Abstract: Antioxidant property of Sasa senanensis Rehd (Kumaisasa) leaf powder was examined both in vitro and in vivo. Kumaisasa is a native Japanese dwarf bamboo plant and the leaves have been traditionally used as tea, functional food, or folk medicine, such as for wrapping sushi and chimaki (rice cake) to prevent putrefaction. When the antioxidant activities were determined by calorimetric and Electron Spin Resonance (ESR) assays for the aqueous and 80% methanol (MeOH) extracts, the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activity was not markedly different between the aqueous and MeOH extract but the Oxygen Radical Absorbance Capacity (ORAC) value of the MeOH extract showed almost twice as strong as aqueous extract. Hydroxyl radical and superoxide radical scavenging activities were also higher than in MeOH than aqueous extract. Consistently, both the content of flavonoids and phenolics, especially of flavonoids was much higher in MeOH than in aqueous extract. Interestingly, both the polyphenols content and the observed antioxidant and radical scavenging activities were significantly low when the sample was extracted for overnight compared to 1 hr. When hydroxyl radical scavenging activity was measured using Fenton reaction and Ultraviolet light (UV) illumination as hydroxyl radical generation, respectively, the observed activity was significantly higher for the Fenton system indicating metal chelating nature of the Kumaisasa extract. Further, the preventive effect of Kumaisasa intake was studied against the restraint stress-induced oxidative stress in mice.

Keywords: Kumaisasa (Sasa senanensis Rehd), Japanese bamboo leaf, antioxidant potential, restrain stress, oxidative stress, GSH

1. Introduction

It is well accepted that the reactive oxygen species (ROS) including free radicals are generated routinely in the body via physiological reactions such as mitochondrial respiration and external stimulus including ultraviolet rays, smoking, exhaust gas, air pollution, and other stressors [1]. Endogenous defense enzymes and physiological antioxidants play a primary role in scavenging ROS [2], but when the production of these species overcomes the defense ability of the system, a condition called oxidative stress is produced in which these species tend to damage tissues to develop variety of pathological conditions such as heart diseases, neurodegenerative diseases, and aging [3]. Therefore antioxidant supplementation is considered to be vital to combat the oxidative damage [4]. The antioxidants not only suppress the generation of free radicals, but also delay or inhibit the initiation and/or propagation of oxidizing chain reactions through quenching the ROS including free radicals [5]. It is suggested that many of the negative effects of oxidative stress are controllable upon supplementation with certain dietary antioxidants such as antioxidant vitamins and non-nutrient antioxidants, typically phytochemicals like flavonoids [6,7]. Therefore, efforts have been focused on finding naturally occurring antioxidants in food and medicinal resources to replace the synthetic antioxidants such as BHT that are being restricted due to the incidence of carcinogenicity [8,9].
Bamboo species are an attractive health food or functional food resource that has been used as a medicinal herb in the oriental medicine and also as a folk medicine [10]. Moreover, bamboos have made a mark in the field of functional and medicinal food with their rich antioxidant contents [11] and specific functional factors [12]. Kumaisasa (Sasa senanensis Rehd) is the native Japanese dwarf bamboo plant, and is also habitating in the eastern Asian territories including China, Korea and Russia [13]. In Japan, Kumaisasa leaves have been used traditionally as tea, functional food, or folk medicine [10] and also for wrapping such types of foods as sushi and chimaki (rice cake) for preservation because of its antimicrobial function. Hence, the pharmacological importance of this plant is attracting attention.

In the present study the antioxidant potential of Kumaisasa leaf powder has been studied both in vitro and in vivo because the antioxidant activity is the basic and common property required for the functional food. Results showed that the Kumaisasa leaf powder not only has high potential as antioxidant in vitro but also prevented in vivo the restrict-stress-induced oxidative stress effectively in mice.

