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Corresponding Address
Karamanoğlu Mehmetbey University, Faculty of Health, Sciences, Department of Nutrition and Dietetics, Karaman, Turkey
E-mail: mtdogan1@gmail.com
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Computational study on anti-inflammatory and anti-hypertensive drug molecules interaction with base pairs

Işılay Öztürk[*1], Armağan Kınal[2], Toomas Tamm[3]

[*1] *Department of Chemistry, Faculty of Science, Ege University, Turkey*
[2] *Department of Chemistry, Faculty of Science, Ege University, Turkey*
[3] *Department of Chemistry and Biotechnology, School of Science, Tallinn University of Technology, Estonia*

*Corresponding author : isilayozturk@gmail.com
Orcid No: https://orcid.org/0000-0002-9134-6917

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Abstract: Non-steroidal anti-inflammatory drugs (NSAIDs) and anti-hypertensive drugs have been in use for a long time for the treatment of inflammation, pain, hypertension. Besides these functions, they also show different types of other activities. Many of them exhibit critical side effects in different types of cancer such as colon, lung, and breast cancer. In the present study, we computationally investigated the interactions of some nonsteroidal anti-inflammatory and anti-hypertensive drugs (acebutolol, naproxen, diflunisal, bisoprolol) with nucleobases and interaction of drugs with nucleobase pairs by optimized at the B3LYP/6-31+G (d), B3LYP-D2/6-31+G (d) and ωB97X-D /6-31+G (d) levels of DFT. The main purpose of this study is was to determine the strengths of drug-DNA-base interactions and drug-DNA-base pair interactions that can provide insights about the side effects of the drugs. The calculations were produced the following results. Acebutolol has the highest interaction between adenine in single base-drug complexes. However, acebutolol has the strongest interaction between the guanine-cytosine base pair. The ωB97X-D method, which accounts for dispersion interaction properly, gives better results than the B3LYP and B3LYP-D2 methods.

Keywords: NSAIDs; beta blockers; nucleobase ; nucleobase pairs; binding energies; DFT methods.

1 Introduction

The DNA - drug interaction has a major role in pharmacology and this interaction has a vital feature for the determination of the mechanisms of drug action. Non-covalent interactions are important for biological systems (Toupkanloo and Rahmani 2 018). Non-covalent DNA- interacting agents can cause a conformational change in DNA. Non-covalent interactions are weaker than a chemical bond and they have a vital role in controling the structure and function of DNA and RNA in understanding of replication and origin of life (Ghosh et al. 2010). Therefore, interactions between drugs and nucleobases play an critical role in determining side effects (Abbas 2017-Kennard 1993). Non-steroidal anti-inflammatory drugs (NSAIDs) are a significant class of compounds to reduce inflammation and pain associated with infection or injury. Nevertheless, when NSAIDs are used for a long time, they cause various side-effects such as kidney failure, gastrointestinal problems, colon, lung, and breast cancer (Azam F et al. 2018). The ß-blockers belong to the antihypertensives class of medications and they are mainly used to manage high blood pressure (hypertension), treat arrhythmia and decrease the risk of heart complications after a heart attack.

The studies performed in the past few years aimed to provide an understanding of the fact that how drugs interact with the nucleobases of DNA/RNA. In 2003, Baik et al. studied how two possible hydrolysis products of cis-platinum complexes bind to adenine and guanine nucleobases. Their findings indicate that guanine is the preferred reactant for platination, and guanine is more suitable both thermodynamically and kinetically. In 2018, Toupkanloo and Rahmani investigated some bicyclic fragments, which may have possible genotoxic effects, interacting with nucleobases. Hence, they focused on the π-π stacking interactions and this interaction leads to both an increase and a decrease in hydrogen bond lengths in the drug-nucleobase interactions. The main objective of this study is to quantitatively determine and scale the interactions of some nonsteroidal anti-inflammatory and anti-hypertensive drugs with nucleobases with quantum chemical methods, especially with DFT methods.

2 Materials and Method

This study consists of two main parts: a) interaction of drugs with nucleobases b) interaction of drugs with nucleobase pairs. All structures have been optimized at the B3LYP/6-31+G (d), B3LYP-D2/6-31+G (d) and ωB97X-D /6-31+G (d) levels of DFT. That each optimized geometry corresponds to a local minimum on the potential energy surface was identified via harmonic vibrational analyses. The effect of implicit water solvation on complex formation was calculated by employing integral equation formalism of the Polarized Continuum Model (IEF-PCM). All calculations were performed using the Gaussian09 program (https://gaussian.com/g09citation/)

3 Results and Discussion

3.1 Binding Enthalpies of Drugs to Nucleobases

In this part, we scrutinized the effects of each drug on a single nucleobase. In order to determine drug-nucleobase interactions, all drugs-nucleobase complexes were optimized at the B3LYP/6-31 G(d), B3LYP-D2/6-31 G(d) and ωB97X-D/6-31 G(d) levels of DFT. The binding energies were calculated exploiting the following equation.

$$\Delta H_{bind} = H_{298}(complex) - \left(H_{298}(drug) + H_{298}(base)\right) \quad (1)$$

Table 1 Binding enthalpy (kcal/mol) values of the drug–nucleobase complexes optimized with the B3LYP, B3LYP-D2 and ωB97X-D methods.

Drug name	Base	B3LYP	B3LYP-D2	ωB97X-D
Bisoprolol	A	2.02	8.3	-8.17
	T	1.7	3.33	-11.33
	G	-2.39	5.72	-11.78
	C	0.17	5.25	-7.22
Acebutolol	A	1.44	9.03	-12.56
	T	1.02	7.74	-7.9
	G	1.57	8.29	-9.51
	C	-2.83	*	-8.32
Naproxen	A	0.91	6.24	-9.7
	T	1.03	7.07	-8.45
	G	*	3.86	-11.11
Naproxen	C	0.94	7.36	-8.37
Diflunisal	A	-5.4	7.62	-7.45
	T	-6.05	7.22	-8.28
	G	-8.59	6.15	-7.59
	C	*	6.37	-6.91

* Calculations did not converge to a stable geometry.

Table 1 shows the binding enthalpy values of the drug–nucleobase complexes optimized with the B3LYP, B3LYP-D2 and ωB97X-D methods.

As seen from the table, the most negative binding energy is between adenine and acebutolol while the lowest one is between diflunisal and guanine. The ωB97X-D method predicted that all drug-nucleobase complexes are rather stable. Our expectation was that the B3LYP-D2 method would produce similar results since the D2 part includes the contribution of dispersion interactions that are not incorporated in the B3LYP method. However, this is not the case. Although the B3LYP methods produce somewhat reasonable results, the D2 correction made them worse. Hence, it can be said that the ωB97X-D method gives more reasonable results than the B3LYP-based methods.

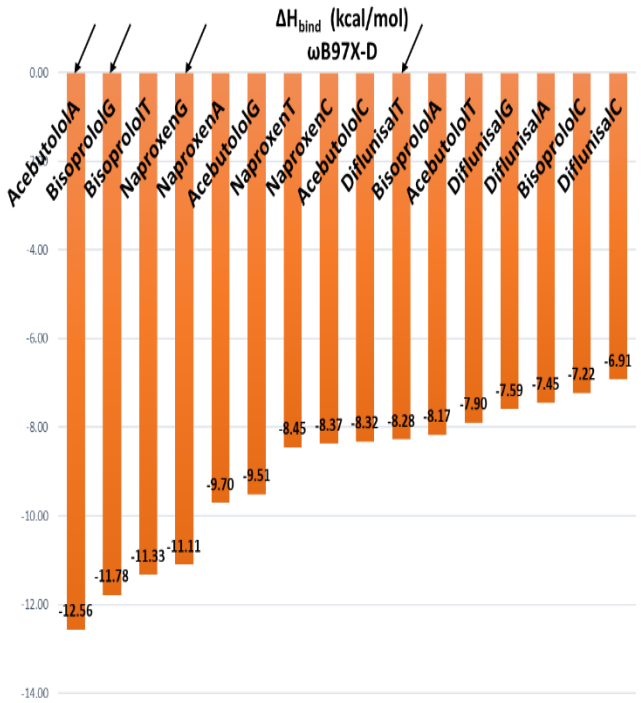

Figure 1 Highly interacting drug-base structures

The figure shows the highly-interacting-drug-nucleobase structures obtained with the ωB97X-D method. As previously stated, it is more likely that this method gives better results than the other two since it includes long-range interactions and it is claimed to give good results in the weakly interacting systems. Therefore, the ranking obtained with this method is presented here. Accordingly, acebutolol and adenine have the most negative binding energy among all single nucleobase–drug complexes. The second most interacting complex is the bisprolol-guanine complex. The next two complexes are naproxen-guanine and diflunisal-thymine complexes, too.

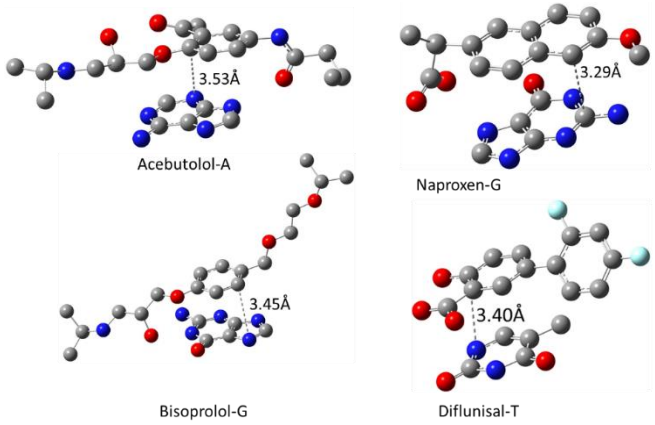

Figure 2 ωB97X-D optimized structures of drug–nucleobase complexes

3.2 Drug-Nucleobase-pair Binding Enthalpies

In this part, we investigated the effects of each drug on nucleobase -pairs. To determine these interactions, all drugs-nucleobase pair complexes were optimized at the B3LYP/6-31G(d), and ωB97X-D/6-31G(d) levels of theory. The binding energies were calculated using equation (1) as in the previous section.

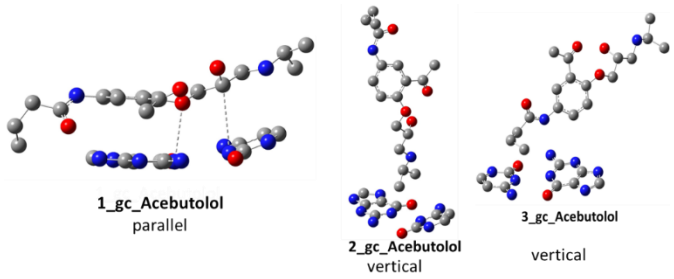

Figure 3 Orientations of drug–nucleobase pair complexes optimized by ωB97X-D

Drug molecules were interacted with nucleobase pairs in the orientations shown in Figure 3, and Table 2 shows the binding enthalpy values for all drugs that interact with the nucleobase pairs in these three orientations. The calculations predict that mostly the parallel oriented drug complexes have more negative interaction interaction than the vertical interacted ones.

As seen from the table, the strongest interaction is between Acebutolol and the G-C base pair. The ωB97X-D method predicted that all drug-nucleobase complexes be more stable than the ones predicted with B3LYP. However, in only one case, the B3LYP method predicts the π-π stacking interaction of the acebutolol drug with the A-T base pair approximately four times stronger than the one predicted by ωB97X-D. This is most probably spurious stability originating from the inability of the B3LYP method in this calculation. Because this high binding energy (-45 kcal/mol) is larger than the covalent bond in the F_2 molecule whose bond dissociation energy is about 37 kcal/mol [Ref F2_BDE]. This clearly shows that B3LYP does not work for this particular case. Figure 4 displays the binding enthalpy order of the drug-nucleobases pairs calculated with the ωB97X-D method.

Table 2 ΔH_{bind} (kcal/mol) values of B3LYP and ωB97X-D optimized drug–nucleobase pair complexes

Interreaction Site	Drug Name	Basepair	B3LYP	ωB97X-D
1_gc	Bisoprolol	G-C	1.26	-13.8
2_gc			1.74	-4.67
3_gc			1.66	-4.82
1_at	Bisoprolol	A-T	*	-13.38
2_at			1.67	-4.56
3_at			1.68	-4.66
1_gc	Acebutolol	G-C	0.63	-17.56
2_gc			0.59	-3.73
3_gc			1.13	-9.62
1_at	Acebutolol	A-T	-45.02	-11.87
2_at			-44.59	-3.67
3_at			-45.34	-6.63
1_gc	Naproxen	G-C	0.19	-9.99
2_gc			0.85	-12.79
3_gc			-1.83	-12.69
1_at	Naproxen	A-T	0.74	-10.31
2_at			1.02	-11.3
3_at			-2.29	-12.97
1_gc	Diflunisal	G-C	-6.15	-14.56
2_gc			-5.9	-11.52
3_gc			*	*
1_at	Diflunisal	A-T	-6.74	-14
2_at			-5.9	-14.76
3_at			-5.87	-10.94

* Calculations did not converge to a stable geometry.

