



## მედიცინისა და მენეჯმენტის თანამედროვე პრობლემები

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შემოთავაზებული სამეცნიერო ნაშრომთა ჟურნალი განკუთვნილია მედიცინის, ეკონომიკის, მენეჯმენტის, ფიზიკური მედიცინისა და რეაბილიტაციის დარგის სპეციალისტებისათვის.

ჩვენ ვიმედოვნებთ, რომ ავტორთა მიერ წარმოდგენილი ნაშრომები ხელს შეუწყობს სამეცნიერო პოტენციალის გაძლიერებას.

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ბიოლოგიურ მეცნიერებათა დოქტორი,  
პროფესორი, აკადემიკოსი,  
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**Dear colleagues!**

**The proposed scientific journal is intended for specialists in medicine, management, physical medicine and rehabilitation, economics.**

**We hope that the works presented by the authors will help to strengthen the scientific potential.**

**Marina Pirtskhalava**

Doctor of Biological Sciences,  
Professor, Academician,  
Rector of University Geomedi


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## Original Research

# Impact of Social Media Use on Academic Performance and Well-Being among the Secondary Level Students in Selected Schools in Nepal

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## Abstract

Concerns regarding Academic Performance (AP) and student Well-Being (WB) have grown as a result of the increased usage of Social Media Use (SMU) among Secondary Level Students (SLS). The main aim of this research is to examine the impact of SMU on academic performance and wellbeing among male and female secondary-level students in Nepal. In this study, an analytical cross-sectional analysis was conducted on 229 students in Grades 8, 9, and 10 at a boarding school in Kathmandu, using probability-based stratified sampling techniques with two boarding schools as strata. The research tool was a self-administered questionnaire with four sections addressing SMU, AP, and WB, as well as well-structured tools utilized by researchers. All ethical approval was obtained before conducting the study. Data were analyzed using descriptive analysis, regression analysis, and the t-test. The study examined the relationship between SMU and AP and WB. Neither hypothesis H1 nor H2 was supported, demonstrating no significant impact of SMU on AP or WB. Though, a significant difference in SMU between male and female students was detected. The majority of students were male students who favored using Facebook. Further examination is recommended to fully comprehend the complex association between SMU, AP, and WB. The study highlights the need for guidance and supervision from parents, teachers, and authority figures to prevent undesirable effects on students' WB and AP. Researchers may suggest models such as emotional intelligence to increase self-awareness, self-management, empathy, and communication. Appropriate policies and models are urgently needed to prevent serious issues related with SMU among students.

**Keywords:** Academic Performance (AP), Well-Being (WB), Social Media Use (SMU), Secondary level Student (SLS)



## Introduction

Social Media (SM) has emerging into a necessary part of daily life and has an influence on people of all ages [1], particularly secondary school pupils [2]. Despite the fact that SM provides a number of advantages, such as improving knowledge and communication [3], it also presents a number of issues that have an impact on AP and WB among students [4; 5; 6]. The following are some issues with SM use among students:

**Distraction** - SM is a significant distraction for students, particularly during study time. Students' study may be interrupted by the constant notifications and updates from social media platforms, which might negatively affect their AP results [7].

**Cyberbullying** - Social media has created a forum where students can bully one another online [8]. CB can lead to emotional pain, which is the root cause of bad AP and has an impact on pupils' WB [9].

**Dependence** - Children who use SM excessively may develop an addiction to the platform<sup>10</sup>, due of their dependence, students may neglect their academics, which will affect their AP [10].

**Lack of Sleep** - Students that use SM at night may have sleep deprivation, which can influence their WB and AP grades [2; 6].

**Mental Health Issues** - SM can result in mental fitness problems [11] including hopelessness, concern, and anxiety, which can negatively impact kids' WB [12] and eventually result in poor AP [13].

## Prevalence on Social Media Use (SMU)

SMU has become an integral part of the lives of adolescents, and research has demonstrated its impact on their psychological and social wellbeing. The prevalence of SMU among SLS has been a topic of interest in recent literature, and several studies have investigated this phenomenon; a study conducted by E-ISSN, (2022) surveyed 150 and found that 73 % of students engaged in SM for non-academic purposes and most of them visited the Facebook site [4]. According to a report by the ITU (2021), there is also a generation gap-71 percent of the world's population aged 15–24 is using the digital divide, compared with 57 percent of all age groups [3]. Likewise, According to reports by Kemp (2020), mobile device activities apps now account for more than 90 percent of our total time spent [14]. The data also shows that people are using apps in an increasingly varied range of everyday activities, but SM still accounts for half of all the time people spend using mobile devices [14]. Similarly, a study by Barrot (2022). The review analyzed 396 documents on SM as a language learning environment, showing popular platforms like Facebook, Skype, WhatsApp, and Twitter are widely used [15]. A study found that all participants were using Facebook, and 96.5 percent were using Facebook Messenger; other popular apps included Telegram and Instagram [16]. The frequency and intensity of SMU have also been investigated. In a study, 74.9 percent of



students accessed the Internet via mobile phones, and 44.7% used it for more than 3 hours a day. Nearly 80% used Facebook daily, and almost 60% used Instagram daily, while only 13.5 percent ever used it [8]. According to the study by Rideout (2016), young people's media utilization is growing, with TV and online videos, social media, gaming, reading, and music all becoming more popular [17], however, concerns arise over excessive screen time on social media [17]. Similarly, study systematic searches conducted by Liu et.al (2022), the aim of scientific databases to examine the link between SMU time and depression risk [18], the finding revealed that for every hour increase in SM use, the risk of depression in adolescents rose by 13% (OR=1.13, 95% CI: 1.09 to 1.17,  $p>0.001$ ) [18]. This study showed that most students visit SM sites at homes and in residential halls, with an average daily use of more than four hours, mainly between 6pm to 6am. The research also revealed that excessive SM use negatively impacts AP [4]. Overall, the literature suggests that SMU is prevalent among students, with a majority of adolescents using SM on a daily basis. While the impact of SMU on mental wellbeing is still being considered, evidence suggests that excessive SMU may be associated with negative consequences [1; 18].

### **Impact of social Media Use (SMU) on Academic performance (AP)**

The impact of SMU on AP has been a topic of concern among researchers, educators, and parents in recent years, while some studies advise a negative relationship

between SMU and AP, some reports mixed of inconclusive findings [19; 12; 20; 21; 7]. Chykowski (2020) conducted a cross-sectional study with 348 participants between the ages of 15 and 25 with the primary goal of examining the effect of social networking on academic achievement. According to the study, more time spent on social networks in general has a negative impact on academic achievement [22]. Similarly, based on the findings of Asemah et al. (2013), it is apparent that SM platforms are easily accessible to college students. However, it has been observed that excessive and negative use of social media can adversely impact their academic performance [23]. Azizi and colleagues (2019) study, which utilized a large sample size of 360 students selected through stratified random sampling, revealed a noteworthy finding. Specifically, the study demonstrated a significant and unfavorable link between students' academic performance and their overall social network usage [13]. Similarly, Researchers also found that students who used Facebook more frequently had lower AP results than those who used the site less frequently. These results suggested that excessive SMU can impair academic performance by increasing distractions, shortening attention spans, and decreasing productivity [8]. However, other studies have believed more positive effect of SMU on academic performances. This study examined the impact of instructor Twitter feeds on college student perceptions of instructor credibility and attitude towards Twitter as a teaching tool. Result showed



that Twitter feeds with professional content were perceived as the most credible, with credibility ratings linked to positive attitudes about instructor Twitter use and tweet frequency [20]. Likewise, Hamid et al. (2015) suggest that social media can effectively enhance virtual education by fostering collaboration, group dialogue, and knowledge exchange among students, thereby strengthening their knowledge and performance [24]. Similarly, a study found that perceived usefulness, ease of use, and peer interactions positively influenced e-learning (OL), which in turn positively impacted academic performance (AP) and engagement. Engagement also positively impacted AP. Social media facilitated collaboration, group discussion, and idea exchange to enhance OL [19]. Although there have been mixed feelings regarding the impact of SM on students' AP, this study shows that SM can be used in a positive and effective way. Despite the potential for SM to act as a distraction and have negative consequences, this study emphasizes that it can also be harnessed as a valuable tool for learning and AP [25; 16; 13; 24; 5]. Students can create online groups, for instance, to organize projects, talk about course material, and stay in touch with absent classmates for updates on their academic progress [5]. Likewise, according to Bartosik-Purgat et al. (2017), SM is a good communication medium for university lecturers, depending on their level of education. It can inform successful instructional strategies. These results add to previous research on the advantages and difficulties of SMU in

education and can help design successful approaches to incorporate social media into instructional practices [26]. In conclusion, while SM can be a useful tool for learning and communication, excessive use of SM can have negative effects on AP.

