±0.01	1.392±0.01	1.472±0.05
UV absorbance	λ_{\max} (200~320nm)	λ_{\max} (200~320nm)	-
Acid value	-	-	0.12
Sap. value	-	-	192
Dry residue	7.8%	3.3%	99.12%

3.2. Determinations of total polyphenolic compounds

Table II and Fig. 4 showed result that make standard calibration curve. The quantitative method of polyphenol is as following. Put to 100 mL volumetric flask weighing gallic acid 50 mg precisely and fills to indication line after melt suitable amount in water. This solution measures correctly 5 mL, did in standard solution putting to 100 mL volumetric flask and fills water to indication line. Calibration curve about gallic acid made calibration curve measuring absorbance in 1.0, 2.0, 4.0, 8.0 and 12.0 mg/mL concentrations. This gets linearity in result that analyzed regression, correlation coefficient 0.9999, within average deviation 5%, got very good calibration curve. Therefore, polyphenolic compounds showed result that analyzing determination (Fig.5). Polyphenolic compounds could know 0.563% in leaf extract, stem extract could know that 0.325% contains. As anti-oxidative activity, this component is expected that acts in aging prevention in the skin.

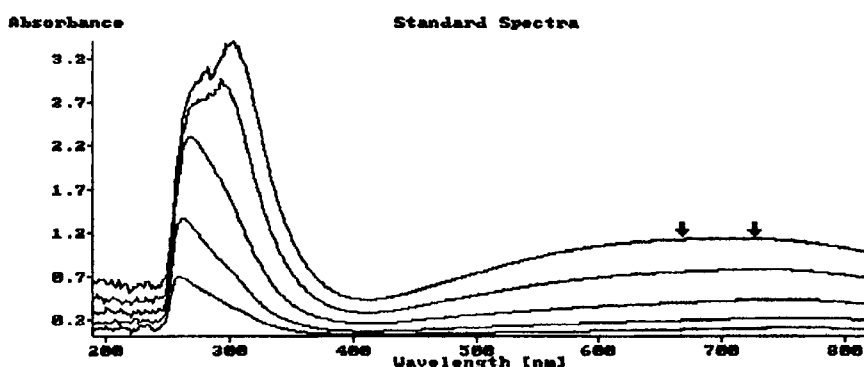


Fig. 4. Standard UV spectrum of gallic acid for measuring the total polyphenols.

Table II. Calibration Curve of Gallic Acid as A Standard Sample

Regressoin Analysis for Polyphenols						
Item	Value		Standard	Conc.($\mu\text{g/ml}$)	Func. Result	Error(%)
Slope	10.6635		Gallic acid	1.0	0.08774	6.21
Intercept	0.0049		"	2.0	0.18546	1.15
Corr. Coeff.	0.9999		"	4.0	0.38571	-2.65
			"	8.0	0.75385	-0.26
			"	12.0	1.12418	0.35

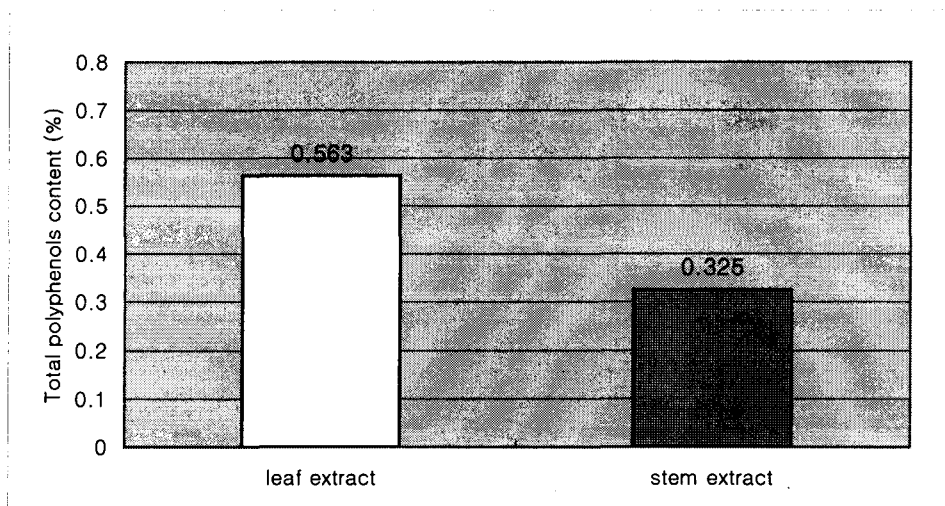


Fig. 5. Determinations of total polyphenols in extracts of yew (*Taxus cuspidata* Sieb.).

