Journal of Digital Convergence
Vol. 16. No. 6, pp. 353-361, 2018

ISSN 1738-1916
https://doi.org/10.14400/JDC.2018.16.6.353

Antimicrobial and Antioxidant Activity of Hot Water and Ethanol Extracts of *Ricinus communis L.* Leaves and Fruits

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피마자(*Ricinus communis L.*) 잎과 열매의 열수(熱水) 및 에탄올 추출물의 항균 활성과 항산화 효능

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Abstract RFW and RFE of castor fruit selected as part of the development of natural antimicrobials and antioxidants yielded 15.8% and 18.4% respectively. In the results of measuring the antimicrobial activity through paper disc method, the antimicrobial activity of castor fruits in ethanol extracts appeared. Especially, the activity was excellent in *P. aeruginosa* and *S. aureus*, and antimicrobial activity of *C.* was 1.5mm up to 16 hours. However, the proliferation of *C.* was observed again after 24 hours. In the MIC experiment results of RFE, *S. aureus* and P. aeruginosa showed 96% and 93% of antimicrobial activity, respectively. The DPPH radical scavenging activity of RLW and castor leaf ethanol extract showed 1.8±0.6% and 2.1±0.7% free radical scavenging activity at 1000(㎍/㎖). This study is expected to provide basic data for the development of antimicrobial agents and antioxidants using natural products.

Key Words : *Ricinus communis L.*, antimicrobial, Antioxidant, Ricin, Toxic

요 약 천연 항균제 및 항산화제 개발의 일환으로 선택한 피마자 열매의 열수 추출물(RFW)과 에탄올 추출물(RFE)은 각각 15.8%와 18.4%의 수율을 나타냈다. Paper-disc method을 통한 항균활성 측정 결과, 피마자 열매를 에탄올로 추출한 추출물에서 항균활성이 나타났다. 특히 녹농균 및 황색포도상구균에서 활성이 우수하였으며, 캔디다균에서도 배양 16시간까지 1.5mm 정도의 항균활성이 나타났으나 24시간 이후에는 캔디다균의 증식이 다시 관찰되었다. 피마자 열매 에탄올추출물(RFE)의 MIC실험 결과 *S. aureus*와 *P. aeruginosa*에서 각각 96%와 93%의 항균력이 나타났다. 피마자 잎 열수추출물(RLW)과 피마자 잎 에탄올추출물(RLE)의 DPPH radical 소거능 측정을 통한 항산화 효능 결과는 1000(㎍/㎖)에서 각각 1.8±0.6%, 2.1±0.7%의 free radical 소거율을 나타냈다. 본 연구가 향후 천연물을 이용한 항균제 및 항산화제 개발을 위한 기초 자료로 제공되리라 사료된다.

주제어 : 피마자, 항균, 항산화, 리신, 독성

*This paper was supported by the Graduate School of Public Relations at Songwon University in 2018.

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Received January 2, 2018
Revised February 2, 2018
Accepted June 20, 2018
Published June 28, 2018

1. Introduction

Due to the rapid development of living standards, modern people are living in the high technology age, and as the life expectancy increases, they pursue a more affluent and healthy life and express a strong desire for beauty. As the desire for the beauty of modern people is getting complicated and diverse, the beauty industry field is becoming more and more specialized and professionalized[1]. This phenomenon has led to the development of the beauty industry, and in particular, the beauty sector began to establish itself as an independent domain by being a part of fashion as a result of economic growth and improved living standards[2]. In modern society, beauty is becoming a practical science, a field of comprehensive art based on science and crafts[3].

Before expressing the ever increasing beauty freely, the awareness of the health of modern people is rising together. Modern people are unprotectedly exposed to a variety of mental and physical stress in a busy daily life. The risk of cancer is steadily increasing due to factors such as fast food habits, smoking, and drinking, along with stress, and the number of cancer cases per year is increasing not only in Korea but also in the whole world[4]. In addition to early checkup for various cancers, it is possible to live a healthy life by having resistance to various bacteria that are present in daily life of modern people. Drugs with antimicrobial action are widely available in the market, but antimicrobial activity using natural products is relatively ineffective compared to modern medicines. On the other hand, active ingredients that are widely used in medicine and cosmetics are mainly synthetic substances, and it is true that they have many problems such as mutagenicity, chronic toxicity, tolerance and induction of carcinogens[5]. Therefore, it is urgent to develop substitute materials that can complement various side effects of artificial synthetic materials and as a solution to this problem, various studies are being actively conducted as natural substances are mentioned.