### **Impact of SMU on WB**

The impact of SMU on the WB of school level student has drawn more attention as a study area in recent years. The increasing use of SM among students poses a significant risk to their overall mental and emotional health [27; 28; 29]. This review will examine the most recent studies on SMU's impact on WB in SLS. Although SMUs can offer many advantages, such as improved social connectivity and information availability, concerns have been raised about the potential adverse consequences on WB. This review will look at the most recent research on how SMU affects WB among SLS. While some studies suggest a negative relationship between SMU and WB, others, like Baltacı and colleagues' (2021) study observed at the relationship between internet addiction, social anxiety, and coping strategies. The study's findings showed an important link b/t internet addiction and social anxiety, based on data collected from 481 university students [27]. Similarly, another cross-sectional study conducted by Jia et al. (2022) among 1307 Chinese college students, there has been an increasing incidence of social anxiety among social media users, particularly young adults, in recent years as a result of their intensive use [30]. Facebook is a popular SM that people use for communication and entertainment. Yet,

frequent use leads to addiction and has an effect on many users' daily lives, particularly those of young people [28]. According to a cross-sectional study done by Blachnio et al. (2016) on 381 Facebook users, regular users have higher life satisfaction and self-esteem than addicts or intensive users [12]. Facebook addiction was found to be associated with lower self-esteem and decreased life satisfaction [12]. Likewise, increased anxiety and depression are side effects of excessive use. A study conducted by Fardouly et al. (2015), teenage girls may have negative consequences from SM due to social comparison and fear of Missing out (FOMO) [21]. On the other hand, social media use has been found to have a positive impact on WB by promoting bonding and bridging social capital [29]. An Empirical evidence suggests both positive and negative effects exist, reconciling inconsistencies in

the study [29]. Overall, the research points to the possibility that SMU may affect the WB of SLS in both favourable and unfavourable ways. While SMU can have positive impacts for numerous, it's crucial for teenagers to be aware of potential drawbacks, participate actively in SMU activities, and utilize coping mechanisms to lessen bad consequences.

### Objective

- To examine the prevalence of SMU among SLS.
- To examine the impact of SMU on AP and WB among SLS.
- To examine the different between male and female students.

### Hypothesis

- H1: Impact of SMU on AP.
- H2: Impact of SMU on WB.
- H3: There is a significant difference between male and female students in terms of SMU.

### Conceptual Framework on Impacts of SMU on AP and WB

Fig 1. Impact of Social Media Use (SMU) on academic Performance (AP) and Student Well-Being (SWE).



### Material and Methods

The study mainly aims to investigate the impact of SMU on AP, and WB among SLS in selected Kathmandu boarding schools and socio-demographic factors. The research design employed was an analytical cross-sectional study conducted in a selected boarding school in Kathmandu. The study population consisted of students in Grades 8, 9, and 10 at the boarding school, with a total

of 473 individuals. Two boarding schools were employed as strata in the probability-based stratified sampling techniques. From each stratum, the sample size was determined using the formula:

The population of strata  $\times$  sample size (n)  
Total population size.

For one boarding school, the sample size was calculated as  $381/473 \times 229 = 184.4 \approx 184$ , while for the other boarding school, the



sample size was  $92/473 \times 229 = 44.5 \approx 45$ .

Thus, the total sample size was  $208 + 21 = 229$ , utilizing the Cochran formula.

A self-administered questionnaire method was used as the research tool, with the questionnaire developed in the English language. The research tool consisted of four parts: questions related to the use of social media (a standard tool developed by Sandeep Lahiri, 2019), questionnaires about academic performance and well-being, and instruments developed by researchers.

Pretesting of the tool was done among 10% of the total sample size, i.e., 23 students currently studying in Grades 8, 9, and 10 of Mount Glory Boarding School. Modifications were made to the tool as needed. This study was conducted after obtaining approval from the research guide and an approval letter from the Institutional Review Committee (IRC) of YHSA.

Data were analyzed using simple descriptive statistics, regression analysis and independent t- test to draw conclusions and implications from the study findings.

## Results

The descriptive statistics data analysis of the study reveals some interesting findings. A majority of the respondents were male students, comprising 56.3% of the sample. Furthermore, 83% of the respondents were below 15 years of age, indicating that the study primarily focused on a younger population. In terms of ethnicity, the majority of respondents were Brahmin and Chhetri, representing 49.3% and 60.3% of the sample, respectively. Similarly, 37.1% of the respondents were grade 10 students.

When it comes to religion, the study found that 60.3% of the respondents followed Hinduism. Meanwhile, the average monthly income of families was above 50,000 Nepalese rupees among 61.1% of respondents, suggesting that a majority of the participants were from relatively well-off families. Interestingly, playing games was the favorite leisure activity among 46.3% of the respondents.

These findings shed light on the socio-demographic characteristics of the study population, highlighting the importance of considering such factors when analyzing the impact of SMU on AP and WB among SLS in Kathmandu boarding schools.

Based on the results of the descriptive study, it can be determined that a majority of the respondents, precisely 60.7%, have an inclination for mobile devices. Additionally, it was revealed that 57.6% of the respondents have been utilizing SM platforms for a period of 1-5 years.

Further investigation of the data shows that Facebook is the favored platform for 41% of the respondents, with 36.7% preferring Instagram and 66.4% using YouTube regularly. Surprisingly, 39.3% of the respondents do not ever use Viber.

Furthermore, it was perceived that a substantial number of the respondents, precisely 45.9%, use SM at night. Remarkably, 29.7% of the respondents were informed using SM or both knowledge-seeking and entertaining devotions.

Generally, these results deliver appreciated insights into the SM usage patterns and favorites of the respondents, the prominence



of the domination of mobile devices, and the admiration of certain SM platforms.

**Table 1. Information Related to Academic Performance of Respondents**

Statements	SD <sup>1</sup>	D <sup>2</sup>	N <sup>3</sup>	A <sup>4</sup>	SA <sup>5</sup>	Mean ± SD <sup>6</sup>
	%	%	%	%	%	
1. Use subject matter.	0.9	7	24.9	40.2	27.1	3.86±0.92
2. Aware of educational value.	7.9	1.7	11.4	39.3	39.7	4.01±1.13
3.Discovery information	2.2	4.4	13.5	34.1	45.9	4.17±0.97
4. Creation of study timetable.	5.2	10.9	17	24.9	41.9	4.17±0.97
5. latest updates.	0.9	3.1	14.4	32.8	48.9	3.87±1.22
6. Sharing opinions and ideas.	0.9	3.1	14.8	28.4	52.8	4.26±0.87
7. Academic success.	2.6	2.2	10.9	32.3	52	4.29±0.89
8. Express originality and ingenuity.	4.4	10.9	21	26.6	37.1	3.81±1.17
9. Inappropriate use affects academic	0.9	10.9	18.8	27.5	41.9	4.29±0.93
10.Writing, speaking	10.5	20.1	16.2	27.1	26.2	3.38±1.34
11. Heavy use affects grade.	9.6	21.8	24.5	13.1	31	3.34±1.36

<sup>1</sup>SD = Strongly Disagree; <sup>2</sup>D=Disagree; <sup>3</sup>N = Neutral; <sup>4</sup>A=Agree; <sup>5</sup>SA = Strongly Agree

<sup>6</sup>SD = Standard Deviation

**Table 1** Shows that the respondents who were aware about inappropriate use of social media with highest mean ± SD 4.29±0.93.

Likewise, the respondents whose grades were affected by frequent use of social media having mean± SD of 3.34±1.36.

