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CONTENT


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TYROSINASE INHIBITION, ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITY OF ACTIVE SUBSTANCES AND CREAMS USED IN THE TREATMENT OF HYPERPIGMENTATION (KOJIC ACID, NIACINAMIDE AND GRAPEVINE EXTRACT)

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:
Hyperpigmentation is a skin disorder caused by increased melanin production. It appears in the form of dark spots and makes the complexion uneven. Pigmentation spots, such as age spots (also known as sun spots), manifest themselves most often on the palms, face and hands. The aim of this work was to investigate and evaluate which of the active substances used in hyperpigmentation treatments has the highest degree of tyrosinase inhibition, and the best antioxidant and antimicrobial activity. Kojic acid, niacinamide and grapevine extract were used as active substances in the formulations. When summarizing the results, kojic acid gave the best results, followed by niacinamide, and finally grapevine extract. However, it is necessary to perform a long-term *in vivo* study on volunteers that will show the effectiveness and efficiency of the mentioned substances. In addition to the treatment of hyperpigmentation, the mentioned substances can also be used in antiaging skin treatments due to the presence of antioxidant activity.

KEYWORDS: kojic acid, niacinamide, grapevine exctract, hyperpigmentation, tyrosinase inhibition

INTRODUCTION

Hyperpigmentation is a skin disorder caused by increased melanin production. It appears in the form of dark spots and makes the complexion uneven. Pigmentation spots, such as age spots (also known as sun spots), manifest themselves most often on the palms, face and hands.

Pigmentation disorders, dyschromia, acne scars and other disorders, along with a host of other dermatoses, are treatment challenges that dermatologists face every day. Through a detailed review of the literature, as well as recent research, we can conclude that the use of preparations for hyperpigmentation is very popular among all populations, but it is not always safe. The therapy that is most often prescribed to the patient, in addition to a positive effect on hyperpigmentation, also shows a cytotoxic effect. The goal of the therapy is that, in addition to the expected therapeutic effect, it is also safe, especially if the use of the drug requires a longer period of time.

The pigment melanin determines the color of the eyes, hair and skin of each person. Certain internal and external factors, e.g. sun exposure, genetics, hormonal

changes, inflammation and age can affect melanin production. For melasma, topical treatment options include retinoids, azelaic acid, hydroquinone, chemical peels and cosmetic medications.

Hydroquinone is considered the gold standard for the treatment of hyperpigmentation, but with long-term use, its cytotoxic effect is possible.

During the last decade, numerous cases and studies have been conducted that emphasize the use of products such as zinc, arbutin, kojic acid, basic compounds of vitamin C and green tea extracts, as newer therapies for the treatment of patients with melasma.

Kojic acid is a by-product of the Japanese rice wine, sake, and is a safer, natural ingredient, although its effectiveness in inhibiting melanin production is disputed. This acid (5-hydroxy-2 hydroxymethyl-4-pyrone) is a hydrophilic fungal product that occurs naturally in some species, such as *Aspergillus* and *Penicillium*. Kojic acid reduces hyperpigmentation by inhibiting the production of free tyrosinase, and is also a powerful antioxidant. Tyrosinase plays a pivotal role in the biosynthesis of melanin pigment in various organisms, including mammals [1]. It is predominantly localized within melanocytes, specialized cells responsible for synthesizing and secretion of pigment granules [2]. Kojic acid is used in a concentrations ranging from 1% to 4% [3].

Kojic acid presents a variety of applications for human use, especially as a depigmenting agent. Besides the depigmenting activity, kojic acid and derivatives can act as antioxidant, antimicrobial, anti-inflammatory, radioprotector, anticonvulsant and obesity management agents, and present potential as antitumor substances. Depigmenting activity is due to the molecules, after penetrating the cell, binding to tyrosinase active site, regulating melanogenesis factors, leucocytes modulation and free radical scavenging activity. Hence, polarity, size and ligands are also important factors for activity. Kojic acid and derivatives present cytotoxicity to some cancerous cell lines, including melanoma, hepatocellular carcinoma, ovarian cancer, breast cancer and colon cancer [4].

Niacinamide, an active form of vitamin B3, is recognised for its significant dermal benefits including skin brightening, anti-ageing properties and the protection of the skin barrier. Its widespread incorporation into cosmetic products, ranging from cleansers to serums, is attributed to its safety profile and proven efficacy. Topical niacinamide is used for some skin disorders, including dermatitis, acne vulgaris and actinic keratosis [4].

Niacinamide has been verified in treating almost every skin disorder, hyperpigmentation, acne, psoriasis, pruritus, dermatitis, fungal infections, epidermal melasma, non-melanoma skin cancer, etc. Long term use of niacinamide, regardless of the skin type, paves the way for new skin cells, making skin healthier, brighter, and hydrated [5,6,7]. It is used in creams in concentrations ranging 4% to 5%.

The inhibitory effect of leaf extracts from a few *Vitis vinifera* varieties concerning tyrosinase has been recorded in previous studies [8,9]. Therefore, investigating the potential of bioactive compounds of grapevine leaves becomes an attractive research direction due to the widespread occurrence of skin hyperpigmentation and the demand for natural and efficacious treatments [1].

MATERIALS AND METHODS

All chemical reagents were purchased from Semikem (Bosnia and Herzegovina) and Merck (Germany). Active substances used in this study were kojic acid (Terra Organica d.o.o., Zagreb, Croatia), niacinamide (Volimo prirodno d.o.o., Mostar, Bosnia and Herzegovina) and grapevine water-ethanol extract. Spectroscopic measurements were performed on a Perkin Elmer Lambda 25 spectrophotometer.

TYROSINASE INHIBITION ASSAY

The sample solution (120 μL) and 50 μL of tyrosinase solution in phosphate buffer (16 mM pH 6.8) were left at room temperature in the dark. After 10 minutes, 50 μL of L-DOPA solution (0.8 mg/mL in phosphate buffer) was added. After 10 min, the absorbance at 492 nm was measured.

Tyrosinase inhibitory activity (TyInh) was calculated as:

$$TyInh\ (\%) = (A0\text{-}As)/A0 \times 100\ [10]$$

where A0 is the absorbance of the negative control (where buffer was used instead of the extract) and As is the absorbance of the corresponding sample.

The concentration of the extract that inhibits 50% of tyrosinase activity (IC50) was calculated.

DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical inhibition assay was performed according to the published method [11]. Substances were mixed with absolute methanol and then mixed with a DPPH radical solution. Absorbance measurements were performed at 517 nm, after which DPPH radical inhibition was calculated according to the equation:

$$I = Ac - As / Ac \times 100\ [\%]$$

where As is the absorbance of the solution containing the sample at 517 nm, and Ac is the absorbance of the DPPH solution.

Results are expressed as IC50 value. Vitamin C was used as a positive control.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The ferric reducing antioxidant power of the substance, which reflects the antioxidant activity, was determined following the protocol [12]. 3 mL of prepared FRAP reagent was mixed with 100 µL of diluted substance. Absorbance at 593 nm was recorded after a 30 min incubation at 37°C. The FRAP value was calculated from the iron (II) sulfate heptahydrate calibration curve.

IN VITRO ANTIBACTERIAL ACTIVITY TESTING

Antibacterial activity was investigated by diffusion method [13] on reference microbial strains *E. coli*, *Listeria sp.*, *S. aureus*, *B. subtilis*, *P. aeruginosa and C.albicans*. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared. The strains were then placed on the surface of the nutrient substrate Mueller-Hinton agar, dispersed in sterile Petri dishes. Drill-shaped holes were made ("wells") in the agar, into which 100 µL of substances solutions in different concentration were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, and incubated at 37°C/24 h. After the incubation period, the inhibitory zones were measured.

FORMULATION AND CHARACTERIZATION OF O/W CREAMS

The formulations are given in Table 1. The cosmetic creams of the O/W type were made by using the dissolution method or the English method. The creams were made by dissolving the emulsifier (Phytocream) in the inner (fatty) phase and heating it in steam at a temperature of 40˚C with gentle mixing, and in a chemical beaker the ingredients of the outer (aqueous) phase were measured and heated to temperature of 40-50˚C with stirring. The active components (kojic acid, niacinamide or grapevine extract) were added to the heated water phase.

Table 1. Formulation of O/W creams

Components	Kojic acid cream	Niacinamide cream	Grapevine extract cream
Rose hydrolat	60.0	60.0	60.0
Almond oil	27.2	12.5	25.0
Geogard	0.8	0.8	0.8
Phytocream	11.0	11.0	10.0
Grapevine extract	-	-	5.0
Niacinamide	-	4.0	-
Kojic acid	2.0	-	-

After the creams were made, the color, feel on the skin, pH and electrical conductivity (by immersing the electrode directly into the creams) were recorded.

DETERMINATION OF THE ANTIOXIDANT CAPACITY OF ACTIVE COMPONENTS ISOLATED FROM O/W CREAMS

The antioxidant activity of the active components isolated from the creams involved testing and isolation after 72 hours after production.

The sample for determining the antioxidant capacity using the DPPH method was prepared by heating 5 g of the sample with 25 mL of purified water at a temperature of 60°C for 10 minutes. After cooling, the aqueous layer was separated by decantation or filtration. In this way, the fatty and aqueous phases of the cream were separated, and the aqueous layer was used for testing the antioxidant capacity of the cream [14].

RESULTS AND DISCUSSION

RESULTS OF TYROSINASE ACTIVITY

Niacinamide and grapevine extract did not show tyrosinase inhibition, which was confirmed by other authors. Hakozaki and others found that niacinamide had no effect on the catalytic activity of mushroom tyrosinase or on melanogenesis in cultured melanocytes. However, niacinamide gave 35-68% inhibition of melanosome transfer in the coculture model and reduced cutaneous pigmentation in the PREP model. In the clinical studies, niacinamide significantly decreased hyperpigmentation and increased skin lightness compared with vehicle alone after 4 weeks of use [10]. Kojic acid showed tyrosinase inhibition with IC_{50} 39.55 µg/mL.

Table 2. Tyrosinase inhibition of active substances

Sample	Tyrosinase inhibition IC_{50} value [μg/mL]
Kojic acid	39.55 μg/mL ± 0.86 μg/mL
Niacinamide	did not show tyrosinase inhibition
Grapevine extract	did not show tyrosinase inhibition

RESULTS OF ANTIOXIDATIVE ACTIVITY

Table 3 shows the results of the antioxidant activity of the tested samples. The antioxidant potential of kojic acid, niacinamide, and grapevine extract was determined *in vitro* using DPPH and FRAP colorimetric assays.

According to the obtained results, grapevine extract showed the best reducing properties in the FRAP assay. The obtained results can be explained by the presence of polyphenolic compounds in the grapevine extract [15]. Phenolic compounds act as electron donors, which reduce the yellow-colored ferric tripyridyltriazine complex (Fe(III)-TPTZ) to the blue-colored iron complex (Fe(II)-TPTZ). Thanks to their reducing properties, phenolic compounds are correlated with antioxidant activity. A lower IC_{50} value in the DPPH assay means a better antioxidant activity of the sample. An IC_{50} value of 1.31 mg/mL was recorded for grapevine extract, which confirms the antioxidant properties proven by the FRAP method.

In the DPPH and FRAP assay, niacinamide showed a weaker antioxidant activity compared to grapevine extract. Also, the IC_{50} value for niacinamide is higher than for kojic acid, which makes niacinamide a weaker antioxidant. Kojic acid is used as an antioxidant in cosmetic preparations, because it shows the ability to chelate iron ions. The lowest IC_{50} value was recorded for kojic acid, which means the best antioxidant activity of all tested samples. However, the ability to reduce ferric ions was unmeasurable, since, when mixed with the FRAP reagent, a dark yellow color appeared, with no subsequent visible changes during incubation. In the FRAP assay, a blue-colored Fe (II)-TPTZ complex appears, as a consequence of the reduction of ferric ions in the presence of antioxidants, which was not the case when testing kojic acid. It was assumed that a reaction occurs between some of the components of the FRAP reagent and the kojic acid itself. In addition, the results of antioxidant activity, measured by the FRAP assay, are affected by the concentration of kojic acid, as well as the pH value.

Table 3. Antioxidant activity of active substances

Sample	FRAP (μmol/g)	IC_{50} (mg/mL)
Kojic acid	-	0.38
Niacinamide	26.2	2.60
Grapevine extract	77.9	1.31

RESULTS OF ANTIMICROBIAL ACTIVITY

The most common causes of skin infections are *S. aureus, S. pyogenes* and *P. aerugionsa*. *S.aureus* and *S.pyogenes* often causes infections such as impetigo, folliculitis and cellulitis and necrotizing fasciitis. *P.aeruginosa* is known to cause infections in people with damaged skin, such as infections after injuries or surgery.

Kojic acid showed the largest inhibition zones, and grapevine extract did not show inhibition zones for any bacterial strain. A concentration of 1% kojic acid has shown excellent results on all strains except *B. subtilis* and *C. albicans*, and a concentration of 10% showed zones larger than 20 mm for all bacterial strains, including *C. albicans.*

Niacinamide is otherwise used in creams in a concentration of 5%, and has shown a zone of inhibition for *Listeria sp.* and *P. aeruginosa*. In a concentration of 10%, it showed zones of inhibition on *E.coli* and *S.aureus*. Grapevine extract did not show antimicrobial activity, which may depend on many factors such as chemical composition, geo-climatic location and growing condition of the plant, etc.

Table 4. Antimicrobial activity of the active substances

Name of the organism	*Kojic acid ZI (mm) 1% sol.*	*Kojic acid ZI (mm) 10% sol.*	*Niacinamide ZI (mm) 5% sol.*	*Niacinamide ZI (mm) 10% sol.*	*Grapevine extract ZI (mm) Concentrated*	*Grapevine extract ZI (mm) 20% sol.*
***E.coli* WDCM 00012**	**24**	**28**	**0**	**15**	**0**	**0**
Listeria sp. WDCM 00017	**10**	**30**	**10**	**18**	**0**	**0**
***S.aureus* WDCM 00034**	**18**	**30**	**0**	**14**	**0**	**0**
B. subtillis WDCM 00003	**0**	**23**	**0**	**0**	**0**	**0**
P.aeruginosa WDCM 00025	**21**	**33**	**15**	**25**	**0**	**0**
***C.albicans* WDCM 00054**	**0**	**20**	**0**	**0**	**0**	**0**

RESULTS OF THE CHARACTERISATION AND ANTIOXIDANT CAPACITY OF ACTIVE COMPONENTS ISOLATED FROM O/W CREAMS

The formulations differed in color, which depended on the active substance. pH ranged around 5, which corresponds to the requirements of the European pharmacopoeia (pH of semi-solid preparations for the skin is 3.5-8), and the electrical conductivity was above 50 μS/cm, which indicates O/W emulsions/creams.

Table 5. Characterisation of O/W creams

Sample	Color	Feel	pH	Eletrical conductivity (μS/cm)
Kojic acid cream	Light yellow	Smooth	5.57	51.4
Niacinamide cream	White	Smooth	5.80	58.9
Grapevine extract cream	Light brown	Smooth	4.84	59.3

Figure 1 graphically presents the antioxidant activity of creams made with kojic acid, niacinamide and grapevine extract.

The highest inhibition of DPPH radicals was recorded in the formulation with niacinamide, and the lowest in the formulation with kojic acid. Although kojic acid alone showed the lowest IC_{50} value in the DPPH assay, the formulation with kojic acid showed the weakest inhibition of DPPH radicals. Kojic acid is unstable at high temperature, so during technological and analytical processes, its decomposition and consequent reduction of antioxidant activity may occur [16]. Also, elevated temperature can lead to structural changes and degradation of polyphenols in plant extracts, which correlates with a weaker antioxidant activity of cream with grapevine extract [17, 18].

The 4% niacinamide cream showed the highest percentage of DPPH radical inhibition, although niacinamide individually showed a higher IC_{50} value compared to kojic acid and grapevine extract. Niacinamide is widely used as an antioxidant in topical antiaging formulations, usually in a concentration range between 4% and 5%. It has been shown to protect the integrity of cell membranes from oxidation, as it contributes to reducing the concentration of superoxide radicals in keratinocyte cultures [19].

Changes in the antioxidant activity of creams may be due to interactions with other ingredients of the creams and the amount of the starting substance in the creams. In creams, antioxidants can be dispersed in a matrix that can limit their freedom of movement and interaction with free radicals. The created O/W creams (aqueous phase) showed an antioxidant activity above 20% and have the potential to fight free radicals and prevent skin aging.

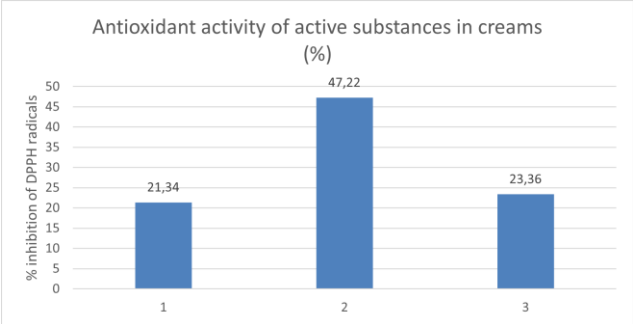

Figure 1. Antioxidant activity of active substances in creams

CONCLUSION

When summarizing the results, kojic acid gave the best results, followed by niacinamide, and finally grapevine extract. However, it is necessary to perform a long-term *in vivo* study on volunteers that will show the effectiveness and efficiency of the mentioned substances. In addition to the treatment of hyperpigmentation, the mentioned substances can also be used in antiaging skin treatments due to the presence of antioxidant activity.

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REFERENCES

[1] S. Shecori, M.M. Kher, A. Azagury, E. Drori, "The Potential of Grapevine Leaf Extract in Treating Hyperpigmentation", Cosmetics, vol. 11, no.2, pp. 1-17, 2024.

[2] K.E. Oh, H. Shin, M.K. Lee, B. Park, K.Y. Lee, "Characterization and Optimization of the Tyrosinase Inhibitory Activity of Vitis amurensis Root Using Lc-q-Tof-Ms Coupled with a Bioassay and Response Surface Methodology", Molecules, vol. 26, pp.446, 2021.

[3] A.M. Mayer, "Polyphenol oxidases in plants and fungi: going places? A review." Phytochemistry; vol. 67, no.21, pp.2318-2331, 2006.

[4] J.C. Zilles, F.L. Dos Santos, I.C. Kulkamp-Guerreiro, R.V. Contri, "Biological activities and safety data of kojic acid and its derivatives: A review", Exp Dermatol; vol. 31, no. 10, pp. 1500-1521, 2022.

[5] RR Ong, CF Goh, "Niacinamide: a review on dermal delivery strategies and clinical evidence", Drug Deliv Transl Res; 2024.

[6] P. Madaan, P. Sikka, D.S. Malik, "Cosmeceutical Aptitudes of Niacinamide: A Review". Recent Adv Antiinfect Drug Discov; vol 16, no. 3, pp. 196-208, 2021.

[7] H.M. Rolfe, "A review of nicotinamide: treatment of skin diseases and potential side effects", J Cosmet Dermatol; vol. 13, no. 4, pp. 324-8, 2014.

[8] N. Thanh, T. Nhi, T.G. Khiem, T. Doan, T.T. Luan, N. Hoang, L. Thi, T. Lan, N. Thanh, T. Nhi, "Inhibition of TyrosinaseActivity by Grape Leaf Extract *Vitis vinifera* L. (Vitaceae)", TNU J. Sci. Technol, vol. 227, pp. 10-15, 2022.

[9] F. Labanca, I. Faraone, M.R. Nolè, R. Hornedo-Ortega, D. Russo, M.C. García-Parrilla, L. Chiummiento, M.G. Bonomo, L. Milella, "New Insights into the Exploitation of *Vitis vinifera* L. Cv. Aglianico Leaf Extracts for Nutraceutical Purposes", Antioxidants, vol. 9, pp. 708, 2020.

[10] T. Hakozaki, L. Minwalla, J. Zhuang, M. Chhoa, A. Matsubara, K. Miyamoto, A. Greatens, G.G. Hillebrand, D.L. Bissett, R.E. Boissy, "The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer", Br J Dermatol, vol. 147, no. 1, pp. 20-31, 2002.

[11] E. Horozić, A. Zukić, L. Kolarević, D. Bjelošević, Z. Ademović, B. Šarić-Kundalić, D. Husejnagić, A. Kudumović, S. Hamzić, "Evaluation of an-tibacterial and antioxidant activity of methanol needle extracts of *Larix Decidua* Mill., *Picea Abies* (L.) H. Karst. and *Pinus Nigra* J. F. Arnold", Technics Technologies Education Management, vol. 14, no.1, pp. 14-19, 2019.

[12] I.F.F. Benzie, J.J. Strain, "Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration", Methods in Enzymology, vol. 299, pp. 15-27, 1999.

[13] M. Ibišević, "A comparative study of antimicrobial activity of *Melaleuca alternifolia, Achillea millefolium* and *Cinnamomum camphora* vaginal suppositories", Technologica Acta, vol. 16, no. 1, pp. 1-6, 2023.

[14] D. Vasiljević, S. Savić, Lj. Đorđević, D. Krajišnik, "Priručnik za kozmetologiju", Data Medika, Beograd, 2009.

[15] N. Zeghad, E. Ahmed, A. Belkhiri, Y. Vander Heyden, K. Demeyer, "Antioxidant activity of *Vitis vinifera, Punica granatum, Citrus aurantium* and *Opuntia ficus* indica fruits cultivated in Algeria", Heliyon, vol.5, no. 4, 2019.

[16] M.L. Gonçalez, D.G. Marcussi, G.M. Calixto, M.A. Corrêa, M. Chorilli, "Structural characterization and *in vitro* antioxidant activity of kojic dipalmitate loaded W/O/W multiple emulsions intended for skin disorders", BioMed research international, vol. 1, 304591, 2015.

[17] S. Mapoung, W. Semmarath, P. Arjsri, S. Umsumarng, K. Srisawad, P. Thippraphan, S. Yodkeeree, P. Limtrakul, "Determination of phenolic content, antioxidant activity, and tyrosinase inhibitory effects of functional cosmetic creams available on the Thailand market", Plants, vol. 10, no. 7, 2021.

[18] M. Guaita, L. Panero, S. Motta, B. Mangione, A. Bosso, "Effects of high-temperature drying on the polyphenolic composition of skins and seeds from red grape pomace", LWT, vol.145, 2021.

[19] C. Marques, F. Hadjab, A. Porcello, K. Lourenço, C. Scaletta, P. Abdel-Sayed, N. Hirt-Burri, L.A. Applegate, A. Laurent, "Mechanistic insights into the multiple functions of niacinamide: Therapeutic implications and cosmeceutical applications in functional skincare products", Antioxidants, vol. 13, no. 4, pp. 425, 2024.

CYTOTOXIC, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF THE METHANOLIC EXTRACT OF SPEEDWELLS (*VERONICA OFFICINALIS L.*)

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:
Speedwells (*Veronica officinalis* L.) is a plant species used in traditional medicine for the preparation of teas, tinctures and other preparations for the treatment of diseases of the skin, respiratory and digestive organs. In this paper, the biological activity of the methanolic extract of the speedwells was examined. To assess the cytotoxic potential, a tetrazolium salt reduction (MTT) viability assay was performed. The experiments were carried out on 3 human cell lines: lung carcinoma (H460), cervical adenocarcinoma (HeLa) and colorectal carcinoma (HCT116). Antimicrobial potential was tested using the diffusion technique on three bacterial strains: *S. aureus*, *E. faecalis* and *S. enterica*. Inhibition of free radicals was tested using the ABTS and DPPH methods, and the reduction potential of the extract of the speedwells was confirmed by the FRAP method. The treatment of HeLa, H460, and HCT116 cell lines with the methanolic extract of speedwells demonstrated a dose-dependent decrease in cell growth. The extract showed a high inhibition of the growth of *S. aureus* but also a complete absence of activity in the case of *E. faecalis*. A high efficiency of inhibition of DPPH and ABTS radicals, as well as reducing ability, was recorded.

KEYWORDS: polyphenols, flavonoids, cytotoxicity, antibacterial activity, antioxidant potential

INTRODUCTION

It is evident that the biologically active components of the plant become an inspiration for the treatment of diseases and the development of medicines in healthcare every day [1]. Interest in the development of natural antioxidants is growing due to their positive impact on health, but also due to the fact that some synthetic antioxidants act as endocrine disruptors and carcinogenic agents [2]. The genus *Veronica L.* is the largest genus of the *Plantaginaceae* family. It includes up to 500 species that are distributed throughout the Northern Hemisphere and in certain parts of the Southern Hemisphere. Their habitats are diverse, from dry steppe habitats to alpine regions [3]. Apart from chemotaxonomic and phytochemical importance genus, *Veronica* species are of particular interest considering their traditional use and biological activities [4]. *Veronica officinalis* is a popular medicinal plant species. It is part of the traditional medicine of many European countries. In Turkish flora 26 of *Veronica* species are endemic. It is important to emphasize that different parts of the plant are often used for different medicinal purposes [5]. Species from the genus *Veronica* represent a valuable source of biologically active compounds.

Extracts show antioxidant, antimicrobial, antifungal, anti-inflammatory and anticarcinogenic effects. The inhibitory potential on acetylcholinesterase, tyrosinase, lipoxygenase and xanthine oxidase has also been proven [6]. *Veronica officinalis L.*, in Balkan traditional medicine is used for the treatment of eczema, liver, wound healing and skin lesions, and also for the treatment of snake bites

[5]. In traditional Chinese medicine, *Veronica* species are used as expectorants, restoratives, tonics, and for the treatment of influenza and other respiratory diseases [7]. *Veronica officinalis L.* has a long history of medicinal use as a diuretic and diaphoretic. In Romanian traditional medicine, it was used for kidney diseases, cough and catarrh, wound healing, and for the treatment of lung diseases and hypercholesterolemia [8,9,10].

Several *Veronica* species are used to treat cancer, influenza, hemoptysis, laryngopharyngitis, hernia, cough and respiratory diseases in different countries [11]. Extracts obtained from the aerial parts of certain species of *Veronica* are used as folk remedies for the treatment of various inflammatory diseases, including rheumatism [12]. In addition, the stems and leaves of some species of *Veronica* are edible, raw or cooked [13]. *Veronica officinalis* extract shows an antimicrobial effect on *Staphylococcus aureus, Listeria monocytogenes and Listeria ivanovii* [3]. There are also reports that extracts of *Veronica officinalis L*. can potentially be used as good natural anti-phytoviral agents [14]. In comparison with V. *peduncularis Bieb., V. baranetzkii Bordz., V. orientalis Miller, V. hederifolia L., Veronica officinalis L*. shows the most significant antioxidant potential due to its rich composition of phenols [12]. Previous phytochemical tests showed that the herb of this plant species contains iridoids (veronicoside, catalpol, aukubin, veproside, musaenoside, landroside): flavonoids (luteolin derivatives): triterpene saponosides, tannins, and phenolic acids (chlorogenic and caffeic acid). It has been proven that the most abundant biologically active ingredient in this species is Acteoside [15].

Of particular importance for medicine is the proven gastroprotective activity of the extract of *Veronica officinalis L*. Namely, the extract of this plant significantly inhibits the formation of stomach ulcers [16]. The molecular docking method proved the potential of Cyclododecane and 2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone in the treatment of lung cancer. Both biologically active components are present in methanolic extracts of *Veronica officinalis L* [17].

MATERIALS AND METHODS

The aerial part of the dried plant material was purchased in a local market in Tuzla. The dry sample was pulverized in an electric mill and immediately used for extract preparation. Methanol, glacial acetic acid, hydrochloric acid, sodium carbonate were purchased from Merck (Darmstadt, Germany). 2,2`-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), dimethyl sulfoxide (DMSO), gallic acid and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Iron(II) sulphate heptahydrate, vitamin C, iron(III) chloride hexahydrate and potassium persulfate were purchased from Honeywell (Charlotte, North Carolina, USA). Folin & Ciocalteu's reagent was purchased from Semikem (Bosnia and Herzegovina). Spectroscopic measurements were performed on a Perkin Elmer λ25 spectrophotometer.

PREPARATION OF EXTRACT

40 grams of dry crushed plant material was transferred to a flat-bottomed flask and mixed with 160 mL of methanol. The mixture was mixed on a vibromix at 250 rpm/minute. After 24 hours of mixing, the mixture was filtered through filter paper, and the filtrate was immediately evaporated on a rotavapor. The dry extract was collected and further used for analysis. Extract solutions were prepared in DMSO.

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

Total phenolic compounds presented in the extract were quantified spectrophotometrically using the Folin-Ciocalteu test following the protocol [18], with some modifications. 200 μL of extract solution was mixed with 2.54 mL of 10% Folin-Ciocalteu reagent. After 5 min 420 μL of 10% sodium carbonate was added. The absorbance of the resulting blue-coloured solution was measured at 765 nm after incubation at room temperature for 1 hour. Quantitative measurements were performed, based on a standard calibration curve of gallic acid. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gram of dry extract.

DETERMINATION OF TOTAL FLAVONOID CONTENTS (TFC)

Total flavonoid content in the extract was determined by the previously described method [19], with some modification. 1 mL of extract solution were mixed with 0.3 mL of 5% sodium nitrite. 0.3 mL of 10% aluminium chloride was added after 5 minutes. After 6 minutes incubation at room temperature, 1 mL of 1 M sodium hydroxide was added to the reaction mixture. Immediately the final volume was make up to 10 mL with distilled water. Absorbance of sample was measured against the blank at 510 nm using a spectrophotometer. The results were derived from the calibration curve of quercetin and expressed in quercetin equivalents (QE) per gram of dry extract.

