Studii şi Cercetări Ştiinţifice 2023, **24** (2), pp. 173 – 181
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

URTICA DIOICA AND *ZINGIBER OFFICINALE* EXTRACTS WITH AGRO-FOOD AND PHARMACEUTICAL APPLICATIONS

Ioana-Adriana Ștefănescu*, Irina-Loredana Ifrim

[1]*"Vasile Alecsandri" University of Bacau, Faculty of Engineering, Department of Chemical and Food Engineering, 157, Calea Mărăşeşti, Bacău, Romania*

*Corresponding authors: ioana.stefanescu@ub.ro

Received: May, 26, 2023
Accepted: June, 29, 2023

Abstract: The present work is an attempt to highlight the effect of aqueous extracts of *Urtica dioica* (nettle) and *Zingiber officinale* (ginger) in *in vitro* studies. The insights from current research would be helpful to have an overview of the antioxidant potential of nettle and ginger and provide direction for optimization and development of these extracts for phytosanitary applications against vegetable diseases as well as preservation role in the agri-food industry. The antioxidant activities of nettle and ginger are found to be protective for vegetables diseases with *Alternaria solani*. The study also highlighted the potential of a green corrosion inhibitor for metal food packaging.

Keywords: *Alternaria solani, catalase and urease activities, food packaging, green corrosion inhibitor, Urtica dioica, Zingiber officinale*

INTRODUCTION

The nettle, under its scientific name *Urtica dioica*, as well as *Zingiber officinale*, known as ginger, has a varied chemical composition, a fact that determines a wide spectrum of uses in the food, cosmetic, pharmaceutical and even agricultural industries.
The properties that stand out in the case of nettle are antifungal, antibacterial and antioxidant. Nettle and ginger extracts can be considered an alternative to chemical additives used in the control of fungal diseases in plants due to its antifungal activity [1].
The properties of ginger are concentrated in the sphere of natural medicine, showing chemopreventive, neuroprotective effects, as well as antioxidant, anti-inflammatory, analgesic activity, but it is also an increasingly popular spice plant in gastronomy.
Oxidants such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are continuously formed in living organisms and have a very short half-life. They can cause the degradation of macromolecules such as DNA, proteins and lipids, which can lead to disease [1, 2]. Antioxidants can neutralize these reactive species through nonenzymatic and enzymatic antioxidant activities, such as catalase for ROS and urease for RNS.
From an economic point of view, synthetic antioxidants show greater stability and availability, as well as a low cost, but long-term consumption can lead to adverse reactions, allergies, increases the risk of cancer and gastrointestinal diseases. Natural antioxidants are preferred over synthetic antioxidants [3, 4]. Thus, the use of natural antioxidants is always sought and in this context, nettle and ginger could be an important source of natural antioxidants through their enzymatic activity [5].
The accumulation of ROS and RNS in plants and in animal bodies is both determined by pollutants and other chemical stressors and by the activity of molds and pathogenic bacteria. The specialized literature does not provide information about the effect of the extracts of these plants on the mold species *Alternaria sp*., a species that affects a wide range of legumes, among tomatoes, cucumbers, cabbage, beans, peppers. More than that, *Alternaria sp*. is responsible for diseases of the respiratory tract and skin diseases in humans [6]. On the other hand, literature reports that nettle aqueous extract reduces lipid peroxidation in minced meat preparations [7, 8] and increases the warranty period of vacuum-packed beef [9].
On the other hand, oxidizing species can induce corrosion phenomena on food packaging. Two of the most preferred materials for food packaging are aluminum and tinplate (tinplate is steel with a thin layer of tin for corrosion protection). These materials have been used for over two centuries. These materials have been used for over two centuries. Over time, ways of improvement have been found so that the packaging is efficient and safe from a corrosive point of view. Tin like aluminum is not essential for human consumption, but neither is it dangerous in its modified form for commercial packaging when it comes into contact with food. In its pure form, however, aluminum can pose risks due to corrosion. To make pure aluminum safe, it is mixed with copper, zinc, iron, chromium or manganese. As with tin used for food packaging, the enamel coating is added to aluminum as a safety measure.
Both tin and aluminum can pose health risks when used in their pure forms, but are perfectly safe for food packaging when processed with other protective materials.
Inorganic tin can be released in acidic preserved foods such as stewed fruit and cheeses. The resin coating helps prevent this release of tin into food products. To avoid gastric

