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Evaluation of Antioxidant and Anti-inflammatory Potential of *Alpinia officinarum* with Different Ionic Solutions

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ABSTRACT

Alpinia officinarum, which attracts attention with its antioxidant and anti-inflammatory properties, is used in traditional medicine, especially to relieve stomach and digestive system disorders. Although many studies have revealed the various pharmacological effects of *Alpinia officinarum*, the effect of different ionic solvents on its biological activities has yet to be investigated. In this study, the effects of homogenization of *Alpinia officinarum* roots with potassium chloride (KCl), sodium chloride (NaCl), and phosphate (PBS) buffer solutions on the antioxidant and anti-inflammatory properties of the plant were investigated. *Alpinia officinarum* plant was collected from the Adana region during the season, and fresh root parts were separated and analyzed. Superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO) enzyme activities, and malondialdehyde (MDA) levels of plant homogenates prepared with KCl, NaCl, and PBS were determined by spectrophotometric analysis. The highest MPO and CAT enzyme activities were observed in the KCl solution, while lower levels were observed in NaCl and PBS solutions, respectively. The highest MDA level was observed in the PBS solution. Moreover, SOD enzyme activity showed a decreasing trend in NaCl, KCl, and PBS solutions, respectively. These findings suggest that the biological activity of plant extracts may vary depending on the solvent used. Determination of the conditions under which the antioxidant and anti-inflammatory effects of *Alpinia officinarum* in different ionic solvents are the highest supports increasing the bioavailability of the plant.

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Introduction

Alpinia officinarum is mainly grown for medicinal purposes in Türkiye since it prefers tropical climates (Abd Rahman et al., 2024; Ozkan et al., 2016). It is widely used against stomach and intestinal diseases with its digestive system supportive feature. *Alpinia officinarum*, a member of the ginger family (Zingiberaceae), shows similar biological properties to ginger (Lei et al., 2024)(Herbarium code: LINN-HS 6.3). This plant has attracted attention due to its antioxidant, anti-inflammatory, and anticancer properties due to its high flavonoid and phenolic compound content, and it has been included in scientific research (Lin et al., 2020).

Previous in vitro and in vivo studies have demonstrated the effects of *Alpinia officinarum* in alleviating oxidative stress and inflammation by changes in superoxide dismutase (SOD), catalase (CAT) , malondialdehyde (MDA), glutathione, myeloperoxidase (MPO), interleukin, and nuclear factor kappa B (NF-κB) levels (Ashtari et al., 2023; Lin et al., 2023; Rajendiran et al., 2018; Suja & Chinnaswamy, 2008; Xin, 2011). It was reported that *Alpinia officinarum* extract caused a decrease in oxidative stress markers by modulating SOD and CAT enzyme activities in various organs in a hypertensive rat model (Javaid et al., 2021). Similarly, it was reported that hydro-alcoholic extract of *Alpinia officinarum* suppressed cisplatin-induced testicular toxicity by decreasing MDA levels and increasing SOD activity. It was emphasized that the antioxidant effects of the plant extract may be supportive in increasing the safety of cancer treatments (Ashtari et al., 2023). In addition, other in vivo studies on the antioxidant effects of *Alpinia officinarum* show that the plant extract reduces oxidative stress markers, alleviates lung damage by increasing SOD activity, and prevents diseases associated with the digestive system (Lei et al., 2024; Xin, 2011; Zhao et al., 2019). In vitro studies investigating the chemical and biological effects of *Alpinia*

officinarum extract have generally focused on the cytotoxicity of the plant in various cancer cell lines (F et al., 2019). It was reported that the ethanolic extract of the plant suppressed cell proliferation by causing DNA damage in cell lines derived from prostate adenocarcinomas (Suja & Chinnaswamy, 2008) It was also reported that the plant extract exhibited antiproliferative effects by activating the caspase-dependent cell death pathway in breast cancer cells (Ghil, 2013).