3.3. Determinations of total tannins

So that tannin does quantitative analysis, Table III and Fig. 6 showed result that make tannin's standard calibration curve. Tannin's content made calibration curve measuring absorbance in 540 nm using spectrophotometer in 1.0, 2.0, 3.0, 5.0 mg/mL concentrative ranges, with tannic acid by standard sample. Result that this analyzes regression, correlation coefficient got calibration curved line that linearity is very good in 0.9994, average deviation 5.0%.

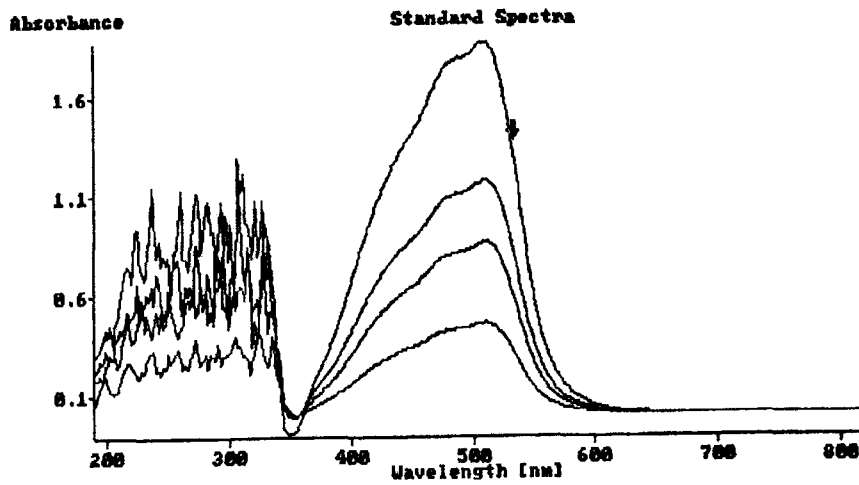


Fig. 6. Standard UV spectrum of tannic acid by measuring the total tannins.

Table III. Calibration Curve of Tannic Acid for Yew (*Taxus cuspidate* Sieb.)

Regressoin Analysis for Tannins						
Item	Value		Standard	Conc.($\mu\text{g/ml}$)	Func. Result	Error(%)
Slope	5.5809		Tannic acid	1.0	0.23003	4.91
Intercept	-0.3328		"	2.0	0.43396	-4.45
Corr. Coeff.	0.9994		"	3.0	0.59184	0.99
			"	5.0	0.95372	0.20

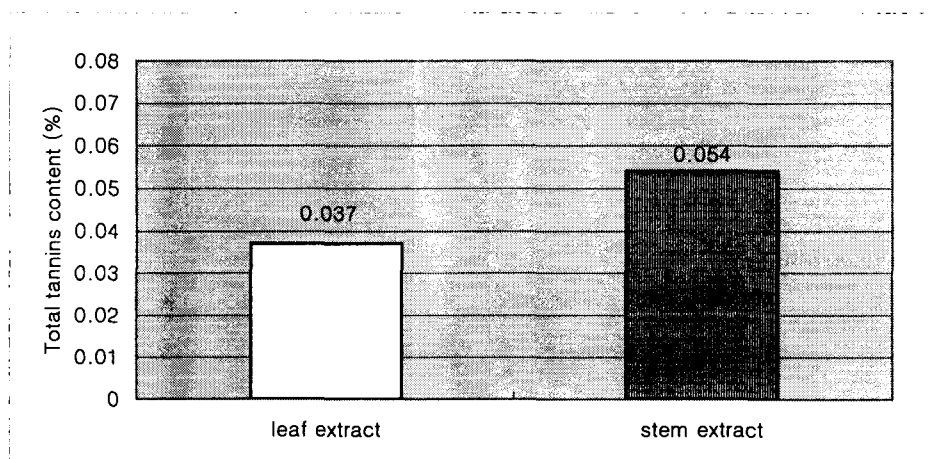


Fig. 7. Determinations of total tannins in extracts of yew.