**Table 2. Information Related to Wellbeing of Respondents**

Statements	SD <sup>1</sup>	D <sup>2</sup>	N <sup>3</sup>	A <sup>4</sup>	SA <sup>5</sup>	Mean ± SD <sup>6</sup>
	%	%	%	%	%	
1. Avoid feeling lonely.	4.4	8.3	14	32.3	41	3.97±1.13
2. Stress reliever.	7.9	7	16.2	36.2	32.8	3.79±1.19
3. Utilized bored.	3.5	4.8	11.4	45.4	34.9	4.03±0.98
4. Solutions.	5.2	6.6	29.3	34.1	24.9	3.66±1.08
5.Health suffers using heavy SM.	7.4	13.1	11.8	33.6	34.1	3.73±1.33
6. Excessive usage social problems.	9.6	19.7	14.8	27.9	27.9	3.44±1.25
7. Affects exercises.	10.9	21	16.2	31.4	20.5	3.29±1.30
8. Problems eating habits.	19.2	26.2	14	21.4	19.2	2.95±1.42
9. Issues sleeping.	16.6	15.7	17.9	22.7	27.1	3.28±1.43

<sup>1</sup>SD = Strongly Disagree; <sup>2</sup>D=Disagree; <sup>3</sup>N = Neutral; <sup>4</sup>A=Agree; <sup>5</sup>SA = Strongly Agree

<sup>6</sup>SD = Standard Deviation



**Table 2** shows the information related to wellbeing of respondents, the respondents who utilized social media to remove boredom have highest mean±SD 4.03±0.98

Meanwhile, respondents, whose daily eating habits has been affected by use of social media has lowest mean ± SD 2.95±1.42.

**Table 3. The Effect of Socio Media Use (SMU) on Academic Performance (AP) (Coefficient Model)**

Model	UC		SC	t	LLCI	ULCI	SIG
	B	SE*	Beta				
Constant	4.196	0.099		42.27	4.00	4.39	0.000
Mean SMU	-0.086	0.038	-0.147	-2.24	-0.162	-0.10	0.026

Dependent variable: Mean Academic Performance (AC)

\*SE stands for Standard Error, UC for Unstandardized Coefficient,

SC for Standardized Coefficient, LLCI for Lower Level Confidence and ULCI for Upper Level Confidence.

**Table 3** reviews and offers evidence for Hypothesis H1, which suggests that AP is dependent on SMU. The study used regression analysis to examine this relationship, with AP being the dependent factor and SMU serving as the independent factor. The outcomes of the analysis disclose a constant beta value of -.147, by a t-value of -2.24 and a level of confidence between -.162 and -0.10. The p-value was greater than 0.005, demonstrating that the results were not statistically co-relationship at this level. Though, the study did discovery a significant

level of .026, which was less than 0.005. This recommends that while the beta value did not demonstrate a positive level in this examination, there was a significant level of relationship between the two variables. Generally, the suggestion from Table 3 proposes that while there may be an association between SMU and AP, further examination is essential to determine the nature and strength of this connection. However, the study's discoveries do deliver important intuitions into the potential impact of SMU on AP.

**Table 4. The impact of Social Media (SMU) Use on Well-Being (WB) (Coefficient Model)**

Model	UC		SC	t	LLCI	ULCI	SIG
	B	SE	Beta				
Constant	3.613	0.119		30.431	3.379	3.847	0
Mean SMU	-0.015	0.046	-0.022	-0.33	-0.106	0.075	0.741

Dependent Variable: Mean Well-Being (WE)

The acronyms SE, UC, SC, LLCI, ULCI, and Mean SMU stand for Standard Error,

Unstandardized Coefficient, Standardized Coefficient, Lower Level Confidence, and Upper

Level Confidence, respectively



**Table 4** revealed the regression analysis model for Hypothesis H2, which suggests that SMU is an independent factor that impacts WB, the dependent factor. The outcome discloses a constant beta value of -0.022, with a t-value of -0.33 and a level of confidence between -0.106 and 0.075. The p-value was greater than 0.005, proposing that the results were not statistically significant at this level.

Though the study establishes a significant level of 0.741, this value was upper than  $p > 0.005$ , demonstrating that the relationship

between SMU and WB was not significant. In assumption, the regression analysis proposes that both the beta value coefficient and the significant level did not show a positive association in this study.

Hence, the results suggest that SMU may not have a positive influence on WB, showing possible problems associated with SMU at the SLS. While the study's effects do not provide definite evidence, they do focus on the importance of additional research in this area to well understand the possible impact of SMU on WB.

**Table 5. Male students and Female Students Independent Sample T-tests**

		Levene's Test for Equality of Variances		t-test for Equality of Means		Sig. (2-tailed)
		F	Sig.	t	df	
Mean SMU	Equal Variances Assumes	0.006	0.94	2.46	227	0.014
	Equal variances not assumed			2.45	210.3	0.015

**Hypothesis 3:** Here is a significant difference between male students and female students in terms of SMU. An independent t-test was showed to test this hypothesis. The statement of equal variances was inspected with Levene's test, which resulted in  $F = 0.006$  and  $P$  value (2-tailed) = 0.014. Established on these outcomes, it was expected that the variances were equal at  $df=227$ . Though, the result of the test presented that the  $P$  value is lower than 0.05. Thus, the hypothesis is recognized.

**Discussion**

The increasing habit of SM platforms amid young people has risen up concerns about the possible effects on their WB and AP.

Current study examined SMU's impact on WB and AP among 229 SLS who were belonging to in a boarding school. The researchers established objectives and hypotheses to discover the relationship between SMU and AP and the impact on WB, as well as to examine the association between SMU and demographic factors such as gender.

The current study discoveries also demonstrate that SMU had both positive and negative impact on students' WB and AP. Positive things included developed communication with peers and access to information, while negative things included increased worry, depression, and decreased



AP. The study things to see the essentials for mediation aimed at encouraging healthy SM habits amongst young students. For example, parents and instructors can inspire responsible SMU by setting guidelines and limits on usage. Additionally, SM platforms can present features that encourage an improved online setting by limiting excessive practice and filtering unsafe content.

**The prevalence of SMU among SLS** was acknowledged using descriptive analysis in this current study, and the outcomes showed important information about SMU. The current study findings showed that 60.7% of students preferred using mobile devices to access social media platforms. Moreover, 90% of students used Messenger, which has been extensively researched. Amongst them, 53.7% used at least 2-3 SM sites regularly. The current study found that 48% of students spent 1-3 hours daily on SM, which is alike previous study where 36.2% of participants spent 2.4 hours per day [22], another evidence reported that; spend time on average more than four hours daily [4]. Additionally, 29.7% of students reported searching for informative and entertaining content while surfing online in the current study where previous study revealed that 40.5% of students use social media as a source of recreation and relaxation, while 21.8% use it for educational purposes [22]. Likewise, 73% of student's engaged in SM for non-academic purpose [4].

### **H1: Impact of SMU on AP**

The relationship between SMU and AP has been a subject of attention among

researchers in recent ages. The present study's hypothesis H1 proposes that AP is dependent on SMU. The results from the regression analysis discovered a negative beta value of -0.147 and a significant level of 0.026, demonstrating a relationship between the two factors. Though, it is main to note that additional examination is needed to define the nature and strength of this relationship. Numerous previous studies have also observed the impact of SMU on AP [16; 4; 7]. A study by E-ISSN (2022) found that time spent on SM and excessive usage of SM negatively impact students' AP [4]. Similarly, Alahmar (2016) found that 42% of students reported that SOME have a positive effect on their AP [16]. Conversely, a study by Azizi et al. (2019) found that there was an adverse and substantial correlation b/t addiction to social networking and AP [13]. Even though some variations in the results, these previous studies highlight the potential impact of SMU on AP [25; 22; 4; 5]. The current study's findings provide vital insights into this relationship and suggest that further investigation is needed to fully understand the nature and strength of this association.

