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Article

Detection of Electron Beam Irradiated Crude Drugs by Electron Spin Resonance (ESR)

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Perillae Herba, Sennae Folium, Cinnamomi Cortex, Phellodendri Cortex, Ginseng Radix, Glycyrrhizae Radix, Paeoniae Radix, and Zingiberis Rhizoma were irradiated with electron beam (5 MeV) and organic radicals were detected by ESR measurement, before and after irradiation (10 kGy). A single line spectrum was detected at around $g = 2.005$ in non-irradiated crude drugs, and radical concentrations were high in the leaf varieties of crude drugs. After irradiation, the signal intensity around $g = 2.005$ increased, and a new subsignal was detected as a 3 mT shoulder of this signal. Broad, asymmetrically divided signals were also detected in irradiated root varieties of crude drugs. The free radical localized on the organic components of irradiated crude drugs tended to decrease with the water content. After irradiation, signal intensity reduced and reached a steady state after about 1 to 2 months. However, specificity of the ESR signal shape appearing after irradiation continued to be detectable for 6 months in leaf varieties and for a year in bark and root varieties of crude drugs. Consequently, it was concluded that ESR could be applied as an initial screening procedure to detect irradiated crude drugs.

Key Words: crude drug, ESR, free radical, cellulose, sugar, electron beam irradiation

1. Introduction

Because crude drugs (herbal medicines) are natural substances, insect and microbial contamination is unavoidable. As a result, sterilization is a necessity in most cases in which crude drugs are used as ingredients in pharmaceutical products. To date, crude drugs have been sterilized by heating methods. However, discoloration and loss of volatile components can readily occur with these methods. In contrast, irradiation is a non-destructive method of sterilization, which is known to result in less alteration of the components in comparison to heating[1].

X-rays, gamma rays or electron beams (EB) are used in irradiation. The excitation effect of these types of radiation produces free radicals in the irradiated substances. The free radicals produced react with water and quickly disappear. However, some are trapped in the solid phase of the irradiated substances, and can remain present there as stable radicals for a long period of time. These stable radicals can be used as indicators to identify whether or not irradiation has been performed. Currently, electron spin resonance (ESR) is used in methods established in the EU for detecting irradiated food products, using bone-derived radicals (bone-in meat, fish bone)[2], and cellulose-derived radicals (pistachio nuts, paprika, berries)[3] as indicators.

In this study, 8 common medicinal herbs were irradiated by EB, and the behavior of organic free radicals produced in the crude drugs after irradiation was investigated using ESR. Various types of stable radicals that had been trapped in plant tissues were detected. In addition, the pros and cons of detecting irradiated crude drugs according to the physical properties of the organic free radicals were verified.

2. Materials and Methods

2・1 Specimens

The following substances were obtained from crude drug wholesalers between 1999 and 2005 : Perillae Herba (Perilla Herb, China), Sennae Folium (Senna Leaf, India), Cinnamomi Cortex (Cinnamon Bark, China), Phellodendroni Cortex (Phellodendron Bark, China), Ginseng Radix (Ginseng root, China), Glycyrrhizae Radix (Licorice root, China), Paeoniae Radix (Peony Root, China), and Zingiberis Rhizoma (Ginger root, China). The crude drugs were stored at room temperature in the dark.

2・2 Loss on Drying Test

The crude drugs were pulverized (<850 μm), and approximately 2 g were placed in a weighing bottle and accurately weighed. After drying at 105 ℃ for 6 h (Phellodendri Cortex : 60 ℃, 8 h), the sample was accurately weighed again, and the loss in mass was calculated.

2・3 Irradiation

Irradiation was performed by Japan Electron Beam Irradiation Services using a Dynamitron Electron Beam Accelerator, 5 MeV (Radiation Dynamics Inc.). The crude drugs were cut into 5 mm square pieces, and sealed in 160×100 mm polyethylene bags to a depth of no more than 10 mm. These bags were lined up on the support base, and irradiated on one side from above by EB under ambient atmospheric conditions at an absorbed dose of 10 kGy. Dosimetry was performed using a Cellulose Triacetate (CTA) dosimeter (FTR-125, Fuji Photo Film Co., Ltd.), and a Radiochromic (RC) dosimeter (FWT-60, Far West Technology Inc.). Dosimeters measured across both sides of the irradiated sample, the absorbed dose was calculated from the difference in absorbance before and after EB irradiation, and mean values were calculated.