DPPH RADICAL SCAVENGING ACTIVITY

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method [20]. A series of dilutions of the extract was made, after which 500 uL of 0.5 mM DPPH radical solution was added to each test tube. The samples were incubated for 30 minutes. The absorbance was measured at 517 nm with methanol as a blank sample. 0.5 mL of 0.5 mM DPPH dilution, diluted with 4 mL of methanol, was used as a control sample. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$$[(Ac - As) / Ac] \times 100$$

where As is the absorbance of the solution containing the sample at 517 nm and Ac is the absorbance of the DPPH solution. Results are expressed as IC_{50} value.

ABTS (2,2-AZINO-BIS-3-ETHYLBENZOTHIAZOLINE-6-SULPHONIC ACID) ASSAY

The ABTS scavenging activity was evaluated according to the method of Almeida et al. [21] with some modifications. A stock of ABTS radical cation ($ABTS^{\bullet+}$) was prepared by the reaction of 7 mM ABTS solution (5 µL) with 140 mM potassium persulfate (88 µL), and incubated for 16 h in the dark. The $ABTS^{\bullet+}$ solution was diluted with 95% ethanol to obtain absorbance 1.0 at 734 nm. For the analysis, a series of diluted extract solutions was made and mixed with $ABTS^{+}$ reagent working solution. After 6 minutes of incubation, the absorbance is measured at 734 nm. Results are expressed as IC_{50} value.

FERRIC-REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The reducing powers of the extracts that reflected their antioxidant activity were determined following the protocol [22]. 3 mL of prepared FRAP reagent is mixed with 100 µL of extract solution. Absorbance at 593 nm is recorded after a 30 min incubation at 37 °C. The FRAP value was calculated from the calibration curve of iron (II) sulfate heptahydrate and expressed in µmol per gram of dry extract.

ANALYSIS OF ANTICANCER POTENTIAL

The experiments were carried out on 3 human cell lines. The following cell lines were used: H460 (lung carcinoma, large cell lung cancer (ATCC®HTB-177™), HeLa (cervical adenocarcinoma, ATCC® CCL-2™), and HCT116 (colorectal carcinoma ATCC® CCL-247™).

Cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37˚C.

The panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 1.5×10^4 cells/ml. Extract was added at 10 µg/ml, 50 µg/ml, 100 µg/ml, 500 µg/ml and 1000 µg/ml concentration and incubated for a further 72 hours.

After 72 hours of incubation the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. The MTT Cell Proliferation Assay is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. For this purpose the substance treated medium was discarded and 40 µl of MTT reagent was added to each well at a concentration of 0.5 µg/µl. After four hours of incubation the precipitates were dissolved in 160 µl of DMSO. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the cell viability. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

$$\text{If } (A_{test} - A_{tzero}) \geq 0 \text{ then:}$$
$$PG = 100 \times (A_{test} - A_{tzero}) / (A_{cont} - A_{tzero})$$
$$\text{If } (A_{test} - A_{tzero}) < 0 \text{ then:}$$
$$PG = 100 \times (A_{test} - A_{tzero}) / A_{tzero})$$

where:
A_{tzero} = the average absorbance before exposure of cells to the test compound,
A_{test} = the average absorbance after the desired period of time (72 h),
A_{cont} = the average absorbance after 72 hours with no exposure of cells to the test compound.

Each test point was performed in quadruplicate in three individual experiments. The results are expressed as concentration-response graphs. A negative percentage indicates cytotoxicity following drug treatment where -100% shows no cells survived the treatment at the specific drug concentration. The results are also expressed as GI_{50}, a concentration necessary for 50% of inhibition.

ANTIBACTERIAL ACTIVITY *IN VITRO*

Antimicrobial activities were investigated by diffusion method for reference bacterial strains *Enterococcus faecalis* (ATCC 51299), *Staphylococcus aureus* (ATCC 25923) and *Salmonella enterica* (ATCC 13076). In the agar sterile

drill-shaped holes were made ("wells") into which 50 and 100 µL of exstract solutions of concentration 100 mg/mL were added. After the plates were left at room temperature for 15 min, the substance was diffused into agar, incubated at 37 °C/24 h. A solution of ciprofloxacin with a concentration of 0.5 mg/mL was used as a control.

RESULTS AND DISCUSSION

CONTENT OF BIOACTIVE COMPONENTS AND ANTIOXIDANT ACTIVITY

Tables 1 and 2 show the results of the analysis of the content of polyphenols and flavonoids, and antioxidant activity of the extract of speedwells. Fig. 1 graphically shows the dependence of free radical inhibition on the concentration of extract and vitamin C.

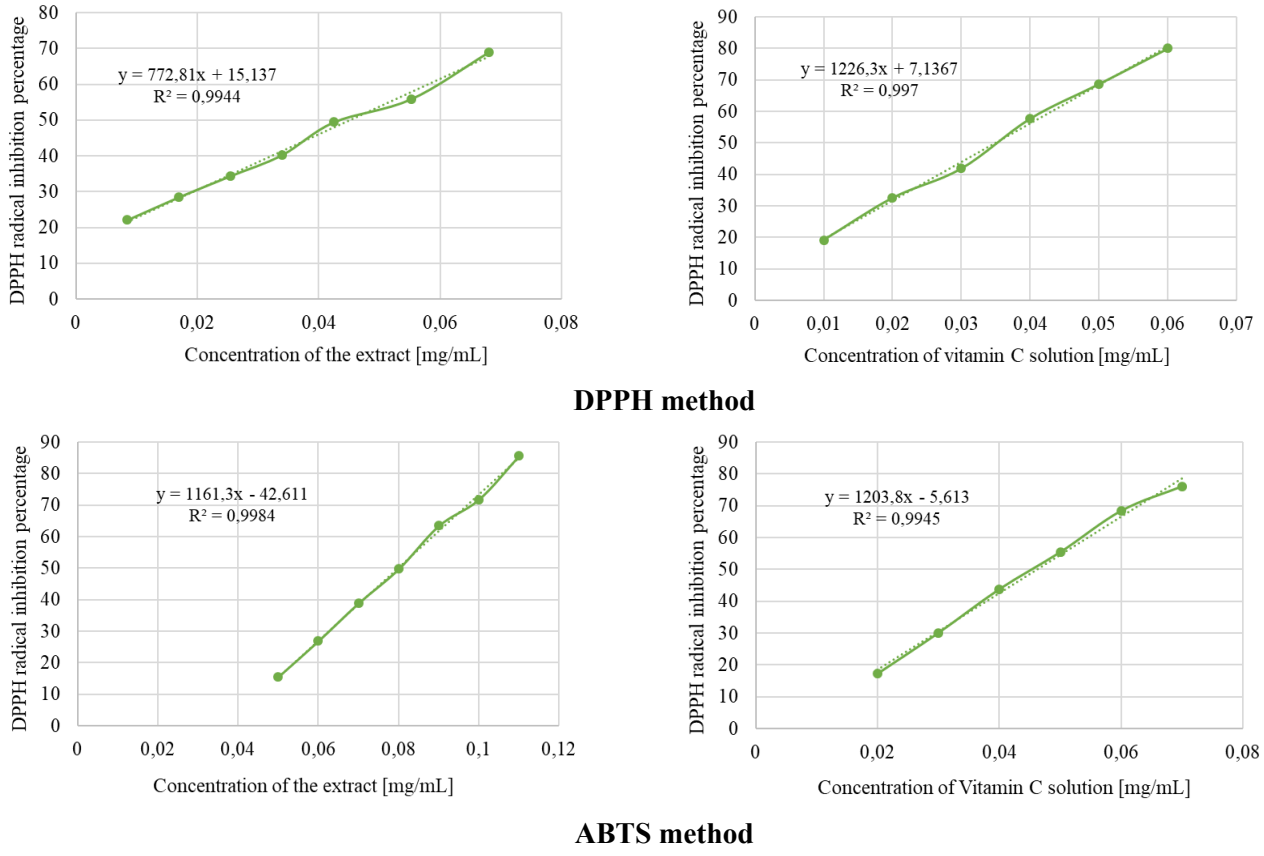

Figure 1. Graphic representation of the dependence of free radical inhibition on the concentration of extract and vitamin C

These graphs were used to calculate IC_{50} values. The content of polyphenols and flavonoids in the methanol extract is high and is correlated with a high ability to inhibit free radicals, and a somewhat weaker reduction potential of the extract. Vitamin C, which was used as a control, showed a higher efficiency of free radical inhibition and a significantly higher reduction potential.

Table 1. Content of bioactive components in the methanolic extract of speedwells

TPC [mg GAE/g]	TFC [mg QE/g]
43,64	2.75

Table 2. Results of the antioxidant capacity of the methanolic extract of speedwells

Sample	DPPH [mg/mL]	ABTS [mg/mL]	FRAP value [µmol/g]
Extract	0.045	0.079	1526.11
Vitamin C	0.035	0.046	14250.05

By looking at the literature data, the presence of numerous polyphenolic components was confirmed by HPLC analysis of the extracts of the speedwells. According to research conducted by Mocan et al. the most abundant are quercitrin, luteolin, ferulic acid, p-coumaric acid and apigenin, while the other components are mostly present in lower

concentrations [23]. This depends on the type of solvent used for extraction, the extraction technique, and the origin and treatment method of the plant material and the resulting extract.

Similar studies of the content of bioactive components and antioxidant capacity were carried out by other scientists for extracts of the mentioned plant species, prepared with different solvents and extraction techniques. Mocan et al. [23] whether the content of bioactive components and the antioxidant capacity of the ethanol extract prepared by ultrasonic extraction at room temperature were examined. The content of polyphenols and flavonoids and their research is lower, and the reason for this can be explained by the use of a different extraction technique and solvent, as well as the different geographical origin of the sample. Žugić et al. [24] examined the content of polyphenolic components and the antioxidant activity of several plant species. For the extract of *V. officinalis*, the polyphenol content was found to be lower than 30 mg GAE/g extract, which is lower compared to the results obtained in our research.

CYTOTOXIC ACTIVITY

The graph below shows the dose-response profiles of a methanolic extract of speedwells on three human cell lines: H460, HeLa and HCT116 (Figure 2). Results were obtained after a 72-hour incubation period with different extract concentrations, as it follows: 10 µg/ml, 50 µg/ml, 100 µg/ml, 500 µg/ml and 1000 µg/ml. The HCT116 and HeLa cell lines exhibited a more complex response, suggesting both stimulatory and inhibitory effects depending on the concentration. At higher doses (above 500 µg/ml), the extract slightly stimulated growth, but the H460 cell line's growth percentage consistently decreased. Altogether, the H460 cells were the most sensitive, showing a significant decrease in PG at lower concentrations compared to HeLa and HCT116 cells. The maximum inhibition of cell growth occurred at a concentration of 500 µg/ml for the HCT116 and HeLa cells, whereas for the H460 cells, it was observed at concentrations higher than 500 µg/ml.

Previous studies correlate with our findings. Several other *Veronica* species have shown an antiproliferative effect on different cell lines (including HeLa and HCT116 cell lines). Authors also suggested potential chemotherapeutic properties [5, 14, 25]. More research is needed to better understand the process and to determine the specific chemicals in the extract that are responsible for these cytotoxic effects.

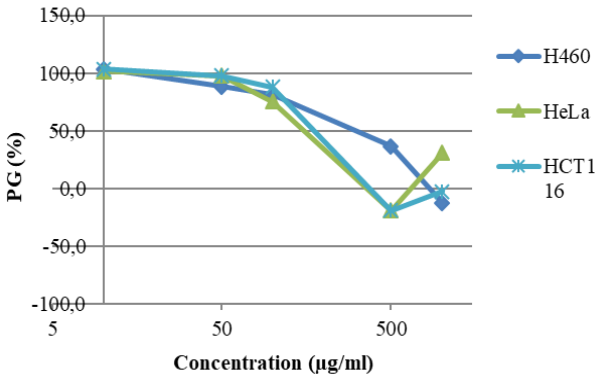

Figure 2 Dose-response profiles for methanolic extract of speedwells on H460, HeLa and HCT116 cell lines

Table 3. GI_{50} values for methanolic extract of speedwells

Cell lines	GI_{50} (µg/mL)
Lung carcinoma (H460)	392±42
Cervical adenocarcinoma (HeLa)	206±99
Colorectal carcinoma (HCT116)	240±15

ANTIBACTERIAL ACTIVITY

The results of the antibacterial activity of the extract of speedwells are shown in table 3. Antibacterial activity was recorded in the case of both tested volumes of the extract solution with a concentration of 100 mg/mL. Greater efficiency of growth inhibition was recorded in the case of *S. aureus*. Ciprofloxacin, which was used as a control, showed a significantly larger zone of growth inhibition on the tested bacterial strains with a zone of inhibition greater than 20 mm.

Table 4. Results of the antibacterial effect of the extract of speedwells

Sample	Inhibition zone [mm]					
	S. aureus		*E. faecalis*		*S. enterica*	
	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL
Extract	14	16	-	-	11	14
Ciprofloxacin	>20	>20	>20	>20	>20	>20

Mocan et al. (2015) investigated the antibacterial activity of ethanolic extract of the speedwells. The research was conducted on a larger number of bacterial strains, which confirmed the antibacterial activity of the ethanolic extract in the case of *S. aureus* and *E. faecalis*, with a minimum inhibitory concentration (MIC) of 7.81 mg/mL.

CONCLUSION

This research has confirmed the biological action of the extract of the speedwells. The content of polyphenols and flavonoids is correlated with the antioxidant capacity of the extract, which was confirmed using three methods. The treatment of HeLa, H460, and HCT116 cell lines with the methanolic extract of speedwells demonstrated a dose-dependent decrease in cell growth. The extract showed high antimicrobial potential in the case of *S. aureus*, and weaker activity against *S. enterica*.

REFERENCES

[1] Wolfender, J.L., Eugster, P.J., Bohni, N., Cuendet, M. „Advanced methods for natural products discovery in the field of nutraceuticals", *Chimia*, vol. 65, no. 6, pp. 400-406, 2011.

[2] Pop, A., Berce, C., Bolfă, P., Nagy, A., Cătoi, C., Dumitrescu, I.B., Silaghi-Dumitrescu, L., Loghin, F. „Evaluation of the possible endocrine disruptive effect of butylated hydroxitoluene and propyl gallate in immature female rats", *Farmacia*, vol. 61, 202–211, 2013.

[3] Mocan, A., Vodnar, D. C., Vlase, L., Crișan, O., Gheldiu, A. M., Crișan, G. „Phytochemical characterization of *Veronica officinalis* L., *V. teucrium* L. and *V. orchidea* Crantz from Romania and their antioxidant and antimicrobial properties", *Int J Mol Sci*, vol. 16, no. 9, pp. 21109-21127, 2015.

[4] Živković, J., Ćebović, T., Maksimović, Z. „*In vivo* and *in vitro* antioxidant effects of three Veronica species", *Open Life Sci*, vol. 7, no. 3, pp. 559-568, 2012.

[5] Salehi, B., Shivaprasad Shetty, M., V. Anil Kumar, N., Živković, J., Calina, D., Oana Docea, A., & Sharifi-Rad, J. „Veronica plants - Drifting from farm to traditional healing, food application and phytopharmacology", *Molecules*, vol. 24, no. 13, 2454, 2019.

[6] Sharifi-Rad, J., Tayeboon, G.S., Niknam, F., Sharifi-Rad, M., Mohajeri, M., Salehi, B., Iriti, M., Sharifi-Rad, M. „Veronica persica poir. Extract - antibacterial, antifungal and scolicidal activities, and inhibitory potential on acetylcholinesterase, tyrosinase, lipoxygenase and xanthine oxidase", *Cell Mol Biol*, vol. 64, pp. 50-56, 2018.

[7] Taskova R.M., Kokubin T., Ryan K.G., Gamock-Jones P.J., Jensen S.R. „Phenylethanoid and iridoid glycosides in the New Zealand Snow Hebes (Veronica, Plantaginaceae)", *Chem Pharm Bull*, vol. 58, pp. 703-711, 2010.

[8] Scarlat, M., Șandor, V., Tămaș, M., Cuparencu, B. „Experimental anti-ulcer activity of *Veronica officinalis* L. extracts", J. *Ethnopharmacol*, vol. 13, pp. 157-163, 1985.

[9] Crișan, G., Krausz, T., Șandor, V., Tămaș, M., Toader, S. „Cercetări asupra efectului hipocolestreolemiant al unor specii de Veronica", *Clujul Med*, vol. 76, no. 4, pp. 937-940, 2003.

[10] Crișan, G., Tămaș, M., Miclăuș, V., Krausz, T., Șandor, V. „A comparative study of some Veronica species", *Rev Med Chir Med Nat Iasi*, vol. 111, pp. 280-284, 2007.

[11] Harput, U. S., Karadeniz, A., Genç, Y., Saraçoglu, I. „Comparative bioactivity studies on four Veronica species", *Fabad J Pharm Sci*, vol. 34, pp. 67-72, 2009.

[12] Harput U.S., Genc Y., Khan N., Saracoglu I., „Radical scavenging effects of different Veronica species", *Rec Nat Prod*, vol. 5, pp. 100-107, 2011.

[13] Küpeli E., Harput U.S., Varel M., Yesilada E., Saracogly I. „Bioassay guided isolation of iridoid glucosides with antionociceptive and anti-inflammatory activities from Veronica anagallis - aquatica L.", *J Ethnopharmacol*, vol. 102, pp. 170-176, 2005.

[14] Nazlić, M., Fredotović, Ž., Vuko, E., Fabijanić, L., Kremer, D., Stabentheiner, E., Dunkić, V. „Wild Species *Veronica officinalis* L. and *Veronica saturejoides* Vis. ssp. saturejoides - Biological potential of free volatiles", *Horticulturae*, vol. 7, no. 9, pp. 295, 2021.

[15] Anđelković, B., Živković, J., Šavikin, K. „Impact of extraction procedure on chemical profile and antioxidant effect of *Veronica officinalis* extract", *Lekovite sirovine*, vol. 35, no. 1, pp. 113-119, 2015.

[16] Omar, A., Yadav, A. K., Rao, C. V. „Investigation of Veronica officinalis whole plant as a gastro-protective and antioxidant in peptic ulcer", *BJMHR*, vol. 4, no. 11, pp. 2394-2967, 2017.

[17] Tüzün, B., Uçar, E., Eruygur, N. Lung Cancer Properties of *Veronica officinalis* L. AS-Proceedings 2023.

[18] Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. „Analysis of total phenol and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent". *Methods Enzymol*., vol. 299, pp. 152-178, 1999.

[19] Olajire, A. A., Azeez, L. „Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables". *AJFST*., vol. 2, no. 2, pp. 22-29, 2011.

[20] Horozić, E., Zukić, A., Kolarević, L., Bjelošević, D., Ademović, Z., Šarić-Kundalić, B., Husejnagić, D., Kudumović, A., Hamzić, S. „Evaluation of antibacterial and antioxidant activity of methanol needle extracts of *Larix Decidua* Mill, *Picea Abies* (L.) H. Karst and *Pinus Nigra* J.F. Arnold". *TTEM*., vol. 14, no. 1, pp. 14-19, 2019.

[21] Almeida, M.M.B., de Sousa, P.H.M., Arriaga, Â.M.C., do Prado, G.M., Magalhães, C.E.d.C., Maia, G.A., de Lemos, T.L.G. „Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil", *Food Res. Int*., vol. 44, pp. 2155-2159, 2011.

[22] Benzie, I.F.F., Strain, J.J. „Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration", *Methods Enzymol*., vol. 299, pp. 15-27, 1999.

[23] Mocan, A., Vodnar, D.C., Vlase, L., Crisan, O., Gheldiu, AM., Crișan, G. „Phytochemical Characterization of *Veronica officinalis* L., *V. teucrium* L. and *V. orchidea* Crantz from Romania and Their Antioxidant and Antimicrobial Properties", *Int J Mol Sci*., vol. 16, no. 9, pp. 21109-21127, 2015.

[24] Žugić, A., Đorđević, S., Arsić, I., Marković, G., Živković, J., Jovanović, S., Tadić, V. „Antioxidant activity and phenolic compounds in 10 selected herbs from Vrujci Spa, Serbia". *Ind Crops Prod*., vol. 52, pp. 519-527, 2014.

[25] Mohadjerani, M., Asadollahi, S. „Veronica crista-galli Steven and Veronica persica Poir. as anticancer and antioxidant plants *in-vitro*", *Trends Phytochem Res*., vol. 3, pp. 61-66, 2019.

COMPARISON OF FIRST-ORDER AND NTH-ORDER KINETIC MODELS FOR COMPOSTING PROCESS OF MUNICIPAL SOLID WASTE

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:

The aim of this study was to propose six first-order kinetic models and then compare with nth-order kinetic model for composting process of municipal solid waste. Kinetic parameters for kinetic models were determined using a nonlinear regression method. For the first-order kinetic models, the reaction rate constant is expressed through experimental variables (time, pH, electrical conductivity, temperature, oxygen concentration, carbon dioxide concentration and moisture content). For nth-order kinetic model, rate constant and reaction order are simultaneously determined. A comparison of simulated and experimental values was performed. Based on statistical indicators (correlation factor, adjusted correlation factor, root mean square deviation, variance and 95% confidence interval), the obtained results between kinetic models were also compared. In order to choose the kinetic model that best describes the experimental data, organic matter content for three reactors were calculated. For first-order kinetic models, maximum differences between experimental and model data based on organic matter content for the first reactor, the second reactor and the third reactor were ranged from 10.30% to 18.64%, from 4.77% to 14.51% and from 6.60% to 14.00%, respectively. Mean differences for the first reactor, the second reactor and the third reactor were ranged from 3.36% to 5.42%, from 4.77% to 14.52% and from 0.43% to 1.62%, respectively. For nth-order kinetic model, maximum differences between experimental and model based on organic matter content for the first reactor, for the second reactor and for the third reactor were 2.30%, 1.22% and 1.50%, respectively. Mean differences for the first reactor, the second reactor and the third reactor were 0.005%, 0.002% and 0.001%, respectively. The nth-order kinetic model showed better agreement with experimental data of organic matter content than first-order kinetic models proposed in this study and in the previous studies.

KEYWORDS: polyphenols, flavonoids, cytotoxicity, antibacterial activity, antioxidant potential

INTRODUCTION

Composting process includes biochemical reactions in which various microorganisms decompose organic matter in the presence of oxygen. The main factors controlling the composting process include environmental parameters (temperature, moisture content, pH and oxygen) and the nature of substrate (C/N ratio, particle size and nutrient content) [1]. There are four phases of the composting process: mesophilic phase, thermophilic phase, cooling phase and maturation phase. In the mesophilic phase, readily biodegradable compounds are consumed, which leads to a slight increase in temperature. In the thermophilic phase, increasing temperature also increases the biodegradation of complex substrates, ultimately destroying most microorganisms. Then, in the cooling phase, microorganisms decompose higher polymers such as starch or cellulose, and finally, in the maturation phase, non-degradable compounds appear, and fungi predominate among microorganisms [2].

Modeling of composting kinetics is necessary for plant operation in accordance with requirements and environmental protection laws. Proper design and operation of composting reactors is essential to obtain good compost and reduce emissions to the environment [3]. Since composting is primarily a microbial process, the main function of a composting reactor is to achieve the optimal conditions. To define these optimal conditions, it is necessary to determine the kinetics of composting process. However, knowing only the optimal conditions is not enough in the most cases. The optimal composting temperature can be up to 45°C, but a higher temperature is needed to reduce pathogenic microorganisms, so here there is a conflict between temperature values. Therefore, it is necessary to choose a temperature that will lead to a reduction of pathogenic microorganisms but will not slow down the rate of the composting process [3].

Mathematical models and simulation of the composting process play an important role in understanding the process and the basic mechanisms that drive the observed process [4]. There are two main

strategies for building kinetic models for the composting process, namely the inductive and deductive strategies. The inductive model is based on a set of equations that describe the dependence of the composting rate on environmental factors (temperature, moisture content, oxygen concentration and free air space). The strategy for deriving the model structure based on theory is called the deductive strategy (classical modeling). The lack of a theoretical framework required to determine the kinetics of composting represents a major obstacle to further development of kinetics. Therefore, a deductive model is necessary to achieve further progress. Several researchers [5] have investigated the kinetics of biodegradation by microorganisms in soil, as first-order kinetics, but studies on kinetics are very limited specifically during composting process. Some researchers used first-order kinetics to investigate the degradation of organic micro-compounds during composting of sewage sludge in a thermophilic environment, although its adequacy for describing degradation has not been investigated [6]. In addition, numerous researchers ([7], [8], [9], [10], [11]) have addressed this issue, i.e. determining the composting kinetics using different types of waste materials. There is much more research on this topic, such as the study of [9] which focus is determination of the composting kinetics of municipal solid waste in three fully mechanical-biological treatments (MBT), concluding that full-scale MBT plants can be successfully modeled with the first-order kinetic model. It has been established that the decomposition of organic matter as a function of time is described by the first-order kinetic model [12].

Some researchers ([3], [13], [14], [9]) have mainly been concerned with determining kinetic parameters: reaction order, decomposition rate constants of the biodegradable fraction of organic material. Due to the complex nature of the composting process, the ability to simulate the kinetics of the process in a simple and generalized manner has proven difficult due to the need to know a large number of environmental factors. Therefore, researchers [15] explored a new approach to modeling with the aim of developing simpler models that do not require many environmental factors, and provide a more accurate description of composting process than previous models. The new models achieved errors ranging from 1.13% to 6.32% and outperformed the traditional first-order model, and only two parameters are required for their determination: the decomposition rate and an estimate of the ratio between the duration of the mesophilic and thermophilic phases. The biochemical reaction processes can usually be expressed using the first-order kinetic model, Monod model, and empirical model, among which the first-order kinetic model is most widely used ([16], [17]).

The aim of this study was to propose new first-order kinetic models for the composting process of municipal solid waste in a reactor. Also, a comparison of the first-order kinetic models with nth-order model as well as with existing kinetic models will be performed.

METHODS

REACTOR SYSTEM

A laboratory-scale composting system was used for this study. The experiment was lasted over the period of 22 days using three identical specially designed reactors made of stainless steel (volume 35 l, height 0.55 m, internal diameter 0.36 m). The reactors were insulated with a layer of polyethylene foam (10 mm of thickness). A vertical rotating axis with blades mixing on intermittent schedule, fixed at perforated plate made of stainless steel (with holes of 5 mm), ensures the complete mixing of compost mass. Mixing was performed once a day.

The reactors were equipped with a valve for dropping the leachate and condensate. On the reactor lid, there were two holes, for the shaft mixer and for the thermocouple. Two stainless steel tubes (tube for gas sampling with valve and tube for discharge of exhausted gases) were welded to the reactor lid. Each reactor was connected with an air compressor (Trudbenik, Bosnia and Herzegovina), which provided air into the reactors at a controlled rate (0.9 l air min^{-1} kg^{-1} OM) based on published recommendations ([1], [4]). Measurement of airflow was carried out using airflow meters (Valved Acrylic Flowmeter, Cole-Parmer, USA). Thermocouple was inserted through a drilled rubber stopper, which is then inserted through a hole in the reactor lid. In all reactors, temperature was measured through thermocouples type T (Digi-Sense, Cole-Parmer, USA), placed in the middle of substrate. Thermocouples were connected through the acquisition module Temperature Data Acquisition Card Thermocouple CardAcq (Nomadics, USA) on a laptop.

At reactor outlet, the gas mixture passed through a gas washing bottle with 1 M sodium hydroxide and a gas washing bottle with 0.65 M boric acid, in order to remove carbon dioxide and ammonia, respectively. The gas washing bottles were changed daily [18].

EXPERIMENTAL DATA, SOFTWARE AND NUMERICAL METHOD

The experimental data from composting process of municipal solid waste (process time, organic matter content, process temperature, moisture content, oxygen concentration, carbon dioxide concentration, electrical conductivity, pH) required for this study were taken from the study [18]. The numerical software package Polymath was used for solving model equations, with kinetic parameters determined by the nonlinear regression method (Levenberg–Marquardt method).

STATISTICAL INDICATORS USED TO ASSESS THE QUALITY OF KINETIC MODELS

In this study, for determination of kinetic parameters the following statistical indicators are used: correlation coefficient R^2 (Eq. (2)), adjusted correlation coefficient R_{adj}^2 (Eq. (3)), root mean square deviation R_{msd} (Eq. (4)) and variance s^2 (Eq. (5)):

$$\bar{y} = \frac{1}{n}\left(\sum_{i=1}^{n} y_{i_{\exp}}\right) \tag{1}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_{i_{\exp}} - y_{i_{cal}})^2}{\sum_{i=1}^{n}(y_{i_{\exp}} - \bar{y})^2} \tag{2}$$

$$R_{adj}^2 = 1 - \frac{(1-R)^2(n-1)}{n-p} \tag{3}$$

$$R_{msd} = \frac{1}{n}\left(\sum_{i=1}^{n}(y_{i_{\exp}} - y_{i_{cal}})^2\right)^2 \tag{4}$$

$$s^2 = \frac{\sum_{i=1}^{n}(y_i - \bar{y})^2}{n-1} \tag{5}$$

where: n – number of observations, p – number of kinetic parameters, y_i – specific observation, $\bar{y}$ – mean of specific observations. The notations "*exp*" and "*cal*" relate to experimental data and calculated data, respectively.