2. Materials and methods

2.1. Plant material and extraction

Dried and ground Sasa senanensis leaves product (Kumaisasa leaf powder) was provided from UNAIAL Co. Ltd., (Tokyo, Japan). For in vitro analysis, 5 g of Kumaisasa powder was dissolved in 50 mL of 80% methanol aqueous solution and kept overnight under gentle stirring. The mixture was centrifuged twice at 830 x g (KUBOTA KN-70) for 15 min and the supernatants were collected. This extract was stored at 4°C for in vitro and in vivo antioxidant activity analysis. The antioxidant activity was also determined in vitro for the samples extracted by 1 hr stirring. For the animal experiments, the aqueous suspension of Kumaisasa powder as gavage was used.

2.2. Chemicals

All chemicals used were of analytical grade. Morpholin-pethanesulfonic acid (MES), ascorbic acid, pyridine, acetic acid, trichloroacetic acid (TCA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Thibarbituric acid (TBA), Folin-Ciocalteu reagent, aluminium chloride (AlCl₃), Gallic acid, Diethylenetriamine-pentaacetic acid (DTPA), 2,2’-azobis(2-amidino-propane) dihydrochloride (AAPH), L(+)-Ascorbic acid and other reagents were obtained from Wako pure Chemicals Co., Ltd, Japan. NADPH, SDS, and n-butanol were supplied from Kanto chemical Co., Inc, Japan. DMPO (dimethylpyroline-N-oxide), and xanthine oxidase (XOD, 20 U/ml, from cow milk) were from LABOTEC Co., Ltd., and Boehringer Mannheim Co., Ltd, Germany, respectively. Quercetin hydrate, Glutathione reductase (GR), and DTNB [5,5-dithiobis(2-nitrobenzoic acid)] were purchased from Sigma-Aldrich Co., Ltd, USA.

2.3. Total phenolic assay

Total phenolic content was measured for the aqueous and MeOH extracts by Folin–Ciocalteu method using Gallic acid as phenolic’s reference [14]. Briefly, 0.25 mL of the extract was added to a mixture of 3.5 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent, vortexed and incubated for 3 min. Following the addition of 1 mL of 1% sodium carbonate, the mixture was vortexed again, and then kept in boiling water for 1 min. After cooling in the dark at room temperature for 30 min, the absorbance at 765 nm was measured using Bio-Rad Model 550 Microplate reader. The total phenolic contents were determined as µg Gallic acid equivalent/100 g Kumaisasa powder using a calibration curve prepared for Gallic acid as the standard reagent.

2.4. Total flavonoid assay

Total flavonoid content was measured by the aluminium chloride (AlCl₃) colorimetric method [15] using Quercetin as the standard. An aliquot (125 µL) of measuring samples were added to 625 µL Distilled water (DW) following addition of 37.5 µL of 5% NaNO₂. At 5 min after the NaNO₂ addition, AlCl₃ (75 µL, 10%) was added to the solution and stood for 6 min at room temperature following addition of 250 µL of 1M NaOH and 137.5 µL of DW. After extended mixing, the absorbance at 490 nm was measured and the flavonoid content was calculated from the standard curve prepared for Quercetin. The results are expressed as µg quercetin equivalent/100 g Kumaisasa powder.

2.5. Measurement of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH radical scavenging activity of Kumaisasa extracts were determined by colorimetric [16] and electron paramagnetic resonance (ESR) [17] methods. Briefly, in a 96-well microplate, an aliquot (50 µL) of Kumaisasa extract was mixed with 150 µL of the reagent mixture containing equal volume of 0.2 M of 2-morpholinopethanesulfonic acid (MES), 0.4 mM of methanolic DPPH and 80% methanol. The plate was covered with an aluminium foil and kept in the dark at room temperature for 10 min, and then the absorbance was measured at 570 nm using Bio-Rad Microplate reader. The inhibitory activity was calculated from the following equation: % inhibition = [(absorbance of blank – absorbance of sample)/absorbance of blank] x 100.