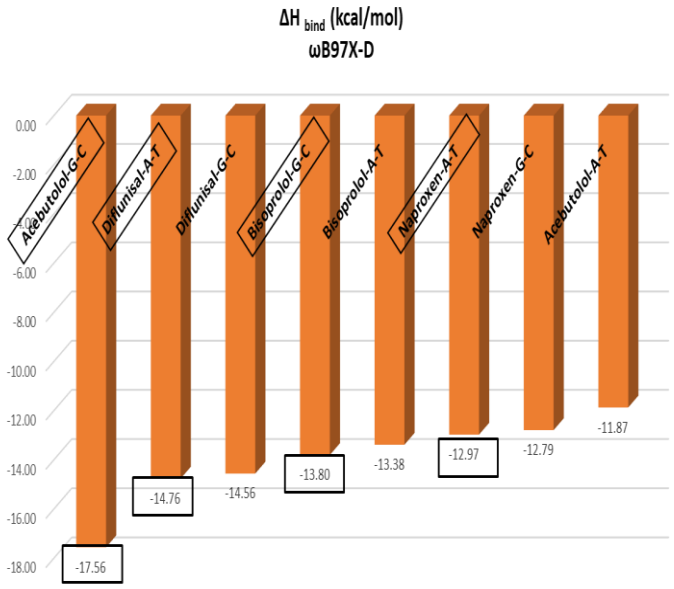

Figure 4 The highly interacting drug-base pair structures

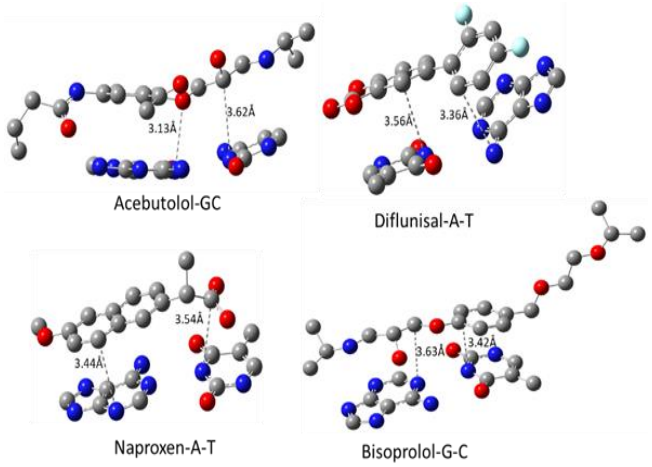

Figure 5 ωB97X-D optimized structures of drug–nucleobase pair complexes

Figure 5 shows the highly-interacting -drug-nucleobase structures obtained with the ωB97X-D method. Acebutolol and Bisoprolol, which are antihypertensive drugs, show the largest interaction with guanine-cytosine base pair. Besides, Diflunisal and Naproxen which are Non-steroidal anti-inflammatory drugs, give the largest interaction with the adenine-thymine base pair.

Table 3. ΔH_{bind} (kcal/mol) values of ωB97X-D and B3LYP, B3LYP-D2 optimized the parallel-oriented drug–nucleobase pair complexes

Interreaction site	B3LYP	B3LYP-D2	ωB97X-D
Bisoprolol-G-C	*	11.39	-13.8
Bisoprolol-A-T	1.26	11.77	-13.38
Acebutolol-A-T	-45.02	11.35	-11.87
Acebutolol-G-C	0.63	*	-17.56
Naproxen-A-T	-2.29	3.68	-12.97
Naproxen-G-C	0.85	13.29	-12.79
Diflunisal-A-T	-5.9	13.39	-14.76
Diflunisal-G-C	-6.15	12.6	-14.56

* Calculations did not converge to a stable geometry.

Table 3 shows the parallel-oriented drug-nucleobase pair complexes that were optimized at the B3LYP/6-31G(d), B3LYP-D2/6-31G(d) and ωB97X-D/6-31G(d) levels of DFT. All of these complexes are π-π stacking complexes. Here, it is seen that the B3LYP underestimates the stability of the complexes as expected except for acebutolol A-T as explained above. However, B3LYP-D2 results are very surprising. Although we performed D2 calculations to improve the results of B3LYP method, B3LYP-D2 made them worse as in the single base-drug interaction case.

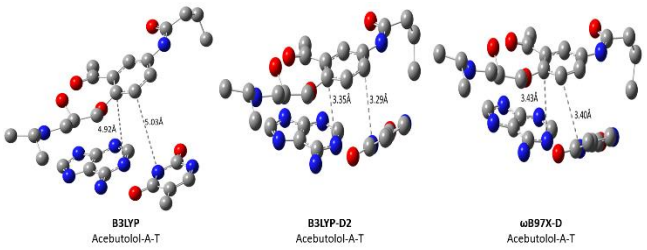

Figure 6 Optimized structures of drug–nucleobase pair complexes

Table 4 Distances between hydrogen bonds of ωB97X-D optimized drug–G-C basepair complexes

Angstrom	O-H	N-H	O-H	difference	difference	difference
G-C	1.840	1.906	1.858	0.000	0.000	0.000
G-C- Acebutolol	1.849	1.905	1.854	0.009	-0.001	-0.004
G-C-Bisoprolol	1.857	1.899	1.866	0.018	-0.007	0.009

Table 5 Distances between hydrogen bonds of ωB97X-D optimized drug– A-T basepair complexes

Angstrom	O-H	N-H	difference	difference
A-T	1.909	1.861	0.000	0.000
A-T-Diflunisal	1.967	1.857	0.058	-0.004
A-T-Naproxen	1.863	1.896	-0.045	0.034

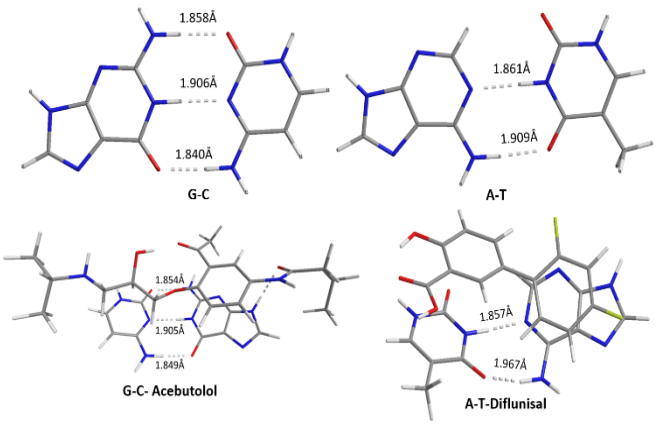

Figure 7 Intermolecular interaction of drug–nucleobase pair complexes

Table 4 shows that the acebutolol affects the bond lengths of the hydrogen bonds between the G-C base pair. On the other hand, the diflunisal affects the bond lengths of the hydrogen bonds between the adenine-thymine base pairs, shown in Table 5 Accordingly, it is likely that these drugs may cause DNA breakage in long-term usage.

4 Conclusion

In this study, we calculated drug-nucleobase and drug nucleobase base-pair interactions with various DFT methods, and we aimed to quantitatively determine the strengths of drug-DNA-base interactions that may improve our understandings about the side effects of the drugs. The most negative binding energy among the drug-single base complexes is between acebutolol and adenine. In the base pair drug complexes, however, acebutolol has the strongest attractive interaction between the guanine-cytosine base pair. Furthermore, we can conclude that the ωB97X-D method gives better results for such non-covalently interacting systems than both the B3LYP and B3LYP-D2 methods since this method accounts for dispersion interaction more accurately.

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Surface sterilization of *Staurogyne repens* (Nees) Kuntze with hydrogen peroxide

Merve Sifa Hane Kose[1], Muhammet Dogan[2], Gokhan Sadi[1*]

[*1]*Department of Biology, Kamil Ozdag Faculty of Science, Karamanoğlu Mehmetbey University, Karaman, Turkey.*
[2]*Department of Nutrition and Dietetics, Faculty of Health Sciences, Karamanoğlu Mehmetbey University, Karaman, Turkey.*

*Corresponding author : sadi@kmu.edu.tr
Orcid No: https://orcid.org/0000-0002-6422-1203

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Abstract: Sterilization is the killing, destruction or removal of all forms of microorganisms with a substance or an object. In tissue culture studies, the environment and equipment are sterilized. Likewise, the plant to be produced must be sterilized. It uses many methods and chemicals for the surface sterilization of plants. In this study, surface sterilization of *Staurogyne repens* (Nees) Kuntze was investigated using hydrogen peroskite (H_2O_2) at different times (10-30 min) and concentrations (3.7- 7.4% v/v). Nodal explants were used in trials. No plant growth regulator was added to the culture medium. Contaminations in food media started to be observed after 5 days and all data were collected after four weeks. While bacterial contaminations were recorded, a few fungal contaminations were also observed. High levels of H_2O_2 negatively affected the regeneration capabilities of explants. Also, some explants died due to H_2O_2. Contamination percentages were recorded between 40-100% in H_2O_2 applied environments. The highest sterilization rate (25%) were obtained in explants exposed to 5.5% H_2O_2 for 20 min. It was then recorded on explants treated with 5.5% H_2O_2 for 30 minutes (20%). As a result, surface sterilization of *S. repens* was accomplished using H_2O_2. These results can be helpful for surface sterilization and tissue culture studies of *S.repens.*

Keywords: Contamination; nodal explan; sterilization; tissue culture

1 Introduction

Humans use various methods to make plants of nature more favourable. They continuously develop these methods depending on the needs and conditions. These methods are called "culture". Plant cultures can be divided into three main groups according to their historical development. The first is classical cultures, which include vegetative and generative reproduction (Boedeltje et al. 2008); the second is water and sand cultures (McCall 1915); the last one is the Plant Tissue Culture which is gaining importance today (Priyadarshan 2019; Phillips and Garda 2019; Rojsanga et al. 2020). Tissue culture is the cultivation of small tissue and organ parts in sterile and appropriate nutrient environments (Ahmad et al. 2020; Gupta et al. 2020).

Tissue culture enables the production of a large number of plants with the same characteristics, both phenotype and genotypic (Dogan 2018; Sax et al. 2019). In this technique, many parts (explants) such as shoot tips (Dogan 2019), node (Mishra et al. 2019) and internode (Koike et al. 2018), hypocotyl (Gutiérrez et al. 2019), leaves (Pathak et al. 2019) and roots (Carvalho et al. 2019) were used to create new plants.

Microorganisms are considered as contamination in tissue culture studies. The most important losses in plant culture are caused by contamination. These microorganisms can be viruses, bacteria, yeast, fungi. If the sterilization is insufficient, fungi, yeast and bacteria may occur (Oyebanji et al. 2009; Mandeh et al. 2012). The presence of microorganisms such as bacteria and fungi in tissue culture techniques is one of the factors preventing plant growth and development. Plants must be stored in sterile conditions to be reproduced *in vitro.* Sterile laboratory conditions are created to reduce the damaging effect of microorganisms. Although surface sterilization is applied, cultures are sometimes not free of bacteria and fungi. Generally, the presence of bacteria in the culture medium may be evident at the first stage. However, microorganism emergence in the nutrient medium can be observed later in the culture. Various chemicals are used in plant tissue cultures to minimize contamination or eliminate it completely. Some of these are ethanol, calcium or sodium hypochlorite, mercury chloride and hydrogen peroxide (Mahmoud and Al-Ani 2016; Orlikowska et al. 2017; Javed et al. 2017).

In this study, surface sterilization of *Staurogyne repens* (Nees) Kuntze was investigated using hydrogen peroxide (H_2O_2) at different times and concentrations. Thus, an important contribution was made to the production of this plant with tissue culture.

2 Materials and Method

Plant material was obtained from the aquarium store. In the studies, MS (Murashige and Skoog 1962) mineral salt and vitamins were used as nutrient media. No plant growth regulator was added to the culture medium. In addition, 3% sucrose (Duchefa) and 0.65% agar (Duchefa) were transferred to the food medium. The pH of the food medium was sterilized at 121°C for 20 min. under 1.2 atmospheric pressure after adjusting to 5.7 ± 1 using 1 N NaOH and 1 N HCl.

The plant was kept under running water for 30 min in order to remove it from the wastes and reduce the microorganism density before surface sterilization. The upper body parts were cut (3-5 cm) and treated with H_2O_2 (35% - Merck Millipore) (3.7%, 5.5% and 7.4% v/v) at different duration (10, 15, 20 and 30 min). Then, rinsing was performed three times with sterile distilled water for five min. Nodal explants were isolated and transferred to test tubes. All values were taken for sterilization after four weeks.