Correlation coefficients are often used to assess whether the investigated model describes the experimental data well. If the values of the correlation coefficients are close to 1, it means that the examined model better describes the experimental data. The correlation coefficients are not sufficient for evaluating the model, because in some situations its values can be close to 1, but the model is still not appropriate for the given experimental data, and it is necessary to take into account other statistical indicators. As correlation coefficients, root mean square standard deviation and variance are used to describe the performance of a model in describing of experimental data. If the values of these indicators are very close to zero, it means that a model excellent describes experimental data.

APPLIED KINETIC MODELS

The following kinetic models were proposed (Eqs. (6)-(13)):

The first-order kinetic models:

$$-\frac{d(OM)}{dt} = k_T \cdot OM \tag{6}$$

(7)

$$k_T = pH^a \cdot \exp\left(\frac{Mc}{T}\right)^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d}$$

(8)

$$k_T = \exp\left(\frac{CO_2}{O_2}\right) \cdot pH^a \cdot \exp\left(\frac{Mc}{T}\right)^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d} \tag{9}$$

$$k_T = \exp(CO_2 + O_2)^a \cdot T^b \cdot pH^c \cdot \exp\left(\frac{Mc}{T}\right)^d \cdot EC \tag{10}$$

$$k_T = \exp(CO_2 + O_2)^a \cdot b^{(T-23)} \cdot pH \cdot \left(\frac{EC}{T}\right)^c \cdot \left[\left(\frac{Mc}{T}\right)^d\right]^{0.5} \tag{11}$$

$$k_T = \frac{O_2}{O_2^{\,a}} \cdot b^{(T-23)} \cdot pH^c \cdot \left(\frac{Mc}{T}\right)^d \cdot EC \tag{12}$$

The nth-order kinetic model:

$$-\frac{dOM}{dt} = k_T \cdot OM^n \tag{13}$$

where: k_T – reaction rate constant (unit depends on reaction order); t – time (days), OM – organic matter content (%); T – process temperature (°C); Mc – moisture content (%); O_2 – oxygen concentration (%), CO_2 – carbon dioxide concentration (%); EC – electrical conductivity (dS m^{-1}); pH – pH value (-); n – reaction order (-); a, b, c, d – constants/kinetic parameters.

In first-order kinetic models, a reaction rate constant is first determined based on measured experimental data, then organic matter content is calculated by model and compared with experimental data. In the nth-order model, reaction rate constant and reaction order are determined simultaneously using the Levenberg–Marquardt method.

RESULTS AND DISCUSSION

FIRST-ORDER KINETIC MODELS

Six kinetic models are used for three reactors, and corresponding kinetic parameters with statistical analysis are presented in Tables 1, 2, and 3.

Table 1. Kinetic parameters and statistical indicators for the first-order kinetic models for reactor 1

No.	Kinetic model	Kinetic parameters	95% confidence	R^2	R_{adj}^2	R_{msd}	s^2
1	$k_T = (\frac{O_2}{(O_2^{\,a})}) \cdot b^{(T-23)} \cdot pH^{c\cdot(\frac{M_c}{T})^d}$	a = -2.6455 b = 0.5555 c = -3.4124 d = 0.6104	1.5121 0.1248 1.5389 0.4229	0.9980	0.9977	0.0021	0.0001
2	$k_T = pH^a \cdot \exp(\frac{Mc}{T})^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d}$	a = -10.5760 b = -2.8448 c = 0.5482 d = -8.1637	2.3763 1.9390 0.1229 2.9797	0.9992	0.9990	0.0014	$5.3\cdot10^{-5}$
3	$k_T = \exp(\frac{CO_2}{O_2}) \cdot pH^a \cdot \exp(\frac{Mc}{T})^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d}$	a = -10.5361 b = -2.1720 c = 0.5762 d = -7.4994	2.4836 2.0224 0.1343 3.1156	0.9991	0.9990	0.0014	$5.7\cdot10^{-5}$
4	$k_T = \exp(CO_2 + O_2)^a \cdot T^b \cdot pH^c \cdot \exp(\frac{Mc}{T})^d \cdot EC$	a = -0.7795 b = 2.5334 c = -0.7415 d = 2.9502	0.0173 0.1119 0.2053 0.0983	0.9992	0.9991	0.0013	$5.1\cdot10^{-5}$
5	$k_T = \exp(CO_2 + O_2)^a \cdot b^{(T-23)} \cdot pH \cdot (\frac{EC}{T})^c \cdot \left[\left(\frac{Mc}{T}\right)^d\right]^{0.5}$	a = -0.7611 b = 1.4219 c = 1.1642 d = 31.2627	$3\cdot10^{-7}$ $1.6\cdot10^{-5}$ $2.3\cdot10^{-6}$ $1.1\cdot10^{-5}$	0.9982	0.9979	0.0020	0.0001
6	$k_T = \frac{O_2}{O_2^{\,a}} \cdot b^{(T-23)} \cdot pH^c \cdot \left(\frac{Mc}{T}\right)^d \cdot EC$	a = -1.2468 b = 0.9079 c = -5.8239 d = 2.2784	0.0433 0.0354 0.0787 0.1188	0.9988	0.9986	0.0016	$7.6\cdot10^{-5}$

Table 2. Kinetic parameters and statistical indicators for the first-order kinetic models for reactor 2

No.	Kinetic model	Kinetic parameters	95% confidence	R^2	$Radj^2$	$Rmsd$	s^2
1	$k_T = (\frac{O_2}{(O_2^{\,a})}) \cdot b^{(T-23)} \cdot pH^{c\cdot(\frac{M_c}{T})^d}$	a = -6.6165 b = 1.2551 c = -15.769 d = -0.2102	0.0246 0.0096 0.0508 0.0031	0.9996	0.9995	0.0010	$2.6\cdot10^{-5}$
2	$k_T = pH^a \cdot \exp(\frac{Mc}{T})^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d}$	a = -10.576 b = -2.8448 c = 0.5482 d = -8.1637	2.3763 1.9390 0.1229 2.9797	0.9992	0.9990	0.0014	$5.3\cdot10^{-5}$
3	$k_T = \exp(\frac{CO_2}{O_2}) \cdot pH^a \cdot \exp(\frac{Mc}{T})^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d}$	a = -11.941 b = 3.0572 c = 0.9698 d = -4.0256	0.0119 0.0079 0.0060 0.0069	0.9997	0.9996	0.0009	$2.2\cdot10^{-5}$
4	$k_T = \exp(CO_2 + O_2)^a \cdot T^b \cdot pH^c \cdot \exp(\frac{Mc}{T})^d \cdot EC$	a = 0.1530 b = 3.4775 c =-12.4632 d = 2.8616	0.2053 0.4568 1.2763 0.2927	0.9998	0.9998	0.0007	$1.2\cdot10^{-5}$

No.	Kinetic model	Kinetic parameters	95% confidence	R^2	$Radj^2$	Rmsd	s^2
5	$k_T = \exp(CO_2 + O_2)^a \cdot b^{(T-23)} \cdot pH \cdot (\frac{EC}{T})^c \cdot \left[\left(\frac{Mc}{T}\right)^d\right]^{0.5}$	a = -1.8570 b = 2.0599 c = -0.8158 d = 67.0533	0.1868 0.1372 1.3642 4.6788	0.9986	0.9984	0.0018	$9.4 \cdot 10^{-5}$
6	$k_T = \frac{O_2}{O_2^{\,a}} \cdot b^{(T-23)} \cdot pH^c \cdot \left(\frac{Mc}{T}\right)^d \cdot EC$	a = -4.2114 b =1.1770 c =-11.9571 d = 5.1288	$8.1 \cdot 10^{-7}$ $1.4 \cdot 10^{-6}$ $1.3 \cdot 10^{-6}$ $2.4 \cdot 10^{-6}$	0.9997	0.9997	0.0008	$1.9 \cdot 10^{-5}$

Table 3. Kinetic parameters and statistical indicators for the first-order kinetic models for reactor 3

No.	Kinetic model	Kinetic parameters	95% confidence	R^2	$Radj^2$	Rmsd	s^2
1	$k_T = (\frac{O_2}{(O_2^{\,a})}) \cdot b^{(T-23)} \cdot pH^{c \cdot (\frac{M_c}{T})^d}$	a = -6.9296 b = 1.5663 c =-22.1121 d = -0.5787	1.1609 0.3153 3.5452 0.2205	0.9981	0.9978	0.0022	0.0001
2	$k_T = pH^a \cdot \exp(\frac{Mc}{T})^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d}$	a = -17.547 b = 0.6596 c = 0.7982 d = -9.5305	2.6001 1.3165 0.0809 2.5209	0.9996	0.9995	0.0010	$2.9 \cdot 10^{-5}$
3	$k_T = \exp(\frac{CO_2}{O_2}) \cdot pH^a \cdot \exp(\frac{Mc}{T})^b \cdot c^{(T-23)} \cdot \frac{EC}{T^d}$	a = -18.966 b = -0.0618 c = 0.7249 d = -10.961	1.0909 0.6368 0.0304 0.9246	0.9993	0.9992	0.0013	$4.8 \cdot 10^{-5}$
4	$k_T = \exp(CO_2 + O_2)^a \cdot T^b \cdot pH^c \cdot \exp(\frac{Mc}{T})^d \cdot EC$	a = -0.0661 b = 4.3394 c =-12.2652 d = 3.7352	0.1113 0.4595 1.2427 0.2155	0.9993	0.9992	0.0013	$4.6 \cdot 10^{-5}$
5	$k_T = \exp(CO_2 + O_2)^a \cdot b^{(T-23)} \cdot pH \cdot (\frac{EC}{T})^c \cdot \left[\left(\frac{Mc}{T}\right)^d\right]^{0.5}$	a = -0.7466 b = 1.6715 c = 3.1398 d = 42.2303	0.4354 0.1254 3.1729 5.7123	0.9988	0.9986	0.0017	$8.4 \cdot 10^{-5}$
6	$k_T = \frac{O_2}{O_2^{\,a}} \cdot b^{(T-23)} \cdot pH^c \cdot \left(\frac{Mc}{T}\right)^d \cdot EC$	a = 0.6343 b = 1.4517 c = -10.049 d = 17.6361	0.8996 0.0726 1.2660 2.3379	0.9991	0.9989	0.0015	$6.4 \cdot 10^{-5}$

Figures 1, 2, and 3 show experimental values and calculated functions of time for three reactors for six first-order kinetic models.

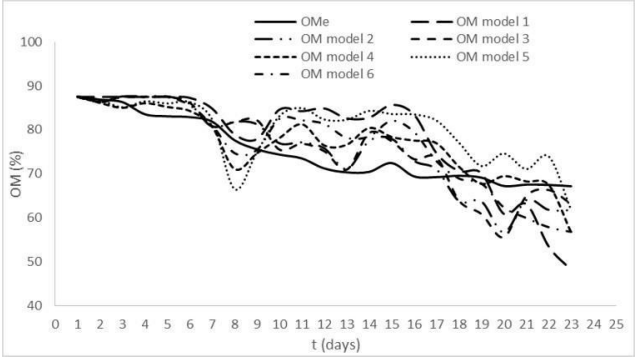

Figure 1. Comparison of experimental data and first-order kinetic models for organic matter content for reactor 1

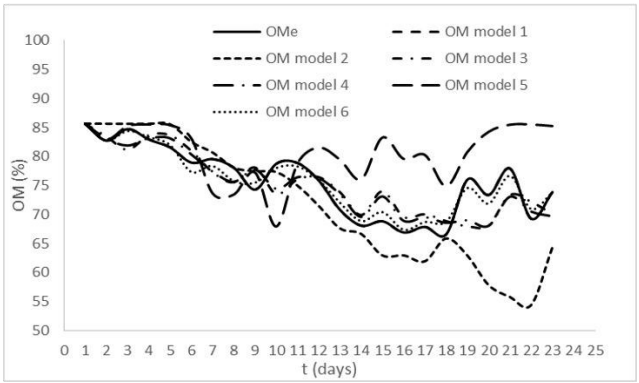

Figure 2. Comparison of experimental data and first-order kinetic models for organic matter content for reactor 2

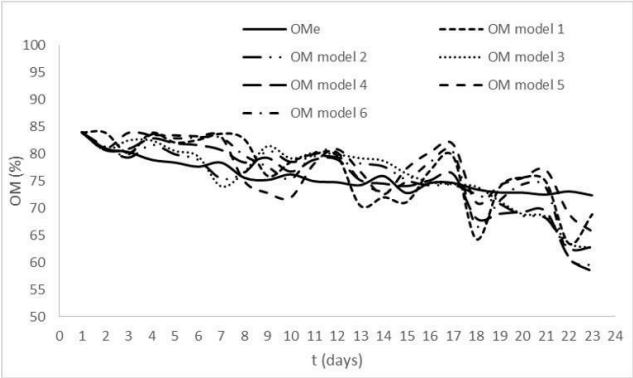

Figure 3. Comparison of experimental data and first-order kinetic models for organic matter content for reactor 3

The maximum and mean differences of experimental data for organic matter content for first-order kinetic models are shown in Tables 4, 5, and 6 for three reactors.

Table 4. Maximum and mean differences between experimental data and first-order kinetic models for organic matter content for reactor 1

Model	Maximum difference				Mean difference			
	0-5 days	6-14 days	15-22 days	0-22 days	0-5 days	6-14 days	15-22 days	0-22 days
1	-0.0009	-1.3853	18.9991	18.9995	-2.4559	-8.8268	2.7461	-3.1395
2	0.4066	1.2253	10.6432	10.6433	-2.0282	-3.5695	3.9628	-0.5475
3	0.4114	1.0184	11.6363	11.6368	-2.0859	-3.6315	3.1154	-0.8815
4	1.2945	6.5188	10.4802	10.4801	-0.6604	-3.3831	-1.1446	-1.8942
5	-1.3340	-6.3339	-6.1358	11.0848	-1.3341	-6.3339	-6.1358	-4.9607
6	0.5097	2.8621	10.3042	10.3047	-1.9616	-5.4603	2.5206	-1.7716

Table 5. Maximum and mean differences between experimental data and first-order kinetic models for organic matter content for reactor 2

Model	Maximum difference				Mean difference			
	0-5 days	6-14 days	15-22 days	0-22 days	0-5 days	6-14 days	15-22 days	0-22 days
1	0.3075	6.5658	5.1973	6.5658	-0.6489	-0.0863	-0.7245	-0.4551
2	-0.0007	9.2216	14.5096	14.5096	-2.9762	1.6864	14.5097	3.3582
3	1.6081	4.7653	3.3458	4.7653	-0.9715	-0.2528	0.7331	-0.0974
4	0.8759	5.0678	3.4912	5.0678	-0.7396	-0.1895	1.0839	0.1099
5	0.0844	8.1269	-3.0903	8.1269	-2.5653	-2.1300	-11.2738	-5.4240
6	1.4935	5.7897	4.2915	5.7897	-0.4384	-0.0880	-0.8312	-0.4378

Table 6. Maximum and mean differences between experimental data and first-order kinetic models for organic matter content for reactor 3

Model	Maximum difference				Mean difference			
	0-5 days	6-14 days	15-22 days	0-22 days	0-5 days	6-14 days	15-22 days	0-22 days
1	0.1682	3.8928	9.5882	9.5882	-2.7203	-1.7795	1.1230	-1.0154
2	1.0352	2.9411	10.4432	10.4435	-0.7903	-2.5281	4.0372	0.2088
3	0.2797	4.4183	9.6891	9.6891	-1.5587	-3.0090	3.5914	-0.3348
4	0.3209	1.3916	14.0051	14.0051	-1.9296	-2.0534	4.9988	0.4319
5	0.0394	4.2286	6.6031	6.6031	-3.0974	-1.1616	-1.0203	-1.6174
6	0.4487	2.8707	12.8509	12.8509	-2.3466	-2.3242	2.6209	-0.6100

NTH-ORDER KINETIC MODEL

In order to more accurately determine the reaction order that best describes the experimental data, the nth-order model was used. Using a nonlinear regression method, the values for k_T and n were simultaneously determined for three reactors, as shown in Table 7. Based on the kinetic parameters, organic matter content was calculated and compared with experimental data, which are shown in Figures 4, 5 and 6.

Table 7. Kinetic parameters and statistical indicators for the nth-order kinetic model for reactor 1

Reactor	k_T	n	95% confidence	R^2	R_{adj}^2	R_{msd}	s^2
1	1.0484	0.9891	$k_T \pm 0.2106$ $n \pm 0.0464$	0.9891	0.9885	0.1514	0.5778
2	1.0695	0.9844	$k_T \pm 0.2670$ $n \pm 0.0577$	0.9830	0.9822	0.1279	0.4118
3	1.0283	0.9935	$k_T \pm 0.1655$ $n \pm 0.0372$	0.9929	0.9926	0.0525	0.0694

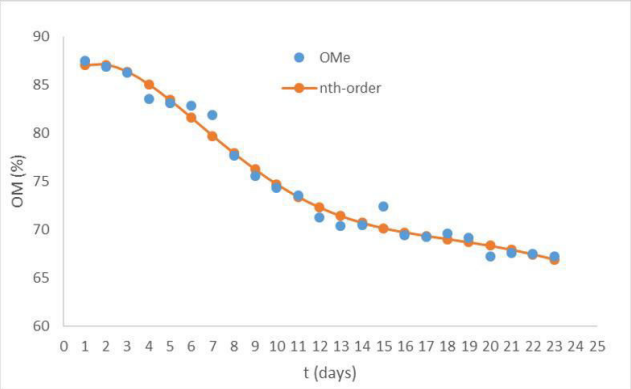

Figure 4. Comparison of experimental data and nth-order kinetic model for organic matter content for reactor 1

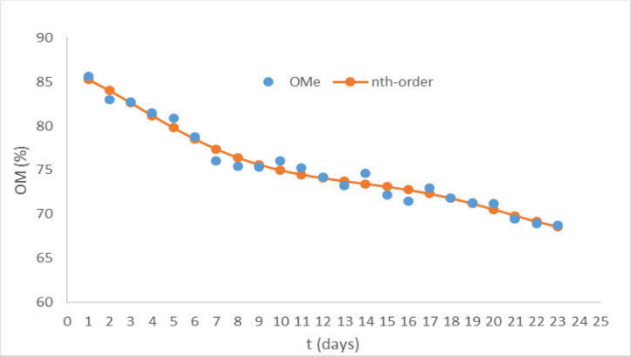

Figure 5. Comparison of experimental data and nth-order kinetic model for organic matter content for reactor 2

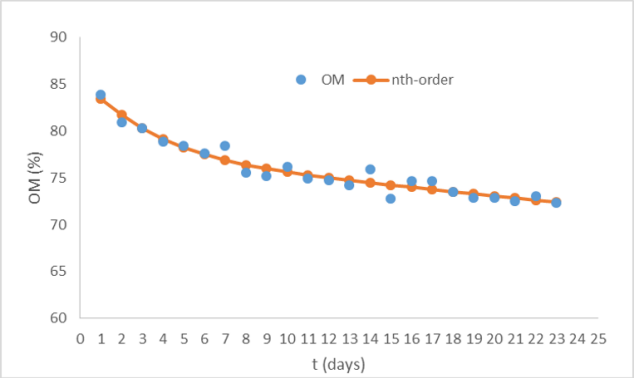

Figure 6. Comparison of experimental data and nth-order kinetic model for organic matter content for reactor 3

The calculated maximum and mean differences for experimental values and calculated values of organic matter are shown in Table 8.

Table 8. Maximum and mean differences between experimental data and nth-order kinetic model for organic matter content

Reactor	Maximum difference				Mean difference			
	0-5 days	6-14 days	15-22 days	0-22 days	0-5 days	6-14 days	15-22 days	0-22 days
1	1.2876	2.2966	0.5391	2.2966	-0.0705	0.0949	-0.0682	-0.0049
2	1.0891	1.2187	0.7047	1.2187	0.2156	-0.1116	-0.0318	0.0015
3	0.4800	1.5013	0.8881	1.5013	-0.0356	-0.0753	0.1079	-0.0012

COMPARISONS OF KINETIC MODELS

Statistical analysis showed that the highest values of the correlation factor and adjusted correlation factor, and the lowest values of the mean and standard deviation and variance, were found in the first-order kinetic models 2, 3, and 4. On the other hand, the 95% confidence interval for almost each kinetic parameters in the first-order and nth-order kinetic models were less than the values of kinetic parameters. Higher values of 95% confidence interval indicated possible

measurement errors or poor estimation of the initial values of the model parameters.

Although the statistical analysis showed very good results with first-order models, when compare the graphs comparing the experimental data and the calculated values for organic matter content for used models, it can be seen that the maximum and mean differences are greater for first-order models than for nth-order model. These deviations can also be seen in Tables 4, 5 and 6, where the maximum difference ranges from 10.30 to 18.64% for the first reactor, from 4.76 to 14.51% for the second reactor, and from 6.60 to 14.00% for the third reactor, and mean difference from -4.96 to -0.55% for the first reactor, -5.42 to 3.36% for the second reactor and from -1.62 to 0.43% for the third reactor for days 0-22. The highest value for the maximum difference was for 15-22 days for the first reactor, 6-14 days for the second reactor, and 15-22 days for the third reactor, and the highest value for the mean difference was for 6-14 days for the first reactor, 15-22 days for the second and third reactor. Large deviations from experimental data can be explained by the fact that the mixture that was composted was very heterogeneous, and therefore it was difficult to take samples that would contain all composted components [7]. Also, it should be taken into account that not all components degrade at the same rate, some slower and some faster, which can lead to errors in experimental data [12].

The nth-order kinetic model showed the best results for three reactors, as it can be seen in the Figures 4, 5 and 6. The maximum difference between the experimental data and the calculated values is 2.30% for the first reactor, 1.22% for the second reactor, and 1.50% for the third reactor, and the mean difference is -0.005% for the first reactor, 0.002% for the second reactor, and 0.001% for the third reactor for days 0-22. In the study [18], the maximum difference was 3.91% for the first reactor, 4.07% for the second reactor, and 3.93% for the third reactor, and the mean difference is 1.30% for the first reactor, 1.60% for the second reactor, and 1.76% for the third reactor for days 0-22, in which first-order kinetics was used. By comparing the statistical analysis and the values for the maximum and mean difference for the experimental data and the calculated values for organic matter content obtained with nth-order model in this study and for the first order models used in the study [18], it can be concluded that the nth-order kinetic model gave the best results.

An n-th order model for composting is often preferred over first-order kinetics because it offers more flexibility in modeling the non-linear nature of the composting process. The composting rate typically decreases over time as substrates are consumed, and the n-th order model can better represent this dynamic process. It allows for the incorporation of various factors, including microbial activity, substrate concentration, and environmental conditions, making it a more realistic choice for describing composting kinetics.

Although the nth-order kinetic model gives better results than the first-order, the n-th order kinetic model offers a simplified approach to understanding the composting process, but it has several limitations when it comes to capturing the complexities and variability inherent in composting. It's often more useful for understanding general trends in decomposition rather than predicting the exact dynamics of the process under varying conditions. For more accurate predictions, more complex models or experimental data might be required to account for factors like temperature, moisture content, microbial communities, and the heterogeneity of the organic material, which are present in the first-order kinetic model.

CONCLUSION

In this study, six new first-order kinetic models nth-order kinetic model for composting process of municipal solid waste were applied to experimental data. Nonlinear regression method was used for evaluation of kinetic parameters. Six kinetic first-order models were proposed based on the following experimental data: organic matter content, pH, electrical conductivity, oxygen concentration, carbon dioxide concentration, temperature and moisture content.

Among six first-order kinetic models, kinetic models 2, 3 and 4 showed better statistical analysis compared to other models. For six first-order kinetic models, maximum differences between experimental and model data based on organic matter content for the first reactor, the second reactor and the third reactor were ranged from 10.30 to 18.64%, from 4.77 to 14.51% and from 6.60 to 14.00%, respectively. Mean differences for the first reactor, the second reactor and the third reactor were ranged from 3.36 to 5.42%, from 4.77 to 14.52% and from 0.43 to 1.62%, respectively. For nth-order kinetic model, maximum differences between experimental and model based on organic matter content for the first reactor, the second reactor and the third reactor were 2.30%, 1.22% and 1.50%, respectively. Mean differences for the first reactor, the second reactor and the third reactor were 0.005%, 0.002% and 0.001%, respectively. The nth-order kinetic model showed better agreement with experimental data of organic matter content than first-

order kinetic models proposed in this study and in the previous studies.

REFERENCES

[1] R. Külcü and O. Yaldiz, "Determination of aeration rate and kinetics of composting some agricultural wastes", *Bioresource Technology*, vol. 93(1), pp. 49-57, 2003.

[2] G. Vidriales-Escobar, R. Rentería-Tamayo, F. Alatriste-Mondragón and O. González-Ortega, "Mathematical modeling of a composting process in a small-scale tubular bioreactor", *Chemical Engineering Research and Design*, vol. 120, pp. 360-371, 2017.

[3] H. Hamelers, "Modeling composting kinetics: A review of approaches", *Reviews in Environmental Science & Bio/Technology*, vol. 3, pp. 331–342, 2004.

[4] I. Petric and V. Selimbašić, "Development and validation of mathematical model for aerobic composting process", *Chemical Engineering Journal*, vol. 139(2), pp. 304–317, 2008.

[5] D. Kulikowska and S. Sindrewicz, "Effect of barley straw and coniferous bark on humification process during sewage sludge composting", *Waste Management*, vol. 79, pp. 207-213, 2018.

[6] M. Shirish Jain, S. Paul and A.S. Kalamdhad, "Kinetics and physics during composting of various organic wastes: Statistical approach to interpret compost application feasibility", *Journal of Cleaner Production*, vol. 255, 2020.

[7] I. Tosun and M.T. Gönüllü, E. Arslankaya and A. Günay, "Co-composting kinetics of rose processing waste with OFMSW", *Bioresource Technology*, vol. 99(14), pp. 6143–6149, 2008.

[8] R. Külcü and O. Yaldiz, "The composting of agricultural wastes and the new parameter for the assessment of the process", *Ecological Engineering*, vol. 69, pp. 220-225, 2014.

[9] M. Baptista, F. Antunes, M.S. Gonçalves, B. Morvan and A. Silveira, "Composting kinetics in full-scale mechanical–biological treatment plants", *Waste Management*, vol. 30(10), pp. 1908-1921, 2010.

[10] K. Ekinci, I. Tosun, B. Bitrak and B.S. Kumbul, "Effects of initial C/N ratio on organic matter degradation of composting of rose oil processing solid wastes", *International Journal of Environmental Science and Technology*, vol. 16, pp. 5131-5140, 2018.

[11] M. Rastogi, M. Nandal and L. Nain, "Seasonal variation induced stability of municipal solid waste compost: an enzyme kinetics study", *SN Applied Sciences*, vol. 1, pp. 1-16. 2019.

[12] R. Külcü, "New kinetic modelling parameters for composting process", *Journal of Material Cycles and Waste Management*, vol. 18(4), pp. 734-741, 2016.

[13] P. Agamuthu L.C. Choong, S. Hasan and V.V. Praven, "Kinetic Evaluation of Composting of Agricultural Wastes", *Environmental Technology*, vol. 21(2), pp. 185-192, 2000.

[14] J.S. Huang, C.H. Wang and C.G. Jih, "Empirical model and kinetic behavior of thermophilic composting of vegetable waste", *Journal of Environmental Engineering*, vol. 126(11), pp. 1019-1025, 2000.

[15] E. Walling and C. Vaneeckhaute, "Novel simple approaches to modeling composting kinetics", *Journal of Environmental Chemical Engineering*, vol. 9(3), 2021.

[16] X.Y. Sun, H. Xu, B.H. Wu, S.L. Shen and L.T. Zhan, "A first-order kinetic model for simulating the aerobic degradation of municipal solid waste", *Journal of Environmental Management*, vol. 329, 2023.

[17] N.A.H. Hassan, Z.B. Mohamed and I.R. Karim, "Compost production from aerobic digestion: a review", The 5th International Conference on Buildings, construction, and Environmental Engineering: BCEE, 21–23 November 2023, Amman, Jordan *AIP Conference Proceedings*, vol. 3219(1): 2024.

[18] I. Petric, A. Helić and E. Avdihodžić Avdić, "Evolution of process parameters and determination of kinetics for co-composting of organic fraction of municipal solid waste with poultry manure", *Bioresource Technology*, vol. 117, pp. 107-116, 2012.

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CHEMICAL PRETREATMENT OF CATTLE MANURE TO IMPROVE BIOGAS PRODUCTION

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:

Great potential in gas production is found in raw materials of organic origin, such as manure, waste sludge, food residues and the like. In order to speed up the process of biogas production, that is, the first stage of hydrolysis, it is necessary to pre-process the substrate. In order to obtain the maximum amount of biogas, the anaerobic digestion process was carried out in mesophilic conditions for 37 days. For the purpose of examining the effects of pretreatment, the substrate was chemically treated with the addition of dehydrated CaO in quantities of 0.75% and 1.5% in relation to the mass of the substrate. The results showed that by treating cattle manure with the addition of 0.75% CaO, higher daily production of biogas with a higher methane content is achieved. The total amount of biogas obtained with the addition of 0.75% CaO was 5247.21 ml, while the highest methane content was 61.24%.

KEYWORDS: anaerobic digestion; biogas; chemical pretreatment; cattle manure; biogas production; methane content.

