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Convergence study of oxidative stress from fraction of Xanthium strumarium L.

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도꼬마리 추출물의 산화적 스트레스에 대한 융합연구

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Abstract *Xanthium strumarium* L. is an annual plant belongs to the family Asteraceae which is is called a 'Cocklebur' that is used for medicinal purposes. Convergent phyto-activity of various extracts of *Xanthium strumarium* L. (Asteraceae) was examined. We estimated antioxidant activity from ground part and fruit extract of *X. strumarium* using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and ABTS assay. The extract of *X. strumarium* was separated each fraction that of ethanol, petroleum ether, and ethyl acetate. It showed potent radical scavenging effect against the DPPH radical and ABTS. The study revealed that *X. strumarium* could be used as a potential source of natural antioxidant.

• Key Words : Antioxidant activity, *Xanthium strumarium* L., DPPH Assay, ABTS Assay, Convergence.

요 약 도꼬마리 (*X. strumarium*)는 국화과에 속하며, '창이자'라는 한약명으로 사용되는 다년생 식물이다. 열매부위와 지상부위의 추출물을 이용하여 융합 식물활성작용을 관찰하였다. 산화적 스트레스에 대항하는 실험법으로는 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), ABTS assay를 사용하였다. 도꼬마리의 추출물은 단일분리가 아닌 ethanol, petroleum ether, 그리고 ethyl acetate로 추출하였다. 산화적 스트레스연구에 사용되는 radical scavenging 능력을 확인하기 위하여 DPPH radical and ABTS를 투여하였다. 본 연구에서 도꼬마리는 천연유래 항산화 효과를 보였다.

• Key Words : 항산화능력,도꼬마리(*Xanthium strumarium* L.), DPPH법, ABTS법, 융합.

1. Introduction

Xanthium strumarium L. is an annual plant belongs to the family Asteraceae which is widely distributed all parts of the country and especially has a strong viability on the roadside and desolated land[1]. *X. strumarium* is spread throughout all part of Asia including South Korea, China, Japan etc[2]. Young leaves are edible and fruit is called a 'Cocklebur' that is used for medicinal purposes[3]. Cocklebur helps to cure diseases such as fever, labor, sweat, ulcerative skin disease, neuralgia, rhinitis, headache, toothache, convulsion, malignant tumor, lesions, hypoglycemia, scrofula, herpes and cancer[1,2,4,5]. The whole plant is

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used as a diaphoretic, sedative, diuretic, and malarial fever[6]. The study of antioxidant activity of the *X. strumarium* L. has indicated their potential to be used as a possible source of antioxidants for medicinal.

Recently, it has been an increasing interest in natural products as medicinal plants in different parts of the world. Because, synthetic antioxidants that side effects are known for exchanging interest and research of natural antioxidant is increasing. There has been an attention to the cytotoxicity of the reactive oxygen as the cause of various pathological conditions.

Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radical, represent molecules that are derived from the oxygen metabolism and exist in all the aerobic organisms[7]. According to the results of other study, these active oxygen or free radical induce various disease such as atherosclerosis, cardiovascular disease, cerebrovascular disease, cancer and aging[1,8]. Free radicals induce oxidative damage to bio-molecules, such as proteins, lipids, lipoproteins and DNA[5]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury and cancer[9,10]. Therefore, study is being carried out to prevent free radical damage to tissue and find natural antioxidants from medicinal plants[11].

The antioxidant effects of properties derived from natural plants, that could be suitable in relation to their role in health and disease of free radicals in unhealthy conditions, anti - stress and neuro - degeneration[12].

Natural antioxidants from plant sources are potent and safe due to their harmless nature, wild herbs have been investigated for their antioxidant properties[13].

Recently, It has been reported that research on the antioxidant and antibacterial activity of *X. strumarium*[14,15]. In our study, we evaluated the DPPH and ABTS radical scavenging activity of several extracts such as Ethanol, petroleum ether, and ethyl acetate fraction of *Xanthium strumarium* L.