Alpinia officinarum extracts contain various bioactive flavonoid components such as quercetin, galangin, kaempferol, and curcumin, strengthening the plant's anti-inflammatory and antioxidant activities. The anti-inflammatory effects of *Alpinia officinarum* rich in flavonoids were tested in the RAW264.7 cell model in which inflammation was induced by lipopolysaccharide (LPS). This study reported that *Alpinia officinarum* and its bioactive compounds suppressed the inflammatory response by inhibiting COX-2, IL-1β, IL-6, NF-κB, NO, and TNF-α production (Li et al., 2021). In addition, in vivo anti-inflammatory and COX-2 inhibitory effects of *Alpinia officinarum*, which may be related to the presence of phenolic content, were investigated, and the relationship between these effects and in vitro antioxidant activities was revealed. It has been reported that the phenolic content of the plant extract with anti-inflammatory and antioxidant activities may provide supportive effects in the treatment of inflammatory diseases (Honmore et al., 2016)

Previous studies have addressed the broad pharmacological profile of *Alpinia officinarum*, particularly its antioxidant, anti-inflammatory, and anticancer activities. However, these studies usually utilize extractions with solvents such as ethanol or methanol, with no specific evaluation of the effects of KCl, NaCl, or PBS solutions on biological activity. This study aimed to investigate the effects of antioxidant and anti-inflammatory activities of *Alpinia officinarum* roots using solutions with different ionic environments. Evaluation of antioxidant/oxidant parameters such as CAT, SOD, MPO, and MDA in various solutions will contribute to the enhancement of the pharmacological efficacy of the plant and potential health applications.

Materials and Methods

The root of the naturally growing *Alpinia officinarum* (galangal) plant used in this study was collected from the borders of Adana province in the Mediterranean Region. Plant roots, flowers, stems, and leaves were removed and used fresh for the analyses. Plant description was carried out by researchers at the Faculty of Agriculture of our university.

Biochemical Analyses

Plant preparation for biochemical analysis

The preparation of plant samples was carried out in the Medical Biochemistry Research Laboratory of KSU Faculty of Medicine. Fresh plant roots brought to the research laboratory were cut into small pieces using a sterile scalpel. Plant homogenate was prepared with 1.15% KCl, 0.9% NaCl, and 0.1 M PBS solutions at indicated concentrations. The solutions were added 1/5 to the plant root pieces and homogenized (lavion mechanical disintegrator). The plant homogenate was filtered and centrifuged (Hettich 420 R) at 5000 rpm for 5 min, and the supernatant was used for analysis. Analyses were carried out in five replicates for fresh plant roots.

Determination of SOD Activity

SOD enzyme activity in plant extracts was measured using the assay method described in detail previously (Fridovich, 1995). The method is based on the interaction of superoxide radicals produced by xanthine and xanthine oxidase with NBT to form a purple formazan dye. The intensity (OD) of formazan dye was measured in a spectrophotometer at a wavelength of 505 nm. A low absorbance value indicates high SOD enzyme activity.

Determination of CAT activity

CAT activity was determined by spectrophotometric measurement of H2O2 concentration at 230 nm wavelength in the reaction in which hydrogen peroxide was the substrate (Beutler, 1984). CAT enzyme catalyzes the conversion of hydrogen peroxide to water and oxygen. CAT activity was expressed as U/mg protein. The rate of decrease in absorbance value (ΔA/min) is considered an indicator of catalase activity.

Determination of MDA level

It is based on the principle that MDA, which is the secondary product of lipid peroxidation formed as a result of incubation of the sample at 90-95 C^{o} with thiobarbituric acid (TBA) at pH 3.40 under aerobic conditions, forms a pink complex with TBA. This color intensity is directly proportional to the MDA concentration in the medium and is evaluated spectrophotometrically at 532 nm (Ohkawa et al., 1979).

Determination of Protein level

Both protein and polyphenol compounds can be determined by the Folin Cioacalteu method. The Folin technique is based on the absorbance measurement at 750 nm by the spectrophotometric method of the color reaction of tyrosine and tryptophan residues contained in proteins in the extract with phosphotungstic-phosphomolybdic acid (Lowry et al., 1951). Bovine serum albumin was used as a standard.