INTRODUCTION

The process by which energy from biomass can be obtained is anaerobic digestion. In addition to the economic benefits it generates, this process of organic waste treatment is extremely current because it directly affects the reduction of greenhouse gas emissions, which ultimately does not lead to negative impacts on the environment. Anaerobic digestion is a biological process of decomposing organic matter without the presence of oxygen, a process that is part of the natural cycles of matter and energy [1].

For the production of biogas, raw materials of organic origin, such as municipal waste, food residues, agricultural waste, are huge potential, but given that most of these raw materials are difficult to decompose, which limits the production of biogas, today biogas is mostly produced from raw materials that can be easily decomposed and largely used [2]. The application of cattle manure for fertilization of agricultural land is limited with application of the Nitrates Directive (91/676/EEC) [3].

Although these raw materials are of enormous importance for gas production, due to their complex physico-chemical structure, they are more difficult to decompose, and ultimately result in less biogas production. In the process of anaerobic digestion by the action of different types of microorganisms, two main products are formed: biogas and digestate [4]. A possible solution for obtaining higher biogas production is the process of pretreatment of incoming raw materials, and their main task is to facilitate the anaerobic digestion process itself [5], [6].

The choice of pretreatment, in addition to being economical, should meet several important conditions to ensure its effectiveness and sustainability. Firstly, it should not require excessive energy consumption, as this would offset the potential benefits of biogas production and lead to an inefficient process. The energy demands of the pretreatment method should be optimized to ensure that the overall process remains cost-effective and energy-efficient. Furthermore, the pretreatment should not have a negative impact on the environment through the generation of harmful by-products or pollutants. Any waste products produced during the pretreatment should be manageable and ideally reusable, minimizing the ecological footprint of the process.

Another crucial aspect is that the pretreatment method should not involve or produce substances that could inhibit the subsequent stages of biogas production. Some chemicals or compounds might interfere with the anaerobic digestion process or hinder the microbial activity necessary for efficient biogas generation. Therefore, careful selection of chemicals and treatment conditions is necessary to ensure that no adverse effects on biogas production are introduced.

Chemical pretreatment typically involves processes that utilize chemical reactions to alter the structure of biomass, breaking down complex compounds and making the organic material more accessible for microbial degradation during anaerobic digestion. These chemical treatments may involve the use of acids, alkalis, or oxidizing agents, all of which can disrupt the lignocellulosic structure of the biomass, enhancing the release of fermentable sugars and other nutrients that are critical for the biogas production process. The ultimate goal of chemical pretreatment is to enhance the efficiency and yield of biogas production, while also ensuring that the process remains sustainable and environmentally friendly.

MATRERIALS AND METHODS

For the purpose of the research, samples from the dairy cow farm "Spreča" were used, which were chemically treated with the addition of CaO in quantities of 0.75% and 1.5% and waste sludge from the wastewater treatment plant Živinice.

To conduct this experimental research, a laboratory reactor system for anaerobic batch-type digestion was used, consisting of six glass eudiometric tubes that are connected to glass bottles with a volume of 500 ml (Figure 1). Eudiometric tubes are used to equalize the level and accept excess liquid, and thus they are used to read the production of biogas. By heating the water, a constant temperature of 35 °C is enabled.

In order to obtain a certain recalculated value of the volume of biogas, a baro-thermohygrometer for measuring pressure and a thermometer for temperature measurement were used, and the mixing of the substrate was carried out using a magnetic mixer.

Figure 1. Laboratory reactor system for anaerobic digestion

In the chemical pretreatment of cattle manure, dehydrated CaO was added in quantities of 0.75 and 1.5% to the total mass of the sample, and then after added CaO, mixing of the substrate was performed to homogenize the sample and added CaO. The samples were left at room temperature for three hours for CaO to act on the substrate.

An analysis of substrate (cattle manure and waste sludge, as well as a mixture of cattle manure and waste sludge) was performed before and after the anaerobic digestion process, which included the measurement of pH value and electroconductivity, determination of dry matter content (TS) and volatile organic matter (VS), content of total Kjeldahl nitrogen (TKN) and chemical oxygen demand (COD).

The methods used in the analysis of physicochemical characteristics are standard methods and modified standard methods for the examination of water and wastewater (APHA).

The pH value was measured by a digital measuring device with direct immersion of the electrodes in samples, using pH meter Mettler Toledo FE20/EL20. Prior to each measurement, the control of the measuring device was performed using standard buffer pH 4.01, 7.01, 10.01.

Chemical oxygen demand was determined according to the modified standard method BAS ISO 6060:2000 [7].

Determination of the total content of dry matter and the total content of volatile organic matter of all samples was carried out according to *Metod 2540-Solid B.Standard Methods for the Examination of Water and Wastewater 21st edition*. APHA, Washington, DC (2005) [8]. The method used to determine the content of total Kjeldahl nitrogen is *Metod 4500-Norg B. Standard Methods for the Examination of Water and Wastewater 20nd edition*. APHA, Washington, DC (1998) [9].

The experimental part of this study lasted 37 days. The composition of the obtained biogas was analyzed daily on the clarus 500 gas chromatograph (PERKIN ELMER, India) equipped with thermal conductivity detector (TCD-R) and gas analyzer "Arnel".

RESULTS AND DISCUSSION

Before the experimental part, a physical-chemical analysis of cattle manure substrate (CM) and waste sludge (WS) was performed, and their mixture was used as a control sample (M). The experiment was conducted under mesophilic conditions at a constant temperature of 35 ± 1°C for 37 days. After the chemical treatment process, two mixtures M0.75 and M1.5 were formed, and in order to achieve a significant value of dry matter content (about 8%) a mixture of waste sludge and cattle manure was formed in a ratio of 1:1.

In chemical pretreatment of cattle manure, dehydrated CaO was added in quantities of 0.75 and 1.5% to the total mass of the sample. After the addition, mixing of the substrate was carried out to homogenize the sample and added CaO. Samples left at room temperature for three hours for CaO to act on the substrate. After the chemical pretreatment, physico-chemical characterization of pretreated samples was performed.

Table 1 shows the parameters of the physico-chemical characteristics of cattle manure and waste sludge, as well as mixtures of waste sludge with untreated and chemically treated bovine manure.

Table 1. Results of physicochemical characteristics of cattle manure mixture, waste sludge and CM:WS mixture

Parameter	Unit	CM	WS	M	M0.75	M1.5
pH	-	6.7	6.79	6.83	8.99	10.47
TS	%	15.35	4.13	9.77	10.97	10.60
VS	%	12.97	2.49	7.95	8.60	8.04
VS/TS	-	0.84	0.60	0.81	0.78	0.75
TKN	g/kg	3.69	1.87	2.27	3.9	3.03
COD	g/kg	118.15	52.92	68.90	102.67	89.89

TS-total solids VS-volatile solids; TKN-total Kjeldahl nitrogen; COD- chemical oxygen demand

In order to obtain a larger amount of biogas, it was observed that by adding a smaller amount of CaO (0.75%) with the previous pretreatment process, a higher production of biogas with a higher methane content compared to the control sample was obtained, as well as a sample in which cattle manure was treated with the addition of 1.5% CaO.

Certain changes in pH values, COD, the total amount of Kjeldahl nitrogen in the content of dry matter and volatile organic matter were also observed. The pH value for waste sludge was 6.79, the value of cattle manure was 6.7, and thus it was lower compared to the mixture of cattle manure and waste sludge that underwent the pretreatment process and which had a value of 7.03. According to literature, for an undisturbed process of anaerobic digestion the optimum pH value ranged from 6.5 to 7.6 [10]. The VS/TS ratio was approximately 0.8 in all formed mixtures, so the requirement for a sufficient amount of organic matter in reactors was satisfied [11]. After chemical treatment, the value of this parameter increased to 8.99 with the addition of 0.75% CaO, and to 10.47 with the addition of 1.5% CaO, which could be expected, because the introduction of dehydrated CaO into the substrate with a high water content resulted in the formation of calcium hydroxide.

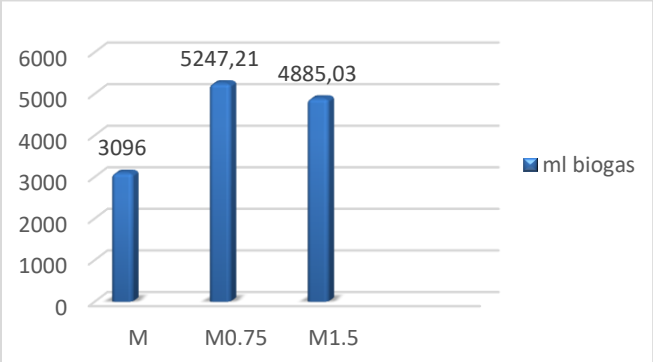

Diagram 1. Amount of biogas of untreated and chemically treated manure and waste sludge in a period of 37 days

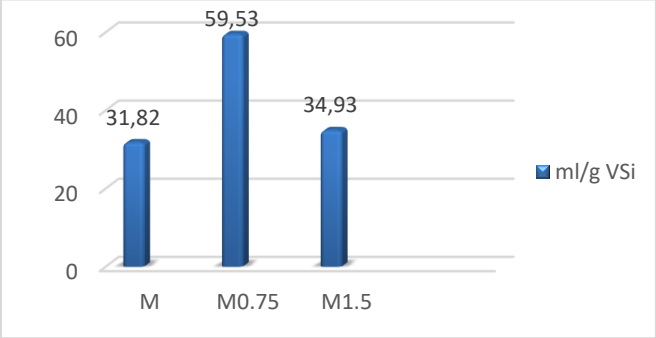

Diagram 2. Specific biogas production

Table 2 shows the characteristics of the remaining digestate after the process of anaerobic digestion, and the amount of biogas produced and specific production of biogas in relation to the parameter of volatile organic matter. From the table, it is noticeable that in the sample M1.5 pH value was significantly lower (7.17) compared to the pH values for samples M (7.3) and M0.75 (7.33).

Table 2. Physico-chemical characteristics of the mixture of cattle soil and waste sludge after conducting the experimental part

Parameter	Unit	M	M0.75	M1.5
pH	-	7,3	7,33	7,17
TS	%	7,44	7,32	6,53
VS	%	5,51	4,92	3,68
TKN	g/kg	2,59	2,74	2,09
COD	g/kg	64,40	74,76	58,11
V biogas	ml	3096	5247.21	4885.03
W methane	%	52.65	61.24	52.35
W CO_2	%	44.02	34.58	41.32
V methane	ml	758.83	1535.93	842.61
Specific production of methane	ml/g VSi	31.82	59.53	34.93

TS-total solids VS-volatile solids; TKN-total Kjeldahl nitrogen; COD- chemical oxygen demand; V biogas – volume of biogas; W methane – share of methane in biogas; W CO_2 – share of CO_2 in biogas, V methane – volume of methane;

CONCLUSION

After conducting the entire experimental part, based on the physical-chemical characterization of waste sludge and cattle manure, the basic parameters of starting raw materials were obtained.

After the process by which the samples underwent pretreatment, in this case chemical pretreatment, and after the characterization of physico-chemical characteristics, results were obtained that determined a decrease in the dry matter content and an increase in the content of organic matter (due to the addition of CaO).

The pH value in both treated samples was significantly increased compared to the value of the untreated sample, which is an indicator of the consequence of $Ca(OH)_2$ due to the reaction of CaO and water present in the substrate.

The best results of daily production of biogas and methane, and specific biogas production were obtained using the addition of 0.75% CaO (5247.21 ml), with a methane content of 61.24% and a CO_2 content of 34.58%. The higher amount of CaO used for the research of 1.5% showed that it did not achieve significant yield and that it adversely affected the production and quality of the biogas obtained.

Mineral substances in the substrate for both treated samples resulted in an increase in the content of volatile organic matter with the addition of CaO, and as a result of the decrease in the proportion of dry matter, in both treated samples was the formation of CO_2.

Chemical pretreatment of cattle manure is a process by which improved biogas production is obtained, as well as the quality of which achieves a higher content of methane in biogas, compared to the same samples that have not been previously treated, and which were used in the process of anaerobic digestion.

REFERENCES

1. J. Ariunbaatar, A. Panico, G. Esposito, F. Pirozzi, and P.N.L. Lens, "Pretreatment methods to enhance anaerobic digestion of organic solid waste", *Applied Energy 123, 143-156*, 2014.
2. S. Achinas, V. Achinas, and G.J.W. Euverink,"A Technological Overview of Biogas Production from Biowaste", *Engineering 3, pp. 299–307*, 2017.
3. J.J. Schröder, H.F.M. Aarts, J.C. van Middelkoop, R.L.M. Schils, G.L. Velthof, B. Fraters, and W.J. Willems, "Permissible manure and fertilizer use in dairy farming systems on sandy soils in The Netherlands to comply with the Nitrates Directive target",*Europ. J. Agronomy 27 (2007) 102–114*, 2007.
4. Z. Osman, "Projekt biolinskog postrojenja", Diplomski rad, 2015.
5. B. AbuabakerandN. Ismail, "Anaerobic Digestion of Cow Dung for Biogas Production",*ARPN Journal of Engineering and Applied Science7 (2):169-172*, 2012.
6. J.B.van Lier, A.Tilche, B.K.Ahring, H. Macarie, R. Moletta, M. Dohanyos, L.W. Hulshoff Pol, P. Lens, andW. Verstraete, New perspectives in anaerobic digestion. *Water Sci Technol*. Vol. 43, No.1, pp 1-18, 2001.
7. BAS ISO 6060:2000, Određivanje hemijske potrošnje kisika, 2000.
8. Method 2540-B and 2540 Solid-Solid E. Standard Methods for the Examination of Water and Wastewater.
9. Method 4500-Norg B. Standard Methods for the Examination of Water and Wastewater 20nd edition. APHA, Washington, DC, 1998.

10. P. Bishnoi, Effects of thermal hydrolysis pre-treatment on anaerobic digestion of sludge, *Master of Science In Civil Engineering*, Blacksburg, VA, 2012.

11. S. Halilović, V. Stuhli, M. Zohorović, Z. Iličković, Determination of the biomethanic potential of corn grain. *TecnologicaActa, 11 (1), 1-4,* 2018.

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EFFICIENCY AND ECONOMY OF THE INDUSTRIAL PROCESS OF WATER TREATMENT - CASE STUDY

PROFFESIONAL ARTICLE

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ABSTRACT:
Water preparation for industrial use commonly involves a multi-stage approach to raw water treatment. The selection of individual treatment operations is based on various factors. Adherence to regulations and standards on water quality for a specific purpose is of key importance, as the entire treatment process should meet or exceed applicable regulatory standards. In the selected plant for the preparation of industrial water, an evaluation of the efficiency and economy of the process was carried out by comparing the results of water analysis before and after each treatment, by reviewing the justification of the location of the process units in the treatment line, and by analyzing the selected process parameters. The results of water analyzes confirmed that adequate selection and location of unit operations in the raw water treatment line can have multiple beneficial effects in meeting water quality requirements for the desired purpose.

KEYWORDS: water treatment; process evaluation; industrial process; treatment operations selection

INTRODUCTION

The role of water used in industry is diverse [1], [2], and the requirements of its quality for each individual purpose can differ significantly [3]. Industrial processes mainly rely on freshwater for water supply [4], [5]. All natural waters contain different amounts of suspended and dissolved substances, gases and microorganisms, which is caused by the type of source, as well as geological specificities of the soil and contaminants with which they come into contact in the hydrological cycle [6], [7], [8]. For the same reasons, raw water most often does not meet the quality requirements for a specific purpose in industry, and it is necessary to subject it to appropriate treatment. The main purpose of the treatment, which may include one or more different operations, is the removal of contaminants, with the ultimate goal of providing the required amount of water of the desired quality with the lowest treatment costs. All alternatives are identified and evaluated in order to select a system that achieves the required treatment at the lowest cost. The multiple effects of different operations should be considered in the broader context of water quality, given that a particular operation selected to meet the requirements of one regulation may cause compliance problems with other regulations. The circumstances are different for each facility and may be different for each individual source of water used by a facility. Due to all of the above, the selection of adequate raw water treatment operations in the industry is a complex task, and requires periodic evaluation even after the establishment of the entire treatment system.

In this paper, a comparative analysis of the quality of water, before and after individual treatment operations, intended for the supply needs of a combined boiler plant (including fire-tube and water-tube boilers) for the production of steam, working pressure 12.5 (bar) and maximum pressure 15 (bar) in a selected food industry, was performed. Additionally, selected process parameters of individual water treatment units were analyzed, all with the aim of evaluating both individual operations and the entire process of raw water treatment, from the aspect of efficiency and economy. The flow diagram of the industrial water preparation plant for steam production is shown in Figure 1.

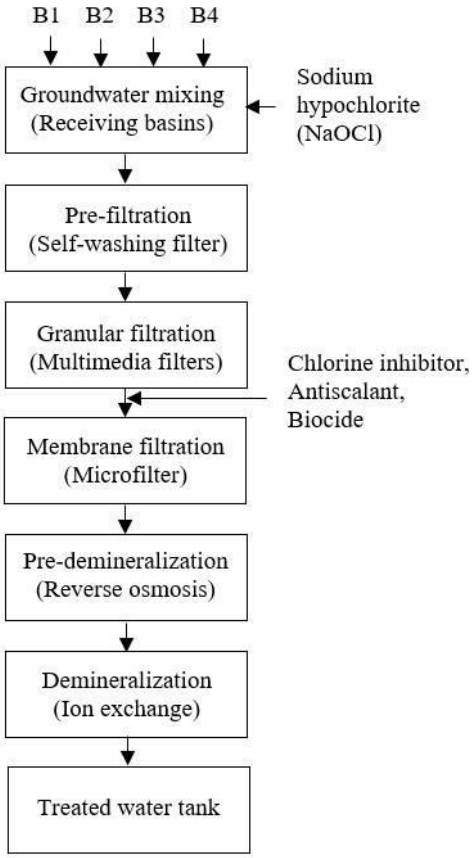

Figure 1. Flow chart of industrial water preparation for steam production

Raw water for industrial needs consists of water from four underground sources (B1, B2, B3, B4), which are fed into receiving basins and from which, as a single, mixed flow, they are subjected to appropriate treatment for supplying the steam boiler plant. Sodium hypochlorite (NaOCl) is regularly dosed into the receiving basins. The mixed flow of water from basins, before entering the multimedia filters, passes through an automatic self-cleaning filter with a pore diameter of 100 (μm). It is possible to adjust the time interval between two filter self-cleanings. The flow of water through the filter does not stop even when the filter is in the self-cleaning phase.

The multimedia filters, which make up the filter battery, are two filters filled with a combination of hydroanthracite, pyrolusite and three granulations of sand. The filtration filling of each filter is placed inside a polyethylene column with a diameter of 122 (cm) and a height of 183 (cm), coated with polyester reinforced with glass fibers. The filters are designed to work in parallel mode. The operation of each filter consists of three phases: filtration, reverse washing of the filter and rejection of the first filtrate. Iron, manganese and suspended matter are removed by means of the mentioned filters.

An oxidation-reduction potential meter is installed behind the multimedia filters, which detects the presence of residual chlorine and gives a signal to the dosing pump for dosing the sulphite-based free chlorine binding agent. In addition, antiscalant and biocide are added to the water after exiting the multimedia filter. The filtered water is further transported to a microfilter (membrane filtration), housing dimensions 1500x388 (mm) and a fineness cartridge of 5 (μm). The maximum working pressure of the filter is 10 (bar). The water flow on the cartridge is 25 (m^3/h), and the cartridge is changed when the pressure difference at the inlet and outlet of the filter reaches a threshold value of 1 (bar).

The outflow of water from the microfilter enters the reverse osmosis (RO) system, which consists of 14 membrane modules. The nominal flow of water entering the system is about 20 (m^3/h); the system works in constant mode and has a capacity of 15 (m^3/h) of demineralized water. Additional technical characteristics of the system are: working pH value 6.5-8, maximum chlorine concentration in the inlet water <0.1 (mg/L), maximum inlet water turbidity 1 (NTU), maximum sludge density index (SDI) value 5, maximum SiO_2 content 25 (mg/L), working temperature 8–30 (°C). The permeate is drained through a separate pipeline and stored in a demineralized water tank. Permeate production is 70-75 (%) of the water input to the membrane system, and its estimated flow rate is 15 m^3/h at 15 (˚C). The permeate tank also provides a reserve of water for occasional flushing during RO operation. The working water level in the permeate tank gives the signal to start or stop reverse osmosis. The tank is equipped with a working pump and a backup pump. The part of the water that did not pass through the membranes is drained through another pipeline. The estimated concentrate flow is about 5 (m^3/h). The RO system is additionally equipped with: a biocide dosing system, equipment for chemical washing of membranes, permeate, concentrate and recirculation flow meters, pressure detection sensors and an electrical conductivity meter. The RO is stopped during the backwash of the multimedia filters, and restarts after the wash is complete.

After the reverse osmosis treatment, the water enters the ion exchange (IEX) water softening system whose function is to neutralize residual hardness in the permeate. The filling of the ion exchanger consists of a strongly acidic cation-exchange resin. During the operation of the IEX system, water passes through a layer of ion-exchange resin, whereby Ca^{2+} and Mg^{2+} from the water are bound to the resin, and cations are released from the resin into the water, which do not

affect the hardness of the water. The water flow through the ion exchange column is 22 (m^3/h). Considering that after passing a certain amount of water through the column, the resin is saturated and needs regeneration, the softening process works in the working and reserve column mode, i.e. while one column is working, the other is regenerating and after regeneration is in stand by mode. Tablet salt is used to regenerate the resin. Each ion exchange unit consists of a polyethylene column, coated with polyester reinforced with glass fibers, diameter 92 (cm) and height 183 (cm). The columns are interconnected by a communication cable and each is equipped with a control system. From the ion exchanger system, the water goes into the permeate tank of 20 (m^3) volume, and at the exit of the water from the ion exchanger there is an automatic hardness measuring device that communicates with the control system so that when the hardness value exceeds 0.3 (°dH), the ion exchanger enters the regeneration phase.

EXPERIMENTAL

Laboratory research of water quality was conducted at:

- samples of raw water from four underground springs, marked as B1, B2, B3 and B4 and samples of their mixed flow,
- samples of the mixed flow of water after individual treatment operations in the observed industrial process.

Research methods included:

1. Laboratory quality analyzes of raw and treated water samples:
   - determination of the conctent of iron (mg/L) and manganese (mg/L) by spectrophotometric method [9],
   - determination of residual chlorine content (mg/L) by iodometric method [10],
   - determination of pH value (dimensionless) using a pH meter [11],
   - determination of electrical conductivity (μS/cm) according to the ISO 7888:1985 method [12],
   - determination of total hardness (mg/L $CaCO_3$ or °dH) by titration with EDTA and eriochrome-black T as an indicator [10],

2. Direct readings of process parameters from measuring equipment in the subject water treatment process.

RESULTS AND DISCUSSION

THE QUALITY OF RAW WATER FROM UNDERGROUND SOURCES

The results of the analysis of individual groundwaters (Table 1) indicate the presence of a certain content of iron, manganese and the total hardness of raw water in all four sources. The industrial system can be supplied from several sources, each of which can have its own characteristics of water quality [3]. Iron and manganese are commonly found in groundwater [13], in different concentrations [14]. Although they can be in dissolved or undissolved form [15], the former is more common in groundwater due to its low redox potential. Manganese typically occurs in lower content compared to iron [16], which is confirmed by the results of the analysis of all four sources (Table 1). Iron content values in the waters of sources B2 and B3, of 0.395 and 0.399 (mg/L), are similar and slightly higher compared to those in the waters of sources B1 and B4, while the water of source B4 has the lowest values of manganese content and total hardness, i.e. 0.240 (mg/L) and 271 (mg/L $CaCO_3$). The maximum value of iron content in the analyzed raw water samples was 0.399 (mg/L), which is in accordance with the statement that groundwater generally contains iron below 5 (mg/L) [17]. The feed water quality requirements for fire-tube boilers of an operating pressure of 0.5-20 (bar) prescribe an iron concentration < 0.2 (mg/L) [18]. Suspended or dissolved iron in the feed water of the boiler by precipitation creates porous deposits of dark color (red-brown or black) on metal surfaces, which promotes the deposition of other impurities [3]. The thermal conductivity (kcal/mh°C) of iron deposits is 1-5, which compared to those of structural boiler metals, such as carbon steel (40-60) or copper (320-360), is significantly lower [19], which negatively affects the overall efficiency of the boiler, and thus the increase in fuel consumption. Taking into account the working pressure of the existing steam boiler, which is 12.5 (bar), the results of the water analysis of all four sources indicate an iron content that exceeds the mentioned requirement.

Manganese, like iron, affects the formation of deposits in boiler plants [20], and its content in feed water for low and medium pressure boilers is limited to a maximum of 0.3 (mg/L) [21]. Among the analyzed water samples, only that of underground source B4 has a manganese content of 0.24 (mg/L), which meets the aforementioned limit.

The values of the total hardness of all tested raw water samples were in the range of 271-307

(mg/L $CaCO_3$). Water with a calcium carbonate content below 60 (mg/L) is considered soft, 60-120 (mg/L) moderately hard, 120-180 (mg/L) hard, and above 180 (mg/L) very hard [22]. If the determined range of hardness of all analyzed water samples is compared with the mentioned classification, the waters of all four underground sources can be classified as very hard. Taking into account the limitation of the total hardness of boiler feed water to below 0.01 (mmol/L $CaCO_3$) [18], which is equivalent to <1.0009 (mg/L $CaCO_3$), the waters of all four underground springs do not meet the mentioned requirement for use in steam boilers, which is why their appropriate treatment is required.

Table 1. Water quality of underground sources for supplying industrial plant

Groundwater source	Quality parameter	Value (mg/L)
B1	Iron	0.373
	Manganese	0.321
	Total hardness (as $CaCO_3$)	302
B2	Iron	0.395
	Manganese	0.302
	Total hardness (as $CaCO_3$)	300
B3	Iron	0.399
	Manganese	0.309
	Total hardness (as $CaCO_3$)	307
B4	Iron	0.375
	Manganese	0.240
	Total hardness (as $CaCO_3$)	271

WATER QUALITY IN THE RECEIVING BASIN

The water at the exit from the receiving basin, created by mixing of four groundwaters and designated as "mixed flow", was analyzed every 4 h for the total hardness content, pH value and residual chlorine content, and the obtained analysis results are shown in Table 2. By comparing the values of the total hardness of individual groundwaters (Table 1) with those of the mixed flow (Table 2), significant differences can be observed, i.e. lower values of the hardness of the mixed flow. Given that the dosing of the chemical agent sodium hypochlorite into the receiving basin has no effect on the total hardness of the mixed flow, the decrease in hardness can be explained by degassing CO_2 from the water. Namely, groundwater is typically 10 to 100 times oversaturated with CO_2 [23]. However, when groundwater is pumped from the source to the surface, it comes into contact with air (O_2) that enters the water and begins an oxidation process that releases carbon dioxide (CO_2) from the water into the atmosphere [24] and thus potentially causes precipitation of calcium carbonate This can result in a decrease in water hardness due to scale formation. Nevertheless, the values of total hardness given in Table 2, according to the descriptive classification of water [22], characterize the water as hard, i.e. the mixed flow of water still does not meet the requirement for the total hardness of boiler feed water of <0.01 (mmol/L $CaCO_3$) [18], i.e. <1.0009 (mg/L $CaCO_3$).

Although it is stated that for iron and manganese content up to 5 mg/L, one-stage water treatment is planned, e.g. filtration [15], this operation can be less effective for their dissolved forms, which justifies the addition of a chemical agent – sodium hypochlorite for their oxidation into insoluble forms. Taking into account the joint mixing of the waters of all four ground sources in the receiving basin, the mean values of their individual iron and manganese contents could be taken as a quality parameter of the mixed water flow. Thus, the content of iron in the mixed flow of water would be 0.3855 (mg/L) and manganese 0.293 (mg/L). However, adding NaOCl to the receiving basin reduces their content. Sodium hypochlorite acts as an oxidizing agent and in the presence of iron and manganese it can facilitate the oxidation of these metals, turning them into a form that is easier to remove from water. Depending on the pH of the water, the oxidation kinetics is more or less rapid. The results of the conducted research [25] showed that NaClO is the most effective oxidant compared to O_2 and $KMnO_4$ and that its addition removes 95 (%) of Fe^{2+} from water at pH 9.8 in less than five minutes, while at pH 7.3 only 60 (%) of Fe^{2+} is removed. The pH values from Table 2 show that the water in the receiving basin has a pH of 7.5-7.8, which is why a high degree of iron removal cannot be expected with the addition of NaOCl. In addition, manganese is more difficult to oxidize compared to iron [26], so water needs to be further treated to remove Fe^{2+} and Mn^{2+}.

Table 2. Mixed flow quality

Time since first sampling (h)	Total hardness (mg/L $CaCO_3$)	pH	Residual chlorine (mg/L)
0	130	7.8	0.19
4	129	7.8	0.21
8	130	7.7	0.21
12	129	7.8	0.20
16	130	7.6	0.17
20	130	7.5	0.19

WATER QUALITY AFTER MULTIMEDIA FILTRATION

Most processes for removing dissolved iron and manganese from water involve their oxidation and precipitation into insoluble compounds that are then separated by filtration [15], [24]. Rapid sand filters are often used to clean groundwater of iron and manganese [27], which justifies the choice of multimedia rapid filters in the treatment plant in question.