Therefore, result that tannin amount does quantitative analysis, appeared in graph to Fig. 7. Included tannin's content was 0.037% to leaf extract of yew. Stem's extract could know that

0.054% was contained.

3.4 Anti-oxidative effect

Fig. 8 showed the result that measures an anti-oxidative activity by DPPH method. Result that measure anti-oxidative activity about leaf and stem's extract 500 ppm, whereas it was 0.21% in the case of placebo, leaf extract was 75.0%. And stem extract displayed vitality of 64.0%. In case of green tea extract that is sold in present market, anti-oxidative activity in equal concentration was shown 52.0%. Therefore, leaf's and stem's extract of yew become that anti-oxidative activity was excellent, and the reason was forecasted by thing by polyphenolic ingredients that is contained to leaf and stem extract.

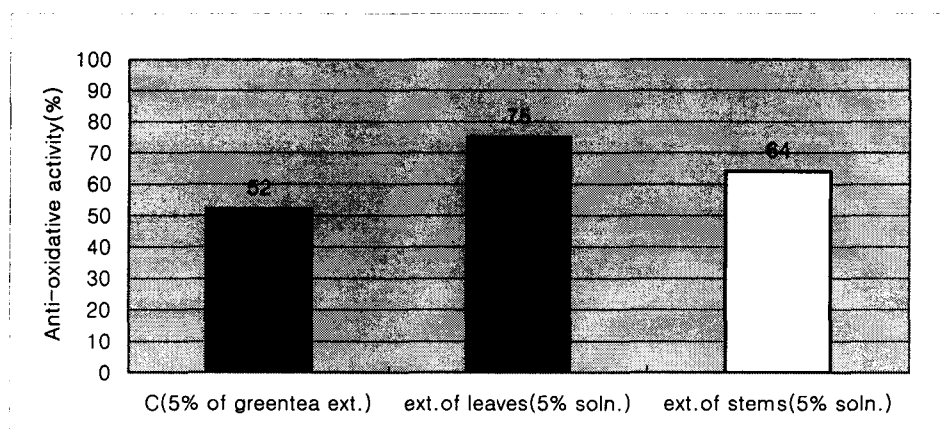


Fig. 8. Anti-oxidative activity of extracts for leaves, stems compared with green tea extract using DPPH method.

3.5. Fibroblast proliferation activity

Effect of a medicine of yew's ingredient is used to anti-cancer medicine and anti-inflammation in herb medicine. Therefore, to use into cosmetics, and search vitality as skin external application that tested about fibroblast proliferation effect. Fig. 9 showed a graph that display fibroblast proliferation rate. As see in Fig. 9, whereas it was 1.7% of placebo sample, could know that 38.18%, stem extract was concerned to fibroblast proliferation by 44.16% in leaf extract. See as above result, this could certify indirectly that contribute to synthesis of collagen. Substantially, practical use value is expected greatly because polyphenolic ingredients are shown effect by a little amount.

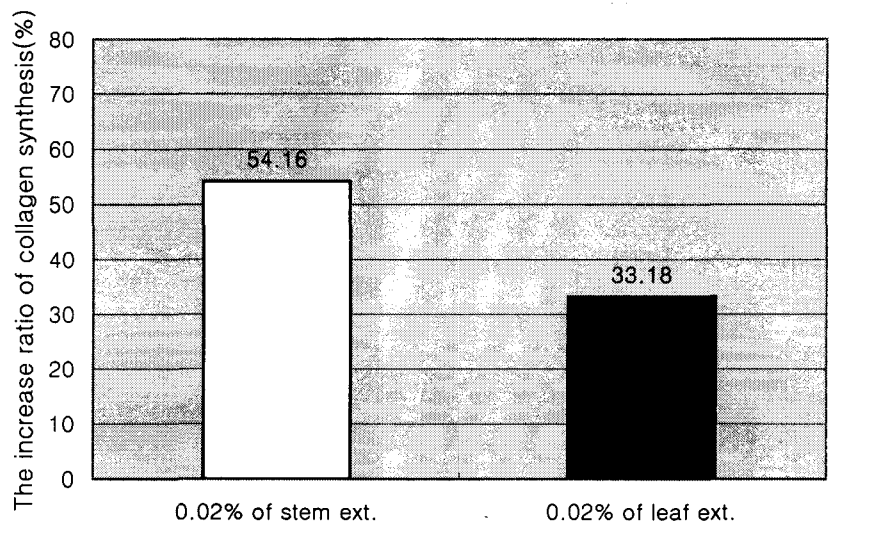


Fig. 9. The increase ratio of collagen synthesis for extracts by activating fibroblast.