2.6. Determination of ORAC value

The ORAC values were measured according to the method reported elsewhere [18] with slight modification. The stock solutions of fluorescein (4.19 x 10⁻³ mM), AAPH as peroxyxl
radical generator (153 mM), and Trolox as reference antioxidant were prepared in 75 mM potassium phosphate buffer (pH 7.4), respectively. To 25 µl of blank solution (75 mM potassium phosphate buffer), the reference antioxidant (Trolox of 5, 10, 25, 50, and 100 µM) or measuring samples (0.25, 5.0, 1.0, and 2.0 mg/mL) and, 150 µL of fluorescein were taken into the wells of a 96-well microplate. Then following the addition of 25 µl of AAPH, and the fluorescence intensity of the reaction solution was recorded at 37°C of incubation temperature every 30 sec for 90 min at the emission of 527 nm and excitation of 485 nm using a fluorescence spectrophotometer (Labsystem Fluoreskan Ascent CF). Final results were calculated using the differences of areas under the fluorescence decay curves between the blank and sample or Trolox. The results were expressed as ORAC units, where 1 ORAC unit equals to the net protection produced by 1 µM Trolox.

2.7. Measurements of Hydroxyl and Superoxide Radical Scavenging Activities

The hydroxyl radical (⋅OH) scavenging activity was determined by the spin trapping ESR method using DMPO as the spin trap reagent, in that the ⋅OH was generated either by Fenton system [19] or UV irradiation [20] using UVGL-58 Handheld UV lamp (Funakoshi Co. Inc, Japan).

When the Fenton system was used as radical generator, the reaction solution was consisted of an aliquot of MeOH extract of Kumaisasa powder, 150 mM DMPO, and 1 mM FeSO₄, and 100mM hydrogen peroxide(H₂O₂) in 300 µL. The reaction was initiated by adding 30 µL of H₂O₂ and then the DMPO-OH formation was measured just 1 min after the addition of H₂O₂ using JEOL LES-TE 200 (X-band Microwave Unit) ESR spectrometer as described previously [19]. The ESR recording condition was as follows: microwave power; 8 mV, microwave frequency: 9.43893 GHz, modulation amplitude: 32 m. time constant; 0.03 s, sweep time; 0.5 min, center fields; 341.0 mT. In the UV irradiation system, the reaction mixture consisting of Kumaisasa extract as sample, DMPO and H₂O₂ was taken into a hematocrit capillary tube and illuminated by the UV lamp for 1 min. Then the ESR spectrum was recorded just 1 min after the irradiation was ceased. ESR recording condition was the same as for the Fenton reaction system.

Superoxide anion radical scavenging activity was measured also by DMPO spin-trapping ESR using Xanthine–Xanthine oxidase system as a superoxide radical generator according to the method reported elsewhere [21]. To the reaction solution containing an aliquot of Kumaisasa extracts, DMPO, 4 mM hypoxanthine, 5 mM DTPA in 300 mL total volume, 30 µL of aqueous solution of xanthine oxidase (20 U/mL) was added to generate the superoxide radicals. Then, the DMPO-OOH signal was determined by ESR just 1 min after the addition of xanthine oxidase. The ESR condition was same as above only with the exception of mod wide (0.2 mT) and the amplitude (100 m).

2.8. Animals and treatments

Male ICR mice, 7 weeks old, were housed in a specific pathogen-free animal room, under standard laboratory conditions of temperature (25±1°C), lighting (12 hr light-dark cycle) and relative humidity (25±15%). The animals were fed standard laboratory chow and tap water. The animals were kept under these conditions for 1 week before the experiment. The restraint stress experiment was carried out according to the method reported elsewhere [22]. Briefly, the restraint stress was given by confining mouse to an oval metal restraint cage prepared with wire mesh in our laboratory for 18 hr. The mice were starved during the stress acquisition. The normal control group was starved also for 18 hr without restraint stress. Water was freely given to all groups during experiment. Kumaisasa powder was suspended in water and orally administered as gavage to animals by intubation at two dosages of 125, and 250 mg/kg body weight, respectively, at 24 hr and at 30 min before the restraint stress was given. The volume of administrated sample was adjusted to 0.1 mL/10 g body weight. (Figure1). Vitamin C as the positive reference of antioxidant was given orally at a dose of 250 mg/kg. All animals were sacrificed after the restraint stress acquisition period.