2・4 ESR measurement

Organic radicals of crude drugs were detected by ESR before and after irradiation. Crude drugs were pulverized (<850 μm) using a compact tabletop grinder (Mini Blender, Mr. Coffee, Inc.). Approximately 0.1 g of the powder was accurately weighed, and placed in a quartz ESR tube (ϕ 5 mm) to a depth of approximately 3 cm. The tube was then sealed with plastic film. ESR measurements were performed using an ESR spectrometer (ES-10, Nikkiso Co., Ltd.). Measurements were conducted under the following conditions. Microwave frequency : 9.4 GHz ; power : 4.05 mW ; sweep width : 330 ± 15 mT ; modulation width : 0.2 mT ; sweep time : 60 s ; and time constant : 0.12 s. Samples were measured at room temperature, and double accumulation was performed to obtain the ESR spectra. The g-values were calculated from the spectra obtained using a Mn^{2+}/MgO standard. The radical concentration was calculated from the area of the double-integrated curve of the ESR spectrum, using 1, 1-diphenyl-2-picrylhydrazyl (DPPH in benzene) as the standard. Measurements were performed twice, the mean values were calculated and corrected based on Mn^{2+}/

Table 1 Dry loss of crude drugs

Sample	Dry Loss %	JP %	Sample	Dry Loss %	JP %
Perillae Herba	9.58	< 13.0	Ginseng Radix	9.19	< 13.0
Sennae Folium	8.03	< 12.0	Glycyrrhizae Radix	7.80	< 12.0
Cinnamomi Cortex	11.7	< 15.5	Paeoniae Radix	9.21	< 14.0
Phellodendri Cortex	5.20	< 9.0	Zingiberis Rhizoma	13.6	

JP : Japanese Pharmacopoeia Fifteenth Edition

Table 2 Characteristics of spectra obtained with crude drugs.

Sample	*g* value		spins/g	
irradiation	before	10 kGy	before	10 kGy
Perillae Herba	2.0056	2.0057	4.49×10^{16}	3.71×10^{17}
Sennae Folium	2.0041	2.0053	2.29×10^{16}	3.37×10^{17}
Cinnamomi Cortex	2.0049	2.0053	1.56×10^{16}	1.37×10^{17}
Phellodendri Cortex	2.0060	2.0059	1.03×10^{16}	3.45×10^{17}
Ginseng Radix	2.0052	2.0062	2.61×10^{15}	1.73×10^{16}
Glycyrrhizae Radix	2.0059	2.0058	9.53×10^{15}	1.90×10^{17}
Paeoniae Radix	2.0067	2.0060	3.40×10^{15}	8.26×10^{16}
Zingiberis Rhizoma	2.0044	2.0042	3.34×10^{15}	3.45×10^{16}

MgO signal intensity. ESR tubes containing the samples were stored at room temperature in the dark in a desiccator at 40 ± 5% humidity.

3. Results

3・1 Water content

Crude drugs were air dried following normal procedures and stored[4]. For quality assurance purposes, the upper limits used for loss on drying values for strongly hygroscopic crude drugs were those established by the Japan Pharmacopoeia (Table 1). Depending on the type of crude drug, the loss-on-drying value may include volatile component loss in addition to water content loss. Results showed that loss on drying was around 10% for each of the crude drugs; these water content rates were within the standard range. Consequently, we determined that the crude drugs used in the tests had in all cases been stored at the appropriate humidity.

3・2 ESR spectra of crude drugs

ESR spectra of the crude drugs were compared before and after 10 kGy EB irradiation (Table 2, Fig. 1). In the crude drugs before irradiation, single line spectra were detected from $g = 2.0041$ to 2.0067. After 10 kGy irradia-