As the awareness of the health of modern people increases, the interest in physiological efficacy such as prevention of disease and aging is proportionally increased, and the interest in active ingredients showing antimicrobial activity and antioxidant effect is increasing. In recent years, the interest in the concept of well being and alternative medicine has been increasing due to changes in the living environment and national income, and research has been actively conducted to discover functional materials from natural substances[6].

In keeping with this trend of the times, as a material to study antimicrobial activity and antioxidant effect of natural substances, this study selected castor leaves and fruits to conduct research on antimicrobial action of five kinds such as *Staphylococcus aureus*, an aerobic gram positive strain, and *Escherichia. coli* and *Pseudomonas aeruginosa*, aerobic gram negative strains, and fungi *Candia albicans* and *Aspergillus niger*. By studying the antioxidant effect through DPPH radical scavenging activity, this study aims to provide basic data for the development of natural substances that can replace drugs composed of artificial compounds.

2. Theoretical background

2.1 *Ricinus communis L.*

Castor is an exotic plant native to India and Asia Minor, imported for cultivated plants and commonly planted in farms throughout the country[7]. Most are cultivated in the wild in tropical and subtropical regions, and they are low in production costs and adaptable to a variety of climates. Humidity is low and temperatures between 20 and 26°C are suitable for maximum yields[8]. It is an annual herbaceous plant belonging to the Euphorbiaceae, which is called "Ajukari" in the countryside, and it forms a perennial shrub in the tropics, reaching 2m in height and branches like a tree. Leaves are alternate, long petiole,

shield like, 30 100cm in diameter, split into 5 11 parts like a palm, lobes ovate or narrow ovate, pointed end, green or brown on surface, no hair, sharp saw teeth on edge[9]. It grows straight as a one year old herb, and the stem is empty. Leaves are alternate and split like a palm and flowers bloom in July and September. The harvest time is from September to October, the leaves are castor leaf, the root is castor root, and the oil is castor oil for medicine. In Korean oriental medicine and private, those leaf, seed, and root are used for hemostasis, laxative, swelling, edema. bruise, and diarrhea remedy, etc.[10]. Castor's flowering period is between May and August, and the condensation period is between July and October[11]. The property is flat, it has a little poison and its taste is very sweet[12]. By oiling the seeds, the oil is widely used in polymers, lubricants, cosmetics, plastics, pomades, printing inks, and seal ink materials[13].

Castor fruits contain between 50 and 60% of fat and 18 to 20% of protein, and when castor fruits are squeezed, the residue contains more than 36% of protein. As castor fruits contain ricin(toxic albumin), allergen and ricinine, they cannot be used for feed or food[14]. However, it is known as a very high value crop of medicinal, food, and agricultural chemicals. Compared to other fat and oil crops, it has a high oil content and is favorably evaluated as a biodiesel crop[15]. Also, castor oil is non drying oil which has high viscosity and high lubrication at low temperature and does not dissolve well in petroleum or other organic solvents, and has been used as a lubricant for large power hydraulic brakes such as airplanes, trucks, and conveyor devices[13].

It is said that the crude fat content and fatty acid content of castor were average 44.6~49.4%, and ranged from 41.4% to 52.2%. In addition, the fatty acid composition of palmitic acid and stearic acid was 41%. In detail, oleic acid is 4.6%, linoleic acid is 5.2%, linolenic acid is 0.6%, and ricinoleic acid is 87.3%. In particular, ricinoleic acid accounts for a large proportion of fatty acid composition[16].

2.2 Ricin

Ricin is a vegetable protein extracted from castor, *Ricinus communis* seeds and consists of two polypeptide chains linked by a disulfide bond[17]. Among them, B chain is involved in cell surface recognition as lectin, and A chain is known to affect tissues by inhibiting protein synthesis by inactivating 60s ribosomal subunit by penetrating into cells as glycosidase[18].

