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Antifungal and Antioxidant Properties of Some Artificial Antioxidants, Generally Recognized as Safe Compounds and Nano-Oxides

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ABSTRACT

In this study, the usage potential of some artificial antioxidants, generally recognized as safe (GRAS) compounds and nano-oxides solutions in wood preservation industry, was investigated. For this purpose, antifungal and antioxidant properties of solutions were determined. Erythorbic acid, ethoxyquin, potassium disulfide, sodium ascorbate, sodium erythorbate and Enginerring and Nature Sciences Faculty (TBHQ) were selected as artificial antioxidants; dehydroacetic acid, sorbic acid and sodium benzoate were used as GRAS compounds and nano MgO, nano CeO, nano ZnO, nano SiO_2 and TiO_2 were investigated as nano-oxides in this study. Three different concentrations (0.5%, 1.0% and 1.5%) were prepared, and anti-fungal test were carried out. The brown rot fungus *Coniophora puteana* (Schumach.) P. Karst. (BAM Ebw. 15) was used for the anti-fungal test. Then antioxidant activity of the solutions were determined. Iron (III) ion reducing antioxidant power method (FRAP) was used to determine the antioxidant activity of solutions. All solutions at 1.5% concentration completely inhibited the growth of *C. puteana* fungus. The antioxidant activity of solutions was sorted as artificial antioxidants>GRAS compounds>nano-oxides, respectively. It was concluded that the tested substances can be used as impregnating agents in wood preservation.

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Introduction

Wood preservation encompasses treatments to increase the durability of wood materials and provide protection against various harmful agents (Calovi et al., 2024). From an economic point of view, decaying wood materials/structures can increase the cost of reconstruction or repair. Environmentally, the use of environmentally friendly preservation methods is important for the sustainability of natural resources and the reduction of environmental pollution (Ahmad et al., 2022). Furthermore, wood preservation is also critical for human health and safety, as the introduction of harmful organisms into wood can compromise structural safety and lead to health problems indoors. For these reasons, wood preservation plays a vital role in improving the durability of materials, protecting the environment, and ensuring human health and safety (Järvinen et al., 2022; Ramage et al., 2017).

Traditionally, natural extracts have been used for wood preservation throughout history. Natural plant extracts have been a potential approach to develop chemical-free wood preservatives against wood-destroying fungi and insects (Broda, 2020; González-Laredo et al., 2015). Since some natural extracts contain tannins or have toxic effects against biotic agents, they are preferred for protection against organisms that destroy wood or wood-based materials (Martín & López, 2023). However, oils, pitches and plant extracts are difficult to obtain and therefore less sustainable, can be economically unfavorable and have many disadvantages, such as being washed from wood (González-Laredo et al., 2015).

It is a fact that the use of chemicals in wood protection will continue until the disadvantages of natural extracts are overcome for a sustainable market. In this case, the search for cheaper, more environmentally friendly chemicals that only show activity against the target organism and do not show toxic effects on non-target organisms (for example, some preservative chemicals used in agriculture or textile industry) will continue (Pizzi, 2016). In this sense, investigating the possibilities of using preservative compounds used in food in the field of wood protection can be considered as one of the alternative solutions to meet the expectations listed.

Artificial antioxidants are mostly considered safe, but many have negative and potentially life-threatening side

effects (Halliwell, 2024). Proteins in the stomach react with nitrites and produce nitrosamines, which are carcinogenic substances. Researchers have reported a significant link between increased levels of nitrates in foods and increased mortality from Alzheimer's, Parkinson's and Type 2 diabetes (Anand & Sati, 2013). The use of these artificial antioxidants, which may be harmful to human health if consumed, in wood preservation is remarkable.

Nowadays, awareness efforts have been initiated worldwide to control post-harvest diseases, with a greater focus on GRAS chemicals. These chemicals, often used in the food industry, are inexpensive, easily acceptable and used by consumers as they are non-toxic and have small environmental impact at effective concentrations (Campos et al., 2013; Palou et al., 2016).