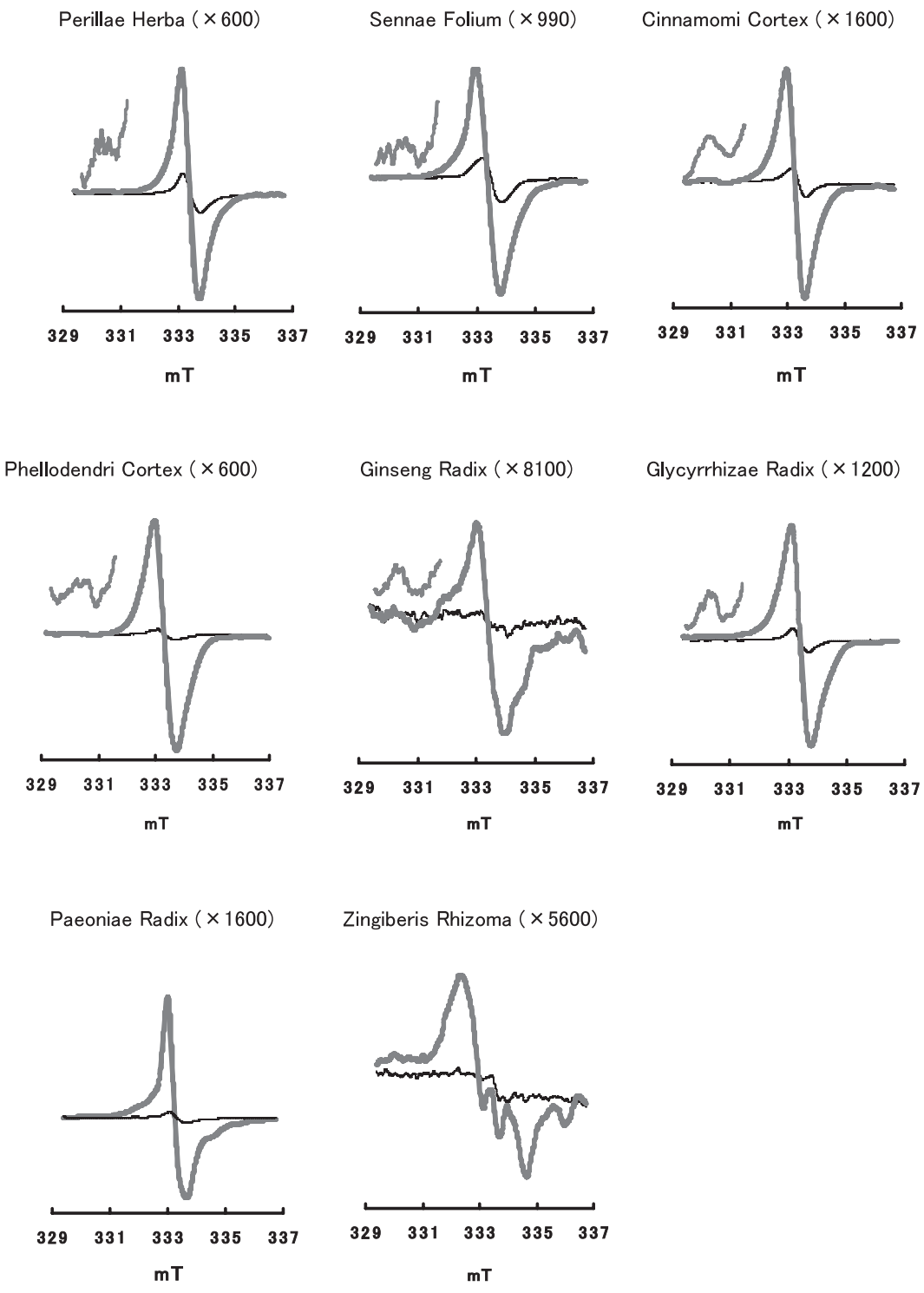

Fig. 1 ESR spectra of crude drugs, un-irradiated (solid line) and irradiated at 10 kGy (bold line) on modulation width 0.2 mT. Inset shows sub-signal on modulation width 1 mT.

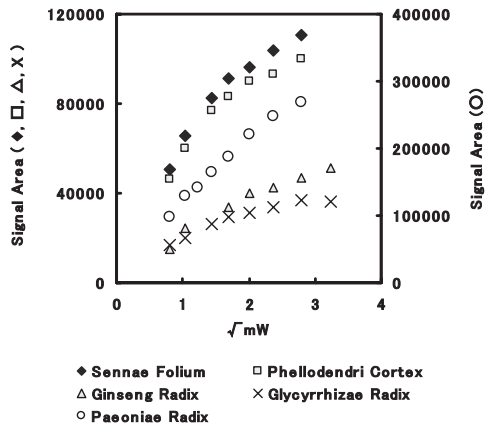

Fig. 2 Relationship between microwave power and ESR signals of the irradiated crude drugs.

tion, the signal became broader and the intensity was also increased. In Perillae Herba, Phellodendri Cortex, and Glycyrrhizae Radix, the g-value of the signal was virtually the same before and after irradiation. In Ginseng Radix, Paeonaie Radix, and Zingiberis Rhizoma, complex asymmetrically divided signals were detected after EB irradiation.