3 Results and Discussion

Plant tissue culture is a modern biotechnological technique for the production of plants or herbal products. But the success of tissue culture depends on maintaining sterile production conditions. Contaminations from microorganisms cause extra time, effort and material spending in tissue culture studies. This is an important monetary problem. It is also the most serious factor responsible for losses in tissue culture. Bacterial or fungal contaminations can be environmentally sourced or endogenic. Many processes are carried out on plants to prevent microorganism pollution (Javed et al. 2017). In this study, H_2O_2 was treated with different time (10-30 min) and concentrations (3.7-7.4 v/v) for surface sterilization of *S. repens*. The nodal explants were used in sterilization studies. All values for sterilization were taken after four weeks (Table 1). Similarly, the use of H_2O_2 for surface sterilization was reported in *Stevia rebundiana* Bertoni (Halim et al. 2016), *Zantedeschia aethiopica* L. (Chen et al. 2017), Phoenix dactilefra L. (Metwaly et al. 2018), *Prunus persica* (L.) Batsch (Al Ghasheem et al. 2018) plants. In addition, the use of $HgCI_2$ in *Musa paradisiaca* L. (Shukla et al. 2019), Sugarcane (Singh and Gupta 2019) and *Catharanthus roseus* L. (Vandana et al. 2020) plants and the use of NaOH in *Phanera sirindhorniae* (Sirimat and Sakulsathaporn 2019) and *Cryptocoryne wendtii* (Klaocheed et al. 2020) plants have been reported for surface sterilization. This showed that different disinfectants can be used for surface sterilization of plants.

Table 1 Surface sterilization data of nodal explants treated with H_2O_2 at different concentrations and time

Hydrogen peroxide (H_2O_2)		Contamination rate (%)	Sterile and dyed explant rate (%)	Sterile and live explant rate (%)
%	Duration (min)			
3.7	10	100	-	-
3.7	15	100	-	-
3.7	20	85	10	5
3.7	30	75	20	5
5.5	10	100	-	-
5.5	15	85	5	10
5.5	20	55	20	25
5.5	30	50	30	20
7.4	10	90	-	10
7.4	15	70	20	10
7.4	20	50	35	15
7.4	30	40	50	5

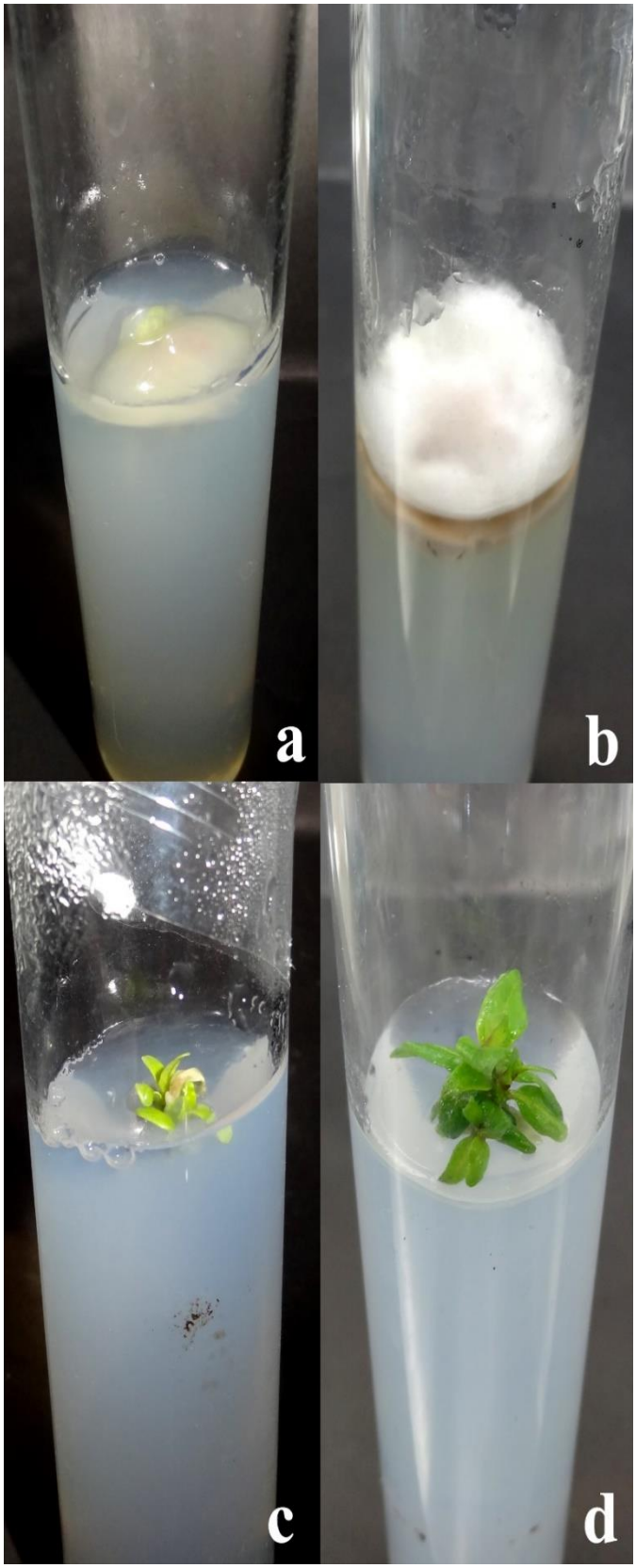

Fig 1 *S. repens* explants treated with H_2O_2 at different rates and periods. (a) bacterial contamination (b) and fungal contamination on the explant (c, d) sterile-strong shoots from the explant treated with 5.5% H_2O_2 for 20 min.

The first contaminations in the culture medium started to be observed on the 5th day. These contaminations became more evident after four weeks. The contaminations covered some explants and caused their death. In general, bacterial contaminations were observed (Fig 1a) and fungal contaminations were also detected in a small number of tubes (Fig 1b). As the H_2O_2 concentration and application time increased, the rate of the contaminations decreased. Some explants were damaged and died with high H_2O_2 concentration.

Contamination percentages were recorded between 40-100% in H_2O_2 applied environments. Maximum contamination levels (100%) were observed in explants treated with 3.7% H_2O_2 for 10 and 15 min. In general, the contamination level detected in cultures treated with high H_2O_2 was low. Minimal contamination (40%) was detected in explants exposed to 7.4% H_2O_2 for 30 min. Sereda et al. (2017) have followed a rather complicated path for surface sterilization of *S. repens.* Before sterilization, the plants were washed for 15 min with 0.01% tween-80 and then rinsed with running water. Plants were washed with sterile water after being treated with 70% ethanol. In addition, 5% chloramine B solution, 1% NaOCl and 0.1% $HgCl_2$ were applied in the sterilization process. The best sterile explants achieved 0.1% and 0.1% with $HgCl_2$ (5 min). In the current study, an easier way was proposed for surface sterilization of *S.repens* than Sereda et al. (2017).

In surface sterilization trials, sterile and live explant levels were determined between 5-25%. The highest sterilization rate (25%) were obtained in explants that interacted with 5.5% H_2O_2 for 20 min (Fig 1c and d). It was then recorded in explants treated with 5.5% H_2O_2 for 30 min (%20). Similarly, Al Ghasheem et al. (2018) applied 5% and 10% H_2O_2 for 10 and 20 min for the surface sterilization of the shoot tip and nodal explants of *P. persica* . They achieved the highest sterile and intact explants after 20 min treatment with 10% H_2O_2 for the shoot tip (25%) and 10 and 20 min treatment with 10% H_2O_2 for the nodal explants (20%).

5 Conclusion

Surface sterilization is an initial and important stage of tissue culture. After this stage, trials are established for reproduction. In this study, successful surface sterilization of *S. repens*, which is important in the aquarium industry, was explained using H_2O_2. The use of low levels of H_2O_2 was not successful for surface sterilization. In general, bacterial contamination has been detected in the food medium. The use of high levels of H_2O_2 also caused the explants to die, although it reduced the level of contamination. Although some explants were sterile, they lost their regeneration ability. It is very important to use disinfectant in optimum concentration. The best results were found in explants treated with 5.5% H_2O_2 for 20 min. These results can be helpful for surface sterilization and tissue culture studies of *S. repens*.

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Antioxidant Potential of *Hypericum spectabile* JAUB. ET SPACH

Falah Saleh Mohammed[*1], Ali Erdem Şabik[2], Muhittin Dogan[3], Zeliha Selamoglu[4], Mustafa Sevindik[5]

[*1] *Department of Biology, Faculty of Science, Zakho University, Zakho, Iraq*
[2] *Department of Chemistry and Chemical Processing Technologies, Bahçe Vocational School, Osmaniye Korkut Ata University, Osmaniye, Turkey*
[3] *Department of Biology, Faculty of Science, Gaziantep University, Gaziantep, Turkey*
[4] *Department of Medical Biology, Faculty of Medicine, Nigde Ömer Halisdemir University, Nigde, Turkey*
[5] *Department of Food Processing, Bahçe Vocational School, Osmaniye Korkut Ata University, Osmaniye, Turkey*

*Corresponding author : falah.sindy@uoz.edu.krd
Orcid No: https://orcid.org/0000-0001-9083-1876

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Abstract: Plants have been indispensable products of nature in human history. People used plants for many purposes such as building shelters, smells, flavors, medicines, warming tools, and weapons. In this study, antioxidant and oxidant potentials of *Hypericum spectabile* Jaub. & Spach were determined. Ethanol extract of the plant was extracted in soxhlet apparatus. Antioxidant and oxidant potentials were determined using Rel Assay kits. Free radical scavenging activity was measured using the DPPH method. TAS value of the plant was determined as 4.215±0.038, TOS value as 23.421±0.161 and OSI value as 0.556±0.001. DPPH free radical scavenging activity increased with increasing concentration. It showed 86.74% inhibition at 2 mg/mL extract concentration. As a result, it was determined that *H. spectabile* has high antioxidant potential.

Keywords: Antioxidant, *Hypericum spectabile*, Medicinal plants, Oxidant

1 Introduction

Humans have used plants as a source of healing in the treatment of many diseases for centuries. Especially in the backward societies that do not have the medical treatment possibilities of societies with high socioeconomic level, millions of people are still taking advantage of phytotherapy, a branch of alternative medicine (Aydın and Sevindik, 2018; Okan et al. 2018; Mohammed et al. 2020a). The genus *Hypericum* L., a member of the *Hypericaceae* family, contains about 400 species in the world, about 80 species in Turkey, all small herbaceous perennials (Robson, 1967, 1988; Dönmez 2000). *Hypericum* (*Hypericaceae*) is one of the plants used traditionally in medicine, crop protection, and flavoring, as well as fragrance in food (Isman et al. 2001; Daferera et al. 2003). Plants of the genus *Hypericum* are known for the production of naphthodianthrones such ashypericin and pseudohypericin possessing antineoplastic, antiviral and antibacterial properties, their proposed precursors emodin or emodin anthrone, as well as phloroglucinols and flavonoids (Nahrstedt and Butterweck 1997). In this study, total antioxidant status, total oxidant status and oxidative stress index of *Hypericum spectabile* Jaub. & Spach plant collected from Gaziantep (Turkey) were determined.

2 Materials and Method

Hypericum spectabile plant was collected from Gaziantep (Turkey) province. The plant was diagnosed using Flora of Turkey Volume 2 (Davis 1967). Aerial parts of the plant samples were collected. 30 g of the collected samples were weighed. It was then extracted with ethanol (EtOH) at 50 ° C in the soxhlet extractor for about 6 hours. The extracts obtained are concentrated with a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

2.1 Total Antioxidant and Oxidant Analyses

The antioxidant and oxidant status of the above-ground parts of the plant were determined using Rel Assay TAS and TOS kits (Erel 2004; Erel 2005). The calibrator Trolox was used in the TAS test. Calibrator hydrogen peroxide (TOS) was used in the TOS test. OSI (Arbitrary Unit = AU) value was determined according to the formula below (Erel, 2005).

$$OSI\ (AU) = \frac{TOS, \mu molH_2O_2\ ^{Equiv.}/_{L}}{TAS, mmol\ Trolox\ ^{Equiv.}/_{L} \times 10}$$

2.2 DPPH radical scavenging activity

Different stock solutions (0.25, 0.5, 1 and 2 mg/mL) were prepared using DMSO (Dimethyl sulfoxide). 50 µL of the prepared solutions were added to 160 µL of 0.039% DPPH. Then, it was incubated for 30 minutes. After the incubation process, absorbance was determined at 517 nm. These processes were repeated for all stock solutions (Shimada et al. 1992). Rosmarinic acid (RA) and ascorbic acid (AA) were used as reference antioxidants. Finally, DPPH free radical scavenging percentages; % inhibition = [(Abs control-Abs sample)\Abs control] x100.

3 Results and Discussion

Imbalance between endogenous antioxidants and oxidant compounds leads to oxidative damage of metabolic reactions (Sevindik, 2018). Antioxidants serve to suppress or eliminate the harmful effects of free radicals on living organisms. However, in cases where endogenous antioxidants are insufficient against reactive oxygen species, the use of supplementary antioxidants is very important. Many herbs used in complementary medicine have antioxidant potential (Sevindik, 2019; Mohammed et al. 2020b). In our study, TAS, TOS and OSI values of EtOH extracts of *H. spectabile* were determined. The findings obtained are shown in Table 1.