Chittenden et al., (2007) investigated the efficacy of chitosan and GRAS (Generally Recognized as Safe) compounds and their effectiveness in combination in wood preservation. They conducted in vitro tests against two blue coloration fungi *Sphaeropsis sapinea* and *Leptographium procerum*. Analysis of the nutrient media showed that some of the GRAS compounds tested, such as sodium benzoate, potassium sorbate and ascorbic acid, had a synergistic effect when combined with chitosan against both test fungi. However, they reported that the degree of efficacy varied according to the concentration used and the species tested.

Nano-based treatments offer superior performance compared to traditional wood treatments because they can easily penetrate and disperse within the wood, maintaining stability and having low viscosity. These treatments enhance scratch and abrasion resistance, provide protection against UV radiation, improve fire resistance, and adjust hygroscopic properties while preserving the natural appearance of wood.

Karimiyan et al., (2015) investigated the antifungal effects of 4 nano-metal oxides (MgO, SiO_2, ZnO and CuO) against *Candida albicans* in vitro and compared them with amphotericin B (antibiotic). It was concluded that ZnO and CuO nanoparticles have higher anti *C. albicans* properties compared to other nano-oxides studied and can be used in the treatment of infections caused by this fungus.

Rosa-García et al., (2018) investigated the antifungal activity of metal oxide nanomaterials (zinc oxide (ZnO), magnesium oxide (MgO) and ZnO:MgO and ZnO:$Mg(OH)_2$ mixtures) prepared under different synthesis conditions against *C. gloeosporioides* strains from avocado and papaya. They reported that all nanoparticles at the concentrations tested significantly inhibited fungal growth and caused structural damage to fungal cells.

Koka et al., (2019) investigated the antifungal activity of magnesium oxide (MgO) and iron oxide (FeO) nanoparticles against *Penicillium expansum*, *Aspergillus niger*, *Alternaria alternata*, *Mucor plumbeus*, *Penicillium chrysogenum*. They investigated against the putrefactive fungi *Trichothecium roseum* and *Rhizoctonia solani* and reported that all concentrations of nanoparticles provided significant inhibition of spore germination and mycelial growth of all putrefactive fungi.

When all the researches are evaluated, it is seen that artificial antioxidants, GRAS compounds and nano-oxides show anti-bacterial and/or anti-fungal effects against many microorganisms tested and these substances will become widespread in the field of wood protection. The aim of this study was to investigate the potential use of artificial antioxidants, GRAS compounds and nano-oxides in wood protection industry. For this purpose, the antifungal activities of the solutions prepared at different ratios of the selected chemicals were measured against the wood destroying fungus *C. puteana*. In addition, the antioxidant activities of the solutions of these chemicals prepared in the same ratio were also determined.

Material and Method

In this study, erythorbic acid, ethoxyquin, potassium disulfide, sodium ascorbate, sodium erythorbate and reversible-butyl hydroquinone (TBHQ) were used as artificial antioxidants. Dehydroacetic acid, sorbic acid and sodium benzoate were used as GRAS compounds. All chemicals used in the study were obtained from a commercial company. MgO (45 nm), CeO (25-45 nm), ZnO (25-35 nm), SiO_2 (28 nm) and TiO_2 (2-28 nm) were used as nano-oxides in this study. All nano-oxides dispersed (activated) in water were obtained from Nanografi A.Ş. (Ankara, Turkey). Chemicals activated in water were diluted with pure water to the desired concentration.