Weak signals, which were not detected before irradiation, were detected in all of the irradiated samples, in 3 mT shoulders of the singlet. Figure 1 shows a subsignal on the low magnetic field side detected at elevated sensitivity (modulation width : 1 mT).

3・3 Saturation of signal intensity according to microwave power

Figure 2 shows the saturation characteristics according to the microwave power of ESR signals of irradiated crude drugs (g = 2.004 to 2.006). Because the number of unpaired electrons produced in the irradiated crude drugs varies in each sample, it is difficult to conduct all measurements at the same sensitivity. The intensity of the signal detected in Glycyrrhizae Radix attained saturation at 4 mW and above. Consequently, ESR measurements were all conducted at a microwave power of 4 mW in this experiment.

3・4 Stability of radicals

Radical concentrations for ESR signals detected in non-irradiated crude drugs near g = 2 were at a level of 10^{15} to 10^{16} spins/g (Table 2). Perillae Herba had the highest spin number, and Ginseng Radix had the lowest. Radical concentrations increased after 10 kGy irradiation to a level of 10^{16} to 10^{17} spins/g. The percent increase in radical following irradiation differed with each specimen ; the largest percent increase in radical number was in Phellodendroni Cortex (33 times pre-irradiation levels).

Figure 3 shows the decay of radicals in crude drugs after irradiation. ESR signals that increased as a result of irradiation had reduced to 60－70% of that increase within 1 day after irradiation. A steady state was attained after approximately 1 month in Perillae Herba, Cinnamomi Cortex, Ginseng Radix, and Zingiberis Rhizoma. In addition, the steady level attained for the ESR signal intensity in Perillae Herba, Senna Folium, Cinnamomi Cortex, Phellodendroni Cortex and Zingiberis Rhizoma was less than two times pre-irradiation levels.

Although signal intensity reduced, the specific ESR signal shapes could be confirmed over a long period of time. In Paeoniae Radix and Zingiberis Rhizoma, asymmetrically divided signals could be detected even approximately 1 year after irradiation (Fig. 4) . In addition, the new subsignals detected on the 3 mT low-magnetic field side of the central signal could be detected for a long period of time after irradiation (Fig. 5) . The peak height of the subsignal from the baseline (S) was observed to

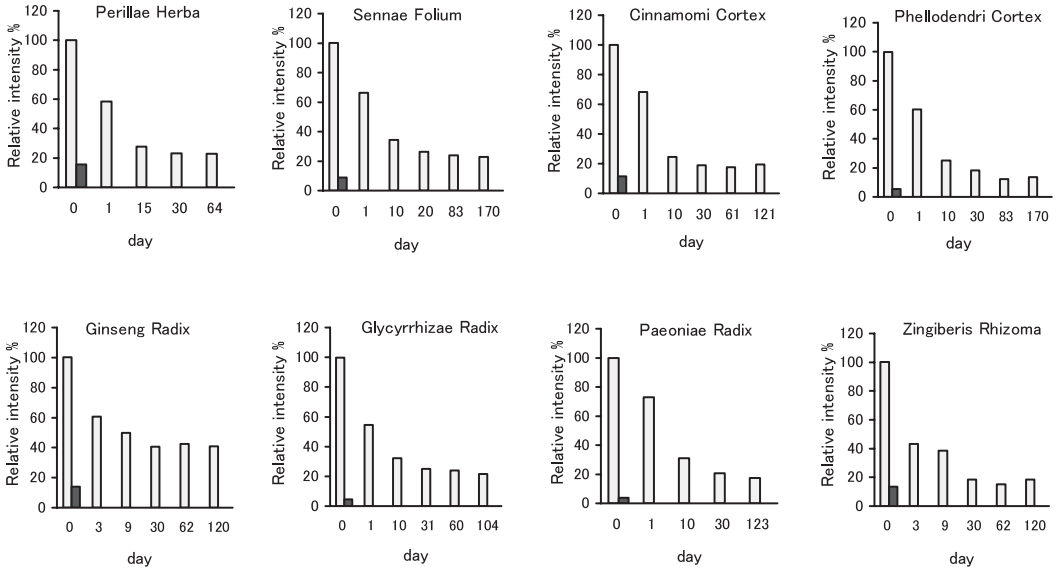

Fig. 3 Time dependence of intensity of ESR spectra amplitude obtained for irradiated crude drugs (10 kGy). The closed bar represents the level before irradiation.