2. Materials & Methods

2.1 Plant materials

The plant was collected at Goesan-gun, Chungcheongbuk-do in Korea. The leaves were freeze-dried (FD5508, Ilsin BioBase, Korea) for two weeks and grounded to powder using pestle and mortar. The powder was kept for further studies. And then they left at room temperature.

2.2 Preparation of plant extracts

The powdered X. strumarium (100 g) was extracted with 70% ethanol (EtOH), at room temperature 7 days. The extraction was repeated two-time and the solvent was evaporated in vacuum (CCA1111, EYELA, USA). The extraction was dried at freeze vacuum drier and dried extracts were stored at deep freezer.

2.3 DPPH assay

The DPPH (1, 1-Diphenyl-2-picrylhydrazyl) assay is based on the ability of the antioxidants to scavenge the free-radical. DPPH-free radical activity by method of Hsu (2006) with some modifications[16]. Each sample (4mg) was dissolved in DMSO (1ml). And then DPPH solution (300μM) by addition of EtOH. Sample addition was 0.32, 1.6, 8, 40 and 200μg/ml, time of reaction 20 min. Absorbance of the solution was measured at 515nm and gallic acid was used as standard[17]. (optizen2120w, Mecasys, Korea)

2.4 ABTS assay

The ABTS (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium) assay by method of Keisari(1980) with some modifications[18]. ABTS (7.4mM) was dissolved in potassium persulfate (2.6mM). The solution in the dark container, room temperature during overnight. Each sample (4mg) was dissolved in DMSO (1ml). Sample dissolved in ABTS solution was 0.32, 1.6, 8, 40 and 200 μg/ml, time of reaction 10 min at dark condition. Absorbance of the solution was measured at 732 nm and gallic acid was used as standard[17].

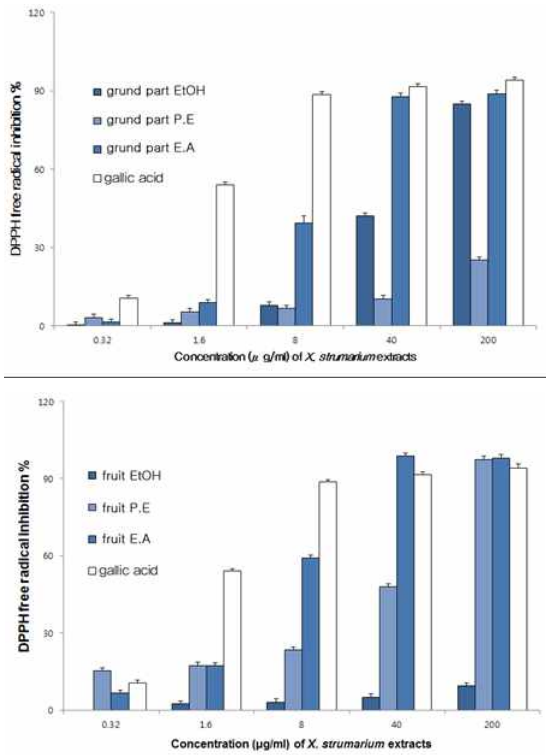
2.5 Statistical analysis

The statistical significance between antioxidant activity values of the extracts was evaluated with Student's t - test. Each value in the table was obtained by calculating the average of six experiments ± standard deviation. ($p<0.05$).

3. Result

3.1 DPPH assay

A Ethanol solution of DPPH free radical was found to be stable for more than 20 min by spectrophotometry at 515 nm. The radical scavenging effects of *X. strumarium*'s extracts and fractions were measured for DPPH free radical. The inhibition concentrations of DPPH was taken as 100%, and the percentage intensity was calculated. The inhibition concentraions is shown in Fig 1.



[Fig. 1] Inhibition concentrations of DPPH (1,1-Diphenyl-2-picrylhydrazyl) activity for *X. strumarium*. It is represented P.E; petroleum ether E.A; ethyl acetate

And also it gave the data in Table 1, the concentration for 50% inhibition (IC_{50}) of DPPH activity for *X. strumarium*'s extracts.