Antifungal Activity

Firstly, solutions were prepared at 0.5%, 1.0% and 1.5% concentrations using the appropriate solvent (water or ethanol) of each chemical and the lowest concentration showing anti-fungal activity was tried to be found. The brown rot fungus *C. puteana* (BAM Ebw. 15) was used for the anti-fungal test and the experiment was carried out according to the method proposed by Singh & Tripathi (1999). First, malt agar mixture was prepared. For this, 49 g of malt-agar was mixed with distilled water to a total of 1000 g and then sterilized in an autoclave at 121°C for 20 min.15 ml of malt agar solution. 2 ml of the solutions prepared at the concentrations studied were mixed separately in sterile petri dishes. After the cooling period, 5×5 mm *C. puteana* fungal mycelium was added to each mixture in the petri dish. As a control sample, the same procedure was performed using 2 mL ethanol for the impregnates dissolved in ethanol. For the impregnates prepared by dissolving in water, the growth of the fungal mycelium added on the malt-agar mixture was observed without any extra treatment. All petri dishes were incubated in an air conditioning cabinet at 22±3°C and 65±5% relative humidity. The diameter of the whole petri dish (mm) and the diameter of the fungal mycelia in the petri dish (mm) were then measured at the end of the 9th day (the day when the control samples completely covered the petri dish) using Digimizer Image Analysis Software version 5.4.1.

Mycelial growth rate (MGR, %) was calculated as a percentage using Equation 1 based on the ratio of mycelial diameter to the entire petri dish diameter.

$$\text{MGR}\ (\%) = (\text{AMD})/(\text{ADW}) \times 100 \quad (1)$$

Where;
AMD: Area of mycelial diameter
ADW: Area of diameter of the whole petri dish

Antifungal activity (AA, %) was calculated using Equation 2.

$$AA(\%) = 100 - \text{Micelle Growth Rate } (\%) \quad (2)$$

All experiments were repeated three times and averaged. When the mycelial growth rate was numerically 1, it was taken as 0 in the antifungal activity equation.

Antioxidant Activity

In this study, iron (III) ion reducing antioxidant power method (FRAP) was used to determine the antioxidant activity of solutions prepared at the selected concentration after anti-fungal activity.

The FRAP method is based on the reduction of the complex (Fe (III)-TPTZ-2,4,6-tris(2-pyridly)-S-triazine) in the presence of antioxidants to form the blue complex Fe (II)-TPTZ, which gives maximum absorbance at 593 nm (Benzie & Strain, 1996). For this purpose, 100 µL of sample was mixed with 3 mL of FRAP reagent [300 mM pH 3.6 acetate buffer: 10 mM TPTZ: 20 mM FeCl3 (10:1:1)] and absorbance was read at 593 nm after 4 min. The results are given in comparison with the standard antioxidant $FeSO_4$. Experimental conditions for FRAP method were given in Table 1.

Table 1. Experimental conditions for FRAP method

	Reagent blind	Standard	Sample
FRAP reagent	3 mL	3 mL	3 mL
Sample	-	-	100 µL
$FeSO_4.7H_2O$	-	100 µL	-
Solvent used	100 µL	-	-

Statistical Analysis

'SPSS 21.0 for Windows' program was used in the statistical analysis of all the investigations performed within the scope of this study. Simple Variance Analysis (BVA) was performed to determine the difference between the groups in the studied tests; Duncan test was applied at the confidence level ($\alpha = 0.05$) to determine the difference between the groups.

Results and Discussion

The results of the anti-fungal test on agar medium to find the lowest concentration of the solutions prepared at three different concentrations (0.5%, 1.0% and 1.5%) inhibiting *C. puteana* fungus are given in Table 2.

All solutions at 1.5% concentration completely inhibited the growth of *C. puteana* fungus. When the solutions prepared with artificial antioxidants at 1.0% concentration are evaluated among themselves, it is seen that the antifungal activity values vary between 86.79% and 100.00%. At 0.5% concentration, these values vary between 32.49% and 80.45%. It is seen that the antifungal activity values of the solutions prepared with GRAS compounds at 1.0% concentration vary between 94.45% and 100.00%. At 0.5% concentration, these values vary between 56.82% and 75.54%. The antifungal activity values of the solutions prepared with nano-oxides at 1.0% and 0.5% concentration ranged from 90.01% to 98.71% and 50.31% to 55.90%, respectively.