Table 1 TAS, TOS and OSI values of *Hypericum spectabile*

Sample	TAS (mmol/L)	TOS (µmol/L)	OSI
H. spectabile	4.215±0.038	23.421±0.161	0.556±0.001

Values are presented as mean±SD; Experiments were made in 5 parallels

Table 2 DPPH radical scavenging activity of *Hypericum spectabile*

Concentration (mg/mL)	Ascorbic acid (%)	Rosmarinic acid (%)	EtOH
0.25	65.47	37.32	36.01
0.5	72.77	43.51	53.09
1	89.1	68.76	73.07
2	94.88	76.32	86.74

In our study, it is seen that the EtOH extract of *H. spectabile* changes DPPH free radical activity depending on the increase in concentration. It was determined that the EtOH extract of the plant has higher activity than the standard Rosmarinic acid. It is seen that it exhibits lower activity than ascorbic acid (Table 2). It has been reported in previous studies that *H. spectabile* has an antioxidant potential using different methods (Zheleva-Dimitrova et al., 2010; Özkan et al., 2018). In this context, the DPPH potential of *H. spectabile* was similar to the literature studies in our study.

In our study, the antioxidant potential was determined for the first time using TAS kits. In studies on different plant species using TAS kits, the TAS value of *Mentha longifolia* L. Hudson ssp. *longifolia* was reported as 3.628 mmol/L, TOS value was 4.046 µmol/L and OSI value was 0.112 (Sevindik et al. 2017). The TAS value of *Rosa canina* L. was reported as 4.602 mmol/L, TOS value was 6.294 µmol/L and OSI value was 0.138 (Pehlivan et al. 2018). TAS value of *Adiantum capillus-veneris* L. was reported as 3.086 mmol/L, TOS value was 21.532 µmol/L and OSI value was 0.698 (Mohammed et al. 2019a). TAS value of *Silybum marianum* (L.) Gaertn. was reported as 5.767 mmol/L, TOS value was 12.144 µmol/L and OSI value as 0.211 (Mohammed et al. 2019b). Compared to these studies, it was determined that the TAS value of *H. spectabile* used in our study was higher than *M. longifolia* ssp. longifolia and *A. capillus-veneris*, but lower than *R. canina* and *S. marianum*. TAS value shows the whole of the antioxidant compounds produced by the plant (Mohammed et al. 2018). This difference between the TAS values of plant species is thought to be due to the plant's potential to produce compounds with antioxidant properties. The TOS value shows the oxidant compounds that the plant produces in its body with environmental effects (Mohammed et al. 2018). It is seen that the TOS value of *H. spectabile* is higher than that of *M. longifolia* ssp. *longifolia*, *A. capillus-veneris*, *R. canina* and *S. marianum*. This difference is thought to be due to the environment in which the plants grow and their potential to produce oxidant compounds. The OSI value shows how much the plant suppresses endogenous oxidant compounds with endogenous antioxidant compounds (Mohammed et al. 2018). It is seen that as the OSI value increases, the antioxidant defense system of the plant is insufficient against oxidant compounds. It was determined that the OSI value of *H. spectabile* was higher than *M. longifolia* ssp. *longifolia*, *R. canina* and *S. marianum*, but lower than *A. capillus-veneris*. As a result, it was determined in our study that the plant has antioxidant potential despite its high TOS value.

5 Conclusion

In this study, the antioxidant and oxidant potentials of *H. spectabile* were determined. As a result of the studies, it was determined that the plant has antioxidant potential. In addition, despite its high oxidant values, it is thought that it can be used as a natural antioxidant source due to its antioxidant potential.

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Investigation of the removal of malachite green and copper ions by dual system using natural and biochar pea shells

Sevda Esma Darama[1*], Semra Çoruh[1]

[*1]*Environmental Engineering Department, Engineering Faculty, Ondokuz Mayıs University, Samsun, Turkey.*

*Corresponding author : sevda.akkaya@omu.edu.tr
Orcid No: https://orcid.org/0000-0002-6747-4679

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Abstract: This study aims to investigate the simultaneous removal of natural and thermally modified (biochar) pea shells, malachite green dye and copper heavy metal. Here, the removal of dye and heavy metal ions at the same time, depending on different adsorption parameters, was studied by using a dual biological system. Adsorption parameters were selected as different contact times (1-120 min), different pollutant initial concentrations (30-400 mg/L) and different adsorbent dosages (0.4-12 g/L). Also, adsorption experiments were applied to different isotherm models to reveal the structure of adsorption better. As a result of all these studies, natural pea shells have a removal efficiency 70% for copper ions and 94% for malachite green ions, while biochar pea shells have a removal efficiency 85% for copper ions and 99% for malachite green ions. Also, natural and biochar adsorbents have adapted to the Freundlich isotherm model. These results showed that pea shells could be an effective and inexpensive adsorbent for dye and heavy metal removal.

Keywords: Adsorption; biochar; copper removal; dye removal; pea shell.

1 Introduction Industrial developments in recent years have left their impression on the environmental society. The dye and heavy metals are two principal concomitant pollutants derived from many industries such as dyestuff, textile, leather, printing, paper and plastic, which continually bring about a severe environmental issue due to their high toxicity to human health (Mohan et al. 2007). Therefore it is essential to verify the water quality, significantly when even just 1.0mg/L of dye concentration in drinking water could significantly impact color and heavy metals, making it unfit for human consumption (Malik et al. 2007). Furthermore, dyes and heavy metals can affect aquatic plants because they reduce sunlight transmission through water. Also, dyes may impart toxicity to aquatic life and maybe mutagenic, carcinogenic. They may cause severe damage to human beings, such as dysfunction of the kidneys, reproductive system, liver, brain and central nervous system (Kadirvelu et al. 2003; Shen et al. 2009). The removal of color and heavy metals from waste effluents becomes environmentally crucial because even a small quantity of dye in water can be toxic and highly visible (Chiou et al. 2004). The wastewaters containing dyes and heavy metals can be diminished; the level wanted with the physical, biological and chemical methods. Moreover, different physiochemical properties of dyes and heavy metals make the disposal of co-polluted effluent more challenging. Therefore, it is urgent to develop novel approaches to eliminate these harmful pollutants simultaneously. However, they have their inherent limitations, such as generating a large amount of sludge, less efficiency, sensitive operating conditions and costly disposal. Thus, the adsorption method is a relatively new process emerging as a potential alternative for removing heavy metals (Agarwal et al. 2017). Various efficient adsorbents, including carbon-based materials, biomass or biomaterials, minerals, polymers and metal-organic frameworks such as peat, wood, barley, rice husk, plant straw, rice bran, peanut shell, almond shell, hazelnut shell, algal biomass, fruit stones, plum kernels, banana pith, soybean, cottonseed hulls, humic acids, pea shell, corn stalk, tree bark, sugar beet pulp, leaves, green algae, activated carbon fibers and coconut have been widely employed to alleviate the ever-increasing reluctant effluent pollution.

Therefore there is a constant need to have an active process that can efficiently remove these dyes and heavy metals (Lee et al. 2006). Adsorption by agricultural by-products used recently as an economical and realistic method for removing different pollutants has proved to be efficient at

removing many pollutants such as heavy metals and dyes (Adegoke and Bello 2015). As a significant class of carbon-based materials, the biomass-derived carbonaceous adsorbent is a promising adsorbing material for effective wastewater treatment due to its low-cost and easy access.

In this paper, a study of simultaneous adsorption of mixture copper (Cu) and malachite green (MG) dyes in a binary system was done using natural and biochar pea shells. The effects of the influential parameters such as contact time, initial metal and dye ions concentration and adsorbent dosages on natural pea shells' adsorption process.

2 Materials and Method

2.1 Materials Pea shells were used in the experiments. These were cut into small pieces (1-2 cm) before use. The pea shells were rinsed thrice with hot water, thrice with cold water, and dried in an oven at 105 °C for 24 hours to remove moisture content. After, they were ground and sieved for a particle size of 0.2–2.0mm.

2.2 Reagent Copper ion solutions used in the experiment were prepared by dissolving the appropriate amount of $CuCl_2.6H_2O$ in distilled water according to Standard Methods 27. All chemicals used were analytical grade reagents of the highest quality available and deionized water was used. The solutions' pH was adjusted with HCl or NaOH solutions using a WTW 330 pH-meter with a combined pH electrode.

The basic dye, malachite green ($C_{23}H_{26}ClN_2$), was selected for adsorption studies. The stock solution of 1000 mg/L was prepared by dissolving accurately weighed amounts of malachite green in 1000 mL distilled water. The initial pH of solutions was adjusted to the required value by using NaOH or HCl solutions. All experiments were conducted in duplicate and the average values were used for data analysis.

2.3 Production of biochar pea shell The sieved pea shells were passed through pyrolysis using a steel reactor under nitrogen gas flow. The pyrolysis conditions; 600°C temperature, 5°C/min slow pyrolysis speed, 1 hour standby time and under 100 mL/min nitrogen gas flow. Pyrolysis conditions were determined by testing similar pyrolysis studies in the literature and selecting average values (Qiu et al. 2018; Demiral and Şamdan 2016; Georgieva et al. 2020; Kaya et al. 2020). From placing in the reactor at 25 g natural pea shell, 6 g of biochar pea shell were obtained as dry granules.

2.4 Adsorption experiments in dye-metal binary mixtures The batch tecnique conducted the experiments in 150 ml Erlenmeyer flasks containing 25 mL of distilled water at the desired level of heavy metal ions and dye. Batch adsorption procedure was used to determine the effect of various operating conditions on the adsorption process. This was performed by the addition of 0.01-0.3 g of pea shells to 25 mL of the binary metal-dye solution at room temperature. The effects of the contact time (1–240 min), adsorbent dosages (0.4-12 g/L) and initial concentration of Cu(II) and dye ions (30-400 mg/L) were investigated. The aqueous media containing desired combinations of heavy metal ions and the dye were prepared by diluting stock solutions of Cu(II) and malachite green dye and mixing them in an aqueous solution. Before analysis, the samples were centrifuged at 8000 rpm for 10 min using a centrifuge and the supernatant fractions were analyzed for the remaining heavy metal ions and dye. All of the biosorption experiments were repeated twice to confirm the results. After adsorption, malachite green concentration was measured with a Thermo brand Aquamate model UV spectrophotometer at 620 nm wavelength. An ATI-UNICAM 929 model Atomic Absorption Spectrophotometer (AAS) was used to determine Cu(II) ions. Concentration values were read with a hollow copper cathode lamp in acetylene flame using the direct aspiration technique. The device gives the concentration value in mg/L.

According to the contact time, the removal efficiency values, adsorbent dosage and initial pollutant concentration after adsorption were calculated with the following Equation 1;

$$E\ (\%) = \frac{(C_0 - C_e)}{C_0}\ x\ 100 \qquad (1)$$

Where; E (%) is removal efficiency, C_0 (mg/L) is initial concentration, C_e (mg/L) is concentration after adsorption. Also, adsorption capacities (qe) for the adsorbents were calculated. Adsorption capacity is the amount of milligram adsorbate held per gram adsorbent and is expressed in mg/g unit.

Besides, adsorption capacities were calculated in all adsorption studies. Adsorption capacity is the amount of adsorbate that the adsorbent's unit mass (or volume) can adsorb. The adsorption capacity (qe) is formulated in Equation 2;

$$qe(mg/g) = \frac{C_0 - C_e}{m} xV \qquad (2)$$

Where; C_0 and C_e (mg/L) are the initial concentration and equilibrium concentration of dye solution, respectively, V (L) is the volume of dye solution and m (g) is the mass of adsorbent.

2.4 Adsorption isotherms In this research, to determine the mechanism of dye and metal biosorption on the natural and biochar pea shells, the experimental data were applied to the Langmuir and Freundlich isotherm equations. The Langmuir sorption isotherm is the best known of all isotherms describing sorption and it has been successfully applied to many sorption processes. Langmuir isotherm is used to describe the single-layer adsorption characteristics of the clinoptilolite. The Freundlich isotherm is an empirical model that is based on adsorption on the heterogeneous surface area.

3 Result and Discussion

3.1 Effect of contact time on dye and metal removal The contact time study was carried out in periods ranging from 1-240 minutes. Removal efficiencies of natural and biochar pea shells are shown on separate charts for malachite green and copper. Also, qe (mg/g) value, expressed as the adsorption capacity for all contact time periods, was also calculated. Figure 1 shows the removal results of malachite green dye. Accordingly, it is seen that natural and biochar pea shells provide similar removal values. Besides, the adsorption reached equilibrium very quickly with a yield of over 90%

starting from the 15th minute. In the malachite green removal study conducted by Khan et al. (2014) using activated pea shell against contact time, adsorption reached equilibrium in a concise time. They quickly achieved 80% removal efficiency at the beginning of the contact time period.

In the graph of the relationship between adsorption and contact time, the increase in percentage removal efficiency seen at the beginning occurs as a result of the high surface area available at the beginning, and it is seen that the dye and metal absorption rate starts to decrease due to the decreasing surface as the time increases. With the occurrence of saturation in the adsorbent, adsorption begins to be held inside instead of the outer surface. Due to the smaller inner surface area, the increased contact time causes the efficiency to decrease or remain constant (Yu et al. 2000).