The animal experiments were performed according to the internationally followed ethical standards and approval from Niigata University of Pharmacy and Applied Life Sciences.

2.9. Plasma and tissue sample collection and preparation

Figure 1: Experimental schedule for administration of Kumaisasa powder, stress acquisition and sample preparation for biochemical assays.
Under Ethyl ether anesthesia, blood was collected in test tubes coated with sodium heparin (50 U/mL blood) by cardiac puncture, and then the liver was quickly removed and dipped into cold saline for rinse. Blood samples were centrifuged at 5000 rpm for 10 min at 4°C by a refrigerated centrifuge apparatus (KUBOTA Micro Mini centrifuge) to obtain the plasma. Liver was chopped into pieces after washing in cold saline and homogenized in chilled 0.01 M PBS (pH 7.4) using a Polytroon mixer (PT-MR 2100, KANEMATICA) and centrifuged at 10000 rpm (KUBOTA Micro Mini centrifuge) for 10 min at 4°C. The supernatant was collected and stored at -20°C until use. The protein concentrations were determined by the Bradford assay with bovine serum albumin as standard.

2.10. Measurement of plasma ORAC value

The plasma ORAC value was determined according to the same method as described above for the Kumaisasa extract using the plasma diluted 500 times with phosphate buffer (pH 7.4).

2.11. Measurement of liver lipid peroxidation

Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa et al [23]. In brief, the tissue homogenate was mixed with 8.1% SDS, 20% acetic acid, and 0.8% TBA solution. Each sample mixture was heated at 95°C in a water bath (Fisher Scientific, Pittsburgh, PA) for 60 min. After cooling to room temperature, n-butanol and pyridine solution at a ratio of 15:1 was added to each sample mixture, vortexed thoroughly, and then centrifuged at 1500 x g for 10 min. The absorbance of the upper organic layer of the centrifuged solution was measured at 532 nm with a spectrophotometer (Milton Roy Co., Rochester, NY). TBARS value was calculated and expressed as nmol MDA (malondialdehyde) equivalents per mg protein.

2.12. Quantification of reduced glutathione (GSH)

GSH was measured by the method of Griffith [24]. Briefly, the precipitation reagent was prepared with 1.67 g metaphosphoric acid, 0.2 g of EDTA, and 30 g NaCl dissolving in one liter of distilled water. An aliquot of tissue homogenate, mixed with 3 mL of the precipitation reagent, was centrifuged and 2 mL of the supernatant was mixed with 4 mL of 0.3 M Na2HPO4 and 1 mL of 1 mM DTNB. After vortexed well at room temperature, the absorbance was measured at 412 nm. The level of GSH was expressed as µg per mg of protein.

2.13. Statistical analysis

Data are presented as the mean ± S.E. Statistical analyses of data were performed with Student’s t-test. Differences were considered to be significant at $p<0.05$.

3. Results

3.1. In vitro anti-oxidant potential of Kumaisasa extracts

The anti-oxidant properties of both aqueous and MeOH extracts of Kumaisasa were determined in vitro for DPPH, hydroxyl and superoxide radicals and ORAC. Since it is well known that plant phenolics, namely flavonoids are highly effective free radical scavengers and antioxidants, and thus contribute to the antioxidant potential of natural resources, the total phenolic and Flavonoid contents were measured first. Data are given for the aqueous and MeOH extracts of Kumaisasa powder obtained by 1 hr and overnight extraction, respectively, in Fig 2. The phenolic contents determined for the 1 hr extraction samples were approximately 566.5 µg and 938.1 µg as Gallic acid/100 g powder in aqueous and MeOH.
Figure 3: DPPH radical scavenging activity and ORAC value of Kumaisasa extracts. H$_2$O and 80% MeOH denote the aqueous and 80% methanol extracts, respectively. The data are given in mean ±SD (n=4). DPPH radical scavenging activities were measured both by colorimetric and ESR methods, and the detail is given in the method section together with ORAC assay.