Türkkan & Erper, (2014) investigated the efficacy of twelve sodium salts as possible alternatives to artificial fungicides for the control of onion root rot caused by *Fusarium oxysporum* f.sp. *cepae*. As a result of in vitro tests investigating the inhibitory effects of sodium salts on mycelium growth, they reported that sodium metabisulphite and sodium fluoride completely inhibited the mycelial growth of the fungus at 2% (w/v) concentration. In this study, 1.5% concentrations of sodium ascorbate and sodium erythorbate, two different salts of sodium, completely inhibited the mycelial growth of *C. puteana*.

In the realm of food and feed preservation, weak acids play a crucial role in prolonging shelf life by impeding the proliferation of microorganisms. Among the widely utilized weak acid preservatives in food items, sorbic acid, benzoic acid, and propionic acid stand out. Among these, sorbic acid, or its sodium or potassium sorbate forms, exhibits remarkable antifungal properties, particularly effective in environments with pH levels up to 5.6 according to Ray & Liewen (2004). It stands as one of the most potent agents in thwarting fungal growth, ensuring the longevity and safety of food products. (Suhr & Nielsen, 2004; Guynot et al., 2005). In this study, the rate of inhibition of *C. puteana* fungus by sorbic acid, one of the GRAS compounds, was found to be 56.82, 94.45 and 100.00% for solutions at 0.5%, 1.0 and 1.5% concentrations, respectively. Ray & Bullerman (1982) reported that a disadvantage of sorbic acid is its limited solubility in water and therefore, the potassium salt of this acid can be used where it is desired due to its generally greater solubility in water. In a study, the effect of sorbic acid and sodium benzoate at concentrations of 0.1%, 0.5% and 1.0% on aflatoxin production by *A. tlavus* growing on maize flour was investigated and the percentage inhibition of sorbic acid was reported as 10.2%, 57.8% and 97.1% from the lowest to the highest concentration. Sodium benzoate was reported to be effective only at 1.0% concentration and 23.6% (Masimango et al., 1978).

Several acids and acid derivatives, which are not typically employed in low-acid medium-humidity foods, have been identified for their antimicrobial properties in laboratory settings. Among these are short-chain saturated fatty acids ranging from C6 to C18, along with dehydroacetic acid. These compounds have demonstrated efficacy against microorganisms, suggesting their potential utility in food preservation despite their less common usage in such environments (Skřivanová et al., 2005). Dehydroacetic acid, one of the GRAS compounds, also showed antifungal activity against *C. puteana* fungus in this study.

Benzoic acid and sodium benzoate are primarily used as antifungal agents (Chipley, 2020; López-Malo et al., 2007). Sodium benzoate, which is also one of the GRAS compounds, showed high antifungal activity against *C. puteana* fungus in this study.

Toolabia et al., (2013) investigated the toxicity and antibacterial properties of nano ZnO, TiO_2 and CuO on bacterial species.