Statistical Analyses

GraphPad Prism 10 software was used to analyze the data obtained in this study. Normality tests were applied to determine whether the data fit normal distribution. An unpaired t-test for independent groups was used to evaluate whether the differences between the groups were statistically significant. The significance level was accepted as $p<0.05$. The results obtained are presented as mean ± standard error.

Results and Discussion

This study showed that the enzyme activities and antioxidant profile of the *Alpinia officinarum* plant may vary depending on the solvent medium. Although there are many studies in the literature demonstrating the antioxidant and anti-inflammatory properties of *Alpinia officinarum* in various disease models (Ashtari et al., 2023; Rajendiran et al., 2018; Xin, 2011), there is no study investigating the effects of specific ionic and buffer solutions such as KCl, NaCl, and PBS on antioxidant and anti-inflammatory enzyme activities and oxidant markers of the plant.

Table 1. Effects of different ionic and buffer solutions on antioxidant enzyme activities and oxidative stress markers of *Alpinia officinarum* Extract

Measurements	KCl	PBS	NaCl
CAT (U/mg protein)	0.470±0.122[a]	0.0940±0.0270[b]	0.418±0.115[a]
SOD (U/mg protein)	1.05±0.187[b]	0.282±0.0259[c]	1.36±0.153[a]
MDA (nmol/mg protein)	1.48±0.195[b]	2.62±0.392[a]	1.09±0.0988[c]
MPO (U/mg protein)	0.136±0.0168[a]	0.0816±0.0109[b]	0.113±0.0151[b]

Data are presented as mean ± standard deviation (SD) (n=5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; Superscript letters (a, b, c) indicate significant differences among the solvents (KCl, PBS, and NaCl) within the same parameter. Groups sharing the same letter are not significantly different (p>0.05).

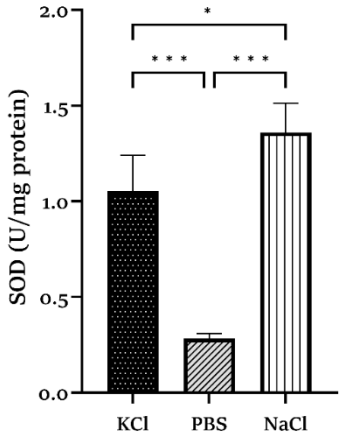

Figure 1. Comparison of CAT enzyme activity changes after Alpinia officinarum plant root homogenization in different solvents (KCl, NaCl, and PBS).

CAT (U/mg protein) levels were measured in root extracts. CAT activities of KCl and NaCl homogenate were significantly higher than PBS homogenate (***p<0.001).

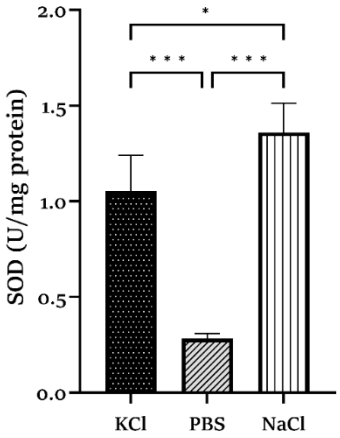

Figure 2. Variation of SOD activity of Alpinia officinarum plant root depending on different solvent media (KCl, NaCl, and PBS)

SOD (U/mg protein) levels were measured in root extracts. SOD activities of KCl and NaCl homogenate were significantly higher than PBS homogenate (***p<0.001). The SOD activity of the NaCl group was significantly higher than that of the KCl group (*p<0.05).

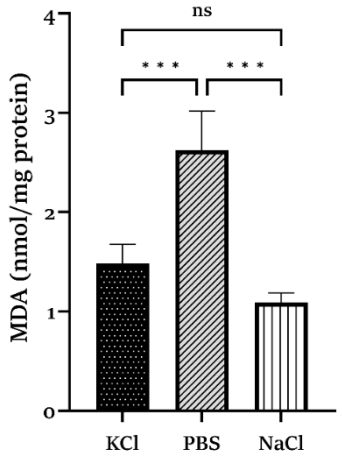

Figure 3. Changes in MDA levels after homogenization of Alpinia officinarum plant root in different solvents (KCl, NaCl, and PBS).