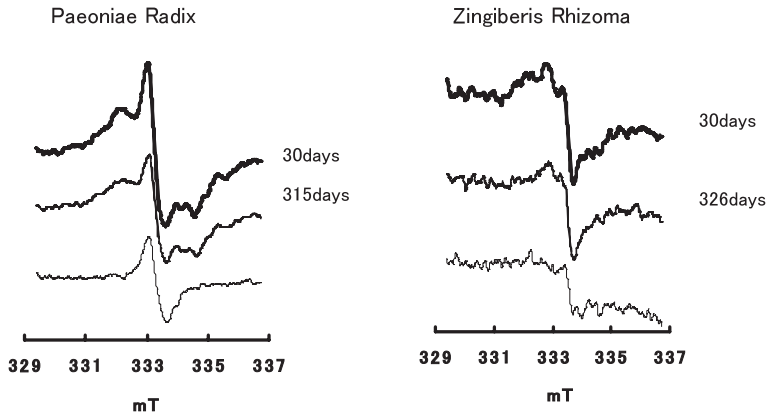

Fig. 4 ESR spectra of Paeoniae Radix and Zingiberis Rhizoma after 10 kGy irradiation. The dashed line represents ESR spectra before irradiation.

be at least twice the noise level (N) of the baseline at the same positions for non-irradiated crude drugs (S/N>2) for approximately 200 days after irradiation in Perillae Herba and Sennae Folium, and for approximately 1 year in the other crude drugs.

4. Discussion

4·1 Organic radicals detected in crude drugs

Figure 6 shows the correlation between loss on drying and the percent increase in radical concentration in crude drugs following irradiation. These results established the existence of