It is noted that the total contents of phenolics and flavonoids were significantly low in the extracts obtained by overnight extraction compared to 1 hr indicating the extracts contains easily oxidizable phenolic components.

DPPH radical scavenging activity was determined both by colorimetric and ESR methods for the aqueous and MeOH extracts of Kumaisasa (Fig.3). The activity was dose dependently increased (data not shown) and the data are shown as the Trolox equivalent/g Kumaisasa powder (Fig.3). There was marked difference between the observed DPPH scavenging activities determined by colorimetric and ESR methods although both results indicated rather high activity for MeOH than aqueous extract. Probably the high background color of the Kumaisasa sample disturbed accurate estimation of net DPPH quenched.

The ORAC values determined also showed marked difference between those in aqueous and MeOH extracts. The MeOH extract showed almost twice as high ORAC value as the aqueous extract.

When the antioxidant activities were compared between the samples obtained by overnight extraction and 1 hr, the activity was considerably low in the overnight extraction samples, consistently with the total phenolic and flavonoid contents in the both samples shown in Fig. 1.

Further the hydroxyl and superoxide radical scavenging activities were determined by ESR spin trapping method. Since the aqueous extract enhanced DMPO-OH signal rather than quenching, the OH scavenging activity was determined only for the MeOH extract. All the data are summarized in Fig. 4 with IC$_{50}$ values. It was revealed the MeOH extract has rather high scavenging activity towards DPPH and superoxide radicals compared to the aqueous extract. The superoxide radical scavenging activity was stronger than DPPH in 1 hr extracted sample. In the overnight extraction sample, both the superoxide and DPPH radical scavenging activities, especially, the superoxide radical scavenging activity, were remarkably decreased in the MeOH extract compared to the 1 hr extraction samples. On the other hand, the OH radical scavenging activity

Figure 4: Comparison of radical scavenging potential of Kumaisasa extracts. H$_2$O and 80% MeOH denote the aqueous and 80% methanol extracts, respectively. The data are given in mean ±SD (n=4). DPPH radical was determined directly by ESR. Hydroxyl radical and Superoxide radical were determined by DMPO spin trapping method using ESR. The hydroxyl radicals were generated both by Fenton reaction with FeCl$_2$ and UV irradiation. Detailed methods are described in the method section.
Figure 6: Protective effect of Kumaisasa intake against restraint stress induced oxidative stress. TBARS (A) and GSH (B) were measured as described in the method section. Data are given in the mean±SE of values obtained from 6 animals in each group. Notations are as follows: SS-125; stress+sasa(125 mg/kg), SS-250; stress+sasa(250 mg/kg), SV-250; stress+vit-C(250 mg/kg). The significance of differences from the stress control at * p<0.05 was determined with Student’s t-test.

3.2. Effect of Kumaisasa extract on plasma antioxidant capacity

In order to know whether the Kumaisasa extract is actually antioxidant active in vivo, the preventive effect of Kumaisasa was examined on the restraint stress-induced oxidative stress in mice. Restraint stress is known to promote the oxidative stress by generating ROS to change the balance between oxidant and antioxidant potential [25].

After 18 hr restraint stress acquisition, the plasma ORAC level was found significantly decreased compared to the normal control. However, the stress-induced decrease of ORAC was markedly suppressed in the mice given 125, and 250 mg/kg Kumaisasa powder as aqueous gavage prior to the stress acquisition, and the effect was fairly dose dependent. On the other hand, Vitamin C as a reference antioxidant did not show significant protection against the stress induced decrease of plasma ORAC level (Fig. 5).