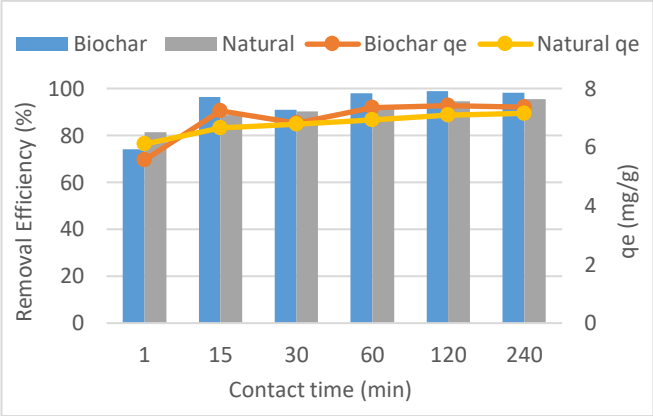

Fig. 1 Effect of contact time on malachite green removal

Figure 2 shows the copper removal results. Biochar pea shell removal efficiency is 5-17% higher than natural pea shell. The highest removal efficiency was achieved as 85% in the 30th minute with the biochar. Haq et al. (2020) obtained the equilibrium at 30 min in the contact time study in which zinc removal with the activated pea shell. After 30 minutes of up to 80 minutes, the efficiency of the removal remained around 70%. Küçükgül and Kutlu (2006), in their copper removal study against the contact time with biochar oak wood, the removal efficiency, which was around 40% at the beginning, reached the balance by increasing to 90% in the 180th minute.

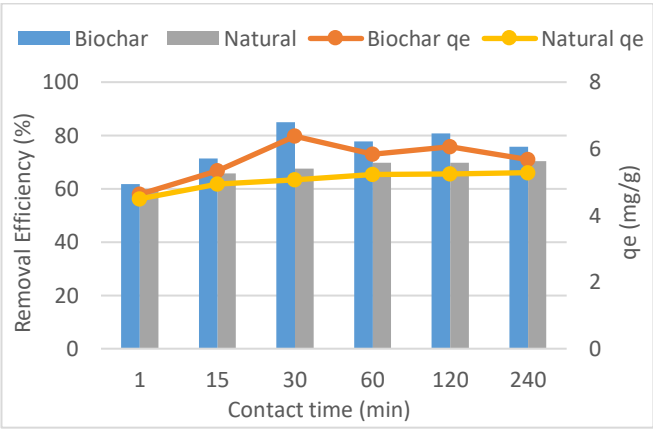

Fig. 2 Effect of contact time on copper removal

3.2 Effect of adsorbent dosage on dye and metal removal

A dosage study was carried out at adsorbent dosages ranging from 0.4 to 12 g/L. Removal efficiencies and qe values are shown in separate figures for malachite green and copper. Figure 3 shows the malachite green removal efficiency graph. According to the graph, it can be said that biochar is more effective in removal. It was observed that the removal efficiency was partially increased with the increasing adsorbent dosages. Similarly, Khan et al. (2014), in their malachite green removal study, which they performed against the adsorbent dosage using activated pea shell, showed that the removal efficiency increased from 76% to 96% with the increasing adsorbent dosage.

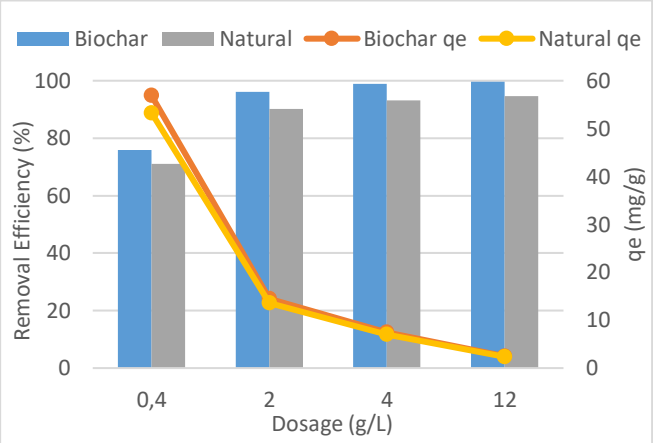

Fig. 3 Effect of contact time on malachite green removal

Figure 4 shows the copper removal graph. Here, the highest yield was seen around 80% with biochar adsorbent at 2 and 4g/L dosage. It can be said that with increasing adsorbent dosage, the removal efficiency begins to decrease. Also, it was observed that the adsorption capacity value above 50mg/g at 0,4g/L dosage for both pollutants decreased to 1-2mg/g at the dosage of 12g/L.

The study of the adsorbent dosage in which Haq et al. (2020) performed zinc removal with activated pea shell increased the removal efficiency from 41% to 64% with the increased adsorbent dosage.

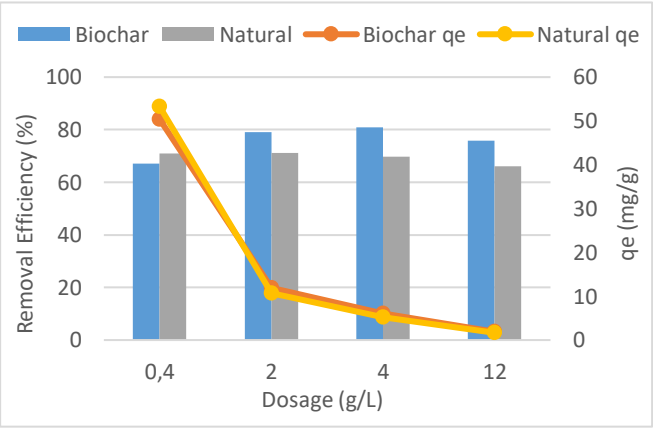

Fig. 4 Effect of adsorbent dosage on copper removal

3.3 Effect of initial metal and dye ion concentrations Studies carried out at concentrations of 30-400mg/L to investigate the effect of the initial pollutant concentration on adsorption are given in two separate graphs.

Figure 5 shows the malachite green removal efficiencies graph. Accordingly, it can be said that initial concentration does not have a significant effect on the removal of malachite green and similar yields are obtained. It was observed that the adsorption capacity increased only with increasing concentration. Çoruh and Gürkan (2018) carried out malachite green removal studies with waste foundry sand at initial malachite green concentrations varying between 25-400 mg/L. Removal efficiency, which was around 95% up to 150mg/L, dropped to 50% at 400mg/L.

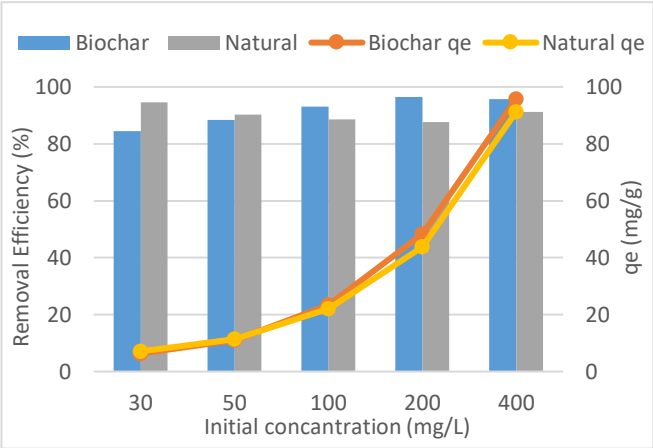

Fig. 5 Effect of initial concentration on malachite green removal

In Figure 6, copper removal efficiencies are given. The biochar has been shown to have higher yields in copper removal. It was observed that the efficiency decreased for both adsorbents at the highest starting concentration. Küçükgül and Kutlu (2006) made a copper removal study with biochar oak wood against the initial copper concentration. While copper concentration has 90% removal efficiency at low initial concentrations, removal efficiency has decreased to 20% at high initial concentrations.

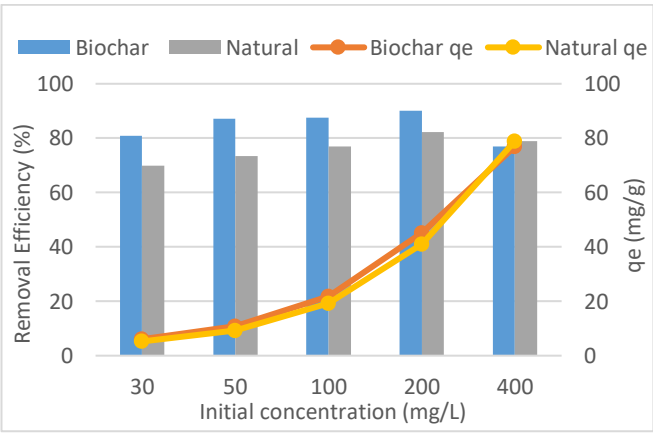

Fig. 6 Effect of initial concentration on copper removal

Malik et al. (2007) achieved a maximum 94% removal efficiency due to their studies of malachite green removal with activated nutshell. Azaman et al. obtained a maximum removal efficiency of 89% due to their studies of malachite green removal with biochar coconut shell under different adsorption conditions. Ali et al. (2020) obtained a maximum removal rate of 91.46% using the modeling method in their malachite green removal studies with active peanut husk. Abdelhadi et al. (2017) achieved a maximum 90% copper removal in their copper removal studies with biochar olive mill. They also achieved 74% copper removal with commercial activated carbon under the same adsorption conditions. As shown in these examples, paint and heavy metal removals are generally considered separately in the literature. When looking at the examples, the removal efficiencies obtained from the same adsorbent or same pollutant removal studies; It is close to or lower than the removal efficiencies obtained in this study.

3.4 Comparison of adsorption isotherms Adsorption isotherms or capacity studies are of fundamental importance in the design of adsorption and ion-exchange systems since they indicate how the metal and dye ions are partitioned between the adsorbent and liquid phases at equilibrium as a function of increasing metal and dye concentrations.

Freundlich isotherm may be expressed as Equation 3:

$$\log q_{e=\ \log K_F} + \frac{1}{n} \log C_e \qquad (3)$$

Where; qe(mg/g) stands for sorption capacity, K_F(mg/g) indicates relative sorption capacity, while n represents affinity between sorbate molecules and sorbent. Usually, the magnitude of n for physical sorption is more significant than that for chemical sorption; its magnitude is less than one (Dönmez and Aksu 2002).

Langmuir isotherm may be expressed as Equation 4:

$$\frac{C_e}{q_e} = \frac{1}{Q_0 x K_L} + \frac{1}{Q_0} C_e \qquad (4)$$

Where; Q_0 and K_L are the constant parameters that indicate the sorption capacity (mg/g) and sorption rate (L/mg), respectively (Rangabhashiyam et al. 2018).

In this study, the Langmuir and Freundlich isotherms for copper and malachite green dye ions removal using natural and biochar pea shells. The calculated results of the Langmuir and Freundlich isotherm correlation coefficient are given in Table 1. The data obtained were well fitted with the Freundlich equation as compared to the Langmuir equation under the different concentrations studied for both natural and biochar pea shells. Freundlich's R^2 values are calculated to be 0.8671 and 0.7895 for copper and malachite green with biochar pea shell, respectively. The R^2 values for Freundlich are calculated to be 0.9785 and 0.9419 for copper and malachite green with natural pea shells, respectively.

Values of qm, which is defined as the maximum capacity of sorbent, have been calculated from the Langmuir plots. The greatest equilibrium sorption capacity qm for copper and

malachite green dye ions were obtained for 163.93mg/g and 277.77 mg/g, respectively.

Table 1 Correlation coefficients of isotherm models

Isotherm	Parameter	Biochar MG	Biochar Cu	Natural MG	Natural Cu
Freundlich	k_F (L/g)	2.106	2.131	3.892	2.665
	n	0.511	1.17	1.255	0.804
	R^2	0.7895	0.8671	0.9419	0.9785
Langmuir	q_{max} (mg/g)	33.783	163.93	277.77	147.05
	K_L (L/mg)	0.047	0.01	0.009	0.004
	R^2	0.3564	0.5135	0.1503	0.3798

4 Conclusion

In this study, malachite green and copper removal efficiency was investigated with pea shell, a natural waste material. The pea shell selected as a natural adsorbent has been used in adsorption with both natural and thermally treated biochar form. Optimum adsorption conditions; 4g/L adsorbent dosage, 2 hour contact time, 150rpm shaking speed and 25mL volume were determined. In general, higher yields were obtained in the removal of copper than the natural pea shell with the biochar pea shell. Malachite green gave similar removal efficiency values in both adsorbents. While the highest yield in malachite green removal was 94.57% with a natural pea shell, it increased to 99.65% with a biochar pea shell. In copper removal, the highest yield with natural pea shell was 69.84%, while the biochar was increased to 85.03% with pea shell. In the literature, dye and heavy metal removals are generally considered alone. In this study, measurements were taken for two parameters simultaneously after adsorption in a single sample. However, very high pollutant removal efficiencies were obtained for both parameters. Also, both adsorbents adapted to the Freundlich adsorption isotherm model for malachite green and copper adsorption. According to these results, the pea shell is an effective adsorbent used for malachite green and copper removal. The malachite green and copper removal efficiencies of natural and biochar pea shell adsorbents are close. This situation may make the thermal treatment of pea shells seem an unnecessary step. However, in the purified sample obtained after the adsorption with a natural pea shell, the natural pea shell leaves a little yellow-green color and causes turbidity. This situation requires a separate treatment step and causes extra cost and time loss. Thermally treated biochar pea shell has high dye and heavy metal removal efficiencies without causing any adverse changes in the sample structure.