### **In the H2: impact of SUM on WB**

The current study outcome demonstrates that; the beta value was found to be -0.022, with a t- value of -0.33 and a confidence level ranging between -0.106 and d0.075. The P-value was higher than 0.005, indicating that the results were not statistically significant. The results suggested that there may not be a positive influence of SMU on WB and that there may be problems



association with SMU at the SLS. The H2 outcome revealed that SMU may not have positive influences on WB, suggesting possible problems associated with SMU. These findings are consistent with numerous studies that have explored the effects of SMU on Students' WB [11; 12; 28; 1]. According to a study by Malviya et al.

(2018), internet addiction is a new issue in the modern period [10]. A previous study found that; SM addiction: strain on eyes, anger, sleep disturbance, smoking, and junk foods. One-third had mild addiction [2]. While the positive indirect impact of SM and psychological WB is due to bonding and bridging social capital. The empirical model explains 45.1%. Evidence shows the coexistence of positive and negative effects [29]. Likewise, a study conducted by Jia et.al and found that; SMU is linked to interaction anxiousness, smartphone addiction, poor relationship with parents, cyberbullying [30]. A study done by Blachnio et. al (2016), and outcome revealed that; addiction is linked to lower self-esteem a life satisfaction [12], the findings highlighted the need for further research to understand the relationship between SMU and WB, and to develop strategies that promote the health of SMU.

### **H3: There is a significant difference between male and female students in terms of SMU**

The present study adds to this body of research by specifically examining the difference in SMU between male and female students. The findings suggest that there is indeed a significant difference in SMU between the genders, with male students

showing higher levels of addiction compared to their female counterparts. These results may have important implications for addressing problematic SMU among students, highlighting the need for gender-specific- intervention and prevention programs. Previous research has also explored the potential difference in SMU. For example, in a study by Liu et.al (2022), a prior study by Liu et al. (2022) highlighted an important finding from a previous meta-analysis. It was found that there is a significant difference in the pooled estimate between boys and girls when it comes to the relationship between time spent on social media (TSSM) and the risk of depression [18]. Previous study evidence revealed that; internet addicts found greater males than females [10]. A previous study by Fardouly et.al. (2015) suggest that women with high appearance comparison tendencies reported more facial, hair, and skin-related [21]. Likewise, another study done by Azizi et al. (2019) and the outcome demonstrate that male students had a higher mean score for social networking addiction than female students [13].

### **Recommendations**

Based on the study's conclusions, it is suggested that students limit their SMU, utilize their phones for AP and WB purposes, and be familiarized to relevant resources. Furthermore, students at threat of addiction should be educated about the threats. Additionally; the researchers recommended the practice of an EI model to enhance AP and WB. This model helps students identify their strengths and



weaknesses, leading to improved self-awareness, Self- management social awareness, and relationship management. By focusing on both personal and professional competencies, students can develop a more comprehensive understanding of themselves and their abilities, resulting in increased success and satisfaction in their academic and WB.

**Conclusion**

The study clarifies the significant influence of SMU on AP and WB among SLS in selected institutions. The study of the descriptive statistics showed that the majority of respondents were male students and that a significant proportion of them were under the age of 15. With a negative beta value and a significant level of 0.026, the study discovered a significant association between SMU and AP. To better understand the nature and strength of this association,

more study is required. The study also emphasized how SMU's detrimental effects on WB contributed to student addiction. The findings have significant ramifications for educators and policymakers as they create plans to deal with students negative SMU and encourage positive behaviors in their AP and WB.

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**Conflict of interest**

The authors declare no conflict of interest.

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**აბსტრაქტი**

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## Original Research

# The role of psycho-physiological characteristics in the realization and sports selection of athletes

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## Abstract

Our article mentions the problem of selection in sports. The psycho-physiological approach proposed by us is based on the method of assessing the inclination to a particular sports specialization, which takes into account the following algorithm of actions: psycho-social characteristics and genetic and typological characteristics of athletes.: psycho-social characteristics of sportsmen, and genetic and typological characteristics. EP Ilyin's motor skills technique; Determination of individual neurodynamic characteristics of a subject and their comparison with known, experimentally identified "model" neurodynamic characteristics that dominate, in terms of frequency of occurrence, among representatives of high-level sports. In modern conditions, when digital technologies are developing rapidly, the methods of evaluating and identifying talented athletes, saving health, and improving the quality of sports selection, which ensure a successful career and self-realization of all participants in sports activities, are of particular importance. We offer a psycho-physiological program, the obtained results provide important information in the form of practical recommendations to specialists, and coaches and help to determine the model characteristics, correctly plan the forecast, increase the selection efficiency, and improve the organization process.

**Keywords:** Selection in sports, typological characteristic, psycho-social characteristic, genetic indicator.

## Introduction

The article mentions that the problem of selection of highly qualified athletes and prediction of results cannot be solved only

by measuring anthropometric indicators, or only by studying and observing typological indicators, which are prevalent in sports science today.



The methodology for determining the individual neurodynamic characteristics of the subject developed by E.P. Ilyin's motor technique, determination of individual psychosomatic characteristics of the subject and their comparison with the known, "model" characteristics of successful athletes, who dominate among the representatives of the mentioned high-level sports. Many authors point to the fact that the changes in the growth process in adolescent athletes are numerous and, in relation to the selection in sports, are essential. Typological data are also important, which confirm the results that with many possible combinations of psychosomatic indicators measurement and evaluation, the share of typological characteristics decreases sharply and mainly dominates among athletes who have reached a high level of training.

The article mentions that the knowledge of the psycho-social and somatic characteristics of athletes, which are important in professional sports, is the basis that makes possible the early selection of potentially talented athletes according to sports specialization. Studies show that this is quite possible if the main psycho-somatic characteristics of the athlete are known. This article also mentions that it is impossible to plan and effectively manage the training process in the selected sport without in-depth knowledge of the psycho-social abilities and typological features of the athlete, and it is also impossible to predict the success and high results of these athletes.

## Material and methods

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The problem of human giftedness has been sufficiently developed in Russian psychology thanks to the research of representatives of the scientific school of D. B. Bogoyavlenskaya [1, 2]. At the same time, the issues of "giftedness" in sports have not yet received due development in academic science. Thus, in the reports of the All-Russian Scientific and Practical Conference "Psychology of Creativity and Giftedness" [3], with a very significant number of them, there are no reports at all that would reflect the study of the problem of giftedness in sports. It seems to us that today this problem cannot be solved only by tests-questionnaires, conversations, interviews, observations, which today dominate in the arsenal of sports psychologists [4, 5]. At the same time, attention is not paid to the psycho-physiological approaches to the problem of selection in sports, which are available in the arsenal of sports science, in particular, the psycho-physiology of sports and, to be more specific, the psychology of mood.

## Statement of the problem

One of the features of this approach is the measurement of neurodynamic characteristics, or, in other words, the properties of the human nervous system. For example, in the books of Serova, L. K. "Professional selection in sports" [6] and "Psychology of selection in sports games" [7], there is no mention of instrumental methods for measuring SNS and there are no instructions on how to take them into account in sports selection. In the book by T. S. Timakova "Factors of Sports Selection or



Who Becomes an Olympic Champion” [8], the researcher, considering methods for determining anthropometric indicators, does not focus on an unfavorable trend - the lack of instrumental methods for measuring NDC in the arsenal of sports psychologists.

In the monograph by N. L. Vysochina “Psychological Support for the Training of Athletes in Olympic Sports” [9], the topic of sports selection is completely absent. From our point of view, the psychological support for the training of athletes should begin with high-quality sports selection, with the solution of the problem of "giftedness" in sports, in other words, with the implementation in practice of the pedagogical principle - an individual approach to the trainee. In the book by Vysochina, N. L. “Sports Talent: Forecast and Implementation”, the issues of athletes’ predisposition to different sports are considered solely on the basis of the results of a survey of Olympic champions and outstanding coaches [9].