3.3. Effect of Kumaisasa on TBARS and GSH levels in liver tissue

Further, the restraint stress-induced oxidative stress and the protection by Kumaisasa were studied by observing malondialdehyde (MDA) formation in the liver. After the restraint stress for 18 hr, the liver MDA level was significantly increased compared to normal control mice (p<0.05) (Fig.6A) indicating that oxidative stress is progressed as was previously reported elsewhere [34]. When Kumaisasa gavage was orally given prior to the stress acquisition at the dose of 125 and 250 mg/kg, respectively, the liver MDA levels were kept low comparable to the level of normal control. L(+) Ascorbic acid (250 mg/kg) as a reference antioxidant also significantly decreased the liver MDA level when compared to the level of restraint stressed mice (p<0.05).

At the same time, the liver GSH level was significantly decreased in the restraint stress mice compared to the normal control mice. Pre-administration of Kumaisasa showed prevention of the stress induced GSH loss in the liver,
especially at its high concentration (250 mg/kg). However, the difference of the GSH level between stressed control and stressed + Kumaisasa group of mice was not statistically significant. Ascorbic acid also showed preventive effect comparable to that in high dose Kumaisasa group.

4. Discussion

In the living system, free radicals and ROS are constantly generated from internal and external sources. Accumulation of damages in tissues and biomolecules lead to various disease conditions such as degenerative diseases and extensive lysis [26]. Since the oxidative stress plays a crucial role in pathogenesis and progression of wide spectra of diseases, antioxidant supplementation attracts attention as a strategy for the prevention of diseases, and a beneficial role of antioxidant intake from diet as either nutrients or non-nutrients is extensively discussed in relation to health outcomes [27].

In this sense, traditionally used herbs and folk medicines are the attractive resource for developing antioxidant health food or supplement because these contain a variety of antioxidant active molecules such as polyphenols [28]. Sasa family is one of such herb having been used in the traditional oriental medicine and folk medicine. For example, alkaline Sasa leaf extract has been reported to have several pharmacological functions, including anti-inflammatory, antiviral, antibacterial and vitamin C-synergized radical scavenging activity [29].

Kumaisasa (Sasa senanensis Rehd.) examined in the present study is a family of Sasa growing in all the areas of Japan islands. The leaf of Kumaisasa has been traditionally used for wrapping of food staff such as sushi because it is considered to have antifungal or antimicrobial activity. It is also known as a popular folk medicine in Japan having variety of functions such as nourishing and tonic functions, deodorizing function, gastro-intestinal stabilization and anticancer functions but a few precise scientific studies have been done to date.

In the present study, we focused our interest on the antioxidant activity of both aqueous and MeOH extracts of the Kumaisasa leaf. Results clearly showed that Kumaisasa leaf extract has strong anti-oxidant potential as evaluated by several in vitro assay systems including hydroxyl, superoxide and DPPH radical scavenging reactions (Fig.5). Further it was demonstrated that the oral intake of aqueous suspension of Kumaisasa leaf powder actually prevented the oxidative stress progression in the restraint stressed mice as evaluated by plasma ORAC and liver GSH levels together with liver oxidative damage (Fig.5, 6).

The antioxidant nature of the Sasa leaf has attracted attention because of its rather high contents of phenolics and flavonoids as the present study showed in Kumaisasa extract (Fig.2). They are the major antioxidant components of many herbs and contributing to the antioxidant activity [28]. Several antioxidant flavone glycosides have been isolated from different species of Sasa family. For example, Kurilensis A,B, and tricin with their glycosides were isolated from Sasa Kurilensis [30], iso-orientin and oreientin glycosides together with apigenin and tricin glycosides from Sasa borealis that is the major Sasa leaf used in Korea [31], and Vitexin, homovitexin, orientin, isoorientin from Sasa argenteastrahtius[32].