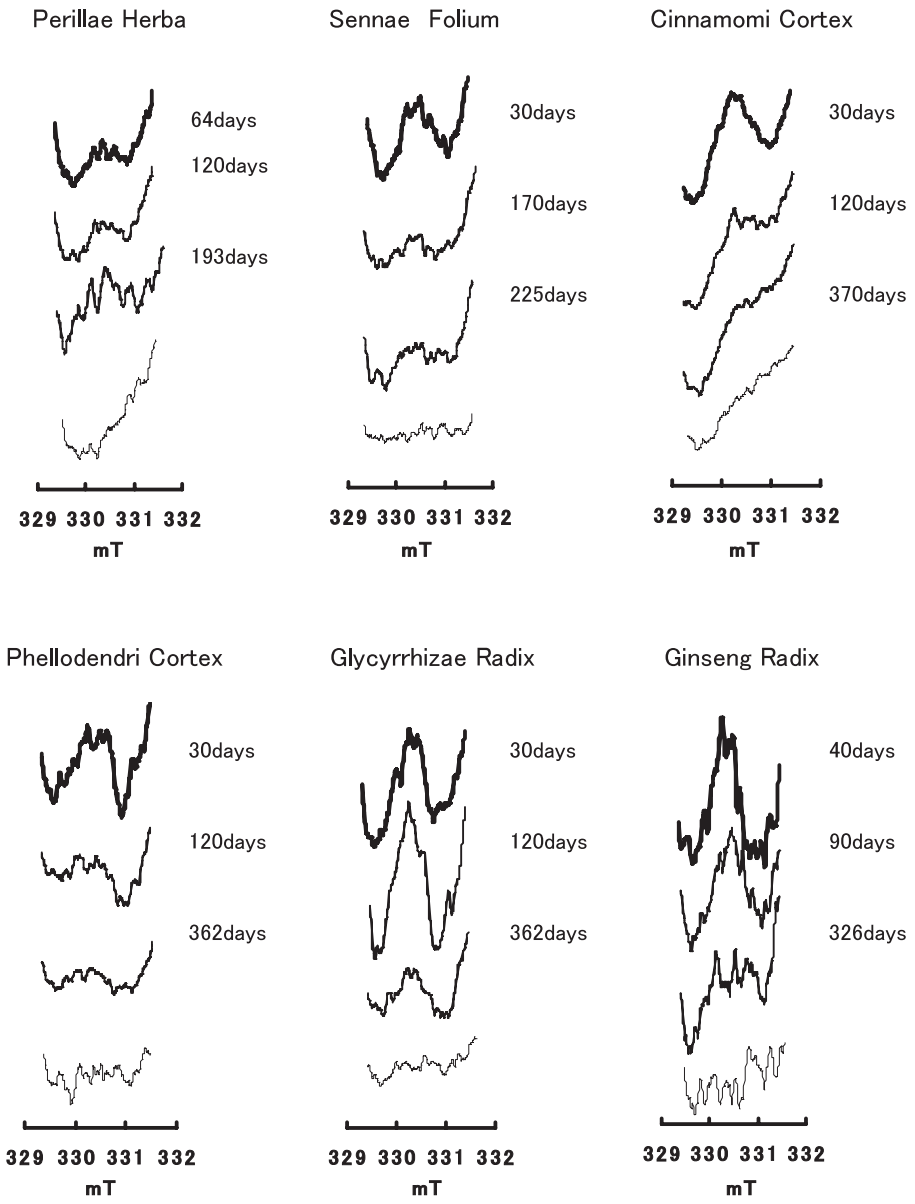

Fig. 5 ESR spectra of sub-signals obtained with crude drugs after 10 kGy irradiation. The dashed line represents ESR spectra of the drugs before irradiation. (modulation width ; 1 mT).

an inverse correlation between these two parameters ($R^2 = 0.55$, $P < 0.05$). In short, if the water content in the tissue is high, the number of radicals trapped in the solid phase after irradiation decreases. This is considered to be because the presence of water molecules disperses the excitation energy at an early stage.

The ESR signal at $g = 2.005$ detected in the dried plants is reported to be derived from semiquinone radicals[5),6)] produced during the course of direct or indirect oxidation of polyphenol by active oxygen. As a result, the

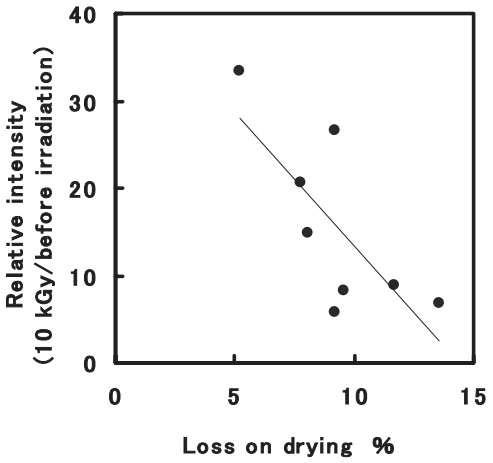

Fig. 6 Relationship between loss on drying and relative ESR signal intensity of irradiated crude drugs.
($Y = -3.03\,X + 43.7$, $R^2 = 0.5541$, $P < 0.05$)

formation of these radicals is not dependent on exposure to radiation, as they may also be produced by factors such as heat or light. Based on Table 1, high radical concentrations were present both before and after irradiation in Perillae Herba and Sennae Folium, which are leaf varieties of crude drugs. On the other hand, radical concentrations were low in Glycyrrhizae Radix, Ginseng Radix, Paeoniae Radix and Zingiberis Rhizoma, which are root varieties of crude drugs. This is considered to be because polyphenols such as anthocyanin are often distributed in leaf epidermal cells. In particular, the g-values of signals before and after irradiation were virtually the same in Perillae Herba. As a result, most of the radicals added to this crude drug as a result of irradiation are believed to be from semiquinone radicals produced from cyanidin.

Broad, asymmetrically divided signals were detected over a long period of time in irradiated Ginseng Radix, Paeoniae Radix, and Zingiberis Rhizoma. The spectra, as shown in Figs. 1 and 4, are similar to those of sugar-derived radicals detected in irradiated dried fruit[7),8)]. Radicals trapped in crystalline sugars are known to remain stable for a very long time[9)]. Storage tissues such as roots have a high sugar content in the form of substances such as sucrose and starch: hence, in root varieties of crude drugs, the formation of sugar radicals takes precedence over that of other radicals, and spectral specificity is thought to be marked.