Studies on castor have been made mainly on letin, a toxic protein with hemagglutinating activity, and the research of lectin began in 1888 when Still mark discovered that castor extracts aggregated in erythrocytes. Since then, similar elements have been found in various plants and animals, and they have been called 'lectin' that has the meaning of 'choose'[19]. It is known that these hemagglutinin aggregates can coagulate cells such as lymphocytes, fibroblasts, spermatozoa, bacteria, and fungi as well as hematopoietic cells. This coagulation phenomenon has been confirmed to be due to the linkage of cells to lectin by binding to sugar exposed on the cell surface[20]. On the other hand, it is known that the rhettic binds only to a sugar specific to the sugar bond, and owing to these features, it has been applied to diverse aspects such as study of sugar and study of structure of cell membrane[21].

The lectin is divided into Type Ⅰ composed of one subunit and Type Ⅱ composed of two subunits[22], and the castor lectin belongs to Type Ⅱ composed of glycoproteins and is named 'Ricin'. In ricin, the A chain, which is an active ring with a molecular weight of 32,000 Dalton, and the B chain, which is a linking ring with a molecular weight of 34,000 Dalton, are bound by a disulfide bond[23]. Ricin activity affects cellular metabolism after the B chain binds to cell surface receptors, and A chain constructs inactivate eukaryotic ribosomes and inhibit protein synthesis. Because of this action, ricin comes to belong to ribosome inactivating proteins (RIPs)[24].

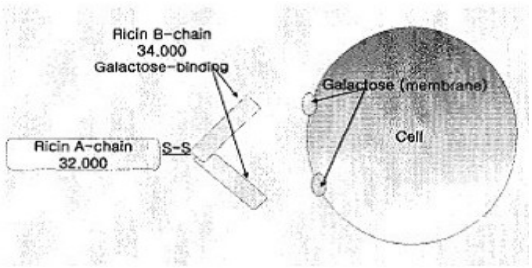

Fig. 1. Bilding reaction of ricin [21]

2.3 Bacteria

2.3.1 *Staphylococcus aureus*

Aerobic Gram positive *Staphylococcus aureus* resides mainly in normal skin and conjunctival mucosa, and corneal damage is often caused by trauma, and if it is not treated properly, it progresses and causes corneal perforation. However, its symptom is more severe than keratitis with *Staphylococcus epidermidis*[25].

2.3.2 *Pseudomonas aeruginosa*

It is known that bacterial keratitis due to aerobic Gram negative *Pseudomonas aeruginosa* has very rapid clinical outcomes, and the bacteria secreted by proteases and toxins destroy the cornea and causes severe corneal perforation [26].

2.3.3 *Escherichiaco*

Escherichiaco is an aerobic gram negative bacterium, which is intestinal bacteria of humans and animals and has motility due to the Peritrichous flagellum. It decomposes glucose, lactose, and maltose to produce acid and gas, and is vulnerable to heat. It is a pathogenic bacterium that survives long in water or soil [27].

2.3.4 *Candia albicans*

It is an ovate or yeast shaped fungus, and when it is cultured isolated from the tissue, satellite mycelium is formed. The fungus may be present as normal fungi in the gastrointestinal tract, the respiratory tract, mucous membranes of the female reproductive system, etc., but often causes diseases. This bacterium parasitizes the skin, throat, and mucous membranes of humans and causes candidasis[28].

2.3.5 *Aspergillus niger*

Aspergillus niger is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of Stachybotrys(species of which have also been called "black mould")[29].

Some strains of A. niger have been reported to produce potent mycotoxins called ochratoxins;[29] other sources disagree, claiming this report is based upon misidentification of the fungal species.[citation needed] Recent evidence suggests some true A. niger strains do produce ochratoxin A.[30,31] It also produces the is of lavone or obol.

3. Experiment materials and methods

3.1 Preparation of experimental materials and extracts

In July 2017, castor leaves and fruit were collected at the same time from the bank of the farm near Pyeongtaek, Gyeonggi province, and dried naturally in the windy shade.

Hot water and RFE of castor leaves were prepared by putting 10g of leaf powder, 200 ml of sterilized distilled water and 200 ml of 80% ethanol into a 1 L Erlenmeyer flask, wrapped in foil, and extracted at 60°C for 24 hours, and hot water and RFE of castor fruits were prepared by putting 7g of powdered beans, 140ml of sterilized distilled water and 140ml of 80% ethanol in a 1 L Erlenmeyer flask, wrapped in foil, and extracted at 60°C for 24 hours.