Table 2. Anti-fungal test results

Group	Chemical	Solvent	Concentration (%)		
			0.5	1.0	1.5
Artificial antioxidant	Erythorbic acid	Water	45.12^{c} (4.71)*	95.68^{cde} (5.72)	100.00 (0.00)
	Ethoxyquin	Ethanol	67.54^{fg} (5.80)	100.0^{e} (0.00)	100.00 (0.00)
	Potassium disulfide	Water	32.49^{b} (3.14)	86.79^{b} (4.23)	100.00 (0.00)
	Sodium ascorbate	Water	70.38^{g} (4.98)	100.0 (0.00)	100.00 (0.00)
	Sodium erythorbate	Water	61.62^{ef} (5.29)	92.43^{bcd} (5.31)	100.00 (0.00)
	TBHQ	Ethanol	80.45^{h} (4.20)	100.0^{e} (0.00)	100.00 (0.00)
GRAS	Dehydroacetic acid	Ethanol	75.54^{gh} (4.03)	100.0^{e} (0.00)	100.00 (0.00)
	Sorbic acid	Ethanol	56.82^{de} (5.19)	94.45^{cde} (5.30)	100.00 (0.00)
	Sodium benzoate	Water	68.42^{fg} (5.20)	100.0^{e} (0.00)	100.00 (0.00)
Nano-oxide	MgO	Water	55.90^{de} (4.82)	92.56^{bcd} (4.82)	100.00 (0.00)
	CeO_2	Water	55.02^{de} (4.78)	90.01^{bc} (4.70)	100.00 (0.00)
	ZnO	Water	52.30^{cd} (5.34)	98.71^{de} (2.04)	100,00 (0,00)
	TiO_2	Water	50.31^{cd} (4.99)	95.56^{cde} (4.95)	100,00 (0,00)
	SiO_2	Water	50.75^{cd} (5.03)	96.73^{de} (3.01)	100,00 (0,00)
Control	Ethanol		$0,00^{a}$	0.00^{a}	0.00
	Only malt-agar mixture		$0,00^{a}$	0.00^{a}	0.00

*The same uppercase letters indicate that there is no statistically significant difference according to the Duncan multiple comparison test. (p>0.05).

Stock nanoparticle suspensions (100 mmol/L) were diluted with Mueller-Hinton agar medium to values in the range of 0.05-75 mmol/L. Growth inhibition of bacteria (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) was determined in the prepared media. It was reported that nano-ZnO at 34.03 mmol/L concentration, nano-TiO_2 at 190.74 mmol/L concentration and nano-CuO solutions at 84.41 mmol/L concentration inhibited all tested bacteria by 100%. In other words, the lower concentration of nano-ZnO showed the same level of antibacterial activity as the higher concentration of nano-TiO_2 solution. In this study, nano-ZnO prepared at the same concentration showed slightly higher antifungal activity than nano-TiO_2.

Since all solutions prepared at a concentration of 1.0% inhibited the fungus by more than 85%, which is a more economical option than the 1.5% concentration. In order to make it easier to compare the results of the tests performed within the scope of the study, the concentration to be studied was chosen as 1.0% and the rest of the study was continued at this concentration.

He et al., (2011) investigated the antifungal activity of zinc oxide nanoparticles and their mode of action against two postharvest pathogenic fungi (*Botrytis cinerea* and *Penicillium expansum*). They used ZnO with dimensions of 70±15 nm and concentrations of 0, 3, 6 and 12 mmol/L. They reported that ZnO solutions at concentrations higher than 3 mmol/L can significantly inhibit the growth of *B. cinerea* and *P. expansum* and reported that nano-ZnO solution can be used as an effective fungicide in agriculture and food safety applications. In another study, it was reported that nano-ZnO solutions showed antifungal properties against *Aspergillus flavus* and *A. fumigatus* molds (Erazo et al., 2019). Other researchers have also reported antifungal activity of nano-ZnO solutions against other microscopic fungi such as *Phanerochaete salmonicolor*, *Botrytis cinerea*, *Penicillium expansum*, *Candida albicans*, *Fusarium oxysporum* (Narendhran & Sivaraj, 2016; Arciniegas-Grijalba et al., 2017) In this study, the inhibition rate of nano-ZnO against *C. puteana* fungus was found to be 52.30%, 98.71% and 100.00% for solutions at 0.5%, 1.0% and 1.5% concentrations, respectively.

Antioxidant Activity

The antioxidant capacities of the solutions of the studied chemicals prepared at 1.0% concentration are given in Table 3.

When the antioxidant values of the solutions prepared with 14 chemicals at 1.0% concentration are examined; it is seen that the highest effect was detected in TBHQ solution (4775.571±13,030 µmol $FeSO_47H_2O$/g) and the lowest effect was detected in titanium dioxide solution (21.871±0.303 µmol $FeSO_47H_2O$/g).