The interviewed specialists expressed the opinion that it takes from 1 to 2 years to reveal sports talent. It should be noted that the terms for revealing sports talents were determined by experienced specialists, but in practice, in most cases, young coaches work with novice athletes, who will certainly face objective difficulties in early assessment of a novice athlete's predisposition to a particular sports specialization. Such diagnostics, based on objective data obtained on the basis of instrumental measurement of psychophysiological indicators, is one of the main tasks of modern sports. Mistakes in the

choice of sports specialization can neutralize, or even nullify, all subsequent efforts of specialists in the implementation of programs for preparing athletes for important competitions, which can significantly reduce the ability of athletes for successful self-realization in sports [1,2,3]. Here it would be appropriate to refer to the collective monograph “Psychological factors of success in sports activities”, dedicated to the problems of elite sports, where the authors ask a reasonable question: “Why does an athlete who has been training for about 10 years in sports never achieve high results. There are several reasons. But one of them is the inconsistency of the athlete's psychocomplex with the chosen type of sports activity” [4].

It seems that the psychophysiological approach to the problem of giftedness and sports selection most of all correspond to the following definitions of the concepts of giftedness, abilities, inclinations, formulated in the encyclopedic dictionary "Man: anatomy, physiology, psychology". “Giftedness is a combination of a number of abilities that ensure the success (level and originality) of performing any activity ... the concept of giftedness can include the psychological (mental) stability of a person if his activity is associated with extreme conditions” [5]. “Abilities are ... innate, but developed in the process of activity: however, it is not unlimited, therefore, differences between people in abilities still remain. The latter are not due to the presence or absence of a particular function ... but the number of innate inclinations that



affect the manifestation of this function: the more inclinations a person has, ... the higher his ability (memory, concentration of attention, quick response to a signal, etc.) [5]. "The makings of abilities are the innate anatomical and physiological characteristics of a person, which determine a high level of manifestation of mental functions. The inclinations include typological features of the manifestation of the properties of the nervous system: strength or weakness, mobility or inertia, balance or the predominance of one of the nervous processes - excitation or inhibition" [9]. From the above definitions, it follows that the solution of issues of giftedness and selection in sports should begin precisely with the inclinations, which include the properties of the nervous system (hereinafter, abbreviated - NDC). In all our studies, in the aspect of the development of a psychophysiological approach to the problem of giftedness and sports selection, SNS was measured by motor methods of E.P. Ilyin [6]. To implement the techniques, the author's software and hardware complex was used [7]. It is appropriate to note here the well-known scientific fact that the human SNS is very conservative to changes in the process of growing up [8, 9]. The noted seems to be essential for the substantiation of the proposed psychophysiological approach to the problem of giftedness and selection in sports. In conclusion of the section, we will refer to the statement of the New Zealand athlete Peter Snell, three-time Olympic champion, Doctor of Physiology, quoted by Serova, L: "We cannot get a complete picture

of an athlete based only on the level of oxygen consumption, glycogen stores and biomechanical measurements ... The main thing what needs to be taken into account is psychology, an understanding of those personal qualities that determine the highest achievements in sports" [5].

### **Evaluation of predisposition to certain sports specializations**

There is a known method for assessing the natural predisposition to certain specializations in sports, which allows, on the basis of knowledge, on the one hand, of individual psycho-social measured by E. P. Ilyin's motor methods, on the other hand, model psycho-somatic characteristics, optimal choices and predict the ability of an athlete to achieve high results in the chosen specialization [9]. For example, in table 1, in digital format, model neurodynamic characteristics of successful representatives of several sports are presented. It also reflects the indicators (in points) of similarity / dissimilarity of the compared neurodynamic characteristics, model and individual, where the latter are given for three randomly selected subjects for whom it is required to determine the sports specialization. Based on the indicator of similarity/dissimilarity (respectively, 0–2 points/8–10 points) of the compared characteristics, represented by digital neurodynamic codes, the level of the subject's predisposition to certain sports specializations is determined. Note that in Table 1, in digital neurodynamic codes, the properties of the nervous system are presented in the following order: 1) the strength of the nervous system: 1 - strong, 2



- medium, 3 - weak; 2) processes of excitation and 3) inhibition: 1 - mobile, 2 - average mobility, 3 - inert; 4) "external" balance and

5) "internal" balance: 1 - excitation prevails, 2 - balance, 3 - inhibition prevails

**Table 1. Measuring the indicator of similarity / dissimilarity of the neurodynamic characteristics of the subject and the "model" characteristics of a typical representative, successful in sports specialization**

Sports	Specializations in sports	TC NDC in the group, neurocode	Index of similarity/dissimilarity of compared neurodynamic codes: high level of predisposition –0–2; medium - 3-4; satisfactory - 5–7; low level of predisposition to specialization – 8-10 points					
			1			2		3
			psychosomatic codes of subjects					
			13333		13311		31111	
Wrestling	freestyle	31122	8	low	8	low	2	high
	Greco-Roman	13322	2	high	2	high	8	low
	Judo	33322	4		4		8	low
Rugby								
	Defender	13311	4		0	high	6	
	striker	31133	6		10	low	4	

From the data presented in Table 1, it is clearly seen that an athlete with certain neurodynamic characteristics can be predisposed to several sports specializations at once, and if it is necessary to choose one, the selection specialist can use additional criteria. For example, it would be appropriate to take into account the results of a number of experimental studies [4, 3,], where statistically significant trends in the severity of balances were revealed, in their combinations. The trends point to the natural predisposition of subjects to more or

less successfully "interact" with the vector of gravity, as one of the fundamental characteristics of the external environment. These trends are presented by us in paragraphs 1–4, where the severity of the external and internal balance of the processes of excitation and inhibition is reflected in digital format. Based on the trends presented in paragraphs 1–4, it can be noted that for athletes of the first group (paragraph 1, combination of balances - 31), characteristic, in the process of activity, are jumping actions directed vertically upwards, that is, against



the vector of gravity. Hypothetically, this group can also include: high jump, pole vault; volleyball (playing on a net), etc. A group of athletes with a combination of balances 13 (item 3) differs from group 1 in the opposite nature of "interaction" with the vector of gravity. Sports are also represented here, where often difficult to predict "forces" of nature (currents, wind, etc.) are present. Hypothetically, the second group can be categorized: wrestling. The identified tendencies need experimental studies for additional verification in the listed sports (points 1–4), as well as in those specializations for which model

neurodynamic characteristics have yet to be identified. Note that in Table 1, the severity of external and internal balances is shown, respectively, by the last two digits in five-digit neurodynamic codes, reflecting individual and model neurodynamic characteristics. Optimizing training programs in the chosen sport is impossible without knowing the psycho-somatic abilities and characteristics of athletes [6, 8]. Such a prediction is needed for athletes who have different playing roles to learn. For example, Table 2 presents data for basketball, rugby, and wrestling.

**Table 2. Prediction of psychological abilities and characteristics of athletes with different playing roles based on model neurodynamic characteristics**

Natural psychological abilities manifested in four aspects of sports activity	Forecast of the severity (in points) of psychological abilities and athletes with different game roles: low - 1 point, medium - 2, high - 3 points		
	wrestler	forward	Defender
psycho-social and somatic characteristics of roles in the form of digital codes	12221	31231	23321
<b>Features of psychomotor</b>			
Movement coordination	2	3	1
Sprint makings	3	2	2
Reaction speed in play activity	2	3	1
The speed of recovery processes	3	3	3
Stayer makings	2	3	2
motor memory	1	1	1
<b>Individual style of activity</b>			



The dominant part of the activity: executive (exec.), indicative (orient.)	exec.	balance	orient.
Learnability (high pace - 3 points)	3	2	2
Tendency to leadership	3	1	3
Adapting to changing, crisis situations	3	3	1
Duration of preparation for the competition (3 points)	1	2	1
<b>Peculiarities of intellectual activity</b>			
Type of thinking: thinking (think); artistic (art)	art.	art.	thinking
The speed of associative and mental processes	3	3	1
Switching attention	3	3	1
Concentration of attention	2	2	3
memory involuntary	3	3	1
Psychonomic characteristics in the form of numerical codes	12221	31333	23322
Memory is arbitrary	2	2	2
Creativity of thinking	1	3	1
Speed of visual image formation	2	3	1
The pace of the beginning of activity (fast - 3 points)	3	3	1
<b>Resistance to crisis conditions, endurance feature (endurance to fatigue)</b>			
Endurance (before fatigue)	3	3	3
Endurance (in the face of fatigue)	2	1	3
Adaptation to crisis environmental factors	1	2	3
Resistance to static postures	1	3	1
Courage	3	2	3
Determination	3	2	3
Tolerance for ambiguity	1	2	2
Emotional and physical resilience	3	3	3



It should be noted that the psycho-social and somatic characteristics of the model for athletes with different playing roles are presented in Table 2, by A. A. Banayan in a study conducted by Banayan on basketball and rugby teams [5]. They are, according to the results of their performances at the World Championships and Olympic Games One of the world leaders. These characteristics, according to our approach, can be taken as "model", which does not exclude the need for additional experimental studies to verify them for the considered game roles. In the study of A. A. Banayan, the task of forming individualized training programs for athletes, taking into account their roles, was also set and solved.