In Sasa senanensis Rehd. (Kumaisasa) leaf, luteolin glycosides (C-glycosyl flavone) together with tricin glycosides were also identified previously [33] and our present study also determined the presence of these flavonoid components together with isoorientin glycoside by HPLC (data not shown). Thus the flavonoid components might be the major components playing roles in the antioxidant activity of Kumaisasa leaf powder observed in the present study both in vitro and in vivo. Although these flavonoids might be the major components contributing to the antioxidant potential, phenolics other than flavonoids (Fig.2) such as chlorogenic acid and caffeic acid also contributed to the high antioxidant potential of Kumaisasa because the aqueous extract also showed high antioxidant potential, which contains phenolics other than flavonoids.

The present study also revealed the superoxide radical scavenging activity of Kumaisasa is relatively high compared to DPPH and hydroxyl radical scavenging activities when the activities were determined for the 1 hr extraction sample. However, the activity was remarkably reduced in the overnight extraction sample, especially in the aqueous extract as shown in Fig.4. This changing pattern was also observed in the DPPH radical scavenging activity. This indicates that certain fraction of the antioxidant molecule(s) present in the extracts were quite susceptible to the atmospheric oxygen. The difference absorption spectra of extracts showed a time dependent decrease of certain component(s) having absorption maximum at around 320 nm in the aqueous extract and in the MeOH extract both at 320 nm and 460 nm after standing under aerobically, especially in the 1 hr extract (data not shown).

Further study is being undertaken to identify this component. Rapid disappearance of radical scavenging potential, especially of superoxide scavenging activity in the Kumaisasa extract allows us to consider that the extract is rich in the compounds with electron donating or H-atom donating properties [34]. Polyphenols, especially the flavonoids having catechol moiety in B ring is a typical of such compounds and are easily oxidized under aerobic condition.

The H-atom donating nature of Kumaisasa leaf powder is also reflected in the ORAC value as a reliable measure of peroxyl radical scavenging potential [35]. This is consistent with the observation that the Kumaisasa MeOH extract containing mainly flavonoids showed rather high ORAC value in vitro.

It is observed that DMPO-OH adduct was enhanced rather than quenched by the addition of the aqueous extract in the spin trapping ESR assay of ‘OH. Since the DMPO-OH formation was observed without hydrogen peroxide presence, the ‘OH was generated by direct interaction of Fe with certain component in the aqueous extract. It is probable that certain hydroperoxide is present in the aqueous extract but further study is necessary to understand the clear reason for the enhanced formation of hydroxyl radical adduct of DMPO in the aqueous extract. It is however interesting that the intake of Kumaisasa aqueous suspension did prevent the restraint stress...
induced oxidative stress in dose dependent fashion without adverse increase of oxidative stress. It is known that a slight oxidative stress enhances cellular antioxidative responses (37). Further study is required to clarify whether this mechanism is also involved in the effective antioxidative function of Kumaisasa in vivo.

The antioxidant property of Kumaisasa shown in the present studies indicates that the intake of flavonoid rich Kumaisasa will be beneficial for protecting and/or balancing the physiological redox system in early stage of oxidative stress. Indeed, the intake of Kumaisasa effectively prevented the oxidative stress induced by the restraint stress (Fig. 6).

5. Conclusion

The present study demonstrated the Kumaisasa extracts with high antioxidant activity in vitro showed effective preventive of oxidative stress induced by restraint stress in mice. Since metabolic fate including absorption and tissue distribution determines in vivo activity of certain functional molecules [36], the high in vitro antioxidant activity is not always reflected in its in vivo activity. In this sense, Kumaisasa extract might be an easily accessible redox modulator and thus could be a significant source of natural antioxidants being helpful in preventing the progress of various oxidative stresses. Thus, the present study has provided scientific credence to the ethnotherapeutic usage of this traditional herb.

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References