irritation from excessive tin ingestion, consumers should not store food in open tin cans, as tin degrades when exposed to air [10 - 13].
Food packaging is covered with a layer of protective varnish to ensure safety. In the case of aluminum and stainless steel packaging, improper storage conditions (high temperature) as well as the chemical composition of the packaged product can cause ions (Al^{+3}, Fe^{+2}, Fe^{+3}, Sn^{+2}, etc.) to migrate from the metal packaging into the food product.
Prevention of the corrosion process can be achieved by adding inhibitors, which reduce the rate of degradation of the packaging and implicitly prevent the loading of food products with metals that affect the health of the consumer. The use of green inhibitors such as plant extracts is toxicologically safe [10, 11].
Considering the complex composition of these plants, the purpose of the research is to valorize the nettle and ginger extracts in two directions, one regarding the antifungal activity with role in the pharmaceutical and agricultural industries, the other with the role of inhibiting the corrosion of metallic materials used in the food industry.
So, the aims of researches are: monitoring the catalase and urease activity of the nettle and ginger aqoueus extracts and their antifungal activity on *Alternaria solani*; studying the corrosion inhibition activity, for the aqueous extracts of nettle and ginger, of different concentrations in the case of aluminum and tinplate food packaging.

MATERIALS AND METHODS

Plant extracts

Plant samples were homogenized with a mortar and pestle in a cooled 0.01 M K-phosphate buffer, *p*H 7.0. Aqueous extracts were obtained in proportions of (1:10, 1:4, 1:2) (w/v) for nettle (U1, U2, U3), respectively 1:1, 1:2 and 1:3 (w/v) for ginger (G1, G2, G3). The extraction time was 30 minutes. After extraction, the samples were filtered and analyzed.

Pathogen collection

In this study, the sporangia of *Alternaria solani* were obtained from freshly infected leaves collected from the tomato plants that were seriously affected by the disease.
The sporangia on the lesion areas of the leaves were rinsed off by using a hand sprayer. The sporangia were collected by centrifugation at 7000 rpm for 10 min (Rotina 38 Hettich Zentrifugen), and then resuspended in sterilized distilled water. Potato dextrose agar medium (PDA) was used to identify and test the inhibitory effect of aqueous extracts [14].

Enzymatic activity

The catalase activity was determined titrimetrically with $KMnO_4$ 0.1N by the method adapted from Troitskaya *et al*. [15]. Catalytic activity was expressed in µmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$.
The urease activity was determined titrimetrically with HCl 0.05 N and expressed in µmol uree$\cdot g^{-1} \cdot min^{-1}$.

Analyzes were performed in triplicate.
All reagents used were analytical purity.

Antifungal activity

The antifungal activity of plant extracts against *Alternaria solani* was determined on PDA (Potato Dextrose Agar) medium. This was evaluated in vitro by the diffusimetric method to determine the inhibition rates. Plant extracts were added by flooding over PDA (1 mL). Then a 1 cm^2 fragment of the tomato leaf was deposited in the center of the Petri dish for which *Alternaria solani* was identified. Incubation takes place in the dark at 25 ± 2 °C. Colony mycelial growth was estimated after 7 days of incubation with an average of two perpendicular diameters. The control is carried out under the same conditions, without the addition of plant extracts [16]. The inhibition rate of mycelial growth is calculated according to the formula of Wang *et al*. (2006) (eq.1) [17]:

$$\text{Inhibitory rate} = (1 - \frac{D_a}{D_b}) \times 100 \quad (1)$$

where D_b - diametric growth of control and D_a - diametric growth of treated fungus.