MDA (nmol/mg protein) levels were measured in root extracts. MDA levels of PBS homogenate. PBS homogenate was significantly higher than KCl and NaCl homogenate (***p<0.001). There was no statistically significant difference between the MDA levels of KCl and NaCl homogenates.

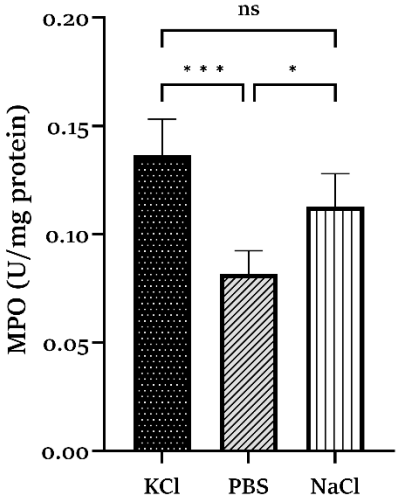

Figure 4. Variation of MPO activity of Alpinia officinarum plant root depending on different solvent media (KCl, NaCl, and PBS)

MPO (U/mg protein) levels were measured in root extracts. MPO activities of KCl and NaCl homogenate were higher than PBS homogenate (***p<0.001 and *p<0.05).

In this context, our study shows that the solvent medium used plays an important role in maintaining the antioxidant and anti-inflammatory enzyme stability of *Alpinia officinarum*. The findings revealed that the activities of plant-derived antioxidant and anti-inflammatory modulators may vary depending on the solution conditions. Changes in CAT, SOD, MPO enzyme activities, and MDA levels after homogenization of galangal root in different solvents (KCl, NaCl, and PBS) are shown in Table 1 and Figure 1, 2 and 3. The results

showed that KCl and NaCl significantly increased CAT enzyme activity. In particular, it was observed that KCl and NaCl stabilized CAT enzyme activity more effectively than PBS (Figure 1). Similarly, SOD enzyme activity was significantly increased in the groups homogenized with KCl and NaCl solutions. The highest SOD enzyme activity was determined in the NaCl group (Figure 2). It is thought that the increasing effect of ionic solutions such as KCl and NaCl on CAT and SOD enzyme activities is due to the stabilization of the three-dimensional structure of the enzymes in the plant root by potassium and sodium ions. In addition, sodium and potassium ions may indirectly affect the function of enzymes by affecting the pH and ionic strength of the medium. High ionic strength can strengthen enzyme-substrate interactions. In addition, sodium or potassium ions are used as cofactors for some enzymes. This facilitates the substrate binding of enzymes (Page & Di Cera, 2006). In support of our study, other studies have examined the changes in antioxidant capacities of different plants after exposure to various ionic environments. In potato shoots exposed to specific concentrations of NaCl, CAT enzyme activity and glutathione levels, which indicate antioxidant capacity, were found to increase (Aghaei et al., 2009). Similarly, the effects of different ionic media such as seawater, KCl, NaCl, CaCl2, and MgCl2 on quinoa seedling emergence and antioxidant properties were investigated. It was reported that CAT, APX, POX, and SOD enzyme activities varied in ionic media such as Na^+, K^+, K^+, Ca^{2+} , and Mg^{2+} and that CAT and SOD antioxidant enzyme activities increased in quinoa plants at specific ionic concentrations (Panuccio et al., 2014). This study analyzed MDA levels after homogenizing *Alpinia officinarum* roots in different solvents (KCl, NaCl, and PBS). MDA levels, an indicator of lipid peroxidation, were highest in the PBS solution and lowest in the NaCl solution. In statistical analyses, MDA levels in PBS solution were significantly higher than in KCl and NaCl solutions (Figure 3). However, no statistically significant difference was observed between KCl and NaCl solutions. The high MDA level observed in the PBS solution suggests that the plant homogenate promotes more lipid peroxidation in the PBS medium. Although PBS is known to increase the stability of the solution due to its buffering properties, it triggered lipid peroxidation by increasing the solubility of oxidation-sensitive compounds in the homogenate of *Alpinia officinarum* root. The observation of lower MDA levels in KCl and NaCl solutions suggests that these ionic environments contribute less to lipid peroxidation and may have a more protective effect against oxidation. In the studies, three different pomegranate varieties were exposed to increasing concentrations of NaCl, KCl, and K2SO4 solutions, and their biochemical responses were evaluated. It was reported that high concentrations of NaCl, KCl, and K_2SO_4 solutions caused an increase in MDA levels, whereas low concentrations of solutions were associated with low lipid peroxidation (Dichala et al., 2022). Similarly, a dose-dependent increase in MDA levels due to salt tolerance was expressed in tobacco seedlings exposed to eight different NaCl concentrations ranging from 0-350 mM (Çelik & Atak, 2012). In addition, oxidative and antioxidative responses of NaCl and KCl solutions on Chenopodium album were examined by superoxide, hydrogen peroxide, MDA concentrations, and SOD, CAT, and POX levels (Yao et al., 2010). It was reported that oxidative stress levels were similar to the control group at 50 mM NaCl and KCl solution concentrations. However, a significant dose-dependent increase in SOD, CAT, and POX activities was observed at 50 and 300 mM concentrations; it was reported that growth parameters were positively affected in plants exposed to 50 mM concentration (Yao et al., 2010). However, these studies focused on the physiological and molecular effects of salt stress on plants rather than the effects of ionic solvents on the antioxidant-oxidant balance of plants. Therefore, in our study, the focus of plant homogenization using ionic solutions and PBS on the plant's enzyme activities and antioxidant defense mechanisms will provide important contributions to the literature in this field.