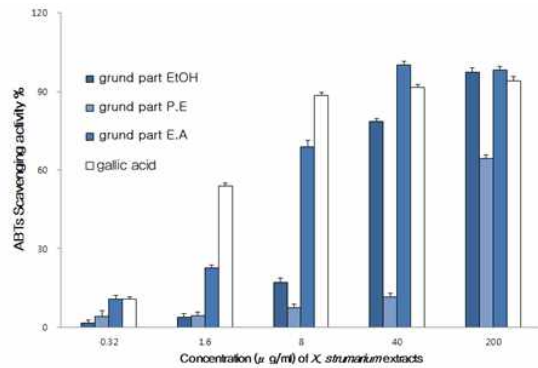
<Table 1> DPPH method of each fractions for *X. strumarium*. 50% inhibition concentrations of DPPH activity for *X. strumarium*. It is represented P.E; petroleum ether E.A; ethyl acetate

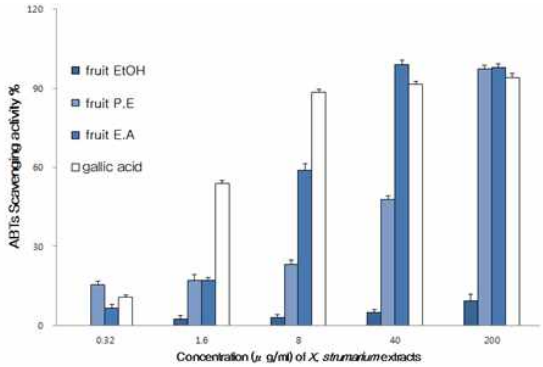
Solution	fraction	IC_{50} (μg/ml)
DPPH	X. Strumarium ground part EtOH	45.78±1.63
	X. Strumarium ground part P.E	9.61±1.20
	X. Strumarium ground part E.A	10.99±0.83
	X. Strumarium fruit EtOH	$11.49X10^5±5.36X10^5$
	X. Strumarium fruit P.E	8.03±0.13
	X. Strumarium fruit E.A	58.45±2.43
standard	gallic acid	0.49±0.03

As shown in Table 1, effects of *X. strumarium*'s extracts on DPPH free radical for oxidants. P.E fraction of X. strumarium fruit showed the strongest effects for oxidants. The IC_{50} was measured at 8.03 ± 0.13 g/ml for DPPH experiments. Each IC_{50} value of P.E fraction of the ground part, EtOH fraction of the ground part, and E.A fraction of the fruit were measured at 9.61 ± 1.20 μg/ml, 45.78 ± 1.63 μg/ml and 58.45 ± 2.43 μg/ml respectively. IC_{50} of standard solution was measured at 0.49 ± 0.03 μg/ml for DPPH experiments.

3.2 ABTS assay

Among the extracts and gallic acid tested for the antioxidant activity using the ABTS assay. The *X. strumarium's* extracts and gallic acid showed antioxidant activity, with IC_{50}.





[Fig. 2] Inhibition concentrations of ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonate)) activity for *X. strumarium*. It is represented P.E; petroleum ether E.A; ethyl acetate

It is shown in Fig. 2 that the in hibition concentrations. And the IC_{50} value of ABTS is shown in Table 2.

<Table 2> ABTS method of each fractions for *X. strumarium*. 50% inhibition concentrations of ABTS activity for *X. strumarium*. It is represented P.E; petroleum ether E.A; ethyl acetate

Solution	fraction	IC_{50} (μg/ml)
ABTs	X. Strumarium ground part EtOH	15.00 ± 0.21
	X. Strumarium ground part P.E	4.24 ± 0.12
	X. Strumarium ground part E.A	4.21 ± 0.21
	X. Strumarium fruit EtOH	-
	X. Strumarium fruit P.E	5.65 ± 0.19
	X. Strumarium fruit E.A	18.33 ± 0.62
standard	gallicacid	0.49±0.03

E.A fraction of *X. strumarium* ground part showed the strongest effects. The IC_{50} was measured at 4.21 ± 0.21 μg/ml. Each IC_{50} value of P.E fraction of the ground part, EtOH fraction of the ground part were measured at 4.24 ± 0.12 μg/ml, 15.00 ± 0.21 μg/ml respectively. And P.E fraction of the fruit, E.A fraction of the fruit were measured at 5.65 ± 0.19 μg/ml, 18.33 ± 0.62 μg/ml.