As shown above (see Table 1), we take a high predisposition as a subject's potential talent for certain specializations. However, before the successful self-realization of an athlete in the chosen specialization [1, 2], even if the optimal choice was made, the subject has a long sports path to the heights of mastery. And here it is advisable to touch on a topic that can be designated by the "coach – method – athlete" scheme, which implies the psychophysiological and psychological compatibility of participants in joint activities [7], when the coach applies such methods of working with a gifted athlete that do not contradict the natural individual style of sports activity of both.

## Conclusion

Based on the data obtained as a result of the research presented in the article, we can

assume that the use of the anthropometric methods we have found will be especially interesting for coaches focused on high sports results. At the same time, the use of somatotype in the process of sports selection is significant.

The selection of athletes and prediction of results should not be done on an intuitive level, but using evidence-based methods (testing, observation, survey, etc.). The main characteristics, which are based on the realization and socialization of athletes in sports, are typological and psychosocial characteristics (motivation, temperament, character). Considering the typology of the athlete's personality, his individual features, allows us to plan the full development of the athlete's abilities, and to effectively use them in predicting the results and realizing the athlete. A special place in the psychological support of sports activities is motivation, which helps a person to engage in sports and achieve success. A successful combination of temperament, motives, and character contributes to the development of capabilities, and their effective implementation. Typological and based on typological and psychosocial characteristics, the coach will be able to individualize the training process, select, and determine the attitude of athletes to responsible games, and make the right choice of motivation. Also, considering psycho-somatic characteristics, plan the career of a sportsman and the process of resocialization in society after the end of a successful sportsman's career.



## ფსიქო-ფიზიოლოგიური მახასიათებლების მნიშვნელობა სპორტსმენთა რეალიზაციასა და შერჩევაში

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### აბსტრაქტი

სტატიაში აღნიშნულია, რომ ნიჭიერი სპორტსმენებისა და ზოგადად, სპორტული შერჩევის პრობლემა ვერ გადაიჭრება მხოლოდ ანთროპომეტრიული მაჩვენებლების გაზომვით, ან მხოლოდ ტესტ-კითხვარებით, გასაუბრებით, ინტერვიუებითა და დაკვირვებებით, რაც დღეს სპორტის ფსიქოლოგთა არსენალში დომინირებს. ასევე, მეცნიერების მხრიდან, იგნორირებულია დიფერენციალური ფსიქოფიზიოლოგიის მეცნიერული საკითხები, რომლებიც აფართოებენ სტატიაში მითითებული პრობლემების გადაჭრის შესაძლებლობებს.

ჩვენს მიერ შემოთავაზებული ფსიქო-ფიზიოლოგიური მიდგომა ეფუძნება სუბიექტის მიდრეკილების შეფასების მეთოდს კონკრეტულ სპორტულ სპეციალიზაციაზე, რომელიც ითვალისწინებს მოქმედებების ალგორითმს: ფსიქო-სომატური სისტემის თვისებების ინსტრუმენტული გაზომვას, ე.პ. ილინის მოძრაობითი უნარების ტექნიკას, სუბიექტის ინდივიდუალური ნეიროდინამიკური მახასიათებლების დადგენასა და ცნობილ, ექსპერიმენტულად იდენტიფიცირებულ „მოდელოზ“ ნეიროდინამიკურ მახასიათებლებთან, რომლებიც დომინირებს მაღალი დონის სპორტსმენებს შორის, მათ შედარებას. დღეს, სპორტული ტექნოლოგიების განვითარების ეპოქაში, განსაკუთრებული მნიშვნელობა აქვს ნიჭიერი სპორტსმენების შეფასებისა და გამოვლენის, ჯანმრთელობის დაზოგვის მეთოდებს, სპორტის შერჩევის ხარისხის გაუმჯობესებას, რაც უზრუნველყოფს სპორტულ აქტივობებში ჩართული ყველა მონაწილის წარმატებულ კარიერასა და თვითრეალიზებას.

შემოთავაზებული ფსიქო-ფიზიოლოგიური პროგრამა მნიშვნელოვან ინფორმაციას აძლევს, პრაქტიკული რეკომენდაციების სახით, სპეციალისტებს, მწვრთნელებს და ეხმარება სწორად დაგეგმოს პროგნოზირება, აამაღლოს შერჩევის ეფექტურობა და გააუმჯობესოს სპორტული წვრთნის ორგანიზების პროცესი.

**საკვანძო სიტყვები:** შერჩევა სპორტში, ტიპოლოგიური მახასიათებელი, ფსიქო-სოციალური მახასიათებელი, გენეტიკური მაჩვენებელი.





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## Original Research

# Hepatoprotective effect of protein extract of the *Potamogeton perfoliatus* L. against carbon-tetrachloride (CCl<sub>4</sub>) - induced hepatic injury in mice

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## Abstract

This article demonstrates the hepatoprotective action of the protein extract of the aquatic plant *Potamogeton perfoliatus* L. based on the histopathologic changes in the liver of mice with CCl<sub>4</sub>-induced liver damage. Using the chromatography, 4 fractions of the protein extract were obtained. It has been suggested that a fourth fraction corresponding to low molecular weight protein should most likely exhibit hepatoprotective properties. SDS-PAGE of *P. perfoliatus* L. revealed a protein with molecular weight of 21 kDa corresponding to the fourth peak of a chromatogram.

**Keywords:** Potamogeton perfoliatus L., proteins, tetrachloromethane, CCl<sub>4</sub>, chromatography, gel filtration, hepatocytes, SDS-PAGE

## Introduction

Plants naturally produce a diverse range of bioactive possessing varied therapeutic properties. In addition to polyphenolic compounds, plant proteins are also important. Proteins produced in medicinal plants exhibit antimicrobial, antioxidant,

anti-cancerous, neuromodulatory and other properties [9].

The therapeutic potential of aquatic plants have not been properly studied. Potamogetonaceae, or the pondweed family, includes 110 species belonging to six genera,



where the genus *Potamogeton* is the largest among them. Twelve species of the genera were revealed in Georgia. The usage of various plants from this genus in folk medicine is reported for curing several pathological conditions [8]. The presence of variety of secondary metabolites, such as glycosides, pentosidases, rosemary acids, quercetin, phenolic acids was demonstrated in *P. perfoliatus* L [6]. The phenolic extract has a degrading effect on CD68 and GFA expression in the brain stem of rats, indicating its possible neuroprotective properties [4].

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1 million due to viral hepatitis and hepatocellular carcinoma [1]. Toxins like carbon tetrachloride ( $\text{CCl}_4$ ) can cause metabolic inefficiency in the liver, which

can lead to liver fibrosis, cirrhosis, or in some cases hepatocellular carcinoma [3]. The injections of  $\text{CCl}_4$  results in the active generation of reactive molecules with cytochrome P450 and formation of trichloromethyl peroxy radical [7]. Trichloromethyl peroxy radical, in turn, causes oxidative stress which promote lipid peroxidation and the damage of hepatocellular membrane and development of inflammation and apoptosis [2]. Despite the large number of hepatoprotective agents, they all have a number of disadvantages, therefore the search for hepatoprotective compounds of natural origin is relevant.

In this study we investigated the hepatoprotective effect of protein extract of the *Potamogeton perfoliatus* L. based on the histopathologic changes in the liver of mice with  $\text{CCl}_4$ -induced liver damage.