Before the multimedia filter, the water passes through a self-cleaning filter, which reduces the load on the multimedia filters with suspended substances. The pore diameter of the filter is 100 (μm), which indicates that it is a macrofilter, because microfiltration membranes typically have nominal pore sizes of the order of 0.1-1.0 (μm) [28]. The advantages of a self-cleaning filter for use in industrial applications, compared to alternative cartridge filters, are proven long service life and the elimination of costs and labor associated with replacement filter cartridges [29]. In the water treatment plant in question, the limit value of the pressure drop (Δp) on the self-cleaning filter is 1 (bar), while the optimal value is 0.5 (bar). Table 3 shows the values of periodic control readings of the water pressure at the inlet (p_1) and outlet (p_2) of the filter, where the results show that the pressure drop values, which are in the range 0.1-0.2 (bar), are slightly lower than the optimal value of 0.5 (bar). A moderate pressure drop is necessary to maintain an adequate water flow rate, but too little pressure drop can lead to insufficient water flow, reducing the efficiency of the filtration process.

Table 3. Pressure drop values on the self-cleaning filter

Time since first reading (h)	p_1 (bar)	p_2 (bar)	Δp (bar)
0	3.3	3.2	0.1
8	3.5	3.2	0.1
16	3.6	3.4	0.2
24	3.9	3.7	0.2

After pre-filtration by self-cleaning filter, the water is treated with multimedia filtration, which is performed by forcing water through a column filled with appropriate filtration materials. Although in industry the filtration process can be carried out either as rapid gravity filtration or pressure filtration, the selection of pressure filtration in the water treatment plant in question is based on the fact that it enables significantly higher flow rates [30]. The selected multimedia filter is of the semicontinuous type, and ensuring continuous operation of the water treatment line is achieved by introducing two parallel-connected filtration units, whereby during the flushing of one unit the other is in operation. The use of semicontinuous operation in industrial practice is the most common [31]. Multimedia filters are those that use three or more different types of filtration media together with a supporting (non-filtering) layer of gravel at the bottom [32], [33]. Multimedia filtration is chosen because of its main advantages over mono-media and dual-media filtration: longer working time and better quality of filtered water [34], [35]. The filtration media in multimedia filters are usually arranged so that with any increase in depth, the specific density of the media particles increases while the particle size decreases [36]. This ensures that after the backwashing of the multimedia filling, the medium with the largest particle size and the smallest density stratifies at the top of the column, the one of medium size and density settles in the middle, and the heaviest but with the smallest particle diameter settles at the bottom of the column [37]. The arrangement of media layers in the considered multimedia filter is, in order from top to bottom: hydroanthracite, pyrolusite and quartz sand, which enables complementary functions of each type of media. Hydroanthracite is a form of anthracite coal that has been specially processed for use as a filter media in water treatment [38]. Its lowest density, 1.4-1.6 (g/cm³), compared to the other two media, enables greater granulation of its particles in the filter, thus allowing better water flow while reducing filter clogging. In addition, it has a large surface area and a porous structure, which makes it effective for the adsorption of certain contaminants [39]. The density of pyrolusite of 4.7-5.0 (g/cm³) compared to hydroanthracite enables a smaller diameter of its particles placed in the layer below. Pyrolusite primarily consists of manganese dioxide (MnO_2), i.e. represents its most stable form [40]. Its role is reflected in the adsorption and oxidation of dissolved iron and manganese [41], [42]. Pyrolusite acts as a catalyst that helps convert dissolved forms of iron and manganese into their insoluble forms, which are then easily filtered out. A layer of quartz sand with

an average particle density of 2.65-2.75 (g/cm³) additionally purifies the water from remaining suspended matter. In fact, quartz sand in a multimedia filter for the removal of Fe^{2+} and Mn^{2+} from groundwater can have several functions [38], [43]: a) it contributes to depth filtration useful in capturing a wide range of particle sizes, including fine particles, b) it contributes to reactions catalytic oxidation, promoting the oxidation of iron and manganese as the water flows through the filter, c) can have a buffering effect on the pH of the water, thereby contributing to maintaining the appropriate pH range for effective removal of iron and manganese. Adsorption of manganese on the surfaces of filtration media in multimedia filters is fast [44], [45] and is accompanied by the release of H^+, as happens with the adsorption of cations on oxide surfaces [46], [47]. Manganese removal in granular media filters has been reported to be enhanced by the development of 'catalytic oxide layers' on the aged media, due to the formation of manganese oxide coatings [48].

In the considered water treatment plant, water analysis showed the presence of free chlorine, which indicates the possibility of oxidation of iron and manganese by chlorine [49]. Iron can be oxidized from Fe^{2+} to Fe^{3+} by free chlorine, however, the oxidation of Mn^{2+} is relatively slow at low pH (lower than about 8 to 8.5) [50] such as that of mixed flow (table 2). However, the oxidation of manganese by free chlorine adsorbed on the oxide-coated surface is very fast (less than seconds to minutes) and can occur even at lower pH and at low temperatures [51].

Table 4 shows the results of periodic water analysis after exiting the multimedia filter. A comparison of the average values of Fe^{2+} and Mn^{2+} contents in each of four groundwater (Table 1) with those after water treatment with multimedia filtration (table 4) indicates the achieved degree of removal of iron up to 92.21 (%) and manganese 100 (%). In this way, the quality of the feed water for reverse osmosis is additionally improved and at the same time it protects the fine pre-filter with a pore diameter of 5 (μm) that is part of the reverse osmosis, that is, the frequency of replacing the filter cartridge is reduced. The slightly weaker effect of removing iron from water can be explained by its higher initial contents in raw water compared to manganese (Table 1). The maximum values of iron content and total hardness in boiler feed water are limited by feed water quality requirements for flue-tube steam boilers [18] and water-tube steam boilers [52], taking into account the boiler's maximum operating pressure. Based on the above, the iron content in the feed water of the combined boiler plant should be < 0.05 (mg/L), and the total hardness should be < 0.01 (mmol/L), i.e. < 5.6·10−4 (dH). A comparison of the mentioned limits with the results of water analysis after treatment with multimedia filtration shows that the iron content in the treated water meets the quality requirements of boiler feed water. In addition to the above, a complete removal of manganese was achieved, which is in accordance with the requirement of its maximum content in feed water for low and medium pressure boilers of 0.3 (mg/L) [21].

The lower value of the residual chlorine content in water after multimedia filtration (Table 4), compared to that in the mixed flow (Table 2), can be explained by a series of chemical and physical processes that take place inside the filter and in the presence of contaminants such as iron, manganese and increased total hardness.

Table 4. Results of water quality analysis after multimedia filtration

Time since first sampling (h)	Fe^{2+} (mg/L)	Mn^{2+} (mg/L)	Residual chlorine (mg/L)
0	0.03	0.00	0.16
4	0.03	0.00	0.16
8	0.03	0.00	0.15
12	0.03	0.00	0.11
16	0.03	0.00	0.13
20	0.03	0.00	0.13

WATER QUALITY AFTER REVERSE OSMOSIS

Reverse osmosis is used to remove dissolved substances from water [53]. In the plant in question, water treatment by reverse osmosis precedes treatment by ion exchange. Although in practice there are also configurations of the reverse order, the chosen configuration is based on the fact that the use of RO before ion exchange (IEX) can significantly reduce the operating costs and the regeneration frequency of the IEX system [54]. According to Rehman and Ahmed [55], the advantages of the mentioned configuration are reflected in the following: a) lower osmotic pressure of reverse osmosis feed water gives lower

pump pressure so that operating costs are low, b) lower chloride content and alkalinity of RO permeate, c) lower concentration of dissolved matter in RO permeate and d) lower concentration of dissolved substances in RO retentate. In order to increase the efficiency and lifetime of the reverse osmosis system, an effective pretreatment is required to minimize membrane clogging, scaling, and membrane degradation [54]. The above explains the pretreatment of water in the plant in question by dosing residual chlorine inhibitor, adding antiscalant and biocide, and microfiltration with a filter cartridge with a pore diameter of 5 (μm). Almost all reverse osmosis systems require pretreatment to reduce particle clogging [3], and the choice of microfiltration as pretreatment in the plant in question is based on the fact that it is a frequently used step before RO [56], which due to its fine porosity ensures a high and constant water quality [57].

Fouling of reverse osmosis membranes is caused by substances that may be present in the incoming water, such as: silicon dioxide, iron and humic acids. However, even typical 5 (μm) microfilters used upstream of a reverse osmosis system may not completely remove these impurities [58], which is why auxiliary chemicals (residual chlorine inhibitor, antiscalant, and biocide) are dosed into the water. Given that the microfilter itself is subject to the development of microorganisms on the surface of the membrane [59], as well as the formation of scale due to the hardness present in the water, and membrane degradation caused by chlorine, the mentioned chemical agents are added upstream of the microfilter in the plant in question. Residual chlorine is neutralized by adding a sulfite-based chemical agent, which reacts with it to form a non-harmful product. Among the common agents for chlorine inhibition, the sulphite-based one has been proven to be an ideal inhibitor [60]. Antiscalant, which is used in the case in question, prevents the formation of deposits of scale and other substances (metal oxides, silicates, etc.), thus enabling operation with hard water. In addition to the above, a biocide is used to control microorganisms that can develop in the previous stage of filtration. Table 5 shows the values of periodic control readings of water pressures at the inlet (p_1) and outlet (p_2) from the microfilter. As in the case of the self-washable mechanical filter, the limit value of the pressure drop (Δp) on the microfilter is 1 (bar), while the optimal value is 0.5 (bar). The read values of the pressure drop in the microfilter (Table 5) are slightly higher than those in the self-washable mechanical filter (Table 3), which can be explained by the lower diameter of the membrane pores of the microfilter compared to those of the self-washable filter, and thus the greater resistance to water flow. In other words, the larger the open area of the filter medium (the sum of all the areas of all the openings in the filter medium through which water can pass) for a given flow rate, the lower the water flow rate and thus the lower the pressure drop on the clean medium [29]. The read value of the pressure drop in the microfilter after 16 and 24 (h) of operation exceeds the limit value of 1 (bar), which indicates the necessary replacement of the filter cartridge, while the other values are within the specified limits.

Table 5. Pressure drop values in the microfilter

Time since first reading (h)	p_1 (bar)	p_2 (bar)	Δp (bar)
0	3.9	3.7	0.2
8	3.9	3.6	0.3
16	2.7	1.2	1.5
24	2.7	1.1	1.6

Table 6 shows the values of the process parameters of the RO system and the electrical conductivity of the treated water (permeate) at the exit from the RO system of the industrial plant. Taking into account that the nominal flow of water entering the system is about 20 (m^3/h), the permeate flow rate (Table 6) is 14-15 (m^3/h). Data related to permeate and concentrate flow rates indicate that it is reverse osmosis with a cross flow. The selection of this configuration of the RO system in the plant in question, compared to the direct flow configuration, is based on its advantage to reduce the accumulation of impurities on the membrane surface [61]. By comparing the results of measuring the permeate flow rate and its conductivity (table 6), it can be seen that at a higher water flow rate through the membrane, its electrical conductivity was lower than that at a lower flow rate. The aforementioned can be explained by the dependence of the electrical conductivity of the permeate on the relative rates of water and salt transport through the membrane [62]. Namely, the applied pressure results in the transport of water through the RO membrane, while most of the dissolved substances are retained on the "inlet" side of the membrane [63]. The transport of dissolved matters through the RO membrane is a consequence of diffusion, it is constant and does not depend on pressure [62]. Therefore, with a higher permeate flow rate, the same amount of dissolved matters (which passes through the membrane) will be diluted by a larger amount of water (permeate) and vice versa.

Table 6. Process parameters of reverse osmosis and electrical conductivity of treated water

Time since first reading (h)	Applied pressure (bar)	Permeate flow rate (m^3/h)	Concentrate flow rate (m^3/h)	Electrical conductivity (μS/cm)
0	8.1	15	6.2	31.3
8	8.1	15	6.2	31.8
16	8.1	14	6.9	32.8
24	8.1	14	6.2	36.6

In other words, as the inlet water flux increases, the concentration of dissolved matters in the permeate decreases and the efficiency of their removal from water increases [3], which is consistent with the results in Table 6.

WATER QUALITY AFTER ION EXCHANGE

The chosen sequence of water treatment in the considered water treatment plant, in such a way that the ion exchange operation is performed after and not before reverse osmosis, is based on the following [55]: reverse osmosis is the best method for water with higher conductivity levels; ion exchange is best for water with low conductivity; the mentioned configuration results in a small amount of wastewater from the ion exchange unit. In the considered ion exchange operation, a strong acid cation exchange resin (Na-based) is used. Na-based ion exchange resin is used more often than H-based resin to reduce the total hardness of water. Namely, sodium ions are easily regenerated from the resin during the regeneration phase of the ion exchange process, and considering that NaCl salt solution is used as a regeneration agent [64], the procedure is low-cost. If H+-based ion exchange resins were used, the regeneration process would be more complex, requiring the use of strong acids (HCl, H_2SO_4). In addition to the above, sodium ions are neutral and their exchange does not lead to acidity or alkalinity of the water, while hydrogen ions can contribute to the creation of excess acidity in the treated water, potentially lowering its pH.

Table 7 shows the results of the periodic analysis of the treated water at the exit from the strong acid cation exchanger. The results of the analysis of the quality of the mixed flow (Table 2) showed that the pH of the water is in the range of 7.5-7.8, while the pH range of the water after the ion exchange treatment is 6.5-6.7. The cause of the drop in the pH value of the water can be attributed to the reverse osmosis operation that preceded the ion exchange, i.e. its potential to remove minerals. The removal of minerals means that there are more free hydrogen ions in the water than before, which lowers the pH level from 6-8 to 5-7 and makes the water more acidic.

Comparison of the electrical conductivity of water after ion exchange (Table 7), whose values are in the range 0.13-0.16 (μS/cm), with those after reverse osmosis (Table 6), which were in the range 31.3-36.6 (μS/cm), indicates that by the ion exchange process a significant degree of removal of ionic species was achieved. A decrease in the electrical conductivity of water after ion exchange was also reported in other water treatment studies [65]. Water softening operation by cation exchange can remove almost all calcium and magnesium from water, and can also remove iron [66]. This is confirmed by the analysis of the total hardness of the water after ion exchange treatment in the considered industrial plant, which is 0.00 (mg/L $CaCO_3$). The obtained results of the water hardness analysis meet the quality requirement of boiler feed water of < 0.01 (mmol/L) [18], which is equivalent to 1.0009 (mg/L $CaCO_3$). Considering that the total hardness of the water has a certain correlation with its electrical conductivity [67], [68], it can be assumed that the total hardness of the water after the reverse osmosis treatment was above the limit for the quality of the boiler feed water [18], which is why the treatment of water by both reverse osmosis and ion exchange was necessary.

Table 7. Results of water quality analysis after ion exchange

Time since first sampling (h)	Total hardness (mg/L $CaCO_3$)	pH	Electrical conductivity (μS/cm)
0	0.00	6.7	0.16
4	0.00	6.7	0.16
8	0.00	6.6	0.15
12	0.00	6.7	0.11
16	0.00	6.5	0.13
20	0.00	6.7	0.13

In that way, it is possible to completely remove the total hardness which was in the range of 129-130 (mg/L) in the receiving basin (Table 2).

CONCLUSION

Given that different types of water sources have specific characteristics in certain parameters of their quality, the type of source has an influence on the selection of treatment operations. Also, in order to select appropriate treatment operations, it is necessary to compare the quality of raw water and its desired quality for final use. In addition, the availability and cost of water treatment equipment and chemicals, maintenance services and training of operating

personnel, as well as waste disposal requirements greatly influence the choice of treatment operations. In this paper, a comparative analysis of water quality before and after individual treatment operations was performed, for the supply needs of a combined boiler plant for steam production, working pressure 12.5 (bar) in the selected food industry. Individual water treatment units and their selected process parameters were additionally analyzed, all with the aim of evaluating both unit operations and the entire process of raw water treatment, from the aspect of efficiency and economy. In raw water samples from four underground sources used to supply the boiler plant, deviations from the requirements of the maximum iron content in boiler feed water were found, with the range of Fe^{2+} content in the samples being 0.373-0.399 (mg/L). Among the analyzed samples, only one groundwater had a manganese content of 0.24 (mg/L) that meets the prescribed maximum limit for low and medium pressure boilers, while the others had excessive values 0.321, 0.302 and 0.309 (mg/L). The determined content of total hardness in all four groundwaters was significantly above the prescribed level, with the decreasing order of their values (mg/L $CaCO_3$) being: 307, 302, 300 and 271. Although natural aeration of water, i.e. contact with air and the consequent degassing of carbon dioxide can partially reduce its overall hardness, the overall effect on water hardness is limited and temporary. The addition of a chemical oxidant, such as sodium hypochlorite (NaClO) to water before its filtration contributes to the oxidation of dissolved iron and manganese and their easier precipitation and separation with a granular filtration medium. Multi-media filters offer improved particle and solute removal efficiency, where each individual filtration media targets specific impurities, including iron and manganese. Analyzes of water quality after the mixing of four underground waters and its treatment with multimedia sand filtration showed a decrease in the content of iron and manganese to 0.03 (mg/L) and (0.00 mg/L), which met the requirements of their maximum content in boiler feed water. In addition to the above, a reduction of the residual chlorine content was achieved, from the initial range of 0.17-0.21 (mg/L) in the mixed groundwater, to the range of 0.11-0.16 (mg/L) after its multimedia filtration. Properly selected industrial processes and their place in the overall raw water treatment line can have multiple beneficial effects on meeting water quality requirements for a specific purpose. The sequence of water treatment after multimedia filtration, in such a way as to first carry out reverse osmosis and then ion exchange, resulted in the complete removal of the total hardness of the water, i.e. from the range of 129 – 130 (mg/L $CaCO_3$) after groundwater mixing to 0.00 (mg/L $CaCO_3$) at the exit from the ion exchange column, which satisfies one of the most important requirements for boiler feed water quality. The efficiency of the selected configuration in removing ions that contribute to water hardness is also confirmed by the results of the electrical conductivity of water, which after reverse osmosis treatment had a range of 31.3-36.6 (μS/cm), and after ion exchange 0.11-0.16 (μS/cm). In addition to the appropriate selection of individual treatment operations and their place in the overall process of industrial water preparation, pre-filtration is a key operation in water treatment processes, especially when multimedia filters and reverse osmosis systems are used. By removing larger particles before the water enters the multimedia filter, pre-filtration allows them to work more efficiently, because a finer filtration medium can more effectively remove smaller particles and dissolved impurities when it is not loaded with larger fractions. Pre-filtration also helps prevent clogging or damage to sensitive equipment, such as membranes in reverse osmosis systems, allowing for high equipment performance and reduced maintenance costs.

REFERENCES

1. M. F. Hossain, "Water," in *Sustainable Design and Build: Building, Energy, Roads, Bridges, Water and Sewer Systems*, Butterworth-Heinemann, 2019, pp. 301-418.
2. D. P. Dupont and S. Renzetti, "The Role of Water in Manufacturing," *Environmental and Resource Economics*, vol. 18, no. 4, pp. 411-432, 2001.[Online]. Available: http://dx.doi.org/10.1023/A:1011117319932. [Accessed: 11-Sep-2024]
3. S. Begić, R. Cvrk, and M. H. Stanić, *Postupci pripreme vode za industriju*. Tuzla, Bosnia and Herzegovina: In Scan Ltd., 2022.
4. H. Ritchie and M. Roser, "Water Use and Stress," *Our World in Data*, 2018. [Online]. Available: https://ourworldindata.org/water-use-stress. [Accessed: 13-Sep-2024].
5. W. Musie and G. Gonfa, "Fresh water resource, scarcity, water salinity challenges and possible remedies: A review," *Heliyon*, vol. 9, no. 8, article e18685, 2023. [Online]. Available: https://doi.org/10.1016/j.heliyon.2023.e18685. [Accessed: 13-Sep-2024].
6. P. Vasistha and R. Ganguly, "Water quality assessment of natural lakes and its importance: An overview," *Materials Today: Proceedings*, vol. 32, pt. 4, pp. 544-552, 2020. [Online]. Available: https://doi.org/10.1016/j.matpr.2020.02.092. [Accessed: Sep. 18, 2024].

7. D. Nelson, "Natural variations in the composition of groundwater," *Drinking Water Program*, presented at Groundwater Foundation annual meeting, Springfield, Oregon, USA, Oregon Department of Human Services, 2002. [Online]. Available: https://www.oregon.gov/oha/ph/HealthyEnvironments/DrinkingWater/SourceWater/Documents/gw/chem.pdf. [Accessed: Sep. 18, 2024].
8. J. I. Drever, *The Geochemistry of Natural Water: Surface and Groundwater Environments*, 3rd ed. Upper Saddle River, NJ, USA: Prentice Hall, 1997.
9. V. Stuhli, M. Zohorović, Lj. Bojanović, A. Đozić, and F. Andrejaš, *Eksperimentalne vježbe sa teoretskim osnovama iz inženjerstva zaštite okoline*, Scan Ltd., Tuzla, 2021.
10. American Public Health Association (APHA), R. Baird, A. D. Eaton, E. W. Rice, and L. Bridgewater, *Standard Methods for the Examination of Water and Wastewater*, 23rd ed. Washington, D.C., USA: American Public Health Association, 2017.
11. British Standards Institution**,** *BS EN ISO 10523:2012 - Water quality — Determination of pH*. 2012.
12. International Organization for Standardization, *ISO 7888:1985 - Water Quality — Determination of Electrical Conductivity*. Geneva, Switzerland: ISO, 1985.
13. N. Manav Demir, "Experimental Study of Factors that Affect Iron and Manganese Removal in Slow Sand Filters and Identification of Responsible Microbial Species," *Polish Journal of Environmental Studies*, vol. 25, no. 4, pp. 1453-1465, 2016. [Online]. Available: https://doi.org/10.15244/pjoes/62679. [Accessed: 13-Sep-2024].
14. H. Khozyem, A. Hamdan, A. A. Tantawy, A. Emam, and E. Elbadry, "Distribution and origin of iron and manganese in groundwater: Case study, Balat-Teneida area, El-Dakhla Basin, Egypt," *Arabian J. Geosci.*, vol. 12, no. 523, 2019. [Online]. Available: https://doi.org/10.1007/s12517-019-4689-1. [Accessed: Aug. 12, 2024].
15. D. Barloková and J. Ilavský, "Removal of iron and manganese from water using filtration by natural materials," *Polish J. of Environ. Stud.*, vol. 19, no. 6, pp. 1117-1122, 2010. [Online]. Available: https://www.pjoes.com/Removal-of-Iron-and-Manganese-from-Water-r-nUsing-Filtration-by-Natural-Materials,88490,0,2.html. [Accessed: Jun. 29, 2023].
16. Araby, R.E.; Hawash, S.; Diwani, G.E. Treatment of iron and manganese in simulated groundwater via ozone technology. Desalination 2009, 249, 1345–1349. [Online]. Available: https://doi.org/10.1016/j.desal.2009.05.006. [Accessed: Aug. 12, 2024].
17. G. Singh, „*Water Supply and Sanitary Engineering*", 5th ed. Delhi: Standard Publisher Distributors, 1999.
18. Viessmann, *"Kvalitet vode u postrojenjima parnih kotlova,"* 2008. [Online]. Available: https://www.scribd.com/document/105600917/Kvalitet-Vode-Parni-Kotlovi-Srpski. [Accessed: Jun. 26, 2024].
19. *Kurita Handbook of Water Treatment*, *2nd ed*. Tokyo: Kurita Water Industries Ltd., 1999. [Online]. Available: https://www.abpsoil.com/images/Books/kurita-handbook-water-treatment.pdf. Accessed: Sep. 18, 2024.
20. Suez, *Industrial Boiler Feedwater - How to Improve Boiler Efficiency and Reduce Operating Costs*, White paper, Sep. 2021. [Online]. Available: https://purite.com/wp-content/uploads/2021/10/SUEZ-Industrial-Campaign-Boiler-Feed-White-Paper-September-2021-v1.3-High.pdf. [Accessed: Jun. 3, 2024].
21. M. S. Jami, M. K. Amosa, M. F. R. Alkhatib, D. N. Jimat, and S. A. Muyibi, "Boiler-feed and process water reclamation from biotreated palm oil mill effluent (BPOME): A developmental review," *Chem. Biochem. Eng. Q.*, vol. 27, no. 4, pp. 477–489, 2013. [Online]. Available: https://core.ac.uk/download/pdf/18622178.pdf. [Accessed: Sep. 5, 2024].
22. W. McGowan, *Water Processing: Residential, Commercial, Light-Industrial*, 3rd ed. Lisle, IL, USA: Water Quality Association, 2000.
23. M. I. Mahmud, S. Sultana, M. A. Hasan, and K. M. Ahmed, "Variations in hydrostratigraphy and groundwater quality between major geomorphic units of the Western Ganges Delta plain, SW Bangladesh," *Applied Water Science*, vol. 7, no. 6, pp. 2919–2932, 2017. [Online]. Available: https://doi.org/10.1007/s13201-017-0581-x. [Accessed: Jul. 10, 2024].
24. M. Ahmad, "Iron and Manganese removal from groundwater - Geochemical modeling of the Vyredox method," M.S. thesis, Department of Geosciences, University of Oslo, Oslo, Norway, 2012. [Online]. Available: https://core.ac.uk/download/pdf/30826276.pdf. [Accessed: Jul. 12, 2024].
25. M. F. Mamadou, M. S. F., E. H. Moussa D., O. T. A., A. M. F., and G. D. M. C., "Iron in Water: Study of Iron Removal Kinetics in Chemically Reconstituted Waters: Application to Groundwater of South Pout (PS2 Site)," *Open Journal of Metal*, vol. 11, pp. 1-10, 2021. [Online]. Available: https://doi.org/10.4236/ojmetal.2021.111001. [Accessed: May. 29, 2024].
26. H. Yang, X. Tang, X. Luo, G. Li, H. Liang, and S. Snyder, "Oxidants-assisted sand filter to enhance the simultaneous removals of manganese, iron and ammonia from groundwater: Formation of active MnOx and involved mechanisms," *Journal of*

Hazardous Materials, vol. 415, p. 125707, 2021. [Online]. Available: https://doi.org/10.1016/j.jhazmat.2021.125707. [Accessed: Jul. 2, 2024].

27. H. Andersson and J. Johansson, "Iron removal from groundwater in Rakai District, Uganda: a minor field study," M.S. thesis, Lulea Univ. of Technol., Uganda, 2002, Master's Thesis 2002:292CIV. [Online]. Available: http://urn.kb.se/resolve?urn=urn:nbn:se:ltu:diva-54321. [Accessed: Jun. 25, 2024].
28. C. Charcosset, "Microfiltration," in *Membrane Processes in Biotechnology and Pharmaceutics*, C. Charcosset, Ed. Amsterdam, Netherlands: Elsevier, 2012, pp. 101-141. [Online]. Available: https://www.perlego.com/book/1834053/membrane-processes-in-biotechnology-and-pharmaceutics. [Accessed: Aug. 6, 2024].
29. M. N. Alhands, "Removing solids with automatic self-cleaning filters," in *Proc. 15th Annu. Produced Water Seminar*, Houston, TX, USA, Jan. 19-21, 2005. [Online]. Available: https://www.environmental-expert.com/articles/removing-solids-with-automatic-self-cleaning-filters-6010/full-article. [Accessed: Jul. 14, 2023].
30. N. F. Gray, *Water Technology*, 3rd ed. London, UK: IWA Publishing, 2010.
31. Metcalf & Eddy, Inc. an AECOM Company, T. Asano, F. Burton, and H. Leverenz, "Removal of residual particulate matter," in *Water Reuse: Issues, Technologies, and Applications*, M. Wright and D. C. D. H. C. Eds., New York, NY, USA: McGraw-Hill, 2007. 373-460.
32. M. L. Gulhane and A. U. Charpe, "Domestic wastewater treatment using multimedia filter technology," *Int. J. for Scientific Research & Development*, vol. 3, no. 01, pp. 1119-1121, 2015. [Online]. Available: https://www.academia.edu/22380344/Domestic_Wastewater_Treatment_using_Multimedia_Filter_Technology. [Accessed: Sep. 2, 2023].
33. V. Anusuya, R. Malathi, P. S. Kumar, and S. S. Barath, "Characteristic study on domestic wastewater by natural absorbents using multi-media filters technology," *Int. J. Engineering Research and Modern Education*, Special Issue, Apr. 2017, pp. 39-42. [Online]. Available: https://doi.org/10.5281/zenodo.570093. [Accessed: Jun. 12, 2023].
34. N. B. Kazemi Noredinvand, A. Takdastan, and R. Jalilzadeh Yengejeh, "Removal of organic matter from drinking water by single and dual media filtration: A comparative pilot study," *Desalination Water Treat.*, vol. 57, no. 44, pp. 20792–20799, 2016. [Online]. Available: https://doi.org/10.1080/19443994.2015.1110718. [Accessed: Jan. 9, 2024].
35. M. A. Al-Ghouti, M. A. Al-Kaabi, M. Y. Ashfaq, and D. A. Da'na, "Produced water characteristics, treatment and reuse: A review," *Journal of Water Process Engineering*, vol. 28, pp. 222-239, 2019. [Online]. Available: https://doi.org/10.1016/j.jwpe.2019.02.001. [Accessed: Jul. 5, 2023].
36. N. B. Kazemi Noredinvand, A. Takdastan, R. Jalilzadeh Yengejeh, and F. Ghanbari, "The efficiency of multi-media filtration in drinking water treatment plants for the removal of natural organic matter," *J. Adv. Environ. Health Res.*, vol. 9, no. 2, pp. 117–128, 2021. [Online]. Available: https://doi.org/10.22102/jaehr.2021.277080.1215. [Accessed: Jan. 10, 2024].
37. K. V. Bhoyar, N. V. Rekkawar, and H. D. Mishra, "Analysis and design of media filter for waste water treatment," *Int. J. Innovations in Engineering and Science*, vol. 4, no. 4, pp. 73-79, 2019. [Online]. Available: https://www.ijies.net/finial-docs/finial-pdf/1204194419.pdf. [Accessed: Jul. 5, 2023].
38. American Water Works Association, *Water Quality and Treatment: A Handbook on Drinking Water*, 6th ed. New York, NY, USA: McGraw-Hill, 2010.
39. D. W. Hendricks, *Water Treatment Unit Processes: Physical and Chemical*. Boca Raton, FL, USA: CRC Press, 2006.
40. J. Jeż-Walkowiak, M. Komorowska-Kaufman, Z. Dymaczewski, M. M. Sozański, and P. Zakrzewski, "Properties of catalyst for iron and manganese oxidation in filter materials," *E3S Web Conf.*, vol. 59, p. 00014, 2018. [Online]. Available: https://doi.org/10.1051/e3sconf/20185900014. [Accessed: Oct. 3, 2023].
41. M. M. Michel, M. Azizi, D. Mirosław-Świątek, L. Reczek, B. Cieniek, and E. Sočo, "Significance of MnO2 Type and Solution Parameters in Manganese Removal from Water Solution," *International Journal of Molecular Sciences*, vol. 24, no. 5, p. 4448, 2023. [Online]. Available: https://doi.org/10.3390/ijms24054448. [Accessed: Oct. 3, 2023].
42. S. Pervez and G. S. Pandey, "Dissolved iron in ground water: Applications of lepidocrocite and pyrolusite for its removal," *Journal of Indian Water Works Association*, vol. 3, pp. 245-247, 1993. [Online]. Available: https://www.researchgate.net/publication/274835483_Dissolved_iron_in_ground_water_Applications_of_lepidocrocite_and_pyrolusite_for_its_removal. [Accessed: Oct. 3, 2023].
43. K. J. Howe, D. W. Hand, J. C. Crittenden, R. R. Trussell, and G. Tchobanoglous, *Principles of Water Treatment*. Hoboken, NJ, USA: John Wiley & Sons, 2012.
44. A. A. Islam, J. E. Goodwill, R. Bouchard, J. Tobiason, and W. Knocke, "Characterization of filter media

MnOx (s) surfaces and Mn removal capability," *J. Am. Water Works Assoc.*, vol. 102, no. 9, pp. 71–83, 2010. [Online]. Available: https://doi.org/10.1002/j.1551-8833.2010.tb10188.x. [Accessed: Sep. 11, 2023].