Authors' contributions: Sevda Esma Darama was interested in performing the experiments, calculating the results, interpreting the data and arranging them according to the format. Semra Coruh was interested in organizing the study.

Conflict of interest disclosure: Our work has not been carried out with any organization or employees.

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Antimicrobial socks for orthosis-prosthesis users

Banu Yeşim Büyükakıncı[1*], Sibel Döşler[2]

[1*] *Istanbul Aydin University, Faculty of Engineering, Textile Engineering Department, Istanbul Turkey*
[2] *Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Istanbul Turkey*

*Corresponding author : byesimb2@gmail.com
Orcid No: https://orcid.org/0000-0001-7597-4406

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This study aims to solve the problem of orthosis-prosthesis users and these socks will be preferred under plaster-splint after body fractures. It might be also a solution for diabetics' wound
Stump socks will keep the users drier during hot days and promote skin integrity and hygiene. As a result, the patients will be offered a better quality of life as it will be reduced the daily care period and prevented fungal infections

Abstract: Orthosis are a device used to correct, accommodate or enhance the use of a body part. Prosthesis is an artificially made limb or part of the body that is used to replace a part of the body that is missing either due to amputation or lack of development. Orthotics and prostheses are usually worn over the cotton stump-socks which should be washed every day. Also the stump-area must be cleaned and maintained regularly. Fungal infections are common on the stump of orthosis-prosthesis users who cannot perform regular care. Considering that 70% of patients using orthosis-prosthesis have such problems, it is understood that patients cannot provide adequate hygiene or care in the problematic areas. Antifungal stockings are aimed to find solutions to this problem and to facilitate patient life. Due to the high price of the existing antibacterial copper and silver ion socks and the presence of heavy metal ions, as an alternative solution, cotton stump-socks might be treated with natural plant extracts such as tragacanth of *Astragalus nitens*, an endemic Turkish-plant. *A.nitens'* extract-treated samples showed antifungal activity against tested three fungus strains, before and after five cycles of washing.
This study aims to solve the problem of orthosis-prosthesis users and these socks will be preferred under plaster-splint after body fractures. It might be also a solution for diabetics' wound. Stump socks will keep the users drier during hot days and promote skin integrity and hygiene. As a result, the patients will be offered a better quality of life as it will be reduced the daily care period and prevented fungal infections.

Keywords: Orthotics, prosthesis, *Astragalus nitens*, tragacanth, antibacterial, stump socks

1 Introduction

Although there are advances in technology and health, orthopedic diseases caused by chronic diseases, aging populations, war, trauma or congenital causes are still an important health problem. Prosthesis-orthotic applications and rehabilitation are important for improving the function and independence for disabilities. For existing problems, socks treated with silver or copper ions are used. However, it is known that such heavy metal ions have a toxic side effect even for healthy people.
Although there are antibacterial effects of these existing socks, there are concerns about their effectiveness against mold and fungi. In addition, because of their high prices, their uses are very limited. Therefore, there is no long-term, hygienic and cheap solution.
In order to find a solution to this problem and facilitate patient life, antifungal socks treated with natural/plant extracts might be a succesfull alternative strategy. There are various naturally antibacterial additives such as clove-, oregano-, cinnamon- and thyme essential oils and components (Saeed 2019). Treating the socks with tragacanth of *Astragalus* would be the prominent one of the plants.
Astragalus, which is named as "geven" is a plant with a wide range of (about 2500) species. Some of the species have white, yellow, purple or pink flowers and fruits in different shapes and sizes.
Astragalus nitens which is a species of the legumes family (Fabaceae) grow in the arid and semi-arid regions of Asia (Fig 1 and Fig 2). They also grow in eastern and the interior of Anatolia in 1300-3500 meters altitude. It is frequently seen in the mountainous regions of Sivas.
The trunk is hollow and grows vertically in the season; as it grows it becomes oblique or flat. It grows 70-120 cm in one season. It consists of a dense in florescence from pale yellow to white. The seeds are in the shape of a sac. The roots and trunk remain intact throughout the winter under snow.
It is resistant to living in wild life due to its structure. It is found in humid regions and stream sizes in Europe (Finland, Sweden, Spain and Russia) (Karadag et al. 2005).

Fig. 1 Astragalus nitens (Geven - in Sivas region)

Fig. 2 The root of *A. nitens* (Tragacanth, in the middle of the root)

The active ingredients of *Astragalus* root are characterized as follows: polysaccharide cycloartane glycoside fractions (astragalosides I-IV and trigonosides I-III), isoflavonoids (formononetin, ononin, calycosin and its glycoside), saponins, several isoflavonoids, biogenic amines and triterpenoids (SigmaAldrich).

Geven plant is effective in strengthening the immune system and increasing the ability of the body to fight diseases. It does not only increase the resistance to flu, but also shortens the duration of the disease. Many species are used among the people for their protective, antioxidant, immunostimulant and antiviral properties. These pharmacological activities were found to be caused by three groups of chemicals: These are Polyholosites, slings and phenolics (Rios and Waterman 1997).

Usage areas: Protecting the soil in sloping areas; maintaining biodiversity; in beekeeping (benefit from the aroma); as animal feed; in medicine sector (pharmacy) and as raw material in the paint and paper industry. In addition, it would also be prefered for socks used for stabilization after diabetes patients and body fractures (Ayubi-Rad 2020, Wang et al. 2019, Wang et al. 2016, Pistelli et al. 2002, Teyeb et al. 2012, Solgar 2020)

The aim of this study was to give antimicrobial properties to the cotton samples by applying the solution of *Astragalus*' tragacanth, and determining the antibacterial and especially antifungal activities of them.

2 Materials and Method

In this study, knitting interlock cotton (100%) fabrics 230 g/m^2 were used and were treated with *A. nitens* solutions according to conventional method (Cotton samples of 5g were used throughout this work).

In June, the plant (*A. nitens*) was collected around Sivas region in Turkey. The tragacanth (gum) which was in the centre of *Astragalus*' root was separated. Then, a solution (10g/L) was prepared by using this tragacanth.

2.1 Exhausting method

Pre-treatment: The 100% cotton fabrics were first scoured with 0.5 % non-ionic detergent in the bath with Fabric to Liquor ratio (F:L) 1:20.

Treatment: The procedures were all conducted in glass beakers (250 mL) in atmospheric conditions.

The fabric to the extract ratio was 1:20 (w:v) and the percentage of the *A. nitens*' tragacanth in the solution was 10%. The temperature was raised from the initial degree of 20°C up to 60°C within a period of 10 minutes (4°C/min) and maintained that condition for 30 minutes. The bath was then cooled; the treated samples were taken out, rinsed with cold water thoroughly and allowed to dry in the open air.

Then, the extract and treated cotton samples were evaluated for their potential antimicrobial activities.

2.2 Antimicrobial Tests

2.2.1. Antimicrobial activities (for extract)

Microorganisms: The American Type Culture Collection (ATCC) fungal standard strains of the yeast, *Candida albicans* ATCC 10231, and the molds *Aspergillus flavus* ATCC 204305 and *Trichophyton rubrum* ATCC 28188 were used in the experiments. Inoculums of fungi were prepared with 2-5 days cultures, for producing a concentration of 1 x 10^7 colony-forming units (cfu/ml).

Media: RPMI-1640 medium (Sigma) buffered to pH 7.0 with morpholine propane sulfonic acid (MOPS, Sigma) were used to determine the minimum inhibitory concentration (MIC) of yeast and molds, and Saboroud dextrose agar (SDA, Difco Laboratories) was used for colony counts.

Determination of minimum inhibitory concentrations (MIC): In vitro antifungal activities of extract against yeast *C. albicans* ATCC 10231, and molds *A. flavus* ATCC 204305 and *T. rubrum* ATCC 28188 were investigated. MICs of extract was determined by microbroth dilution technique as described by the Clinical and Laboratory Standards Institute (CLSI, 2000). Serial two-fold dilutions of extract was prepared in 96 well polistiren microplate, with RPMI-1640 medium. Each well was inoculated with 50 µL of a 2-5 days fresh culture that gave a final concentration of 5×10^3 cfu/mL in the test tray. The trays were covered and placed in plastic bags to prevent evaporation, and incubated at 25°C for 2 or 7 days for yeast and molds, respectively. The MIC was defined as the lowest concentrations of extract producing complete inhibition of visible growth. Ketaconazole was used as reference antifungal for standardization of the study.

In vitro antibacterial activities (MIC values) of extract against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352 were determined by microbroth dilution technique as described by the CLSI (2012). Serial two-fold dilutions of extract were prepared in Mueller-Hinton broth

(MHB, Difco), and each well was inoculated with 50 µL of a 4-6 h broth culture that gave a final concentration of 5×10^5 cfu/mL. The trays were covered and placed in plastic bags to prevent evaporation, and incubated at 37°C for 18-24 h. The MIC was defined as the lowest concentrations of extract producing complete inhibition of visible growth. Ciprofloxacin was used as reference antibiotic for standardization of the study.

2.2.2. Antimicrobial Tests (for treated cotton fabric)

The ISO 20645:2004 method (Agar diffusion method) was used for determining the antimicrobial properties of the cotton samples (ISO 20645, 2004).

The antimicrobial activities of the samples were tested before and after washing (5 times) which were performed by using the Standard Test Method ISO 105-C06 A1S, (2010). The A1S washing test was carried out at 40°C for 30 min in a 150 ml soap solution (4g/L) containing 10 steel balls. After washing, the samples were rinsed in deionized cold water, dried in the open air, and then tested for antibacterial properties as mentioned above (A TERMAL B21606E model washing machine was used for washing of the samples).

3 Results

In this study, it was investigated the antibacterial and antifungal effects of *A. nitens*' extract and extract-treated cotton samples. Untreated cotton samples were prepared for comparison.

Altought there is no significant effect has been observed for the antibacterial activity of *Astragalus* extract, the MIC values of the extract was determined as 1/4 dilution against *C. albicans* ATCC 10231 and *T. rubrum* ATCC 28188, and 1/8 dilution against *A. flavus* ATCC 204305 (Table 1). The MICs of Ketaconazole were within the accuracy range in CLSI quality control breakpoints (CLSI, 2016) throughout the study.

Table 1 Antifungal activities of Astragalus extract (%)

Extract	Fungus		
	C.albicans	***A.flavus***	***T.rubrum***
A. nitens (%100)	1/4 dilution (25%)	1/8 dilution (12.5%)	1/4 dilution (25%)
After five cycles of washing	(10%)	(5%)	(10%)

C.albicans: Candida albicans, ATCC 10231, *A.flavus*: *Aspergillus flavus* ATCC 204305, *T.rubrum*: *Trichophyton rubrum* ATCC 28188

The antifungal properties of the treated samples against *C. albicans, A. flavus* and *T. rubrum* were measured before and after 5 cycles of washing.

When the results were examined, the untreated cotton sample was not effective against any tested bacterial or fungal strains, the extract-treated samples showed antifungal activity against tested three fungus strains.

The treated sample, which exhibited a 16 mm zone against *A. flavus* and an 11 mm zone against *C. albicans* and *T. rubrum* before washing. It was observed that the extract and extract treated sample were more effective against *A. flavus* (Fig 3).

Five cycles of washing decreased the antibacterial properties of all the samples about 40-50%, but all the samples were still antifungal after washing.

Fig. 3 Antifungal effect against *A. flavus*

4 Discussion

According to the 2008 data of the World Health Organization (WHO 2015), 0.5% of any population globally requires prostheses and orthoses and rehabilitation treatment (ICRC 2013). This gives an idea of the challenge countries are facing. It has been estimated that 0.5% of the world population would correspond to 35–40 million people globally who require prosthetics and orthotics services. Over the next few decades, the number of people requiring prosthetics and orthotics services is bound to rise because both the world's population and life expectancy are growing. As a larger proportion of the ageing population will be affected by disability (WHO 2015), he need for services will rise proportionally.

The world is witnessing significant increases in musculoskeletal conditions and noncommunicable diseases such as diabetes and stroke, which will greatly add to the need for prosthetics and orthotics.

Thus, by the middle of this century, the proportion of the world's population that requires services is likely to be closer to 1%.

An estimated 415 million people were reported to have diabetes in 2015, and this figure is expected to rise to 642 million in 2040 (WHO 2016; Diabetes atlas 2016). As 60–70% of people with diabetes lose sensation in their feet, they are at risk for injury. Furthermore, 12–15% of people with diabetes will develop a foot ulcer (Singh et al. 2005; Cavanagh et al. 2005), which increases their risks for infection, amputation or even premature death. According to researches, diabetic finger or leg amputations are more than amputations in accidents.

According to the Social Security Institute's data (Ministry of Labor and Social Security), approximately 30.000 prosthetic and orthotic were made last five years in Turkey.