## Material and Methods

### Plant Material and Extraction

The whole plant, *P. perfoliatus* L., was collected from Tbilisi reservoir (coordinates: 41° 44' 58" N, 44° 50' 44" E). The plant was cleaned using distilled water and air dried. 15 g of leaves (was extracted using 40% PBS (ratio 1:10)). The homogenate was stirred for 60 min using magnetic stirrer and after centrifugated at 3000 rpm for 20 min. The supernatant was used for the protein determination.

### Animals

White mice, 4–6-month-old (average weight of  $25 \pm 0.05$  g), were used in the study. The mice were housed under normal light- dark conditions (12 hours light followed by 12 hours dark) for the entire experiment and had access to food and water, *ad libitum*. The animals were randomly assigned to one of 4 groups:

- First group: control animals
- Second group: 10% oil  $\text{CCl}_4$  solution;
- Third group: 10% oil  $\text{CCl}_4$  solution and *P. perfoliatus* L. extract.
- Fourth group: *P. perfoliatus* L. extract.

Each group contained 6 mice. The mice of the first group were injected with double distilled water for 30 days. The mice of the second group received an intragastric dose of 10% CCl<sub>4</sub> dissolved in olive oil for 30 days. The mice of the third group received an intragastric dose of 10% oil CCl<sub>4</sub> solution and *P. perfoliatus L.* extract (6 g/kg) for 30 days. The mice of the fourth group received an intragastric dose of *P. perfoliatus L.* extract (6 g/kg) for 30 days.

### Biochemical studies

The protein from the plant extract was concentrated using precipitation with 90% ammonium sulfate solution. The total protein concentration was determined using the Lowry method [5]. The protein solution was subjected to gel filtration on a Sephadex G75 column equilibrated with 5 mM KH<sub>2</sub>PO<sub>4</sub>, 150 mM NaCl pH 8. Fractions were collected at a flow rate of 0.5 mL/min.

Protein content was measured. SDS-PAGE was performed accordingly to Laemmli in a 12% polyacrylamide gel.

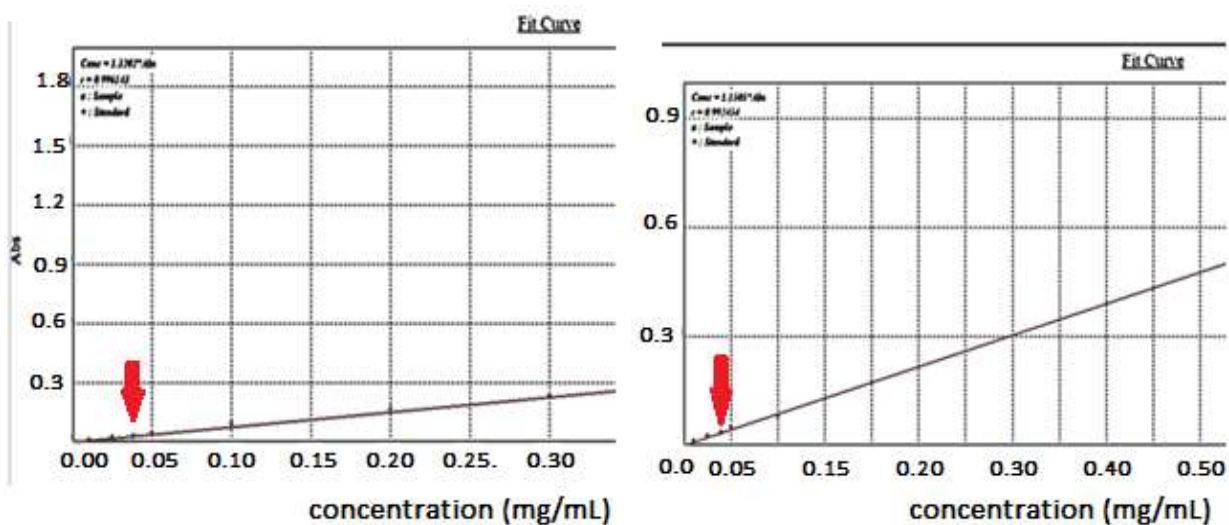
### Histological staining

Van Gieson's Stain of liver extracted from 6 mice was performed for each group. Mice were anesthetized with chloroform; livers were removed and placed in 15% formalin solution. Liver tissues were sliced into coronal sections using a rotary microtome. Images were obtained using AmScope microscope.

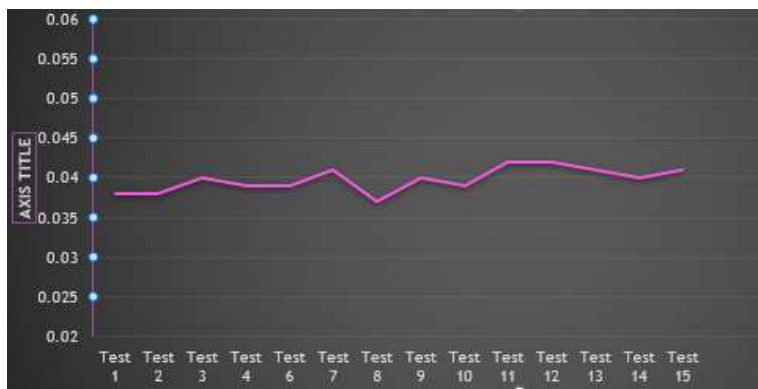
### Discussion

Proteins of the *P. perfoliatus L.* extract retained their initial concentration for 30 days after preparation at +4°C (0.038 mg/mL), which allows the use of the protein extracts in experiments for a long period of time (Figs. 1 and 2).

Figure 1. The concentration of proteins of *P. perfoliatus L.* extract (the concentration of 0.038 mg/mL).



**Figure 2. Concentration of of proteins of *P. perfoliatus L.* extract during 30 days after preparation of the extract at + 4°C. Measurements were performed every 48 hours.**



Histological analysis of liver tissue morphologies confirmed the protective effect of *P. perfoliatus L.* extract in CCl<sub>4</sub>-induced hepatic injury. Liver of mice in the control group (Figs. 3.1 and 3.2) showed normal hepatocytes, obvious sinusoids, and central vein. Liver of mice in the CCl<sub>4</sub> group was characterized by hepatocellular

degeneration and substitution of hepatocytes with connective tissue. Liver of mice of CCl<sub>4</sub> + *P. perfoliatus L.* extract group was characterized by less profound injury, as was evident by not significant development of connective tissue. (Figs.4.1-9.2). Liver of mice of *P. perfoliatus L.* extract group showed no pathological changes.