45. P. Hu, Y. Hsieh, J. Chen, and C. Chang, "Adsorption of divalent manganese ion on manganese-coated sand," *J. Water Supply: Research and Technology-Aqua*, vol. 53, no. 3, pp. 151–158, 2004. [Online]. Available: https://doi.org/10.2166/aqua.2004.0013. [Accessed: Sep. 7, 2023].
46. J. J. Morgan and W. Stumm, "Colloid-chemical properties of manganese dioxide," *J. Colloid Interface Sci.*, vol. 19, no. 4, pp. 347–359, 1964. [Online]. Available: https://doi.org/10.1016/0095-8522(64)90036-4. [Accessed: Apr. 10, 2024].
47. J. W. Murray, "The interaction of metal ions at the manganese dioxide-solution interface," *Geochimica et Cosmochimica Acta*, vol. 39, no. 4, pp. 505–519, 1975. [Online]. Available: https://doi.org/10.1016/0016-7037(75)90103-9. [Accessed: Apr. 11, 2024].
48. D. M. Sahabi, M. Takeda, I. Suzuki, and J.-I. Koizumi, "Removal of Mn^{2+} from water by 'aged' biofilter media: The role of catalytic oxides layers," *J. Biosci. Bioeng.*, vol. 107, no. 2, pp. 151–157, 2009. [Online]. Available: https://doi.org/10.1016/j.jbiosc.2008.10.013. [Accessed: May 6, 2024].
49. M. Elsheikh, H. Guirguis, and A. Fathy, "A comparative study of methods used for Fe and Mn oxidation and removal from groundwater," *J. Engineering and Applied Science*, vol. 63, no. 4, pp. 277–292, 2016. [Online]. Available: https://www.researchgate.net/publication/326990940_A_comparative_study_of_methods_used_for_fe_and_mn_oxidation_and_removal_from_groundwater. [Accessed: Sep. 1, 2023].
50. W. R. Knocke, R. C. Hoehn, and R. L. Sinsabaugh, "Using alternative oxidants to remove dissolved manganese from waters laden with organics," *J. Amer. Water Works Assoc.*, vol. 79, no. 3, pp. 75–79, 1987. [Online]. Available: https://awwa.onlinelibrary.wiley.com/doi/10.1002/j.1551-8833.1987.tb02818.x. [Accessed: Jan. 16, 2024].
51. W. R. Knocke, S. C. Occiano, and R. Hungate, "Removal of soluble manganese by oxide-coated filter media: Sorption rate and removal mechanism issues," *J. Amer. Water Works Assoc.*, vol. 83, no. 8, pp. 64–69, 1991. [Online]. Available: https://www.jstor.org/stable/41293529. [Accessed: Feb. 16, 2024].
52. BS EN 12952-12:2003, "Water-tube boilers and auxiliary installations - Requirements for boiler feedwater and boiler water quality," British Standards Institution, 2003. [Online]. Available: https://img.antpedia.com/standard/files/pdfs_ora/20221211/bs/BS%20EN%2012952-12-2003.pdf. [Accessed: Jan. 16, 2024].
53. J. C. Van der Linde, E. Fosso-Kankeu, G. Gericke, N. Lemmer, and F. Waanders, *"Removal of Total Hardness and Alkalinity from RO – Reject Water,"* in *Proc. 9th Int'l Conf. on Advances in Science, Engineering, Technology & Waste Management (ASETWM-17),* Parys, South Africa, Nov. 27-28, 2017. [Online]. Available: https://www.researchgate.net/publication/321490462_Removal_of_Total_Hardness_and_Alkalinity_from_RO_-_reject_water. [Accessed: Jun. 10, 2024].
54. Dupont**,** *FilmTec™ Reverse Osmosis Membranes Technical Manual* (Form No. 45-D01504-en). 2023. [Online]. Available: https://www.dupont.com/content/dam/dupont/amer/us/en/watersolutions/public/documents/en/RO-NF-FilmTec-Manual-45-D01504-en.pdf. [Accessed: Aug. 28, 2023].
55. H. A. Rehman and K. Ahmed, "Reverse Osmosis and Ion-Exchange Water Treatment for Boilers—A Brief Review," *Sci. Int. (Lahore)*, vol. 27, no. 6, pp. 6147–6151, 2015. [Online]. Available: http://www.sci-int.com/pdf/636372723526902857.pdf. [Accessed: May 7, 2024].
56. V. Bonnelye, L. Guey, and J. Del Castillo, "UF/MF as RO pre-treatment: the real benefit," *Desalination*, vol. 222, no. 1-3, pp. 59-65, 2008. [Online]. Available: https://doi.org/10.1016/j.desal.2007.01.129. [Accessed: Jul. 25, 2023].
57. P. Kajitvichyanukul, N. K. Shammas, Y. Hung, L. K. Wang, and J. Ananpattarachai, *Membrane and Desalination Technologies*. New York: Springer, 2011.
58. G. K. Dhawan, *Solutions to Membrane Fouling*. 1985. [Online]. Available: https://www.appliedmembranes.com/solutions-to-membrane-fouling.html. [Accessed: Aug. 18, 2023].
59. J. Kacprzyńska-Gołacka, M. Łożyńska, W. Barszcz, S. Sowa, and P. Wieciński, "Microfiltration membranes modified with zinc by plasma treatment," *Membranes*, vol. 13, no. 4, p. 387, 2023. [Online]. Available: https://doi.org/10.3390/membranes13040387. [Accessed: Jun. 15, 2024].
60. X. Li, Z. Zhao, Z. Qu, X. Li, Z. Zhang, X. Liang, J. Chen, and J. Li, "A Review of Traditional and Emerging Residual Chlorine Quenchers on Disinfection By-Products: Impact and Mechanisms," *Toxics*, vol. 11, no. 5, p. 410, 2023. [Online]. Available: https://doi.org/10.3390/toxics11050410. [Accessed: Mar. 7, 2024].
61. Q. Li, C. Reeve, L. Martinez, and S. Wright, "Scale-up in Biotechnology," in *Comprehensive Biotechnology*, 3rd ed., M. Moo-Young, Ed. Pergamon, 2019, pp. 919–932. [Online]. Available: https://doi.org/10.1016/B978-0-444-64046-8.00141-5. [Accessed: Feb. 26, 2024].
62. S. G. Salinas-Rodriguez, J. C. Schippers, and M. D. Kennedy, "The process of reverse osmosis," in *Efficient Desalination by Reverse Osmosis*, S. Burn and S. Gray,

Eds. IWA Publishing, 2016. [Online]. Available:https://www.researchgate.net/publication/302909917_The_process_of_reverse_osmosis. [Accessed: May 22, 2024].

63. A. A. Ahuchaogu, O. J. Chukwu, A. I. Obike, C. E. Igara, I. C. Nnorom, and J. B. O. Echeme, "Reverse osmosis technology, its applications and nano-enabled membrane," *International Journal of Advanced Research in Chemical Science*, vol. 5, no. 2, pp. 20-26, 2018. [Online]. Available: http://dx.doi.org/10.20431/2349-0403.0502005. [Accessed: Nov. 4, 2023].
64. K. G. Varshney, N. Tayal, and U. Gupta, *"Acrylonitrile based cerium (IV) phosphate as a new mercury selective fibrous ion-exchanger: synthesis, characterization and analytical applications," Colloids and Surfaces A: Physicochemical and Engineering Aspects,* vol. 145, no. 1-3, pp. 71-81, 1998. [Online]. Available: https://doi.org/10.1016/S0927-7757(98)00657-8. [Accessed: Jun. 21, 2024].
65. N. Ramzan, N. Feroze, M. Kazmi, M. A. Ashraf, and S. Hasan, "Performance analysis of cation and anion exchangers in water treatment plant: An industrial case study," *Polish Journal of Chemical Technology*, vol. 14, no. 2, pp. 35–41, 2012. [Online]. Available: https://doi.org/10.2478/v10026-012-0068-3. [Accessed: Apr. 23, 2024].
66. S. O. Skipton and B. I. Dvorak, "G08-1491 Drinking Water Treatment: Water Softening (Ion Exchange)," University of Nebraska. [Online]. Available: https://www.researchgate.net/publication/282225577_G081491_Drinking_Water_Treatment_Water_Softening_Ion_Exchange. [Accessed: May 30, 2024].
67. A. K. Shrestha and N. Basnet, "Variation of electrical conductivity of different sources of water with temperature and concentration of impurities - The UGC Small RDI Grant Report - 2016," [Online]. Available: http://dx.doi.org/10.13140/RG.2.2.34190.41284. [Accessed: May 29, 2024].
68. K. Jothivenkatachalam, A. Nithya, and S. Chandra Mohan, "Correlation analysis of drinking water quality in and around Perur block of Coimbatore district, Tamil Nadu, India," *Rasayan J. Chem.*, vol. 3, no. 4, pp. 649–654, 2010. [Online]. Available: https://rasayanjournal.co.in/vol-3/issue-4/8.pdf. [Accessed: Oct. 12, 2023].

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ANTICANCER POTENTIAL OF PINK PEPPER FRUIT ESSENTIAL OIL: STUDY ON HUMAN CELL LINES OF LUNG CARCINOMA (H460), CERVICAL ADENOCARCINOMA (HeLa) AND COLORECTAL CARCINOMA (HCT116)

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:
***Schinus terebinthifolius* Raddi is a plant species belonging to Anacardiaceae family, native to South America, with particular abundance in Brazil, Argentina and Paraguay. It is commonly known as Brazilian pepper tree. All plant parts have been used in traditional medicine for the treatment of several pathologies. In this paper, the cytotoxic effects of the essential oil of the commercial pink pepper fruit from the Tuzla market were investigated. To assess the cytotoxic potential, a tetrazolium salt reduction (MTT) viability assay was performed. The experiments were carried out on 3 human cell lines: lung carcinoma (H460), cervical adenocarcinoma (HeLa) and colorectal carcinoma (HCT116). Using GC/MS, 24 components of red pepper essential oil were identified, of which α-pinene, α-phellandrene, δ-3-carene and D-limonene dominate. The essential oil of the pink pepper fruit showed cytotoxic activity in the case of all tested cell lines under *in vitro* conditions.**

KEYWORDS: GC/MS analysis, cytotoxic activity, H460, HeLa, HCT116

INTRODUCTION

The most important role of secondary metabolites in plants is the resistance of plants to adverse external conditions such as climatic variations, mechanical damage, insects, etc. These metabolic end products are stored in leaves, flowers, roots and other plant parts, from which they can be isolated for other purposes, most importantly medicinal. Secondary metabolites are small organic molecules, interesting due to their structural diversity and biological effects. More than 30% of medicinal products derive from natural products [1,2]. Important group of plant-based secondary metabolites are essential oils, which are responsible for the characteristic fragrance and taste of the plant. Plants containing essential oils are known as aromatic. Aromatic plants and essential oils are very often used in medicinal purposes, due to their antiseptic, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic effects [3]. Essential oils generally consist of a variety of chemical compounds, mostly benzene and terpene derivatives. These derivatives contribute to the rich bioactivity of essential oils [4]. Essential oils can be synthesized in all plant organs, mostly buds, flowers, leaves, seeds, fruits, roots. There are several methods for extracting essential oils, and steam distillation is the most common. The isolation method depends on the localization of the essential oils, as they can be stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes. Mostly, essential oils are plant derived but some of them are originate from microorganisms and animals [4].

Schinus terebinthifolius Raddi is a plant species belonging to Anacardiaceae family, native to South America, with particular abundance in Brazil, Argentina and Paraguay. It is commonly known as Brazilian pepper tree. All plant parts have been used

in traditional medicine for the treatment of several pathologies. It has been used for the treatment of ulcers, respiratory problems, wounds and arthritis. Antiseptic, antiinflammatory and haemostatic effects have also been noted. Many of these properties are associated with secondary metabolites of the plant, mainly polyphenols [5,6]. The resin obtained from the bark and stem is traditionally used in Brazil for cleaning the skin and the treatment of mycoses.

Schinus species are characterized by pungent-smell essential oils concentrated especially in fruits. Besides fruits, essential oils from leaves and flowers of *S. terebinthifolius* have been analyzed. Several variations in composition of essential oils from different regions were detected. Characterization of the chemical constituents of *S. terebinthifolius* fruit essential oil revealed the presence of monoterpene hydrocarbons, such as α-phellandrene, limonene, β-phellandrene, myrcene, and α-pinene as major components. Other reports suggested that the main components were sesquiterpenes, with germacrene-D, α-cadinol, and elemol as the main components. Composition of the leaf essential oil differs depending on a geographical position. Limonene, germacrene-D, β- caryophyllene, α-copaene, and α-pinene were reported as the main components of the Brazilian specimens. In Egypt specimen, β-caryophyllene, *cis*-β-terpineol, β-cedrene and citronellal were found as the major components [7,8]. Another research on the composition of essential oil isolated from leaves and twigs has revealed that major components were α-phellandrene, α-pinene, limonene, β-phellandrene and *p*-cymene. The most abundant compounds as second dominating chemical class were sesquiterpene hydrocarbons, precisely germacrene-D, bicyclogermacrene and β-cubebene [9].

*S. terebinthifolius*is promising medicinal plants for the treatment of inflammation, respiratory problems, rheumatism etc. Infusions and tinctures of bark and leaves are used traditionally to treat bacterial infections, to promote healing and for its anti-ulcerogenic effect. Chemical analysis of the bark showed the presence ofanthraquinones, xanthones and free steroids [10]. Some *in vitro* and *in vivo* studies reported antioxidant, antibacterial, and antifungal activities of *S. terebinthifolius* extracts [11]. Ethyl gallate, methyl gallate, quercitrin, myricitrin and myricetin with radical scavenging potential were isolated from leaves extract [12]. An aqueous extract of aerial part of *S. terebinthifolius* has been found effective against *Candida albicans*, but it was not determined which compounds were responsible for antifungal activity [13]. Acetate fraction of *S. terebinthifolius* leaves exhibited anti-inflammatory and anti-allergic effect when administered orally. It was also demonstrated that ethyl acetate extract from the leaves promotes a topical anti-inflammatory effect and antioxidant activity both *in vitro* and *in vivo* [14]. Among afore mentioned, some extracts of *S. terebinthifolius* showed promising antitumour effects. It was suggested that decoction of flowers, stalks and leaves of *S. terebinthifolius*, can be used for the treatment of tumour and leprosy [13]. Cytotoxic activity against four different human tumor cell lines was also detected for hexane and dichloromethane extracts of *S. terebinthifolius* leaves, but further investigations are needed in order to identify and isolate bioactive compounds with cytotoxic properties [15]. Antitumor activity of *S. molle* and *S. terebinthifolius* fruit essential oils was evaluated against the breast cancer cell line (MCF-7), and it was concluded that the investigated oils induced promising *in vitro* antioxidant activity and cytotoxicity on this cell line. *S. terebinthifolius* essential oil, rich in sesquiterpenes, has been found as the most active [11]. In a follow-up study, the crude oil of *S. terebinthifolius* leaves was subjected to chromatographic separation procedures toafford afraction composed of α- and β-pinenes. These compounds were tested *in vitro* against murine melanoma cell line, human melanoma, breast adenocarcinoma, leukemia and cervical carcinoma cell lines. The obtained results indicated that α-pinene, one of the major compounds of the investigated oil, might be responsible for its cytotoxic effect [16].

In this research, the chemical composition and cytotoxic activity of pink pepper essential oil were analyzed *in vitro* on three cell lines: H460 (lung carcinoma, large cell lung cancer), HeLa (cervical adenocarcinoma), and HCT116 (colorectal carcinoma). The aim is to examine the influence of the chemical composition on the cytotoxic activity of the essential oil and to compare the obtained results with previously published studies.

MATERIALS AND METHODS

The pepper sample was obtained commercially. Pink pepper originates from Vietnam. The sample was crushed using an electric mill and kept in a dark and dry place until subjected to distillation.

HYDRODISTILLATION

The crushed pink pepper fruit was subjected to hydrodistillation for four hours on a Clevenger apparatus. The obtained essential oil was separated, dried on anhydrous sodium sulfate and stored at -20°C until analysis.

GC/MS ANALYSIS

The essential oil of the red pepper fruit was analyzed by the gas chromatography with mass detector (GC-MSD) technique. This technique enables the identification of individual organic volatile compounds in essential oils (monoterpene and sesquiterpene alcohols and phenols, esters, aldehydes, ketones, etc.) with the help of GC-MS software with integrated databases or libraries of compounds - Wiley7NIST05 and NIST14. A semi-quantitative method was used to determine the composition of EO, in which individual peaks of chemical components within the obtained chromatogram were identified by comparing their retention indices with the indices of compounds within the databases, and by matching the mass spectrum of the compounds in the sample with the mass spectrum within the databases.

GC/MS analysis of pink pepper fruit essential oil sample was performed on an Agilent Technologies, Inc. gas chromatograph (7820A) with capillary HP5-ms ultra inert column (-60 to 325 °C, 30 m × 250 µm, film thickness 0.25 µm). The gas chromatograph was equipped with an Agilent mass selective detector (MSD-5977E). Helium gas (purity 5.0) was used as carrier gas at a constant flow rate of 1.0 mL/min. The sample was injected in a volume of 1 µL. The oven temperature was programmed from 60 °C (hold 1 min) to 246 °C (hold 0 min) at a rate of 3 °C/min and then to 280 °C at a rate of 10 °C/min. Before and after each injection, three washes of the needle with solvent (n-hexane) were used. The program resulted in a total duration of 86.40 minutes. The mass detector (MSD) was operated in the 40-400 m/z range scan mode. The temperature of the MSD transfer line was 250 °C, and the temperature of the ion source was 230 °C. ChemStation software was used for instrument control and data analysis. The results are expressed as a percentage concentration (% (V/V)) of each component in relation to the entire area of the obtained chromatogram.

ANALYSIS OF ANTICANCER POTENTIAL

The experiments were carried out on 3 human cell lines. The following cell lines were used: H460 (lung carcinoma, large cell lung cancer (ATCC®HTB-177™), HeLa (cervical adenocarcinoma, ATCC® CCL-2™) and HCT116 (colorectal carcinoma ATCC® CCL-247™).

Cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37˚C.

The panel cell lines were inoculated on to a series of standard 96-well microtiter plates on day 0, at 1.5×10^4 cells/mL. Essential oil were then added at 0.01 µL/mL, 0.05 µL/mL, 0.1 µL/mL, 0.5 µL/mL and 1 µL/mL concentration and incubated for a further 72 hours.

After 72 hours of incubation the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. The MTT Cell Proliferation Assay is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. For this purpose the substance treated medium was discarded and 40 µL of MTT reagent was added to each well at a concentration of 0.5 µg/µL. After four hours of incubation the precipitates were dissolved in 160 µL of DMSO. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the cell viability. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

If $(A_{test} - A_{tzero}) \geq 0$ then: $PG = 100 \times (A_{test} - A_{tzero}) / (A_{cont} - A_{tzero})$
If $(A_{test} - A_{tzero}) < 0$ then: $PG = 100 \times (A_{test} - A_{tzero}) / A_{tzero}$

Where:
A_{tzero} = the average absorbance before exposure of cells to the test compound,
A_{test} = the average absorbance after the desired period of time (72 h),
A_{cont} = the average absorbance after 72 hours with no exposure of cells to the test compound.

Each test point was performed in quadruplicate in three individual experiments. The results are expressed as concentration-response graphs. A negative percentage indicates cytotoxicity following drug treatment where -100% shows no cells survived the treatment at the specific drug concentration. The results are also expressed as GI_{50}, a concentration necessary for 50% of inhibition.

RESULTS AND DISCUSSION

GC/MS ANALYSIS

By hydrodistillation, essential oil was isolated from red pepper with a yield of 1.77%. The chemical constitution of the essential oil obtained from the pink pepper are presented in Table 1. The identification of 24 components by GC/MS allowed for 97.1% of the

total pink pepper fruit oil to be identified. Monoterpenes constitute 80.1% of essential oil, whereas the identified sesquiterpenes corresponded to 17.1%. The most abundant components were four monoterpenes δ-3-carene (22.0%), D-limonene (16.5%), α-phellandrene (16.1%) and α-pinene (13.4%) followed by two sesquiterpenes germacrene D (4.9%) and caryophyllene (4.1%). GC/MS chromatogram of Pink pepper fruit essential is shown in Figure 1.

Table 1. Chemical composition of Pink pepper fruit essential oil

Test parameter / Component	Retention index	Retention time	Result v/v (%)
α-thujene	929	5.639	0.329
α-pinene	937	5.842	13.437
sabinene	974	6.971	1.580
β-pinene	979	7.077	2.649
β-myrcene	991	7.500	3.019
α-phellandrene	1005	7.973	16.134
δ-3-carene	1011	8.176	22.027
o-cymene	1022	8.650	2.949
D-limonene	1030	8.819	16.492
α-terpinolene	1088	11.043	1.442
5-isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	1169	17.915	0.389
δ-elemene	1338	21.577	0.725
β-elemene	1391	23.877	0.315
caryophyllene	1419	24.968	4.058
trans-α-bergamotene	1435	25.658	0.249
humulene	1454	26.351	0.411
germacrene D	1481	27.484	4.906
bicyclogermacrene	1495	28.098	0.473
aciphyllene	1499	28.212	0.310
δ-cadinene	1524	29.184	0.764
elemol	1549	30.186	3.526
germacrene B	1557	30.444	0.288
caryophyllene oxide	1581	31.442	0.301
rosifoliol	1600	32.432	0.356

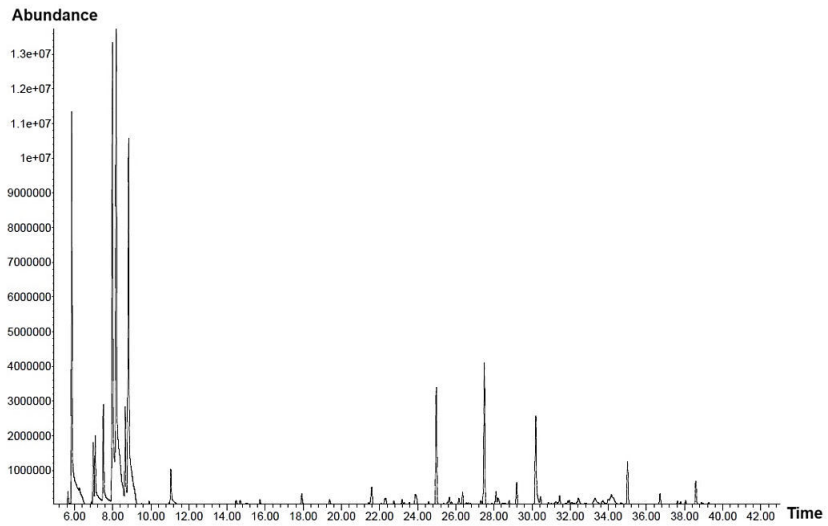

Figure 1. GC/MS chromatogram of Pink pepper fruit essential oil

ANTICANCER POTENTIAL

The treatment of HeLa, H460, and HCT116 cell lines with essential oil from pink pepper fruit demonstrated a dose-dependent decrease in cell growth. At the lowest concentrations of 0.01 µL/mL and 0.1 µL/mL, the essential oil had no effect on cell viability, with the growth percentage (PG) remaining at 100%. However, at a higher concentration of 1 µL/mL, a significant cytotoxic effect was observed, resulting in a 100% reduction in cell growth. This indicates that the essential oil of pink pepper exhibits potent anticancer activity at higher concentrations, effectively inhibiting the proliferation of these cancer cell lines. Dose-response profiles are shown in Figure 2. GI_{50} values for Pink pepper fruit essential oil are shown in Table 1.

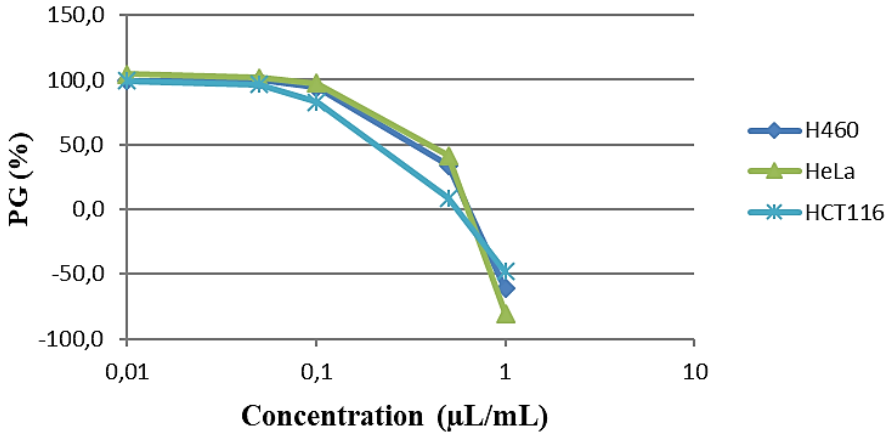

Figure 2. Dose-response profiles for Pink pepper fruit essential oil on H460, HeLa and HCT116 cell lines

Table 2. GI_{50} values for Pink pepper fruit essential oil

Cell lines	GI_{50} (nL/mL)
Lung carcinoma (H460)	423±121
Cervical adenocarcinoma (HeLa)	439±72
Colorectal carcinoma (HCT116)	271±42

Previous studies have shown that fruit essential oil is more cytotoxic on tumor cells than leaf oil, particularly effective in reducing cell viability in MCF-7 cell lines compared to A549 and HT-144 cells [17]. Santana et al. evaluated the activity of *S. terebinthifolius* leaves essential oil against five different tumor cell lines: B16F10-Nex2, A2058, HeLa, MCF-7, and HL-60, with HL-60 cells showing the most sensitivity. The crude oil was separated into a mixture of α- and β-pinenes, which exhibited moderate cytotoxicity. α-Pinene, a major compound (5.71%) isolated from the ripe fruits of *S. terebinthifolius*, was found to induce apoptosis and confer antimetastatic protection, suggesting it might be responsible for the observed cytotoxic effect of the crude oils [16]. The essential oil from *S. terebinthifolius* demonstrated greater anticancer effectiveness against the human breast cancer cells (MCF-7) compared to that from *S. molle* [11]. The ethanol extract of *S. terebinthifolius* fruit inhibited 50% of cell growth, indicating effective *in vitro* cytotoxic activity against the MCF-7 cell line. This cytotoxic activity is likely due to the polyphenolic content, as phenolic compounds are known to protect against cancer development and suppress cancer cell activity [18]. The methanolic extract (MEST) was also tested on ten human tumor cell lines from various tissues (U251, MCF-7, NCI-ADR/RES, 786-0, NCI-H460, PC-3, OVCAR-3, HT-29, K-562, and HaCaT). MEST showed selectivity for prostate, ovarian, breast, glioma, and non-tumoral cells, with the highest inhibitory activities observed in ovarian cancer cells [19]. A 2023 study investigated the chemical composition and cytotoxic activity of essential oils extracted from unripe and ripe pink pepper fruits. Chemical analysis revealed that the major components of unripe-EO were α-pinene (29.16%), dl-limonene (20.65%), and ρ-cymene (15.86%), while ripe-EO was characterized by l-phellandrene (38.91%), sylvestrene (23.02%), and α-pinene (21.62%). The cytotoxic activity of these essential oils was evaluated against HL-60 (acute promyelocytic leukemia) and SK-MEL-28 (malignant melanoma) cell lines, with significant cytotoxic effects, particularly from the unripe fruits

[20]. α-Pinene isolated from *S. terebinthifolius* leaves induces apoptosis and confers antimetastatic protection in a melanoma model. In a study where the viable B16F10-Nex2 murine melanoma cell line was injected into mice and treated with α-pinene intraperitoneally, a significant reduction in lung colonization was observed after 12 days, indicating a potent antimetastatic effect. These oils can also promote the depolarization of mitochondrial membranes, leading to apoptosis and necrosis [21]. Essential oils from the leaves, fruits, and bark of *Schinus terebinthifolius* of Egyptian origin exhibit significant cytotoxic activities against liver (HepG-2) and colon (Caco-2) carcinoma cells. Both cell lines displayed cytopathic effects, including cell distortion and degeneration. These structural changes are consistent with the observed reduction in cell viability, suggesting that the essential oils induce cell death through apoptosis or other cytotoxic pathways. These findings support the potential of these essential oils as sources of novel antitumor agents and underscore the need for further studies to isolate and characterize the bioactive compounds responsible for their cytotoxic effects [22].