Corrosion inhibition activity

The corrosion rates of two food packages (aluminum - Al and tinplate - T) were analyzed in HCl 5 % medium, in the absence (control) and the presence of nettle (U1, U2, U3) and ginger (G1, G2, G3) extracts.
The samples were measured and weighed both at the beginning and after they were removed from the corrosive medium. Also, conductivity, TDS, *p*H and redox potential of corrosion environment were determinate with Multiparameter Orion VERSA STAR PRO, for 335 hours, at different intervals of time. The corrosion rate was calculated according to the equation 2 [13]:

$$v_{cor} = \frac{m_2 - m_1}{S \cdot t}\ g \cdot m^{-2} \cdot h^{-1} \quad (2)$$

where m_1 – the initial weight of the sample [g];
m_2 – the final weight of the sample (after immersion in corrosive medium) [g];
S – area of the sample [m^2];
t – exposure time in corrosive medium [h].

RESULTS AND DISCUSSION

Pathogen collection

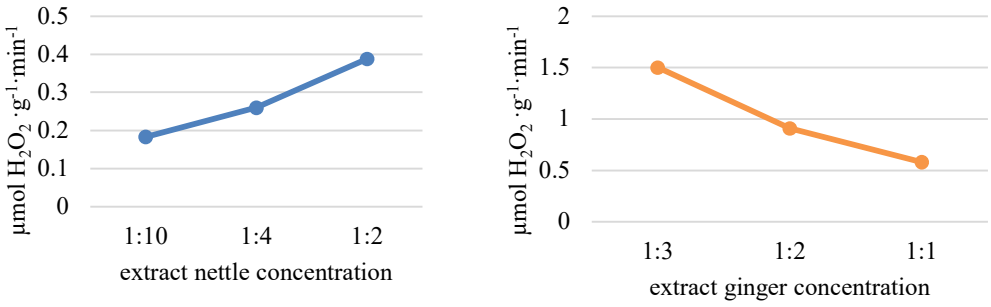
The *Alternaria solani* strain was isolated on PDA medium incubated in the dark for 7 days. Colony morphological characters and microscopic examination are presented in Figure 1. *Alternaria solani* was identified according to the morphological characteristics described by Ellis [18].

Colony morphology *Microscopic image*

Figure 1. *Alternaria solani*

Enzymatic activity

The catalase activity in ginger is approximately three times higher than in nettle, this also highlights the phytosanitary potential for plants (Figure 2).



a *b*

Figure 2. *Catalase activity in nettle (a) and ginger (b)*

Being a component of the cellular antioxidant system, catalase protects an aerobic cell against the toxic effects of hydrogen peroxide that is produced by the metabolism of *Alternaria* sp. [19].

Practically, reactive oxygen species (ROS), implicitly hydrogen peroxide, cause degradation of lipids and proteins in the cell. In this way *Alternaria* sp. causes diseases of leguminous plants. Applying nettle or ginger extracts to the diseased plants brings a supply of catalase that combats the harmful effect of hydrogen peroxide on the plant.

In the case of nettle extract, urease activity could not be detected by the applied determination method. Instead, ginger recorded a high urease activity for the 1:3 extract (Figure 3). Moreover, no studies on this aspect were found in the specialized literature.

Urease activity could not be detected in the nettle extract by the applied determination method. A high urease activity increases the assimilable nitrogen concentration. This is necessary for the plant under stress conditions, such as disease with a pathogen.