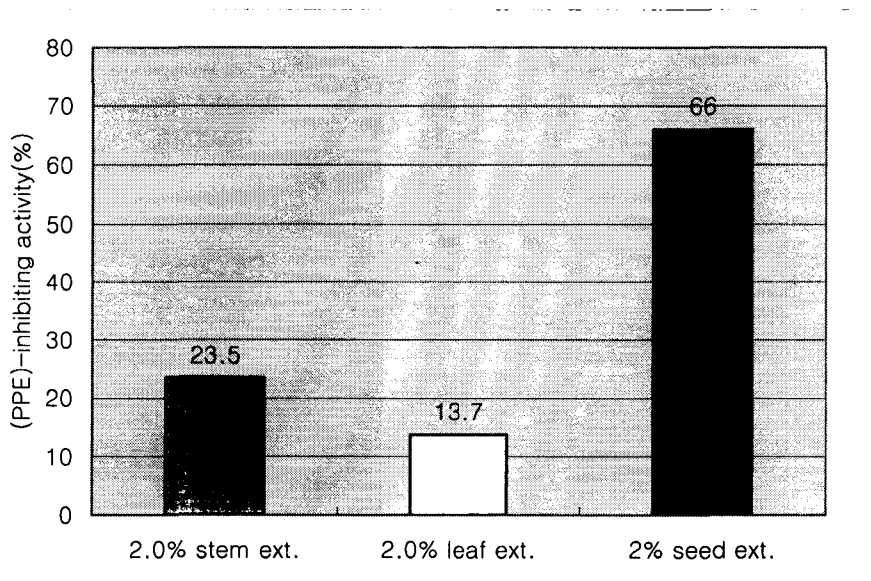


Fig. 10. The PPE-inhibitory activity of yew's extract.

3.6. Efficacy of PPE-inhibition

About yew extract, Fig. 10 showed result that test effect that restrain the elastase vitality that is enzyme that break down the elastin of stratum corneum. PPE-inhibitory activity was *in-vitro* method that break down elastin according to the vitality of elastase, and compared whether have vitality in skin elasticity. As see in Fig. 10, PPE-inhibitory activities for extracts of stem were 42.8%, and extract of leaf was 38.7%, and it was expose that compare for placebo 1.7% and acts greatly. Therefore, by decomposition of elastin that yew extract is concerned in skin

elasticity is controlled, it is expected that give vitality in effect of wrinkle and elasticity.

3.7. Anti-inflammatory efficacy of yew's seed oil

Anti-inflammatory efficacy used dissolving stearyl glycyrrhettinate (SG) 1% that is control to Isopropyl myristate (IPM), and it uses seeds oil 50% solution of yew by sample and that evaluated an effect after take a pathological swelling in guinea pig's both ear. The method of evaluation uses guinea pig, sample group and control group each 10 samples group 20 mL control group IPM 20 mL in ear 1 hour to interval 2 times spread. 1 hour and after, injected arachidonic acid 0.01% aqueous solution that is histamine furtherance agent on ear back part. This estimated control degree of pathological swelling weighing weight cutting mouse's ear by punch that become diameter 0.5 cm after 1 hour. Fig. 11 showed the result that measures an anti-inflammatory effect. Anti-inflammatory effect showed up 41% of the pathological swelling inhibitory activity in oil of yew of 50% that is 24% in 1% of SG sample that control group and sample group. Also, after wound, blood stream confirmed increased thing in inflammatory region in formation change observation, and wound could confirm that fibroblast among process that is cured had vitality. Therefore, oil of yew's seed is expected that excellent anti-inflammatory efficiency is when applying to cosmetics.