X. strumarium fruit EtOH showed weak antioxidant activity, with IC_{50} could not be measured too large. The IC_{50} value for gallic acid was 0.49 ± 0.03 μg/ml.

4. Discussion

These days, interest has increased considerably in finding natural antioxidant. The natural antioxidants to replace synthetic antioxidants, which are being restricted because of their side effects[13], such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT). In high dosages both antioxidants are cause in animal's impairment of blood clotting. In addition to BHA and BHT are bring about tumors in animals's fore-stomach[19]. Natural antioxidants that are present in plants are responsible for inhibiting or preventing the oxidative stress[13]. For example, vitamin C and α-tocopherol are serves to minimize the lipid peroxidation in membranes[20].

The generation of free radicals in the body is the cause of many diseases such as cardiovascular and circulatory disorders, endocrine systemdisorders, central nervous system injury and cancer[9,10].

After isolate the ground part and fruit of *Xanthium strumarium* L., each extract was separated and measured radical scavenging activity of DPPH and ABTS to evaluate the antioxidant activity of them. DPPH assay is founded on the measurement of the scavenging activity of antioxidant. The DPPH radical have an odd electron, which is for a visible violet-colour. DPPH radical is reacting with appropriate for reducing agent. As a result, the solution loses colour depending on the number of electrons consumed. Then the solution colour converted to yellow. It can be quantitatively measured from the changes in absorbance at 515nm using spectrophotometer. The IC_{50} values is the concentration of the sample required to inhibit 50% free radical. In this study, P.E fraction of *X. strumarium* fruit and P.E fraction of the ground part, IC_{50} value is slightly low as compared to gallic acid which is using standard antioxidant. But It is worth as a natural antioxidant.

ABTS assay is based on the reaction between ABTS and potassium persulphate. The reaction produce the ABTS radical cation a blue-green coloured. If in the presence of antioxidant, the coloured is

converted to colourless. That is measured at 732nm using spectrophotometer[4]. This study, E.A fraction of *X. strumarium* fruit and P.E fraction of *X. strumarium*, IC_{50} value is lower than gallic acid. But the reise enough value as a natural anti-oxidant.

In the present study, we have evaluated the antioxidant activity of ethanolic extract of *Xanthium strumarium* L.'s ground part and fruit. That the *X. strumarium* belonging to the Asteraceae, and widely distributed in all parts of the country. It is rich in resources in the country and, fruit is called a 'Cocklebur' that is used for medicinal purposes for skin diseases, including malignant tumors[1,2,4,5]. A recent study on the *X. strumarium* that the following components revealed; xanthostrumarin, xanthol, isoxanthol and xanthumin[21,22]. Recently, studies for the separation of anti-cancer agent and anti-fungal activity from *Xanthium strumarium* L. have been reported[14,15].

The results of our study could be suggested that P.E fraction of *X. strumarium* fruit and P.E fraction of the ground part have antioxidant activity. It could be used as a potential source of natural antioxidant due to it has antioxidant ability.

Our results showed potent radical scavenging effect against the DPPH radical IC_{50}, ground part of *X. strumarium* P.E 9.61 ± 1.20 μg/ml, fruits part of *X. strumarium* P.E 8.03 ± 0.10 μg/ml, respectively.

And also it revealed 50% inhibition scavenging action for the ABTs, ground part of *X. strumarium* P.E 4.24 ± 0.12 μg/ml, fruits part of *X. strumarium* P.E 5.65 ± 0.19 μg/ml, respectively.

This study reveals that the *X. strumarium* could be used as a potential source of natural antioxidants.

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