The antioxidant capacity of solutions prepared with artificial antioxidants were vary between 694.251±8.334 and 4775.571±13.030 µmol $FeSO_47H_2O$/g. When the solutions prepared with GRAS compounds are evaluated among themselves, it is seen that the antioxidant capacity values vary between 107.286±7.071 and 278.001±4.004 µmol $FeSO_47H_2O$/g. Also, solutions prepared with nano-oxides were showed between 21.871±0.303 and 154.429±3.030 µmol $FeSO_47H_2O$/g antioxidant capacity. These values may seem lower than artificial antioxidants and GRAS compounds, but Karunakaran et al. (2013) examined the antioxidant activities of nano and micro ZrO_2 and TiO_2 solutions in their study and reported that nano-sized solutions prepared at the same concentration had higher antioxidant activities than micro-sized ones.

Metal oxide nanoparticles are currently under scrutiny for their possible cleansing capabilities, while natural antioxidant nanostructures are gaining attention for their role as scavengers of free radicals. These functional nanostructures exhibit inherent antioxidant properties and are being explored for their ability to counter oxidative stress. Not only can these nanoparticles serve as carriers, but they also possess the capacity to actively engage in the regulation of oxidative stress. (Manke et al., 2013). The antioxidant capacities of the nano-oxides tested in this study can be ranked from large to small as ZnO, MgO, SiO_2, CeO and finally TiO_2.

Table 3. Antioxidant capacities of the solutions

Group	Chemical	FRAP (µmol $FeSO_47H_2O$/g)
Artificial antioxidant	Erythorbic acid	2810.571±4.041[h]
	Ethoxyquin	1770.001±5.202[f]
	Potassium disulfide	2078.143±3.030[g]
	Sodium ascorbate	2820.571±2.020[h]
	Sodium erythorbate	694.251±8.334[e]
	TBHQ	4775.571±13.030[i]
GRAS	Dehydroacetic acid	107.286±7.071[b]
	Sorbic acid	138.714±1.010[bc]
	Sodium benzoate	278.001±4.004[d]
Nano-oxide	MgO	129.429±13.132[bc]
	CeO_2	70.314±0.202[a]
	ZnO	154.429±3.030[c]
	TiO_2	21.871±0.303
	SiO_2	83.714±10.101

*The same uppercase letters indicate that there is no statistically significant difference according to the Duncan multiple comparison test. (p>0.05).

Sultana et al., (2017), in a study investigating the antioxidant potential of artificial antioxidants, ranked the order of scavenging activity and reducing power as TBHQ > BHA (Butylhydroxyanisole) > Tocopherol > L-ascorbic acid. In this study, based on the iron reducing power investigated as antioxidant activity, the artificial antioxidant solutions studied at the same concentration can be listed as follows: TBHQ> sodium ascorbate> erythorbic acid> potassium disulfide> ethoxyquin> sodium erythorbate. The same order for the GRAS compounds studied is as follows: sodium benzoate> sorbic acid> dehydroacetic acid.

When the 3 groups are compared among themselves, it was seen that the highest antioxidant activity is artificial antioxidants. GRAS compounds and nano-oxide solutions were found to have much lower antioxidant activity than artificial antioxidants.

Conclusion

In the article, evaluation possibilities of some artificial antioxidants, GRAS compounds and nano-oxides solutions as an impregnation agent in wood preservation industry have been researched. According to the results obtained, all solutions at 1.5% concentration completely inhibited the growth of *C. puteana* fungus. The highest antioxidant activity was seen in artificial antioxidants. GRAS compounds and nano-oxide solutions were detected to have much lower antioxidant activity than artificial antioxidants. It was concluded that the tested compounds can be used as an important alternative in wood preservation industry. In future studies, the performance of these types of compounds can also be tested against different biotic and abiotic destructives.

Declarations

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Conflict of Interest

There is no a conflict of interest with any person, institute, company, etc.

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