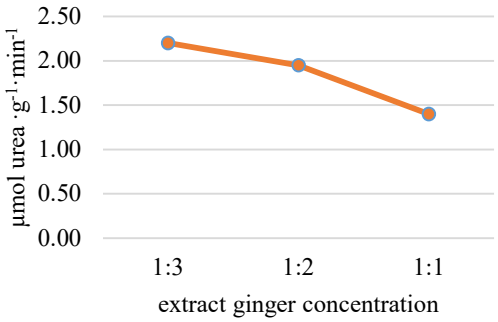


Figure 3. *Urease activity in ginger*

Antifungal activity

The antifungal activity of nettle and ginger extracts was evaluated *in vitro* for *Alternaria solani*, and their results are shown in Figures 4 - 6. The results showed that there is a significant difference between the aqueous extracts action and control. As can be seen in Figure 4, on the control sample (without the addition of nettle or ginger extract) *Alternaria solani* developed on the entire surface of the Petri dish after only 3 days.

Figure 4. *Untreated (control) Alternaria solani mycelia*

The zone of inhibition described by the nettle extracts varies for the three concentrations (U1, U2 and U3) as shown in Figure 5 corroborated with catalase activity (Figure 2) also. The obtained results indicate an increase in the antifungal activity of the nettle extract proportional to the concentration. The calculated inhibition rates were U1 - 52 %, U2 – 29 % and U3 – 25 %.

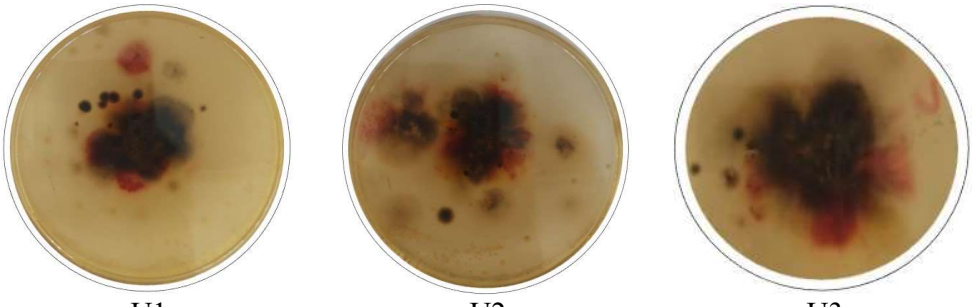


Figure 5. *Antifungal activity of nettle extract*

The ginger extracts show a complete inhibitory effect at all three concentrations, which highlights the possibility of using more diluted extracts as can be seen in Figure 6.

Figure 6. *Antifungal activity of ginger extract*

The complete inhibitory effect of ginger compared to nettle correlates with its catalase and urease activity, which is much higher than that recorded for nettle. More than that, ginger is rich in oxygenated compounds such as oxygenated monoterpenes (92 %), aliphatic hydrocarbons (7.6 %), alcohols and ketones [20], which have fungitoxic action. These compounds, together with catalase and urease, interfere with the components of the cell membrane, changing their structure and thus affecting the permeability of the membrane [21].

Corrosion inhibition activity

The results showed in Figures 7 and 8 that the lowest corrosion rate for aluminum food packaging was obtained in nettle extract U2 and ginger extract G1 respectively compared to the control. Nettle extract has a greater inhibitory effect than ginger.
Regarding tinplate food packaging, the lowest corrosion rate was calculated in the case of nettle extract U1 and ginger extract G1 respectively. In this case, the ginger extract has a greater inhibitory effect than the nettle extract.

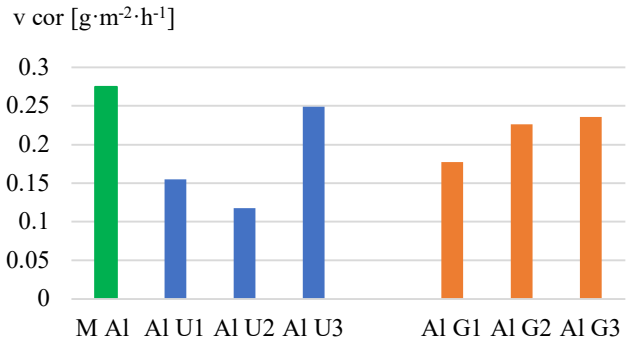


Figure 7. *Corrosion rate of aluminum packaging under the inhibitory action of the aqueous extract of nettle, respectively ginger of different concentrations*