This study investigated the effects of different solutions on the antioxidant content of *Alpinia officinarum* and its anti-inflammatory content. The findings revealed that MPO activity in the plant was higher in KCl and NaCl solutions compared to PBS solution **(Figure 4**). This finding indicates the potential of intracellular potassium and sodium levels to modulate MPO activity in inflammatory processes. Moreover, the increase in MPO activity in the KCl solution was statistically more significant than in the NaCl solution. Although the activity in the NaCl solution was lower than in KCl, it was higher than in the PBS solution, indicating that Na^+ ions also support MPO activity at a certain level. These results suggest that the anti-inflammatory properties of *Alpinia officinarum* roots may vary depending on the solvent medium. Considering that MPO is an enzyme activated during inflammation (Frangie & Daher, 2022), these results suggest that ionic solutions such as KCl and NaCl may provide a suitable medium to evaluate the anti-inflammatory potential of this plant. .In the literature, the anti-inflammatory and antioxidant properties of Alpinia officinarum extracted in different solvents have been evaluated together. In studies, it was reported that hexane extract of Alpinia officinarum showed prophylactic effects in acute and chronic experimental colitis models, and these effects were associated with decreased levels of inflammatory markers and lipid peroxidation (Rajendiran et al., 2018). It has also been reported that Alpinia officinarum exhibits anti-inflammatory and antioxidative effects in gastrointestinal diseases through its flavonoid content, and regulatory mechanisms on MPO, SOD, GSH, and MDA levels mediate these effects (Lin et al., 2023).

In conclusion, this study revealed the varying antioxidant and anti-inflammatory enzyme activities of Alpinia officinarum in different ionic and buffer solution media such as PBS, KCl, and NaCl. The findings indicate that plant sources' antioxidant and anti-inflammatory enzyme activities may vary depending on the solution conditions. Therefore, selecting the appropriate solution for plant extraction or homogenization is important in preserving the plant's antioxidant and anti-inflammatory properties and increasing its effectiveness. This will contribute to optimizing the efficacy of bioactive plants such as Alpinia officinarum and increasing their potential for clinical use, especially in herbal treatment approaches.

Declarations

Ethical Approval Certificate

This research does not involve any data or materials related to humans, animals, or other living organisms. As a result, ethical committee approval and informed consent is not required for this study.

Author Contribution Statement

Nuray Üremiş: Data analysis, formal analysis, and writing the original draft

Figen Güzelgül: Data collection, conceptualization, methodology, and review.

Ergül Belge Kurutaş: Data collection, conceptualization, methodology, and review.

Fund Statement

Not applicable

Conflict of Interest

The authors declare no conflict of interest.

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