Between 2008 and 2012, the number of diabetes patients increased by an average of 17 percent each year, increasing from 2 million 500 thousand to 5 million 200 thousand people. It is thought that the number of orthosis-prosthesis users who have diabetes patients will also increase in these rates. Accordingly, a market size is expected to reach 10 million people in 5 years and 15 million people in 10 years.

The aim of this study was to provide a better quality of life for these patients by shortening the daily care period and preventing fungal growth. For this purpose, we tried to design antifungal socks treated with *A. nitens*.

According to our results, the antibacterial effect of tragacanth-treated cotton fabric was limited against the selected bacteria, whereas the those cotton samples showed antifungal effect against all three fungus. After 5 cycles of washing, the antifungal activities were decreased about 40-50%, but the treated samples were still effective against all the tested fungus (Table 1). Similarly, there are some other researchers were found the *Astragalus* extracts have an in vitro or in vivo antibacterial or antifungal activities against standard bacterial or fungal strains (Kanaan et al., 2017; Mikaeli et al., 2012; Pistelli et al., 2002)

5 Conclusion

In view of these results, it was thought that the *Astragalus* - tragacanth solution can be good candidate for application in the medical textiles sector, especially for the stump-socks of the orthosis-prosthesis users.

The antifungal effect of *Astragalus*' tragacanth will prevent the growth and reproduction of fungi that develop in hot and moisture, while the daily care period of the stump-area will also be reduced. Accordingly, it is aimed to increase patient comfort.

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Risks and benefits of functional foods: an overview

Arzu Altunkaya Dinçay [1,2]

[1]*Ministry of Agriculture and Forestry, General Directorate of Food and Control, Ankara, TURKEY*
[2]*Lokman Hekim University, Faculty of Health Sciences, Human Nutrition and Dietetics Department, Sogutozu, TR-06800 Ankara, TURKEY*

*Corresponding author : arzualtunkaya@gmail.com
Orcid No: https://orcid.org/0000-0001-8211-763X

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Abstract: A direct relationship between foods and health has led to various scientific studies to find out the significance of foods or food components on specific functions in the body. Studies have identified nutrition as a major modifiable determinant playing a role in health promotion and chronic diseases prevention. The term functional food refers to food with specific beneficial functions over their basic nutritional value. We reviewed the factors that have driven the functional food development, various definitions proposed by different authors and their classification. Moreover, we provided an an overview on various functional ingredients in different food sources along with their potential health benefits and risks of adverse effects associated with these products. Lots of research is required to substantiate the potential health benefits of those foods for which the diet–health relationships are not sufficiently validated, and create a strong scientific knowledge base for proper application of naturally present foods in combating various diseases and disorders.

Keywords: functional food, adverse effect, health benefits

1 Introduction

Food is a term which is basically related to the component necessary for several life sustaining functions like production of energy, supply of nutrients, support of various metabolic activities besides growth and maintenance of the body (Kaur and Das 2011). In the early 20th century, nutrition science was engrossed with preventing deficiencies and supporting body growth. In the last decade, studies have identified nutrition as a major modifiable determinant playing a role in maintenance of body and chronic diseases prevention (Yahfoufi et al. 2018). Nutrition concepts today are moving away from prevention to promotion of health and wellness, and due to increased education and awareness to consumers the link between diet and health (Sohaimy 2012). As such, this trend has created a demand for foods are called as functional foods which are traditional foods modified in such a way that they have health benefits compared to the non-modified products. They are prepared by manipulating the formulations or engineered genetically or by other conventional means to provide the desired function (Doyon and Labrecque 2008).

Functional foods are used to improve certain physiological functions, leading to the prevention of disease, reduction of risk factors, and to complement therapies. They provide additional benefits over their basic nutritional value, contributing to the prevention and reduction of risk factors for different diseases or boosting multiple physiological functions (Yahfoufi et al. 2018).

2 Definition of terms

According to Functional Food Center, functional food may be defined as natural or processed foods that contains known or unknown biologically active compounds; the foods, in defined, effective and non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management or treatment of chronic disease (Martirosyan and Singh 2015).

Thus, there is no statutory definition of functional foods, because foods consumed perform some functions in one way or the other. Though, a number of definitions have been given, the general opinion is that functional food is any healthy food similar in appearance to conventional foods, consumed as part of a usual diet, and claimed to have a physiological benefits like health-promoting or disease-preventing properties beyond the basic function of supplying nutrients (Gul et al. 2016). Common characteristics of functional food as follows: consumed as part of a normal food pattern, a capsule or any form of dietary supplement and a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease (Howlett et al. 2008). Besides the above definitions, a number of different terms (Table 1) have come into perspective which sometimes are linked or interchanged with functional foods.

Thus, natural traditional products containing components influencing the health positively are strictly not functional foods, e.g., cranberry juice that influences the urinary tract infections positively is not a functional food when consumed as such. However, if the juice or its health contributing

ingredient in isolated form is added to another food to enhance health positively, the developed one is a functional food (Kaur and Das 2011).

There is a growing overlap between conventional food and food supplements, including energy bars and teas or liquids. This overlap becomes even wider when we consider functional foods and nutraceuticals. What could be considered a functional food under a given set of circumstances may be named a dietary supplement, medical food, food for special dietary use or nutraceutical under different circumstances, depending on its ingredients (active components) and claims (Santini et. al. 2018).

3.Health benefits

Functional foods and nutraceuticals are prepared by manipulating the formulations or engineered genetically or by other conventional means to provide the desired function. Understanding the requirement of food characteristics in tackling specific health problem(s), and contribution of specific food ingredients towards such benefit will definitely help in development of functional foods (Shadidi 2009). Table 2 categorizes such relationship between various functional ingredients with their beneficial role. Consequently, functional components have health-promoting roles at various stages of disease control that are associated with multiple progressive steps, from initiation to development. Thus, in a time when the role of a healthy diet in preventing non-communicable diseases is well accepted, the borderline between food and medicine is becoming very thin (Pravst 2012). As a result, they are claimed to provide a means to reduce the increasing cost on the health care system by a continuous preventive mechanism.

Table 1. Definition for food derived products

Functional Food (Diplock et al. 1999)

Product which is shown in a satisfactory manner that, in addition to adequate nutritional effects, induces beneficial effects on one or more target functions of the organism, significantly improving the health status and welfare or reducing the risk of disease.

Food Supplement (Novellino 2018)

Food product whose purpose is to supplement the normal diet and which consists of a concentrated source of nutrients or other substances with nutritional effects or physiological, single or in combination,
marketed in dosed formulations, such as capsules, tablets, tablets or pills, designed to be taken in small individual quantities measured.

Medical food (Hardy 2000)

a food which is formulated to be consumed or administered etternally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.

Nutrecauticals (De Felice 1995)

Food or part of food that provides medical or health benefits, including the prevention and/or treatment of a disease.

Functional Ingredient (Kauer and Das 2011)

Functional ingredients are the standardized and characterized preparations, fractions or extracts containing bioactive compounds of varying purity, that are used as ingredients by manufacturers in the food.

FOSHU (Martirosyan and Singh 2015)

whole, fortified, enriched or enhanced that should be consumed regularly and at effective amounts in order to derive health benefits.

Prebiotic (Lockyer and Stanner 2019)

a substrate that is selectively utilized by host microorganisms conferring a health benefit.

Probiotic (Kang and Im 2015)

live microorganisms that when administered in adequate amounts provide health benefits to the host.

Colic food (Nocerino et al. 2015)

non-digestible carbohydrate, which provides nutrients for the microflora in the intestines and reaches the column in undigested form.

Phytochemicals (Murano 2003)

plant-derived, non-nutritive and biologically active chemicals that function in the body to prevent the onset of certain noncommunicable diseases.

Table 2 Some functional ingredients of food, their sources and potential benefits

Bioactive components	Source	Potential benefits
Carotenoids		
Alpha-carotene/beta-carotene	Carrots, Fruits, Vegetables	Neutralize free radicals which may cause damage to cells.
Lutein	Green vegetables	Reduce the risk of muscular degeneration.
Lycopene	Tomato products (ketchup, sauces)	Reduce the risk of prostate cancer.
Non-starchy polysaccharide		
Fucoidan (fucose)	Mushrooms (maitake and reshi),brown seaweeds	Immune modulation; apoptosis of cancer cells; stimulates brain development; anti-clotting effect; lower blood cholesterol levels; decrease high blood pressure, stabilize blood sugar.
Insoluble dietary fibre	Wheat bran	Reduces risk of breast or colon cancer.
Soluble dietary fibre (β-Glucans)	Oats, barley	Reduces risk of cardiovascular disease; protects against heart disease and some cancers; lower LDL and total cholesterol.
Soluble Fibre	Psyllium	Reduces risk of cardiovascular disease; protects against heart disease and some cancers; lower LDL and total cholesterol.
Fatty Acids		
Long chain omega-3 Fatty Acids-DHA/EPA	Salmon and other fish oils	Reduce risk of cardiovascular disease; improve mental and visual functions.
Conjugated Linoleic Acid (CLA)	Cheese, meat products	Improve body composition; decrease risk of certain cancers
Phenolics		
Anthocyanidins	Fruits	Neutralize free radicals; reduce risk of cancer.
Catechins	Tea	Neutralize free radicals; reduce risk of cancer.
Flavonones	Citrus	Neutralize free radicals; reduce risk of cancer.
Flavones	Fruits/vegetables	Neutralize free radicals; reduce risk of cancer.
Lignans	Flax, rye, vegetables	Prevention of cancer; renal failure.
Tannins (proanthocyanidines)	Cranberries, cranberry products, cocoa, chocolate	Improve urinary tract health; Reduce risk of cardiovascular disease.
Plant Sterols	Corn, soy, wheat, wood oils	Lower blood cholesterol levels by inhibiting cholesterol absorption.
Stanol ester		
Prebiotics and Probiotics		
Fructo-oligosaccharides (FOS);	Jerusalem artichokes, shallots, onion powder,	Improve quality of intestinal microflora; gastrointestinal health
Lactobacillus;		
Biofidobacterium	Yogurt, other dairy products	Improve quality of intestinal microflora; gastrointestinal health.
Soy Phytoestrogens		
Isoflavones: Daidzein, Genistein	Soybeans and soy-based foods	Menopause symptoms such as hot flashes; protection against heart disease and some cancers; lowering of LDL and total cholesterol.

(Shahidi 2009; , Ferrari 2007; Gry et al. 2007; Plaza, Cifuentes and Ibàñez 2008; Patil et al. 2009; Abuajah et al. 2015)

4.Risks of functional foods

The most important factor in the involvement of functional foods in the diet is a lack of studies on possible mechanisms of action and a lack of in vivo research confirming the claimed beneficial health effects on specific pathological conditions (Santini et al. 2018). Another key aspect is related to the data reported in the literature, which mainly comes from in vitro studies focused on single food constituents (micronutrients); these studies are based on the assumption that micronutrients can be considered safe (or generally recognized as so) because they are derived from commonly used food or food components (Pinto da Costa 2017; Gupta 2016). The use of materials as a food additives can be advantageous in several aspects: their natural origin, health protecting properties, possibility for combination of beneficial physiological properties and the recognition and exploitation of synergistic properties can be used in the production of functional food products. Nevertheless, the ingredients themselves may cause health problems, and proper information on possible unwanted side effects should be provided on the label. The development and commercialisation of novel functional compounds must be pursued to improve the functionality and safety of foods. Moreover, much more work have to be done based on clinical studies rather than only in vitro studies and bioavailability of these products (Santini et al. 2018). Thus, the application of this knowledge might eventually be made food processing to enhance for certain components if, and only if, efficacy and lack of harm are already well established.