CONCLUSION

The treatment of HeLa, H460, and HCT116 cell lines with essential oil from pink pepper fruit demonstrated a dose-dependent decrease in cell growth. At the lowest concentrations of 0.01 µL/mL and 0.1 µL/mL, the essential oil had no effect on cell viability, with the growth percentage (PG) remaining at 100%. However, at a higher concentration of 1 µL/mL, a significant cytotoxic effect was observed, resulting in a 100% reduction in cell growth.

REFERENCES

[1] Hoffmann, K.H. "Essential oils", Z. Naturforsch. C: J Biosciences, vol. 75, no. 7-8, pp. 177, 2020.

[2] Twaij, B.M., Hasan, M.N. "Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses", *Int J Plant Bio*, vol. 13, pp. 4-14, 2022.

[3] Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. "Biological effects of essential oils - A revie",. *Food Chem Toxicol*, vol. 46, no. 2, pp. 446-75, 2008.

[4] Wani, A.R., Yadav, K., Khursheed, A., Rather, M.A. "An updated and comprehensive review of the antiviral potential of essential oils and their chemical constituents with special focus on their mechanism of action against various influenza and coronaviruses", *Microb Pathog*, vol. 152, 104620, 2021.

[5] Sereniki, A., Linard-Medeiros, C.F.B., Silva, S.N., Silva, J.B.R., Peixoto Sobrinho, T.J.S., da Silva JR, et al. "*Schinus terebinthifolius* administration prevented behavioral and biochemical alterations in a rotenone model of Parkinson's disease", *Rev Bras Farmacogn*, vol. 26, no. 2, pp. 240-245, 2016.

[6] da Rocha, P.D.S., Paula, V.M.B., Olinto, S.C.F., Dos Santos, E.L., Souza, K.P., Estevinho, L.M. "Diversity, Chemical Constituents and Biological Activities of Endophytic Fungi Isolated from *Schinus terebinthifolius* Raddi", *Microorganisms*, vol. 8, no. 6, pp. 1-13, 2020.

[7] Santos, A.C., Rossato, M., Agostini, F., Serafini, L.A., Santos, P.L., Molon, R., Dellacassa, E., Moyna, P. "Chemical composition of the essential oils from leaves and fruits of *Schinus molle* L. and *Schinus terebinthifolius* Raddi from Southern Brazil", *J Essent Oil-Bear Plants*, vol. 12, no. 1, pp. 16-25, 2009.

[8] Barbosa, L.C., Demuner, A.J., Clemente, A.D., Paula, V.F., Ismail, F. "Seasonal variation in the composition of volatile oils from *Schinus terebinthifolius* Raddi", *Química Nova*, vol. 30, pp. 1959-1965, 2007.

[9] Ennigrou, A., Casabianca, H., Vulliet, E., Hanchi, B., Hosni, K. "Assessing the fatty acid, essential oil composition, their radical scavenging and antibacterial activities of *Schinus terebinthifolius* Raddi leaves and twigs", *J Food Sci Tech*, vol. 55, pp. 1582-1590, 2018.

[10] de Lima, M.R., de Souza Luna, J., dos Santos, A.F., de Andrade, M.C., Sant'Ana, A.E., Genet, J.P., Marquez, B., Neuville, L., Moreau, N. "Anti-bacterial activity of some Brazilian medicinal plants", *J Ethnopharmacol*, vol. 105, no. 1-2, pp. 137-147, 2006.

[11] Bendaoud, H., Romdhane, M., Souchard, J.P., Cazaux, S., Bouajila, J. "Chemical composition and anticancer and antioxidant activities of *Schinus molle* L. and *Schinus terebinthifolius* Raddi berries essential oils", *J Food Sci*, vol. 75, no. 6, pp. 466-472, 2010.

[12] Ceruks, M., Romoff, P., Fávero, O.A., Lago, J.H. "Constituintes fenólicos polares de *Schinus terebinthifolius* Raddi (Anacardiaceae)", *Química Nova*, vol. 30, pp. 597-599, 2007.

[13] Schmourlo, G., Mendonça-Filho, R.R., Alviano, C.S., Costa, S.S. "Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants", *J Ethnopharmacol*, vol. 96, no. 3, pp. 563-568, 2005.

[14] da Silva Nascimento, M., dos Santos, P.H., de Abreu, F.F., Shan, A.Y., Amaral, R.G., Andrade, L.N., Souto, E.B., Santos, M.I., de Souza Graça, A., Souza, J.B., Raimundo e Silva, J.P. "*Schinus terebinthifolius* Raddi (Brazilian pepper) leaves extract: *in vitro* and *in vivo* evidence of anti-inflammatory and antioxidant properties", *Inflammopharmacology*, vol. 31, no. 5, pp. 2505-2519, 2023.

[15] de Mesquita, M.L., de Paula, J.E., Pessoa, C., de Moraes, M.O., Costa-Lotufo, L.V., Grougnet, R., Michel, S., Tillequin, F., Espindola, L.S. "Cytotoxic activity of Brazilian Cerrado plants used in traditional medicine against cancer cell lines", *J Ethnopharmacol*, vol. 123, no. 3, pp. 439-445, 2009.

[16] Santana, J.S., Sartorelli, P., Guadagnin, R.C., Matsuo, A.L., Figueiredo, C.R., Soares, M.G., da Silva, A.M., Lago, J.H. "Essential oils from *Schinus terebinthifolius* leaves–chemical composition and *in vitro* cytotoxicity evaluation", *Pharm Bio*, vol. 50, no. 10, pp. 1248-1253, 2012.

[17] Magalhaes, M.L., Ionta, M., Ferreira, G.A., Campidelli, M.L.L., Caetano, A.R.S., Brandao, R.M., Nelson, D.L., Cardoso, M. "Genotoxic, cytotoxic and fungicidal activity of the essential oil extracted from the leaves and fruits of the pink pepper ("*Schinus terebinthifolius*" Raddi)", *Aust J Crop Sci*, vol. 15, no. 7, pp. 997-1004, 2021.

[18] Alqathama, A., Abdelhady, M.I.S., Al-Omar, M.S., Barghash, M.F., Shallan, A.I. "Antioxidant, Anti-inflammatory and Cytotoxic Activity of Schinus terebinthifolia Fruit and Isolation of a New Immunomodulatory Polyphenolic Compound", *Pharmacogn Mag*, vol. 19, no. 1, pp. 13-22, 2023.

[19] da Silva, M.M., Iriguchi, E.K.K., Kassuya, C.A.L. *et al.* "*Schinus terebinthifolius*: Phenolic constituents and *in vitro* antioxidant, antiproliferative and *in vivo* anti-inflammatory activities", *Rev Bras Farmacogn*, vol. 27, pp. 445-452, 2017.

[20] Reis, M.Y.F.A., Xavier, L.M.A., de Souza, R.M., Ramalho, Í.M.M, Nascimento, Y.M.D., Ferreira, M.D.L., Damasceno, B.P.G.L., Sobral, M.V., Sampaio, F.C. "Pink pepper (*Schinus terebinthifolius* Raddi) essential oil: Phytochemical composition and cytotoxic activity", *Nat Prod Res*, vol. 22, pp. 1-7, 2023.

[21] Matsuo, A.L., Figueiredo, C.R., Arruda, D.C., Pereira, F.V., Scutti, J.A., Massaoka, M.H., Travassos, L.R., Sartorelli, P., Lago, J.H. "α-Pinene isolated from *Schinus terebinthifolius* Raddi (Anacardiaceae) induces apoptosis and confers antimetastatic protection in a melanoma model", *Biochem Biophys Res Commun*, vol. 411, no. 2, pp. 449-454, 2011.

1. El-Nashar, H., Mostafa, N., Hafez, M., Eldahshan, O., Singab, A.N., Singab, B. "Chemical composition, antimicrobial and cytotoxic activities of essential oils from *Schinus terebinthifolius* Raddi growing in Egypt", *Int J Pharmacogn Phytochem Res*, vol. 11, pp. 235-239, 2019.

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APPLICATION OF MYCORRHIZAL FUNGI IN THE PRODUCTION OF TOMATOES IN A CLOSED SPACE

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:
Agricultural production requires alternative solutions for the application of pesticides and mineral fertilizers. One, reliable solution is definitely the application of mycorrhiza, because in addition to reducing the use of pesticides and mineral fertilizers, it increases the resistance of plants to biotic and abiotic stresses. Mycorrhizal inoculation improves nutrient and water uptake by many host plants. Arbuscular mycorrhizal fungi (AMF) grow in close association with plant roots and play an important symbiotic role in the uptake and transfer of water and nutrients in the root system; in exchange, the plant supplies the fungus with sugars. The aim of this research is the possibility of controlled application of mycorrhizae and examination of the influence of the symbiosis of mycorrhizae and tomato plants on achieving better yields, resistance to diseases and stress factors, as well as on plant growth itself. The main hypothesis of this field study was that the symbiosis of an arbuscular mycorrhizal fungus with tomato roots would increase crop growth and yield under stressful abiotic conditions. The test was conducted in a 300 m^2 greenhouse on an agricultural estate in Ustikolina with the application of mycorrhiza in the amount of 1g, 3g and 6 g., for each plant. The achieved results gave a clearer picture of the importance of the symbiotic action of mycorrhizal fungi and tomatoes in the form of improved growth and fruiting.

KEYWORDS: mycorrhization, symbiotic relationship, yield, stresses

INTRODUCTION

In agricultural production, the application of beneficial microorganisms, such as arbuscular mycorrhizal (AMF) fungi, reduces the use of pesticides, artificial fertilizers and increases the tolerance of plants to abiotic factors. About 96.5% of the global rural area is under the influence of abiotic stresses [1]. Drought can cause a yield loss of 13% to 94% under several conditions depending on the intensity and duration of the drought [2]. World agriculture is in the process of transition to sustainable production, and this again requires the reduction of chemical inputs and the preservation of the richness of the microbiome and biodiversity. Plants should be viewed through their association with microorganisms and positive effects on the condition and health of the plants themselves [3]. In nature, there are communities between plants and microorganisms that can be beneficial or harmful to the host plants. One of the ways towards ecologically acceptable and sustainable crop production is the establishment of a beneficial interaction between plants and microbes [4]. Mitigation of damage from stress can be achieved with a symbiotic mixture of mycorrhizal fungi. Studies have shown that when the plant was faced with multiple abiotic stress factors (lack of nutrients and high concentrations of various heavy metals), co-inoculation of fungi belonging to different families was more effective than mono-inoculation in improving biomass, mineral nutrition, Ca/Mg ratio and heavy metal tolerance of plants in soil [5]. Mycorrhizal symbiosis is formed by fungi with plant roots due to a mutually beneficial relationship. It is one of the first examples of mutual relationship on Earth. In addition to providing plants with nutrients and water, mycorrhizae provide additional benefits in combating biotic and abiotic stresses [6]. The fungus develops hyphae that take up carbohydrates produced by the host through photosynthesis, the fungi help the plant take up water and immobile soil nutrients such as phosphorus, copper and zinc. In most natural environments, symbiosis is a normal occurrence. In terrestrial environments, approximately 85-90% of plant species form a symbiosis with soil fungi (symbiosis defined as mycorrhiza) [7]. The use of mycorrhizal

fungi has a positive effect on the concentration of potassium and calcium in the fruit and a better water potential [8]. in vegetable production, the use of mycorrhizal fungi is desirable, even necessary, because it can have a positive effect on yield and quality even though smaller doses of mineral fertilizers are applied. Inoculation of the root system with mycorrhizal fungi, sweet pepper, allows to reduce the amount of mineral fertilizers without significantly reducing the yield and quality of fruits [9].

The use of mineral fertilizers has theoretically reached its maximum use and there will be no increase in yield due to the use of fertilizers. Poor fertilizer management poses a threat to the environment. In order to avoid negative consequences for the environment, the efficiency of fertilizers must be significantly increased [10]. Increasing the yield by applying a larger amount of chemical fertilizers to the soil is a danger to the health of people and the environment, so that the soil and plants cannot maintain healthy production for a long time. Reducing the impact on the negative effects of chemicals on human health and the environment are the main reasons for increasing restrictions on the use of chemicals and increasing biocontrol [11,12]. Beneficial microorganisms play an important role in high-quality agricultural production, and reducing the use of chemical inputs also helps prevent yield reductions. Although bacteria and fungi have been repeatedly demonstrated over the past 150 years to promote plant growth and suppress plant pathogens, this knowledge has not been widely used in agricultural biotechnology. Despite extensive research on biocontrol and the potential of BCA use as an alternative to chemicals, global reliance on BCA use remains relatively insignificant [13].

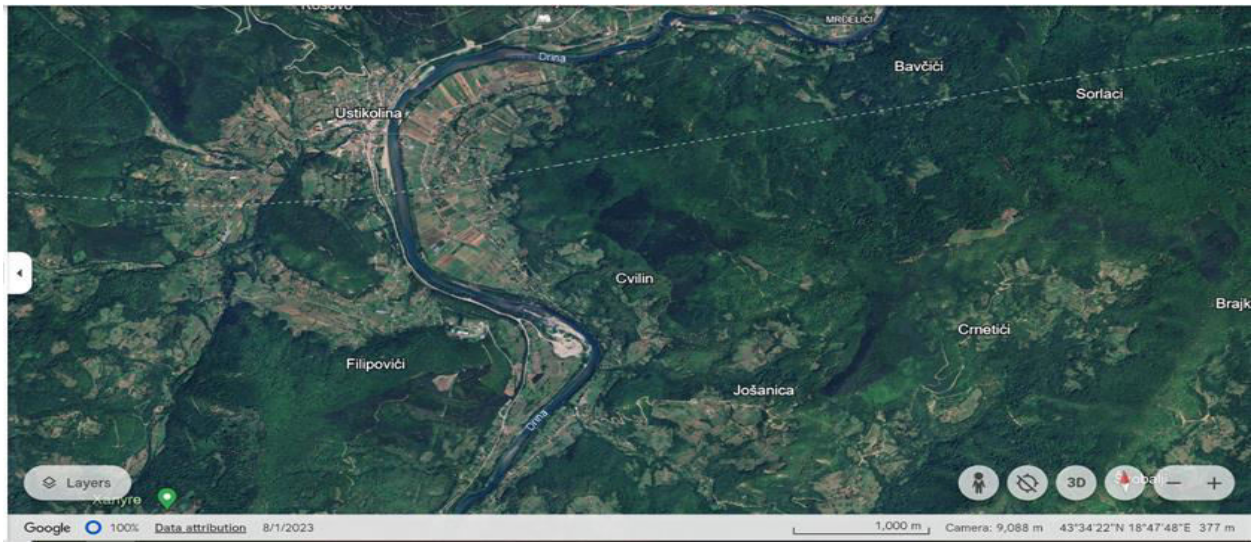
Mycorrhizal fungi that were applied to the root system during seedling production had a positive effect on the yield and biometric characteristics of peppers, with the fruits of the thickest pericarp and the largest mass. Vegetables (*Solanaceae and Cucurbitaceae*) are often under the influence of abiotic factors, which have changed with climate change, and pressure is exerted on the plants to survive and to give good yields [14]. Mycorrhizae improve plant tolerance to several abiotic stresses by various physiological, functional and biochemical changes in plants.

As a result of research on the effects of mycorrhizal inoculation with two species (*Glomus clarum* and *Glomus caledonium*) and three different inoculation treatments (sowing, transplanting and sowing + transplanting) applied to peppers grown hydroponically on a perlite substrate, the effect of *G. clarum* and *G. Caledonium* increased the yield by 29% and 21%, respectively, compared to control plants [15]. On mycorrhized tomato plants, shoot, root and total seedling dry biomass increased significantly with AMF (Arbuscular mycorrhizal fungi) inoculation compared to the control at 14, 28 and 42 days of measurement after inoculation [16]. Arbuscular mycorrhizal fungi (AMF) are widespread soil fungi that form associations with the roots of most (>80%) terrestrial plant species [17].

Tomato plants grown in substrates receiving 50% mineral fertilizers showed the highest level of mycorrhization, showing a frequency (F) of 100% and an intensity (M) of 63%. Importantly, the combination of inoculation with a reduced dose of NPK fertilizer (50% of the recommended amount) resulted in significantly increased concentrations of calcium, potassium, iron, zinc, and phosphorus in plants, which can be attributed to enhanced microbiome root colonization [18]. Numerous previous studies have confirmed the effective use of AMF inoculum either alone or in combination with NPK fertilizers or different levels of phosphorus to improve plant growth, improve mineral nutrient uptake and increase crop yield [19,20].

MATERIALS AND METHODS

The survey was carried out on an agricultural estate in the Municipality of Foča in FBiH, the place of Ustikolina. The place is located in the valley of the river Drina, surrounded by mountains, with a rich and fertile plain, Cvilinsko polje with geographical coordinates: 43°34'22"N; 18°47'48"E.



Picture 1. Display of test location- Ustikolina.
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Sandy soil prevails in this locality, formed as alluvium of the old course of the Drina river. The analysis of the physical and chemical characteristics of the soil, i.e. the analysis of the soil, was done at the Faculty of Agriculture and Food of the University of Sarajevo. The analysis of the chemical properties of the soil indicates that the soil has a neutral to slightly alkaline reaction, medium humus content, but very rich in available potassium and very poor in available phosphorus (table 1).

Table 1. Results of physical and chemical characteristics of soil

Characterictics	Measure unit	Result
pH in H2O	-	7,31
pH in KCl	-	7,05
K2O	mg/100 g	108
P2O5	mg/100 g	< 1,0
Humus	-	2,22%
Carbonates	-	Soil without carbonates

After the own production of seedlings, the planting of seedlings was started. The seedlings were planted in two rows with a spacing of 40 x 50 cm and on three beds. The distance between the beds is 60 cm. During planting, mycorrhiza was applied, in the amount of 1g, 3g and 6 g. for each plant. In the test, 20 plants were used for each variant of mycorrhiza, 20 control plants. At the time of planting, tomato seedlings had an average height of 18 cm with 4 to 5 developed leaves and were about 60 days old.

The test was conducted in a greenhouse 5 meters wide and 60 meters long (300m2), three beds were formed with a planter inside the greenhouse. The space between the rows is mulched with straw. Tomatoes of the Marathon F1 variety from the Superior company, in the apple type, were used in the test. The mycorrhizal agent under the trade name Mycoriza-Rhizo-vam Basic contained the spores of *Glomus intraradices* 10^6 cells/g.

Picture 2. Micoriza- Rhizo-vam Basic

Morphometric measurements included; growth height measurements, average and total yield.

Measurements were made on each of 20 plants, 3 treatments of 20 plants and 20 control plants by measuring the plants with a meter to determine their growth. The yield was measured from each plant (on a digital scale) and then the average and total yield was determined.

Monitoring the presence of diseases on tomatoes was carried out by frequent visual inspection. Frequent visual inspection of tomato plants allows

for rapid recognition of disease symptoms, such as changes in leaves, stems or fruits.

Early recognition of fungal plant diseases is crucial for timely treatment, which can prevent significant losses in agriculture. Molecular analysis offers high accuracy, is often expensive and time-consuming[21].

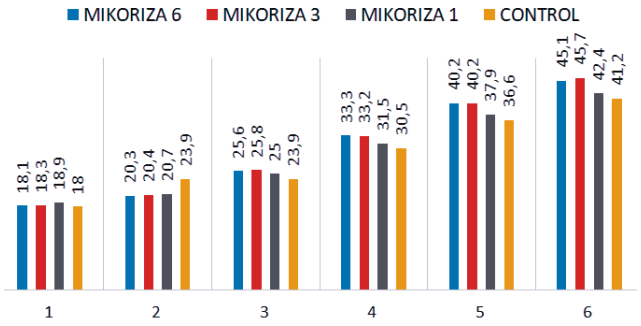
Good and experienced experts are required to diagnose plant diseases with the naked eye. This method can be accompanied by significant error [22].

RESULTS AND DISCUSSION

Three concentrations of mycorrhizal agent 1g, 3g 6g and a control group of plants were used in the work.

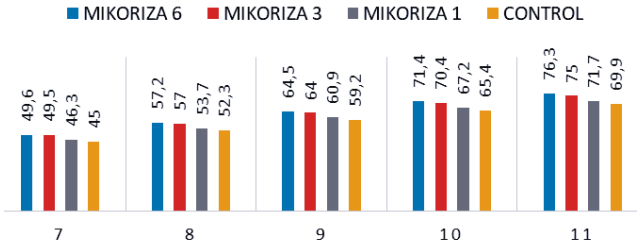
In the first two weeks of growth, from planting and applying the product, no difference in growth was noticed and the plants looked quite uniform.

The difference in height starts to be noticed already in the third week in which the plants from the "mycorrhiza 6 g" and "mycorrhiza 3 g" groups show a better growth tendency compared to the plants from the "mycorrhiza 1 g" and "control" groups, which can be seen from graph 1.



Graph 1. Tomato groups growth in first six weeks

At the end of the sixth week, the plants from the "mycorrhiza 6 g" and "mycorrhiza 3 g" groups had better growth, where the difference in plant height was 3 to 4 cm compared to the other two groups.



Graph 2. Tomato groups growth in period from 7 to 11 weeks

Towards the end of the 11th week, a significant difference in the growth of the tested groups can be observed, where the plants from the "mycorrhiza 6 g" group showed better adaptability, which resulted in an average plant height of 26.7 cm compared to the plants from the control group, where the growth was an average of 24.9 cm, i.e. the difference between these two groups is 1.8 cm, which is proven by Graph 2.

The standard deviation during this period ranged from 9.98 cm (control) to 10.72 cm (MYCORHIZ 6), indicating uniform growth within each group, without drastic oscillations between plants.

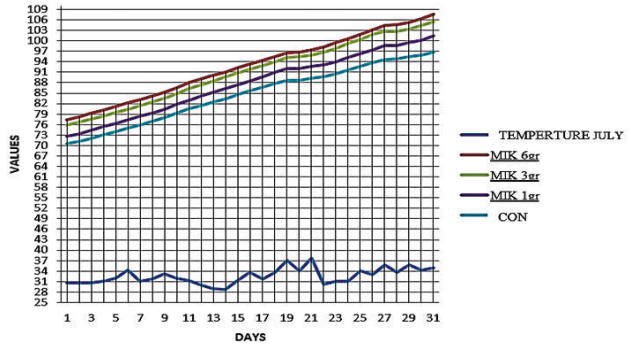
In order to determine the statistical significance of the difference between the treated groups and the control, an independent samples t-test was applied. The results showed that none of the differences between the treatments and the control were statistically significant (all $p > 0.05$), although a tendency for better growth was observed in mycorrhizal plants.

In the July, the first higher air temperatures were recorded, and in order to reduce the stress factor for the plants, foliar biostimulation and one fertigation supplement were performed.

The symbiosis between AMF and plants plays a key role in alleviating the stressful impact of drought, as it enables better availability of water and nutrients, reduces water loss through transpiration and increases plant resistance to stressful conditions. According to literature data, AMF inoculation increased the rate of photosynthesis and transpiration by 40% to 70%, doubled the leaf area and increased the relative water content in the leaves of the plant by 25%. By studying the effect of mycorrhizal inoculation on the total yield, a 23% increase was found in inoculated plants (57.1 t ha) compared to non-inoculated (43.93 t ha) plants. Quantitative yield estimates of fruit number escalated to 35 numbers in inoculated plants (30.6 fruit plants) instead of non-inoculated (19.9 fruit plants) [23].

The application of commercial AMF inoculum (*F. mosseae* and *Septoglomus constrictum*) caused an increase in the total height of tomato plants despite chemical fertilization in inoculated plants (48.4 cm plant -1) compared to non-inoculated plants (39.7 cm plant -1) with an increase of 18% [24].

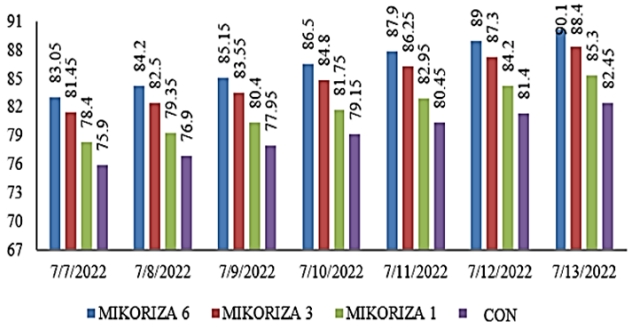
From Graph 3, in which the maximum temperature peak of around 37ºC was recorded, we can see that the plants treated with mycorrhizal fungi more easily tolerated the temperature stress, where they maintained their tendency to grow.



Graph 3. Growth stability in relation to the maximum recorded temperature

At the end of the first week of July, foliar top dressing was carried out with the preparation Slavol, which is a liquid microbiological fertilizer and growth stimulator, certified for use in organic and traditional agricultural production in a concentration of 1.5% according to the manufacturer's recommendation and through an irrigation system with nettle decoction 0.01%.

Urtica dioica is considered one of the best plant liquid nitrogen fertilizers that promotes the formation of chlorophyll. It grows very quickly and absorbs minerals and other phytonutrients, so it is considered a bioaccumulator. In addition to the already mentioned elements such as nitrogen, sulfur and boron, it is rich in iron, silicon, calcium, potassium and phosphorus, which are generally substances that plants need for growth. Foliar nutrition and fertigation were carried out in the morning hours, during which the response of plant stimulation to growth caused by the above-mentioned two agents was monitored for seven days, supported by mycorrhizal fungi, which can be seen in graph 5. The plants from the "mycorrhiza 6 g" group showed the greatest increase at the end of the examined week, where the total weekly increase was 7.05 cm, and showed a better response to foliar nutrition compared to the control group, where the weekly increase was 6.55 cm.



Graph 4. Plant growth (from 7th to 13th day) after fertirigation

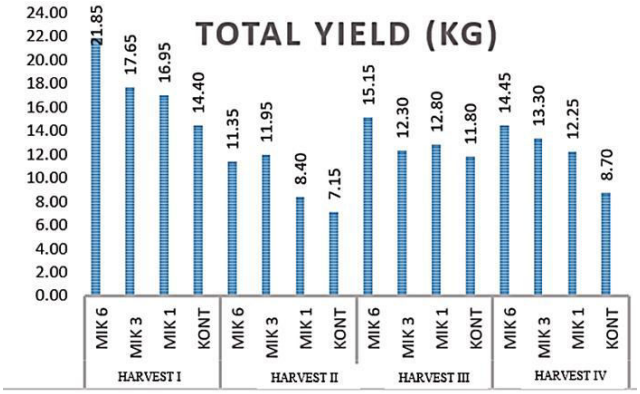
The plants from the groups "mycorrhiza 3 g" and "mycorrhiza 1 g" had a smaller increase caused by foliar feeding compared to the group "mycorrhiza 6 g" by 0.1 cm and 0.15 cm, which is negligible considering the difference. Comparing these two groups with the control, the difference in growth increases by 0.4 and 0.35 cm in favor of mycorrhizae.

In addition to growth, the influence of mycorrhizae on the quality of fruiting was investigated. Four harvests were carried out, during which the values of the total yield of the examined group, the average yield of an individual plant and the average weight of the fruit were monitored.

The total yield of individual groups varied widely, at the first harvest the highest yield was achieved by plants from mycorrhiza 6g with an amount of 21.85 kg, which we can see from graph 6. Compared to mycorrhiza 6g, mycorrhiza 3g and mycorrhiza 1g, achieved lower yields by 4.2 kg and 4.9 kg, but had a higher yield compared to the control by 3.25 kg and 2.55 kg.

In the first harvest, the mycorrhizae showed their potential and produced an average of 0.5 to 1.5 kg more fruits compared to the control group.