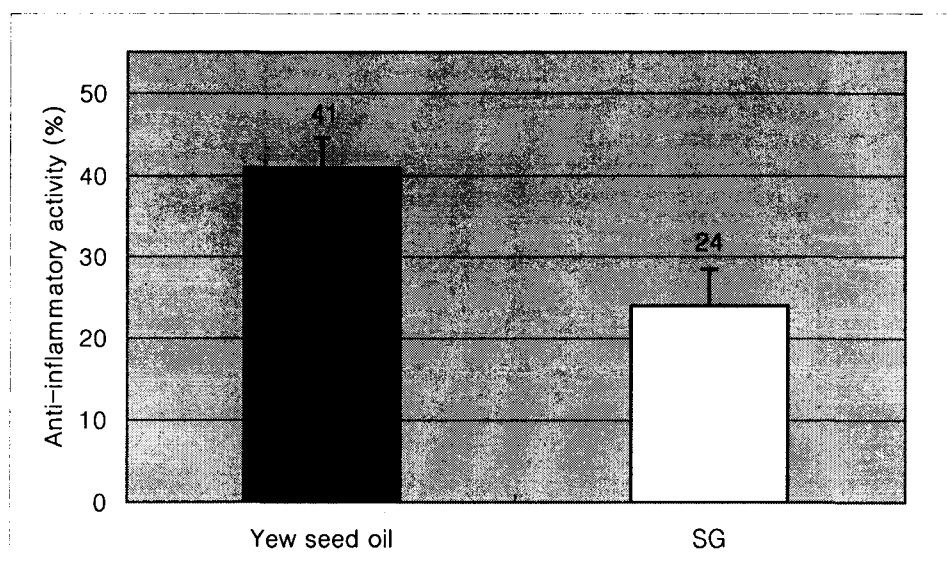


Fig. 11. Anti-inflammatory effect of oil of yew compared with SG. Yew's seed oil: 50% in CCTG, SG: 1% in CCTG. Shown bar: S.D, n=3.

4. Conclusions

To apply in cosmetics industry, used stems, leaves, seeds and fruits of *Taxus cuspidata* Seib. Results about their effect and extracting methods are as following. Appearance of sample that

extract from 250 g of leaf was term brown clear liquid, and pH was 5.3 ± 0.5 , the specific gravity was 1.012 ± 0.05 and the refractive index was 1.375 (20°C). Appearance of sample that extract from 300g of stem was term brown clear extract, and pH was 5.4 ± 0.5 , the specific gravity was 1.016 ± 0.05 and the refractive index was 1.358 (20°C). From seeds, result that refined and separated oil, by slightly brown transparent oil, specific gravity was 0.987, and yield was $40\pm 5\%$. The content of polysaccharide got 5~6% of yield from fruits, and the range of molecular weight was distributed extensively by $1\times 10^4 \sim 5\times 10^5$ dalton. Result that Polyphenolic compounds did quantity of determination, 0.563% in leaf extract, 0.325% in stem extract contained. Also, content of tannic compounds were contained 0.037% in leaf extract and 0.054% in stem extract. When effect of yew extract did by means of 500 ppm, while anti-oxidative effect is 0.21% in placebo's occasion, leaf extract was 75.0%, and stem extract displayed 64% of inhibitive effect. Effect of yew extract of leaf and stem extract 500 ppm of yew result that measure anti-oxidative effect by standard, while it is 0.21% in the case of placebo, 75.0% of leaf extract displayed vitality in and 64.0% stem extract. To measure synthesis rate of collagen, whereas it is 1.7% wave and placebo sample that measure fibroblast proliferation rate, could know that 38.18%, stem extract is concerned to synthesis of collagen by 44.16% in leaf extract. Also, to measure the wrinkle creation of depression effect, result that test PPE-inhibitory effect, stem extract was 42.8%, was expose that leaf extract acts greatly by 38.7% than placebo 1.7%. Result that measure anti-inflammatory effect about oil of seeds using guinea pig, when it is licorice root extract powder of 1% that is comparison sample, was expose that inflammation improvement effect of 41% is in occasion of oil that was shown inflammation improvement effect of 24%, and gets from seeds of 50% of yew. This study develops cosmetics that have anti-oxidation, anti-aging and anti-inflammatory effect and that is expected that contributes greatly in cosmetics industry by good material.

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