The newly detected weak signals seen in all irradiated crude drugs (3 mT shoulder of central signal), as shown in Fig. 1, are derived from cellulose radicals[10),11)]. Cellulose-derived radicals have specific signals produced by the exposure of dried plants to radiation. Consequently, despite the occurrence of differences in signal intensity depending on the plant part (root, leaf, bark, etc.), signals can be detected when crude drugs derived from plants are exposed to radiation.

4·2 Detection of irradiated crude drugs

When examining products, it is crucial to rapidly determine whether or not a product has been irradiated, and whether or not the radiation dose was within allowable limits. In the ESR spectra of irradiated crude drugs derived from plants, specific signals, that were not present before irradiation, were produced by organic radicals derived from sugar and cellulose. In the case of crude drugs that comprise storage tissue with a high sugar content, after irradiation, the ESR spectra presented broad, asymmetrically divided signal shapes that were very durable, making it easy to discriminate between irradiated and non-irradiated samples. In addition, cellulose-derived radicals are detectable in almost all crude drugs de-

rived from plants. However, depending on the plant organ and tissue structure, signals intensity from these radicals may be weak. Moreover, they also may overlap signals of radicals derived from other components of the plant. Consequently, measurement methods that have increased sensitivity for detecting radicals are needed for obtaining more distinct ESR spectra. For example, magnetic field modulation is used in ESR to detect sensitive signals. To obtain the optimal, distortion-free ESR spectra, magnetic field modulation should be set to about 1/4 to 1/2 the width of the ESR absorption peak[12]. In this study, in order to further increase absorption intensity, measurements were attempted with the magnetic field modulation set to the same level as the absorption peak width (1 mT). As a result, signal intensity increased with slight distortion of the absorption peak. However, because the intensity of cellulose-derived signals became more distinct, the signals could be detected for 6 months after 10 kGy irradiation in leaf varieties of crude drugs, and for a year after irradiation in crude drugs that use other parts of plants. In countries where the irradiation of spices is permitted, the maximum allowable absorbed dose is 10 kGy or more[13]. Consequently, if the allowable absorbed dose of 10 kGy is established as the indicator, irradiated crude drugs can also be discriminated in the same way, based on the signal shape characteristics of the ESR spectra.

5. Conclusion

Particularly with ESR methods, irradiated products can be measured rapidly and non-destructively with a high degree of sensitivity. In this respect, the detection of irradiated crude drugs by ESR is a suitable initial screening technique to discriminate whether or not irradiation was performed within permissible limits.

On the other hand, the quantity of organic radicals produced in crude drug tissues as a result of irradiation is significantly affected by the water content of the tissue. Moreover, by 1 or 2 months after irradiation, organic radicals reduce to about twice pre-irradiation levels, and later, frequently attain a steady state. Consequently, the date of irradiation must be specified and the storage conditions confirmed when using an organic radical as an indicator for estimating the amount of radiation an irradiated product has been exposed to. Moreover, the majority of organic radicals will have disappeared 2 or more months after irradiation. Consequently, it is necessary to be careful to discriminate whether an increase in radicals is attributable to irradiation, or is due to some other physical stimulus such as pulverization or heating.

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要　旨

電子線照射した生薬の ESR 法による検知

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ソヨウ，センナ，ケイヒ，オウバク，ニンジン，カンゾウ，シャクヤク，ショウキョウに EB 照射（5 MeV，10 kGy）し，発生する有機フリーラジカルを ESR 測定により検出した。未照射の生薬には $g = 2.005$ 付近に一重線スペクトルが検出され，葉類生薬でスピン濃度が高かった。照射後，$g = 2.005$ 付近のシグナル強度は増大し，その 3 mT 脇に新たなサブシグナルが検出された。また，照射した根類生薬では，線形の広がった非対称な分裂シグナルが検出された。照射生薬の有機成分に捕獲されるラジカル数は，含水量に対して減少傾向を示した。照射後，シグナル強度は減衰し，約 1 ～ 2 か月で定常状態となった。しかし，照射後に見られる ESR シグナル形状の特異性は，葉類生薬では 6 か月間，樹皮及び根類生薬では 1 年間にわたり検出された。したがって，ESR 法は照射生薬の検知の第 1 スクリーニング法として適用し得ると考える。