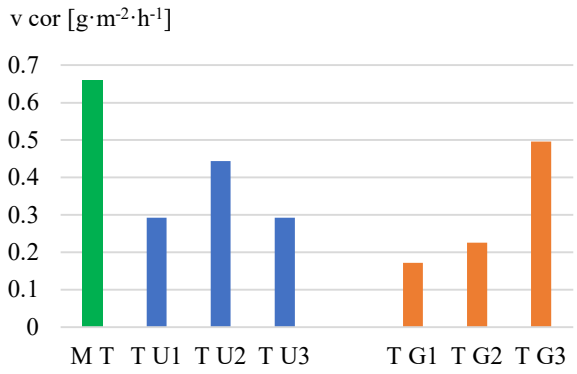


Figure 8. *Corrosion rate of the tinplate packaging, under the inhibitory action of the aqueous extract of nettle, respectively ginger of different concentrations*

So, nettle extract had an inhibitory effect of maximum 57.1 % (U2), and the of ginger 35.4 % (G1) for the packaging from aluminum. In the case of packaging from tinplate, maximum inhibitory effect of nettle extract was 55.66 % (U1) and 74 % for the ginger one (G1).

CONCLUSIONS

It can be concluded that the aqueous extracts of ginger can be used as a phytosanitary treatment for plants showing the symptoms of the disease produced by *Alternaria solani*, and the aqueous extract of nettle can be used as a preventive treatment, considering the inhibition rate between 25 – 52 %. The aqueous ginger extracts show a complete inhibitory effect on the pathogen for all concentrations studied. This suggests that it has the same effect at lower concentrations, being an advantage for the pharmaceutical industry from an economic and logistical point of view.
Also, the results obtained indicate that the use of green inhibitors, such as plant extracts, in the case of metal food packaging significantly reduces the corrosion rate, these being toxicologically safe for the food industry.

REFERENCES

1. Ali, S.S., Ahsan, H., Zia, M.K., Siddiqui, T., Khan, F.H.: Understanding oxidants and antioxidants: Classical team with new players, *Journal of Food Biochemistry*, **2020**, **44** (3), DOI: 10.1111/jfbc.13145;
2. Mehta, K., Van Thiel, D.H., Shah, N., Mobarhan, S.: Nonalcoholic fatty liver disease: Pathogenesis and the role of antioxidants, *Nutrition Reviews*, **2002**, **60** (9), 289–293, DOI: 10.1301/002966402320387224;
3. Liu, R., Mabury, S.A.: Synthetic phenolic antioxidants: A review of environmental occurrence, fate, human exposure, and toxicity, *Environmental Science and Technology*, **2020**, **54** (19), 11706–11719, DOI: 10.1021/acs.est.0c05077;
4. Xu, X., Liu, A., Hu, S., Ares, I., Martínez-Larrañaga, M.-R., Wang, X., Martínez, M., Anadón, A., Martínez, M.-A.: Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action, *Food Chemistry*, **2021**, **353**, DOI: 10.1016/j.foodchem.2021.129488;