During the second harvest, the total yields fell due to high temperatures during the day, and the yields of mycorrhiza 6g ranged around 11 kg, and a slightly higher yield was achieved by the mycorrhiza 3g group with an average of 11.95 kg, which is 4.8 kg more than the control plants.



Graph 5. Total yield of examined group of plants

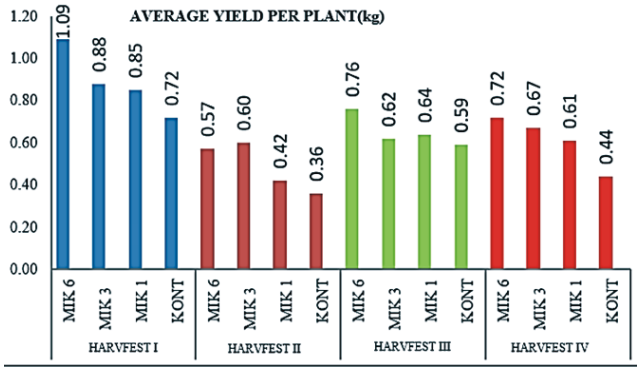
Due to the partial improvement of temperature conditions in the greenhouse and stimulation with foliar nutrition, the yields of all mycorrhizae increased slightly in the third harvest. Plants inoculated with mycorrhiza in the amount of 6g produced an average of 15.15 kg of fruits in the third harvest, and comparing them with the control group, they achieved a higher yield by 3.35 kg.

Analysis of the total yield, standard deviation, shows that MIK 6 generally gives a higher yield than the others, but the differences are not statistically significant.

In addition to the total yield, in order to get a clearer picture of the benefits of mycorrhiza application through the yield, the average yield per plant and the average weight of the fruit were considered.

The average yield per plant varied during the entire growing season through four harvests, so in the first harvest, according to Graph 6, the highest yield per plant was achieved by mycorrhiza 6g with an average of 1.09 kg and an average fruit weight of 300 grams. Compared to the control during the same harvest, it is a difference of 0.37 kg per plant and 70 grams per fruit, converted into fruits, it is 1.48 fruits with an average weight of 250 grams.

According to the standard deviation, MIK 6 generally gives a higher yield than the others, but with visible variability.



Graph 6. Average yield per plant

In the second harvest, yields fell due to unfavorable temperature conditions prevailing in the protected area despite the shade net, where we achieved the highest average yield per plant in mycorrhiza 3g, with the highest average fruit weight of 0.20 kg for that harvest.

By stabilizing the temperature, the yields improved significantly in the third and last harvest, where in the last harvest a stabilization was recorded in terms of the average fruit weight, where the fruits in the mycorrhizal group were almost uniform.

CONCLUSION

Mycorrhizae help tomatoes better withstand stressful conditions, such as drought and high temperatures.

The difference in the height of the plants was 3 to 4 cm. After foliar feeding and irrigation, after heat stress, plants inoculated with 6g each had an average growth of 7.05 cm in one week compared to the average growth of control non-inoculated plants of 6.55 cm.

The use of mycorrhiza resulted in higher tomato yields. In the first harvest, the inoculated plants showed their potential and produced an average of 0.5 to 1.5 kg more fruits compared to the control group. In the second harvest, the total yields are higher by 4.8 kg, in the third harvest by 3.35 kg and in the last fourth harvest by 5.2 kg.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

REFERENCES

1. Cramer et al., (2011, G.R. Cramer, K. Urano, S. Delrot, M. Pezzotti, K. Shinozaki, Effects of abiotic stress on plants: a systems biology perspective, BMC Plant Biol., 11 (2011), p. 163, 10.1186/1471-2229-11-163
2. Bulgari et al.,(2019), R. Bulgari, G. Franzoni, A. Ferrante, Biostimulants application in horticultural crops under abiotic stress conditions, Agronomy, 9 (2019), p. 306, 10.3390/agronomy9060306
3. Sandrini M, Nerva L, Sillo F, Balestrini R, Chitarra W, Zampieri E. Abiotic Stress and Belowground Microbiome: The Potential of Omics Approaches. Int J Mol Sci. 2022 Jan 19;23(3):1091. doi: 10.3390/ijms23031091. PMID: 35163015; PMCID: PMC8835006.
4. Emmanuel OC, Babalola OO. Productivity and quality of horticultural crops through co-inoculation of arbuscular mycorrhizal fungi and plant growth promoting bacteria. Microbiol Res. 2020 Oct;239:126569. doi: 10.1016/j.micres.2020.126569. Epub 2020 Jul 31. PMID: 32771873.
5. Crossay T, Majorel C, Redecker D, Gensous S, Medevielle V, Durrieu G, Cavaloc Y, Amir H. Is a mixture of arbuscular mycorrhizal fungi better for plant growth than single-species inoculants? Mycorrhiza. 2019 Jul;29(4):325-339. doi: 10.1007/s00572-019-00898-y. Epub 2019 Jun 15. PMID: 31203456.
6. Jung et al., (2012), S.C. Jung, A. Martinez-Medina, J.A. Lopez-Raez, M.J. Pozo, Mycorrhiza-induced resistance and priming of plant defenses, J. Chem. Ecol., 38 (2012), pp. 651-664,10.1007/s10886-012-0134-6
7. Giovanni Cafà, (2008)- Monitoraggio molecolare di funghi micorrizici arbuscolari in radici di Medicago sativa, GIOVANNI CAFA'6 October 2048'Pisa University Press'

8. Pasković I., Palčić I., Radić T., Klanjac J., Grozić K., Goreta B., S., (2019), Optimization of fertilization during the cultivation of industrial tomatoes using mycorrhizal fungi. Poreč: Institute for Agriculture and Tourism Poreč.
9. Franczuk, J.,Tartanus, M.; Rosa, R., Zaniewicz-Bajkowska, A., Dębski, H., Andrejiová, A., Dydiv, A. , (2023),The Effect of Mycorrhiza Fungi and Various Mineral Fertilizer Levels on the Growth, Yield, and Nutritional Value of Sweet Pepper (Capsicum annuum L.). Agriculture 2023, 13, 857.
10. Shaviv, A., Mikkelsen, R.L. Controlled-release fertilizers to increase efficiency of nutrient use and minimize environmental degradation - A review. Fertilizer Research 35, 1–12 (1993). https://doi.org/10.1007/BF00750215.
11. Alavanja, MZP; Hoppin, JA; Kamel, F. Health effects of chronic exposure to pesticides: Cancer and neurotoxicity. Annu. Rev. Public Health 2004 , 25 , 155–197.
12. Epstein, L. Fifty years since Silent Spring. Annu. Rev. Phytopathol. 2014 , 52 , 377–402.
13. Trejsi, EF The promise of biological control for sustainable agriculture: A stakeholder-based analysis. J. Sci. Poly Goverance 2014.
14. Raaijmakers, Jos M., et al. "The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms." Plant and soil 321.1-2 (2009): 341-361.
15. Ikiz, O.; Abak, K.; Daşgan, H.Y. and Ortaş, I. 2009. Effects of mycorrhizal inoculation in soilless culture on pepper plant growth. Acta Hort., 807: 533-593.
16. Rhodes, L. H., and J. W. Gerdemann. "Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions." New Phytologist 75.3 (1975): 555-561.
17. Smith SE, Smith FA. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu Rev Plant Biol. 2011;62:227-50. doi: 10.1146/annurev-arplant-042110-103846. PMID: 21391813.
18. Chafai, Wissame, Kaoutar Haddioui, Hana Serghini-Caid, Hicham Labazi, Mashail N. AlZain, Omar Noman, Mohammad Khalid Parvez, Mohamed Addi, and Ahmed Khalid. 2023. "Impact of Arbuscular mycorrhizal Fungal Strains Isolated from Soil on the Growth, Yield, and Fruit Quality of Tomato Plants under Different Fertilization Regimens" Horticulturae 9, no. 9: 973. https://doi.org/10.3390/horticulturae9090973.
19. Mujica, PY; de la Noval, B.; Dell Amico, RJ Respuesta del cultivo de tomate a la applicación de dos inoculantes de hongos micorrízicos arbusculares por vías diferentes de inoculación. Agron. Trop. 2010 , 60 , 381–388.
20. Karagiannidis, N.; Thomidis, T.; Lazari, D.; Panou-Filotheou, E.; Karagiannidou, C. Effect of three Greek arbuscular mycorrhizal fungi in improving growth, nutrient concentration and essential oil production of oregano and mint plants. Sci. Hortic. 2011,
21. Seyed Mohamad Javidan, Ahmad Banakar , Keyvan Asefpour Vakilian, Yiannis Ampatzidis, Kamran Rahnama(2024), Early detection and spectral signature identification of tomato fungal diseases (Alternaria alternata, Alternaria solani, Botrytis cinerea, and Fusarium oxysporum) by RGB and hyperspectral image analysis and machine learning, doi: 10.1016/j.heliyon.2024.e38017.
22. D. Mohammad Zamani, S.M. Javidan, M. Zand, M. Rasouli, Detection of cucumber fruit on plant image using artificial neural network, Journal of Agricultural Machinery, 13 (1) (2023), pp. 27-39, 10.22067/jam.2022.73827.1077.
23. G. Leventis, M. Tsiknia, M. Feka, E.V. Ladikou, I.E. Papadakis, I. Chatzipavlidis, K. Papadopoulou, C. Eha liotis, (2021), Arbuscular mycorrhizal fungi enhance growth of tomato under normal and drought conditions, via different water regulation mechanisms, Elsiver, Volume 19, September 2021, 100394.
24. Chandrasekaran M., Boopathi T., Manivannan P., (2021), Comprehensive Assessment of Ameliorative Effects of AMF in Alleviating Abiotic Stress in Tomato Plants, Nuria Ferrol, doi: 10.3390/jof7040303.

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EVALUATION OF THE EFFECTIVENESS OF NIACINAMIDE-BASED COSMETIC PREPARATIONS IN REDUCING FACIAL SKIN SEBUM LEVELS

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:
Sebum is an oily liquid produced by the sebaceous glands. It serves as a protective barrier for the skin, keeping it hydrated and enhancing its elasticity. Sebum contains triglycerides, fatty acids, wax esters, antioxidant squalene, and cholesterol. While the highest concentration of sebum is produced on the face, it is also present on other parts of the skin, including the scalp. Excessive sebum secretion leads to oily skin, but even dry and normal skin types produce a certain amount of sebum. Cleansing foams and tonics for oily skin have an advantage over other pharmaceutical products, such as creams, soaps, and liquid powders, as they can be applied to all areas of the skin. Niacinamide ($C_6H_6N_2O$) is a pyridinecarboxyamide and organic molecule belonging to the vitamin B group. In recent years, niacinamide has become widely used in cosmetology for the formulation of various pharmaceutical products, primarily for dermal application. However, it is also incorporated into shampoos and hair tonics. Niacinamide is effective in reducing sebum levels on the skin, providing hydration, strengthening the skin's protective barrier, and minimizing visible wrinkles. This study explores the effectiveness of niacinamide as an active component in tonics and foams, along with the stability of these formulations, their microbiological purity, and *in vivo* testing on volunteers. The MPA 6 device, used in skin bioengineering, was employed to measure the amount of sebum on volunteers' facial skin before and after using a cleansing foam for oily skin.

KEYWORDS: oily skin, niacinamide ,sebum, cleansing foams, cleansing tonics

INTRODUCTION

Niacinamide, the amide form of vitamin B3 (niacin), is a hydrophilic endogenous substance and a derivative of nicotinic acid, also known as nicotinamide. It is an organic compound with the molecular formula $C_6H_6N_2O$ and a molar mass of 123.11 g/mol. White crystalline powder, odorless and slightly acidic. Figure 1 shows the structure of the niacinamide molecule.

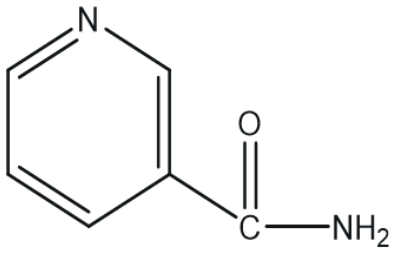

Figure 1. Chemical structure of niacinamide

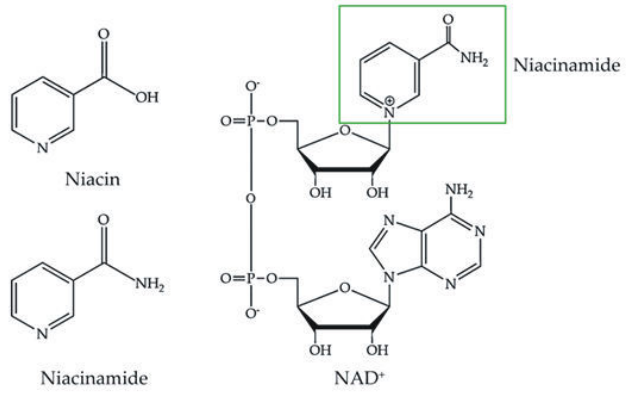

Figure 2. Molecular structures of niacin and niacinamide in the B3 vitamin complex and their molecular constitutive role in NAD+ synthesis

It belongs to the group of B-complex vitamins, specifically one of the eight essential B vitamins [1]. Within the complex metabolic system, niacinamide regulates NFκB-mediated transcription of signaling molecules by inhibiting nuclear poly (ADP-ribose) polymerase-1 (PARP-1).

Vitamin B3 plays a crucial role as a component of the coenzyme NAD (nicotinamide adenine dinucleotide). In living organisms, niacinamide is a vital part of two important coenzymes: NAD+ (nicotinamide adenine dinucleotide) and NADP+ (nicotinamide adenine dinucleotide phosphate). Their reduced forms, NADH and NADPH, act as electron carriers in oxidation and reduction reactions. NAD+ functions as a direct oxidant in glycolysis, fatty acid oxidation, and the citric acid cycle, where it is reduced to NADH. During these reactions, carbon atoms are released as carbon dioxide (CO_2), and NADH transfers electrons to the final oxidant—oxygen. In the phosphogluconate pathway (an alternative glucose metabolism pathway), NADP+ is reduced to NADPH, which then participates in biosynthetic reduction reactions [2].

Vitamin B3, particularly niacinamide, is naturally present in many foods. It is commonly used as a dietary supplement and as an active ingredient in pharmaceutical formulations [3]. Many organisms cannot synthesize niacinamide, making dietary intake or supplementation essential. Pharmaceutical and food-grade niacinamide is used to treat vitamin B3 deficiency and nicotinic acid deficiency-related conditions. It is also effective in managing inflammatory skin diseases, such as acne and pellagra.

In dermatology, extensive research has been conducted on niacinamide and its analogs for the prevention and treatment of cancer, blistering disorders, acne vulgaris, psoriasis, wound healing, and pigmentation disorders. Niacinamide has also been widely used in cosmetics for decades to prevent skin aging and improve skin tone.

Sebum is secreted by the sebaceous glands, which are most numerous on the face, with a density of 400–800 glands/cm^2. Sebum is secreted by the sebaceous glands, which are most numerous on the face, with a density of 400–800 glands/cm^2 [4].

Various dermocosmetic formulations are used in the care of oily skin, with foams standing out as a newer pharmaceutical form. Medicinal foams offer an advantage over other cosmetic products as they can be applied to all areas of the skin, including those covered with hair. Cleansing foams for oily skin are designed to reduce excess sebum on the skin's surface, leaving it looking firmer and less shiny [5].

The aim of this study was to explore the effectiveness of niacinamide as an active component in tonics and foams, along with the stability of these formulations, their microbiological purity, and *in vivo* testing on volunteers.

MATERIALS AND METHODS

All raw materials used in the research were donated by the company (Volimo prirodno d.o.o., Mostar, Bosnia and Herzegovina). The effectiveness of the cosmetic preparations was tested using the MPA 6 skin bioengineering device (Courage+Khazaka) with a sebumeter SM 815.

FORMULATION OF FACIAL CLEANSING FOAM

The production of the facial cleansing foam began by heating an appropriate amount of lavender hydrolate while maintaining a controlled temperature of approximately 40–50°C. This process ensured the proper dissolution of niacinamide, which was then added to the heated hydrolate. Once the niacinamide had completely dissolved, the mixture was blended with the remaining ingredients to achieve a uniform formulation.The specific quantities of raw materials used in the formulation of the niacinamide-based cosmetic foam are detailed in Table 1.

Table 1. Quantities of raw materials used in the formulation of facial cleansing foam with niacinamide

Components	Amount (g)
Niacinamide	4.0
Coconut glucoside	40.0
Lavender hydrolate	45.0
Geogard 221	1.0
Aqua destillata	ad 100.0

FORMULATION OF FACIAL CLEANSING TONIC

Niacinamide was first dissolved in lavender macerate at an elevated temperature (approximately 40–50°C). The lavender macerate was then mixed with the preservative Geogard 221, aloe vera gel and panthenol to create the toner. The quantities of raw materials used for the preparation of the cosmetic toner are shown in Table 2.

Table 2. Quantities of raw materials used in the formulation of the niacinamide-based tonic.

Components	Amount (g)
Niacinamide	4.0
Lavender macerate	85.0
Panthenol	3.0
Aloe vera	7.0
Geogard 221	1.0

TESTING THE pH VALUE OF NIACINAMIDE-BASED FOAM AND TONIC

Seventy-two (72) hours after the preparation of the formulations, the pH values of the tested foam and tonic samples were determined potentiometrically at room temperature (22±2°C). The measurement was repeated after two months to assess the chemical stability of the formulations. The foam and tonic samples were stored at room temperature. The recommended pH range for cosmetic skin cleansing products is between 3.5 and 9 [6].

TESTING THE EFFECTIVENESS OF NIACINAMIDE-BASED FOAM AND TONIC ON SEBUM LEVELS

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethical Committee for Scientific Research University of Tuzla (03/7-6990-1-2/24). It was conducted at the Faculty of Pharmacy, University of Tuzla.

PARTICIPANTS AND PROCEDURE

The study involved 13 participants, women aged between 17 and 36 years, with normal to oily skin and skin prone to hyperpigmentation. Participants were volunteers who had previously signed an informed consent prior to the study, in which the objectives, methods, and procedures of the research were detailed [7]. Each participant received verbal instructions on how to use the formulations, as well as the scheduled times for skin parameter measurements.

The verbal instructions included the regular application of the cosmetic formulations twice daily until the next measurement appointment. First, participants were instructed to clean their faces using a specific facial foam and tonic at least 3 hours before arriving at the testing facility. After cleaning, no additional skincare or cosmetic products were to be applied.

Measurements were taken after a 15-minute acclimatization period in the testing room (with a constant temperature of 20°C ± 2°C and humidity of 50% ± 5%) to ensure that sweating had subsided, which is critical for accurate sebum measurements.

SEBUM MEASUREMENT PROCEDURE

The Sebumeter® SM 815 was used to measure sebum levels on the skin surface. This probe utilizes a transparent film that absorbs sebum from the skin. The light transmission through the film is measured before and after contact with the skin using a photometer, providing an accurate quantification of sebum in μg/cm2 [8].

A probe containing a plastic strip is pressed against the measurement point, and the device is left for 15 seconds. Afterward, the probe is returned to the device, and the amount of lipids is determined by measuring the transparency of the plastic film using a photodetector.

After the objective assessment of sebum level, participants were asked about their personal experiences with the foam and tonic, including their subjective perceptions and any potential side effects such as itching, irritation, etc. The study lasted for a total of four weeks, during which three measurements were conducted. The study was conducted under the supervision of a dermatology specialists.

DETERMINING THE MICROBIOLOGICAL PURITY OF FOAM AND TONIC

The microbiological purity of the foam and tonic formulations was evaluated by testing for the presence of microorganisms. The smear method was used, in which a sample suspension was prepared and then applied to the surface of a solid nutrient medium. The samples were incubated for 24 hours at 37°C [9].

RESULTS AND DISCUSSION

DETERMINATION OF pH VALUE

pH of the facial cleansing foam after 72 hours of production was 5.14, which corresponds to the pH values of formulations that are close to the

physiological pH of the skin [12]. After two months from production, the pH of the foam was 4.96, with no significant deviation observed, indicating that the formulations maintained stability in terms of pH value *(Table 3).*

pH of the facial cleansing tonic after 72 hours of production was 4.76, and after two months from production, the pH was 4.65, with no significant deviation observed, indicating that these formulations also maintained stability in terms of pH value *(Table 4).*

Table 3. Changes in pH value for the niacinamide-based facial cleansing foam sample

Time	pH value
After 72 hours	5.14
After 2 months	4.96

Table 4. Changes in pH value for the niacinamide-based facial cleansing tonic sample

Time	pH value
After 72 hours	4.76
After 2 months	4.65

RESULTS OF THE MICROBIOLOGICAL PURITY OF FOAM AND TONIC

After 24 hours of incubation, no visible colonies were observed on the plates. This indicates that there was no microbial growth, and the products (foam and tonic) can be considered microbiologically stable. Niacinamide itself has potentially antimicrobial effects [10] and prevents the development of microorganisms.

Figure 3. Plates with niacinamide foam and tonic after 24 hours of incubation

MEASUREMENT OF SEBUM LEVELS ON THE SKIN

Table 5 presents the characteristics of the participants, including age and skin type. Table 6 shows percentage results obtained after measuring sebum levels. Table 7 presents the degree of sebum level reduction after one month, and the presence of side effects following the application of the foam and toner.

Table 5. Characteristics of study participants

Characteristic		%
Age	17-30	90
	>30	10
Skin type	Oily	80
	Mixed	10
	Normal	10

Following the application of the foam and tonic, sebum levels decreased in all participants. However, after one month of use, sebum levels remained lower than the initial measurement but higher than the second measurement. The sebum level before applying the foam and toner ranged from 78 to 223 g/cm², and after application, it ranged from 11 to 56 g/cm². One month after the application of the products, the sebum level ranged from 42 to 112 g/cm². These fluctuations may be attributed to factors such as stress, diet, hormonal imbalance, or irregular use of the foam and tonic.

Table 6. Percentage Results Obtained After Measuring Sebum Levels

Participant	Sebum level before applying foam and tonic	Sebum level after using foam and tonic	Sebum level after one month	Percentage of sebum reduction after application of foam and tonic	Percentage of sebum reduction after one month
1.	197	11	75	94%	62%
2.	82	39	51	52%	63%
3.	112	40	42	64%	36%
4.	88	19	56	78%	61%
5.	220	33	87	85%	73%
6.	223	56	60	75%	32%
7.	127	33	87	74%	49%
8.	134	56	68	58%	33%
9.	78	24	52	69%	48%
10.	89	27	46	70%	40%
11.	187	45	112	76%	51%
12.	195	23	95	88%	60%
13.	221	45	88	80%	50%

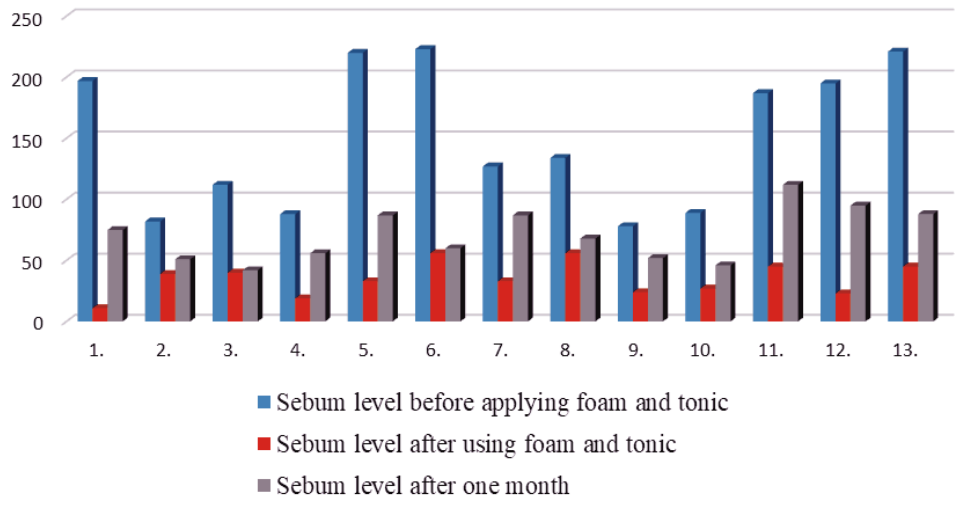

Figure 4. Sebum level on facial skin

Beyond sebum level measurements, participants also provided feedback through a survey. Notably, 80% of them reported that the formulated products improved their overall skin condition. After applying the foam and tonic, the volunteers' skin became softer and smoother, while sebum levels on the facial skin decreased in most participants.

Table 7. Skin condition after applying the foam and toner (subjective feeling)

Characteristic		%
Reduced appearance of oily skin	Yes	80
	No	20
Side effects	Itching	15
	Irritation	15
	No side effects	70

Previous studies have demonstrated that niacinamide reduces sebum production on the skin, which aligns with the findings of our research [11, 12, 13]. The study conducted by Draelos [14] shows that a 2% niacinamide gel reduces sebum levels on the facial skin. The study was conducted in Japan. Volunteers began noticing the effects of niacinamide after two and four weeks of using the cream, with a significant reduction in sebum levels.

CONCLUSIONS

Niacinamide (heterocyclic aromatic amide) as the active ingredient in the foam and tonic, played a crucial role in significantly reducing sebum levels on the facial skin. This reduction in sebum was accompanied by a noticeable improvement in skin texture, making the skin softer, smoother, and more refined to the touch. These findings suggest that the foam and tonic, with their niacinamide content, offer an effective solution for managing oily skin and improving overall skin quality. This study highlights the potential of niacinamide as a key ingredient in skincare products designed to control sebum production while promoting healthier, more nourished skin.

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REFERENCES

1. C. Marques, F Hadjab, A Porcello, K Lourenço, C Scaletta, P Abdel-Sayed, N Hirt-Burri, L. A. Applegate, Alexis Lauren, "Mechanistic Insights into the Multiple Functions of Niacinamide: Therapeutic Implications and Cosmeceutical Applications in Functional Skincare Products", Antioxidants, vol.13, 425, 2024.
2. A.J. Covarrubias, R. Perrone, A. Grozio, "NAD+ metabolism and its roles in cellular processes during ageing", Nat Rev Mol Cell Biol, pp. 119–141, 2021.
3. "Final Report of the Safety Assessment of Niacinamide and Niacin", International Journal of Toxicology, vol. 24, no. 5, pp. 1–31, 2005.
4. Z.D. Draelos, A. Matsubara, K. Smiles, "The effect of 2% niacinamide on facial sebum production", Journal of Cosmetic and Laser Therapy, vol. 8, no. 2, pp. 96-101, 2006.
5. R. Porter, "MSD Manual for Physicians", 2019.
6. D. Vasiljević, S. Savić, Lj. Đorđević, D. Krajišnik, "Priručnik iz Kozmetologije - drugo izdanje", Beograd, 2009.
7. D. Rusić, M. Ivić , A. Slugan, D. Leskur, D. Modun, T. Durdov, D. Vuković, J. Bukić, J. Bozić, A. Š. Perišin, "Pilot Study on the Effects of a Cosmetic Serum Containing Niacinamide, Postbiotics and Peptides on Facial Skin in Healthy Participants: A Randomized Controlled Trial", Life, vol. 14, 1677, 2024.
8. Courage+Khazaka. Sebumeter® SM 815; Manual Instruction; Courage+Khazaka: Köln, Germany, 2016.
9. M. Ibišević, D. Husejnagić, R. Kazanović, I. Arsić, "Antibacterial activity of *Origanum compactum* essential oil tested on vaginal and cervical clinical bacterial strains", Acta Facultatis Medicae Naissensis, vol. 36, no3, pp. 219-228, 2019.
10. N. Ziklo, M. Bibi, L. Sinai, P. Salama, "Niacinamide Antimicrobial Efficacy and Its Mode of Action via Microbial Cell Cycle Arrest", Microorganisms, vol. *12*, 1581, 2024.
11. H.M. Kim, K.A. Byun, S. Oh, J.Y. Yang, H.J. Park, M.S. Chung, K.H. Son, K.A. Byun, "Mixture of Topical Forms of Polydeoxyribonucleotide, Vitamin C, and Niacinamide Attenuated Skin Pigmentation and Increased Skin Elasticity by Modulating Nuclear Factor Erythroid 2-like 2", Molecules, vol. 27, 1276, 2022.
12. E. Araviiskaia, B. Dréno, "The role of topical dermocosmetics in acne vulgaris", J. Eur. Acad. Dermatol. Venereol", vol. 30, 926–935, 2016.
13. S.Y. Lee, Y.H. Yun, G. Ahn, S.D. Yoon, "Preparation of niacinamide imprinted starch-based biomaterials for treating of hyperpigmentation", Int. J. Biol. Macromol, 232, 123382, 2023.
14. Z.D. Draelos, A. Matsubara, K. Smiles, "The effect of 2% niacinamide on facial sebum production", Journal of Cosmetic and Laser Therapy, vol. 8, pp. 96–101, 2006.

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Patent	[11] J. P. Wilkinson, "Nonlinear resonant circuit devices," U.S. Patent 3 624 125, July 16, 1990.
Standard	[12] *IEEE Criteria for Class IE Electric Systems*, IEEE Standard 308, 1969.
Thesis/Dissertation	[1] J. O. Williams, "Narrow-band analyzer," Ph.D. dissertation, Dept. Elect. Eng., Harvard Univ., Cambridge, MA, 1993.

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