Each extract was firstly filtered through gauze and then subjected to secondary filtration with No.2 filter paper (Adventec, Japan). The filtrate was centrifuged at 4℃ and 4500 rpm for 20 minutes with a swinging rotor centrifuge (VS 550, VISION, KOREA), and only the upper part of the filtrate was combined and concentrated under reduced pressure using a rotary evaporator (N 1000, EYELA, Tokyo, Japan) at 45℃ in a constant temperature water bath (NB 301L, N BIOTEK, Korea).

After freezing for 24 hours in an ultralow temperature freezer (Isotemp Upright Freezers, Fisher Scientific, USA), the sample was used in the freeze dried state at 80℃.

The RFW (*Ricinus communis* L. Extract ; RFW) and RFE(*Ricinus communis* L. Extract; RFE) of castor fruits were 1.104g and 1.29g, respectively at 7g, and so the extraction yields were 15.8% and 18.4%, respectively.

3.2 Experiment devices

Table 1. Experiment machine

	model	manufacture company	manufacture country
Centrifugal separator	VS-550	VISION	KOREA
incubator	VS-1203P3N	VISION	KOREA
constant temperature bath	NB-301L	N-BIOTEK	KOREA
Low Temperature Freezers	Isotemp	Fisher Scientific	USA
rotary evaporator	N-1000	EYELA	JAPAN

3.3 Experimental strains and culture medium

The strains used in the experiment were three strains consisting of one Gram (+) strain and two Gram (-) strains. The aerobic Gram (+) was *S. aureus* (KCTC3881), the aerobic Gram (-) includes *E. coli* (KCTC1039) and *P. aeruginosa* (KCTC1637). *C. albicans* (KCTC7965) causing candidiasis was the fungus, and *A. niger* (KCTC6910), known as black germ, was purchased from the BRC (KCTC) and used.

S. aureus, E. coli, P. aeruginosa, C. albicans, and *A. niger*, which were stored at 80℃, were taken out and melted for the antimicrobial activity experiment, and *S. aureus* and *P. aeruginosa* were inoculated into TSA (Trypic soy agar), *E. coli* inoculated into LB (Luria Bertani media), *C. albicans* inoculated into YEPD, and *A. niger* inoculated into PDA (Potato Dextorse agar) respectively were cultured in incubator (VS 1203P3N, VISION, KOREA) for 18 48 hours at 30℃. During the experiment, they were used in subculture at intervals of 7 days so that the activity of the strains did not deteriorate.

Table 2. List of microorganisms used for antimicrobial experiment

Strain	Strain No.	Media	gram strain
Escherichia coli	KCTC 1039	LB	(-)
Staphylococcus aureus	KCTC 3881	TSA	(+)
Pseudomonas aeruginosa	KCTC 1637	TSA	(-)
Candida albicans	KCTC 7965	YEPD	
Aspergillus niger	KCTC 6910	PDA	

3.4 Paper-disc method of castor leaf and bean hot water and RFE

The antimicrobial activity experiment of castor leaf and bean extracts was conducted to measure the prepared four samples with the paper disc method. First, a single colony of each strain with good activity is collected through subculture, and after uniformly streaking over the entire solid medium, the sterilized paper disc is placed on the smear plate and 50㎕ of each extract is collected. As a positive control group, propolis (10 times dilution) is used, and after culturing under optimal conditions of each strain, clear zone is confirmed and measured.

3.5 Minimum inhibitory concentration (MIC) measurement of castor fruits RFE

The antimicrobial activity of castor fruits RFE (hereinafter referred to as RFE) was measured by modifying the minimum inhibitory concentration (MIC) assay. First, sterilize the TSA medium and cool to 45~50℃ after sterilization. Add castor fruits RFE, mix well with a magnetic stirrer, dispense to Petri dish, and set. Prepare the strains to be used for MIC measurement by sequentially diluting them in an EP tube containing sterilized water. Dissolve 0.5㎖ each of the plates so that the strain is well spread throughout the plate, and then cultivate each strain at an appropriate temperature for 24 hours to measure the number of colonies.

$$\text{Antimicrobial activity}(\%) = \left(\frac{\text{Number of bacteria after proliferation of blank} - \text{Number of bacteria after proliferation of sample}}{\text{Number of bacteria after proliferation of blank}}\right)\times 100$$

3.6 DPPH free radical scavenging experiment of castor leaves hot water and RFE

The antioxidant experiment of castor leaves hot water and RFE was measured by DPPH radical scavenging ability.