5. Jaiswal, V., Lee, H.-J.: Antioxidant Activity of *Urtica dioica*: An Important Property Contributing to Multiple Biological Activities, *Antioxidants*, **2022**, **11** (12), DOI: 10.3390/antiox11122494;
6. Randhawa, S., Bahna, S.L.: Hypersensitivity reactions to food additives, *Current Opinion in Allergy and Clinical Immunology*, **2009**, **9** (3), 278–283, DOI: 10.1097/ACI.0b013e32832b2632;
7. Oz, F.: Effects of Water Extract of *Urtica dioica* L. on the Quality of Meatballs, *Journal of Food Processing and Preservation*, **2014**, **38** (3), 1356–1363, DOI: 10.1111/jfpp.12097;
8. Latoch, A., Stasiak, D.M.: Effect of water extract of *Urtica dioica* L. on lipid oxidation and color of cooked pork sausage, *Journal of Food Processing and Preservation*, **2017**, **41** (2), DOI: 10.1111/jfpp.12818;
9. Erbay, E.A., Aksu, M.I.: Effects of water extract of *Urtica dioica* L. and modified atmosphere packaging on the shelf life of ground beef, *Meat Science*, **2010**, **86** (2), 468–473, DOI: 10.1016/j.meatsci.2010.05.036;
10. Hampshire, W.B.: Tin Alloys: Alloying, Properties, and Applications in Encyclopedia of Materials: Science and Technology (Second Edition), **2001**, 9351-9354, DOI: 10.1016/B0-08-043152-6/01686-7;
11. https://www.desjardin.fr/en/blog/aluminum-and-tinplate-for-food-safe-packaging-of-chocolates-biscuits-and-confectionery accessed on February 24, 2023;
12. Khan, G., Newaz, K.Md.S., Basirun, W.J., Ali, H.B.M., Faraj, F.L., Khan, G.M.: Application of Natural Product Extracts as Green Corrosion Inhibitors for Metals and Alloys in Acid Pickling Processes: A review, *International Journal of Electrochemical Science*, **2015**, **10**, 6120-6134;
13. Semeniuk, I., Karpenko, O., Banya, A., Lubenets, V., Shapovalenko, S., Pengxiang, G.: New environmentally friendly compositions as inhibitors of metal corrosion, *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2023**, **24** (1), 41-48;
14. Meena, M., Swapnil, P., Upadhyay, R.S.: Isolation, characterization and toxicological potential of *Alternaria*-mycotoxins (TeA, AOH and AME) in diferent *Alternaria* species from various regions of India, *Scientific Reports*, **2017**, **7**;
15. Troitskaya, G.N., Zhiznevskaya, G.Ya., Ismailov, S.F.: Catalase Activity of Nodules in the Ureideand Amide-transporting Legume Plants, *Russian Journal of Plant Physiology*, **2000**, **47** (6), 721–727;
16. Sehari, M., Kouadria, M., Amirat, M., Sehari, N., Hassani, A.: Phytochemistry and antifungal activity of plant extracts from Nettle (*Urtica dioica* L.), *Ukrainian Journal of Ecology*, **2020**, **10** (1), 1-6;
17. Wang, J., Zhu, F., Zhou, X., Niu, C.Y., Lei, C.L.: Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Journal of Stored Products Research*, **2006**, **42**, 339-347;
18. Ellis, M.B.: *Dematiaceous hyphomycetes*, Commonwealth Microbiological Institute, Kew, England, **1971**;
19. Wu, P.C., Chen, Y.K., Yago, J.I., Chung, K.R.: Peroxisomes Implicated in the Biosynthesis of Siderophores and Biotin, Cell Wall Integrity, Autophagy, and Response to Hydrogen Peroxide in the Citrus Pathogenic Fungus *Alternaria alternate*, *Frontiers in Microbiology*, **2021**, **12**, DOI: 10.3389/fmicb.2021.645792;
20. Husein, K.A., Joo, J.H.: Antifungal activity and chemical composition of ginger essential oil against ginseng pathogenic fung, *Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)*, **2018**, **8** (2), 194–203, DOI: 10.5943/cream/8/2/4;
21. Sur, S., Romo T.D., Grossfield, A.: Selectivity and mechanism of fengycin, an antimicrobial lipopeptide, from molecular dynamics, *The Journal of Physical Chemistry B*, **2018**, **122** (8), 2219–2226, DOI: 10.1021/acs.jpcb.7b11889.