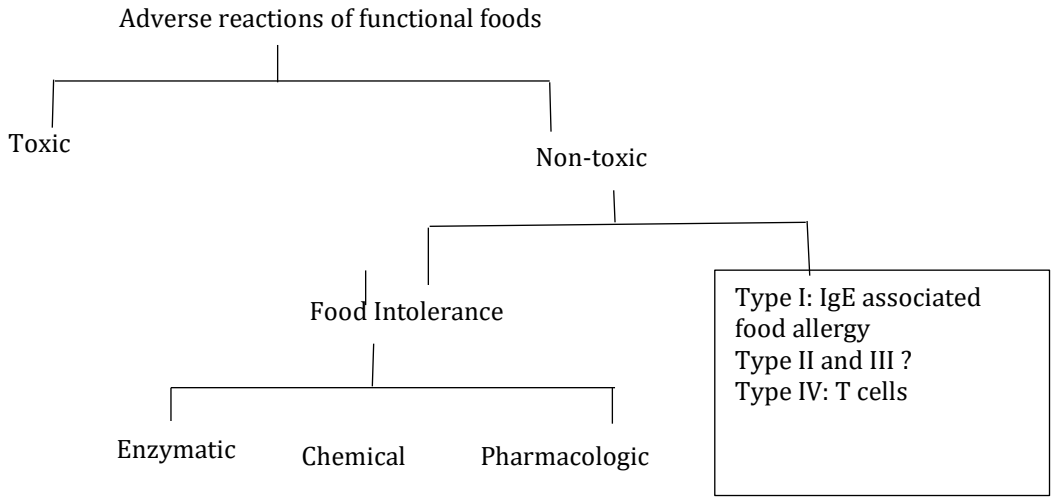
Claims about the nutritional or health benefits of foods can provide information to help consumers adopt a healthy diet (Roberto and Khandpu 2014). Concomitantly, an improved appreciation of the potential beneficial or adverse effects of nutrients and other components in the diet has led to the realization that it is possible to create food items with specific characteristics that are capable of influencing body function over and above meeting the basic nutrition needs. By the way, a direct measurement of the effect a food has on health and well-being and/or reduction of disease risk is often not possible. This may be because the endpoint (the state of health and well-being) does not always lend itself to quantifiable measurement (Keservani et al. 2011). Also, in the case of a disease like cancer, the time frame for development of the disease is very long or it would be unethical to monitor its development under the conditions of a controlled study. Instead, functional food science works from knowledge of the key processes in the attainment of optimal health or in the development of a disease to identify markers that can be used to monitor how those key processes are influenced by foods or food components. Provided that the role of those key processes in the attainment of optimal health or disease development is well established and the markers are chosen accurately to reflect the process, it is possible to study the effect of consuming the food on the final endpoint (the improved state of health or reduction of disease risk) by measurement of the markers (Gallagher et al. 2011). The markers could be chosen to reflect either some key biological function (markers of a target function) or a key stage in development that is unequivocally linked to the endpoint under study, in which case they serve as markers of an intermediate endpoint. Measurements made in the short term on carefully chosen markers of intermediate endpoints can be used to make inferences about effects on final endpoints that would only otherwise be accessible through long-term study. Where the underlying target functions or intermediate endpoints are unequivocally linked to the risk of disease, the markers are also referred to as risk factors for the disease (Howlett et al. 2008).

All foods can cause adverse reactions. The adverse reactions to functional foods are classified in the same way as adverse reactions to conventional foods (Figure 1). By the way, functional foods are biologically active and therefore may cause different effects in the ranges that can vary from the therapeutic effect to the toxic effect depending on the levels. The reactions may occur to the food or to added or enhanced ingredients. Reactions to added ingredients can be toxic or non-toxic. Toxic reactions include carcinogenicity. Non-toxic adverse reactions to foods can be due either to intolerance or allergy (Ameratunga et al. 2016).

Toxic reactions usually occur in products that contain carcinogenic substances or added ingredients at higher doses. Irwin et al. (1996) have observed di acyl glycerol based fatty acids. The response observed in that study was indicative of the carcinogenic potential of glycidol.

Pharmacologically unfavorable reactions may occur when consumed in excess of the recommended amounts for some substances. There is concern that the consumption of large amounts of fish oil may increase the risk of hemorrhage (Komaroff, 2009). Of ore concern have been reports of chronic toxicity to vitamin D supplements. Consumers may be at risk of life threatening hypercalcemia if they consume food supplements with high concentrations of vitamin D (Lowe et al. 2011; Kaptein 2010). It has been seen that many adverse effects such as headache, vomiting, abnormalities in the bones, damage to the liver occur in high doses of vitamin-carotene or vitamin A, which is one of the functional food components with positive effects on health. Moreover, despite its known antioxidant capabilities, ascorbic acid supplements, by themselves, are not consistently associated with decreased oxidative damage. It may be that the water soluble nature of this antioxidant limits its ability to protect against free radicals in lipophilic compartments. Therefore, we may be more likely to observe benefits when this antioxidant is combined with lipid soluble antioxidants such as vitamin E (Wildman and Kelle 2001).

Figure 1. Classification of adverse reaction of functional foods (Valenta et al. 2015)



In the case of functional foods, effects of each component were related to the concentration as well as synergistic or antagonistic effect of some molecules. Many studies have shown that high genistein levels, soy phytoestrogen, were observed to promote certain tumor types in animals. This is contrary to popular opinion on the health benefits of genistein and needs (Marcelo et al. 2019; Nakamura et al. 2011; Kwack et al. 2009). Morever, Xia et al. (2010) has investigated the potential toxicity of some polyphenols from grape, such as epicatechin to the fibroblast, and keratinocyte cell lines. Noticeable DNA damage was observed in mice spleen cells by incubating with higher concentration (150 μmol/L) of catechin.

According to the guidelines of FAO/WHO (2002),"Probiotics are live microorganisms when administered in adequate amounts confer a health benefit to the host such as maintaining bowel mobility, improving the immune system, reducing serum cholesterol levels, and preventing various types of cancers. Nevertheless, patients with suppressed immune systems should avoid them to prevent various infections, although, they have been used safely for years (Suez et al. 2019). Furthermore, prebiotics can potentially serve as functional food ingredients to improve or maintain gut health. They can increase the population of beneficial gut microbiota while suppressing the harmful ones thus help to develop immunologic structures of the intestinal mucos resulting in an improvement in enteric inflammatory disorders and the systemic immune response (Mundi et al. 2017; Johnson et al. 2015). However, inulin, a prebiotic, has been linked to anaphylaxis in at least one reported study (Franck et al. 2005). The safety of probiotics or prebiotics is still among the subjects that science continues to investigate. More information is needed, in particular, on how safe they are for young children, elderly people, and people at risk.

Nutrient hypersensitivity refers to any reaction of the organism to any component of the nutrients including allergy and intolerance. Food allergy is a special form of food hypersensitivity that activates the immune system, an exaggerated response (Valenta et al. 2015). Immunological reaction is a reaction against foreign substances entering the body. Allergen is a protein that is usually contained in nutrients, which triggers the immunological reaction of its release in its antibodies (De Silva et al. 2014). There are 4 major types of allergic reactions based on pathogenesis mechanisms. The most common forms of immune-mediated adverse reactions to foods (type I reactions) always are characterized by the development of IgE against food allergens. It can be accompanied by inflammation, induced by cellular components, and mediated by T cells and eosinophils. Patients with IgE-associated food allergy can be identified based on the detection of food allergen–specific IgE in serum and body fluids, and by measuring IgE-mediated cellular and in vivo responses. Milk, eggs, wheat, peanuts, nuts, sesame, fish, fruits, and vegetables are common inducers of IgE-associated food allergy (Longo et al. 2013).

Similarly, the addition of fish proteins to experimental ice creams was described in Iceland (Shaviklo 2011). Persons allergic to fish are at risk of severe reactions to these products. Ice creams have previously not contained fish proteins and fish allergic consumers may be at risk if they inadvertently consume such products. The health promoting effects of kiwifruit have been described (Stonehouse 2013) as causing severe allergic reactions in some consumers. Moreover, there is no solid experimental evidence to support the adverse reactions via type II or type III hypersensitivity reactions to food allergies that develop in patients .

Type IV hypersensitivity, which mainly involves food antigen–specific T-cell responses and can damage the gut mucosa, is associated with disorders such as celiac disease.

Celiac disease is characterized by a hypersensitivity reaction against the wheat gluten fraction comprising alcohol soluble gliadins and acid-, alkali-soluble glutenins, accompanied by an autoimmune component (Schuppan et al. 2009). Type IV hypersensitivity reactions also might be involved in food protein–induced enterocolitis. Studies have shown that certain food proteins can induce inflammation via direct activation of the innate immune system (Junker et al., 2012). For example, wheat amylase trypsin inhibitors and certain milk oligosaccharides can cause intestinal inflammation via activation of Toll-like receptor 4 and certain allergens have been shown to stimulate the innate immune system. Innate immune mechanisms might mediate nonceliac gluten sensitivity (Catassi et al. 2012).

Food intolerance, also known as non-allergic food hypersensitivity, refers to difficulty in digesting certain foods. There are different types of food intolerances, including enzymatic, chemical and pharmacologic reactions (Losurdo et al. 2018). Pharmacological intolerances involve reactions to certain naturally occurring substances in foods such as vaso active amines - of which histamine is one example, salicylates - substances chemically similar to aspirin found in a wide variety of plant foods, and caffeine or theobromine - found in chocolate. Non-coeliac gluten intolerance and fructose intolerance are also recently recognised conditions which can cause symptoms such as abdominal disturbance (usually bloating but sometimes other symptoms as well) and occasionally malaise and tiredness (Akoğlu and Oruç 2018).

The most common type of enzymatic food intolerance is lactose intolerance, which occurs because these individuals have either too little or no lactase – the enzyme which helps to digest milk sugar lactose (Akoğlu and Oruç 2018).

Another rare and important enzymatic food intolerance type is Phenylketonuria (PKU). PKU, is a genetically inherited birth defect that causes an unwanted buildup of the amino acid phenylalanine in the blood. This buildup occurs because the enzyme that routinely converts one amino acid, phenylalanine, to another amino acid, tyrosine, is absent or deficient. Phenylalanine then accumulates in the blood and is toxic to brain tissue (Schuck et al. 2015).

Chemical intolerances are intolerances to such food additives. The additives most commonly linked to food intolerance are artificial colours, eg tartrazine and preservatives such as sulphites and benzoates. Sulphites have to be declared on all packaged products under the NSW Food Act 2003 (Franck et al. 2005). They are preservatives and are commonly found in wine and dried fruit. Sulphite reactions cause asthma, rashes, irritable bowel syndrome and headaches in sensitive people. Monosodium glutamates (MSG) also occur naturally in such foods as camembert cheese, Parmesan cheese, tomatoes, soy sauce and mushrooms. MSG stimulates nerve endings, perhaps accounting for its function as a flavour enhancer when it is added to food. Many people find digesting certain foods difficult, or that certain foods will make an existing condition – such as irritable bowel syndrome (IBS) - worse. (Losurdo et al. 2018).

There are many other adverse reactions to foods, apart from allergy and intolerance, including: • Feeling unwell after eating from other causes such as heartburn after a fatty or spicy meal or a hangover after too much red wine. • Food aversion is a condition where a person not only dislikes a food, but also experiences unpleasant physical symptoms when they see or smell the food. Symptoms are triggered by emotions associated with food rather than the food itself. This does not usually occur if the food is disguised. • Underlying anxiety can result in unconscious over-breathing or hyperventilation. The symptoms that result (dizziness, tight chest, blurred vision or numbness) can be very distressing, and can sometimes resemble food allergy (Lyra et al. 2013).

5.Conclusion

Global demand for functional foods is expanding dramatically because of technological innovations, development of new products (Granato et al., 2010a) coupled with increasing consumer's consciousness about health (Szakaly et al. 2012) and demand for healthy foods (Bigliardi and Galati 2013). Functional food products resemble conventional food in terms of appearance but are composed of bioactive compounds that may offer physiological health benefits beyond nutritive functions (Arora et al. 2013).

Over the last few decades, tremendous increase in the demand of functional foods have evoked the food processing industries to develop novel methods for maintaining the nutritional quality and functional characteristics of food (Das et al. 2012). Of the various issues being faced by the mankind, diet linked diseases are among the most significant one and need serious efforts to pull up the danger of these physiological maladies. Keeping in view the present conditions, novel health strategies have been devised aimed at highlighting the positive aspects of such healthy diet (Ahmed and Rashid 2019).

This concept has been proposed as a modern approach to food science, and the area of possible use has been defined as beyond the diet, but before the drugs. The potential functions of nutraceutical/functional food ingredients are so often related to the maintenance or improvement of health that it is necessary to distinguish between a food ingredient that has function and a drug. However, because of the complex matrices, the lack of validated analytical methods and the limited availability of reference compounds, the analysis of raw material and finished products may pose a challenge. Thus, current information in this regard is insufficient and hazy. Consequently, there is a need to provide consumers with more information to effectively guide them in making wider choices of diets that contain optimal levels of health-promoting functional food components (Sohaimy 2012).

Success of functional food is influenced by a number of factors like 1) focus on general wellbeing, 2) health benefit for common complaint, 3) mass distribution and market

positioning, 4) effective communication of health benefit, 5) extension of existing brand/food company, and 6) focus on taste, convenience, and appropriate pricing. A successful functional food along with its health benefits must be competitive in all these arenas (Martirosyan & Singh, 2015). The consumers need to be better informed with active ingredient in food products and its health benefits addressing general wellbeing issues (Kaur and das, 2011).). Thus, this exercise may require: (i) appropriate target identification; (ii) safety assessment; (iii) a clear understanding of the mechanism of action; (iv) efficacy assessment substantiated by clinical studies; (v) an evaluation of possible unwanted side effects; and (vi) an evaluation of possible interactions with other products (e.g. food, food supplements and drugs (Santini et. al. 2018).

As a result, one of the most important issues is the lack of deceiving consumers by presenting functional foods as miraculous foods. Due to the a wealth of information in the field of functional foods and their impact on human health, additional efforts of governments and diverse organisms related with human health are necessary in order to highlight the benefits and risks of these food types. Likewise, further investigation is required to understand the mechanisms associated with several biochemical and physiological processes induced by functional foods. Manufacturers and regulatory authorities will need to agree on optimal methods for assessing the quantity and quality of health promoting ingredients in functional foods.

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