Castor leaves distilled water extract (hereinafter referred to as RLW) and castor leaves RFE (hereinafter referred to as RLE) used in this antioxidant experiment were supplied by Soongsil University, and was used after the selection in TBRC RIC of Daejeon University.

The final concentration of castor leaves hot water and RFE was diluted to a concentration of 1, 10, 100, 1,000 (㎍/㎖). And 150 μl of 0.2 mM DPPH solution dissolved in ethanol and the extract were mixed in 100μl of each, and reacted at 37°C for 30 minutes.

After the reaction, the absorbance was measured at a wavelength of 517nm. Distilled water was added to the control group of the sample and ethanol was added to the control group of the DPPH solution to obtain the corrected value. The DPPH free radical scavenging rate was calculated according to the following equation.

$$\text{Scavenging rate}(\%) = \left(\frac{\text{Absorbance of control group} - \text{Absorbance of sample group}}{\text{Absorbance of control group}}\right)\times 100$$

4. Results and Discussion

4.1 The paper-disc method of castor leaves and fruits hot water and RFE

From the results of antimicrobial activity measurement of castor leaves and fruits hot water and RFE (80% EtOH) through the paper disc method, antimicrobial activity appeared in extracts of castor fruits with 80% ethanol. Especially, the activity was excellent in *P. aeruginosa* and *S. aureus*. In *C. albicans*, antimicrobial activity of 1.5mm was observed up to 16 hours after the culture, but the proliferation of *C. albicans* was observed again after 24 hours. On the other hand, antimicrobial activity was not observed in RFW and RFE of castor leaves.

Table 3. Experiment results of antimicrobial activity of paper disc method

Sample \ Strain		*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*	*A. niger*
Leaves	RLW	-	-	-	-	-
	RLE	-	-	-	-	-
Fruits	RFW	-	-	-	-	-
	RFE	-	++	++	+	-

+ : 0.5~1.5 mm, ++ : 1.6~2.5 mm, +++ : 2.6 mm or more

4.2 Minimum inhibitory concentration (MIC) measurement of castor fruits RFE

From the results of minimum inhibitory concentration of castor fruits RFE, *E. coli, C. albicans,* and *A. niger* did not exhibit any significant antimicrobial activity while *S. aureus* and *P. aeruginosa* showed outstanding 96% and 93% of antimicrobial activity, respectively.

Table 4. Results of MIC measurement of castor fruits RFE

Sample	*S. aureus*		*P. aeruginosa*	
	Number of individuals after culture of 24 hours (cfu/mL)	antimicrobial activity [%]	Number of individuals after culture of 24 hours (cfu/mL)	antimicrobial activity[%]
Blank	1.86×10^2		1.78×10^3	
RFE	7	96	1.32×10^2	93

4.3 The results of DPPH free radical scavenging experiment of castor leaves hot water and RFE

Table 5 shows the antioxidant effect of castor leaves (RLW) and castor leaves (RLE) by measuring DPPH radical scavenging ability.

Table 5. DPPH free radical scavenging activity of RLW and RLE

	1 (㎍/L)	10 (㎍/L)	100 (㎍/mL)	1000 (㎍/mL)
RLW	1.4±0.4%	1.4±0.8%	1.7±0.5%	1.8±0.6%
RLE	1.6±0.6%	1.8±0.4%	2.0±0.6%	2.1±0.7%

4.4 Since parts of the castor fruits, such as skin, seed meat, and seeds as a whole were used as test samples and only the simple sensory evaluation was carried out in this experiment, this study failed to provide clear data on the safety and efficacy of ricin, a typical toxic of castor beans, as well as lack of progress in the efficacy of the extract. There is also a limit to the fact that no harmfulness to the human body has been verified.

Therefore, even if a specific ingredient is developed with the entire part of a castor bean, the issues of ricin non detection test results, basic cytotoxicity, and other toxicity of castor fruits may still arise. Therefore, further studies should clarify the safety and progress of ricin contained in castor beans.

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