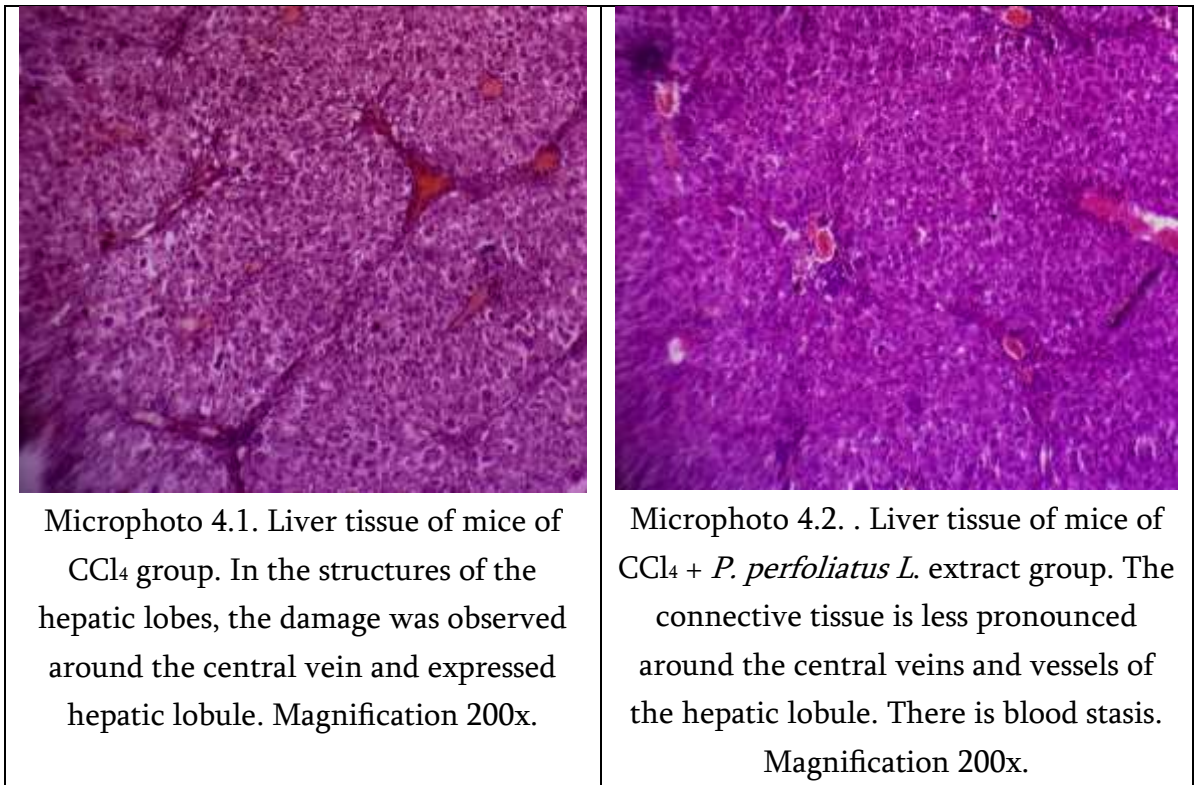


Microphoto 3.1. Liver tissue of mice of control group. Magnification 200x.

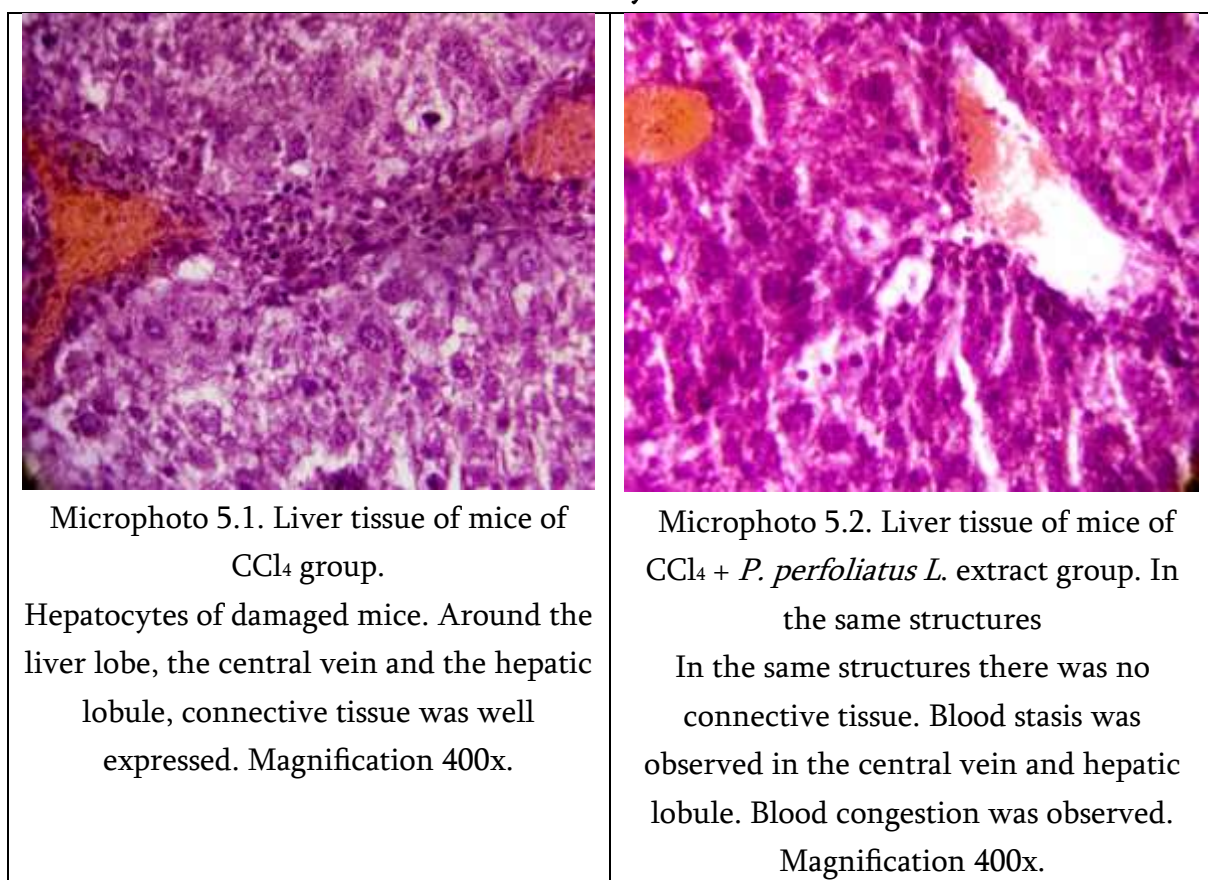


Microphoto 3.2. Liver tissue of mice of *P. perfoliatus L.* extract group. Magnification 200x.

Figure 3.1-9.2. The effect of the *P. perfoliatus L.* protein extract on the liver tissue of mice

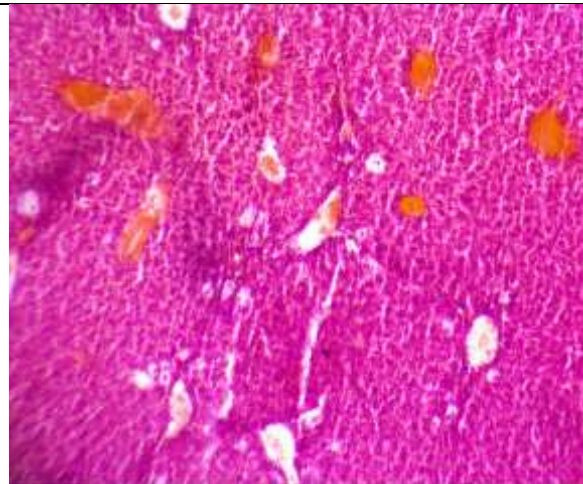


affected by CCl<sub>4</sub>.

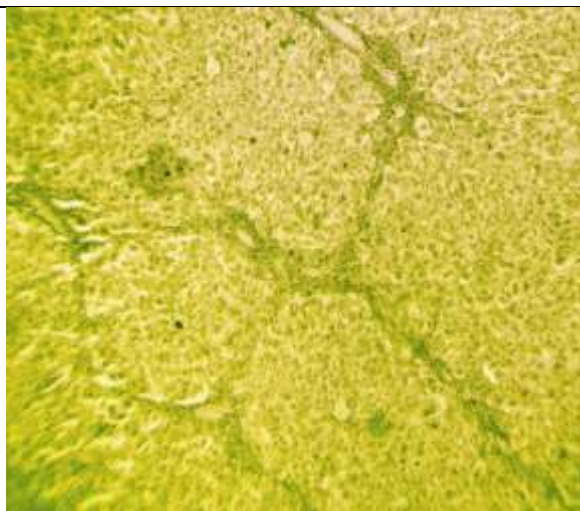




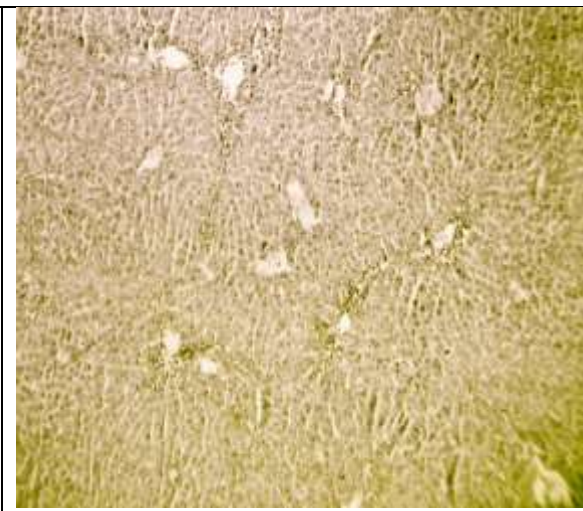
Microphoto 6.1. Liver tissue of mice of CCl<sub>4</sub> group. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



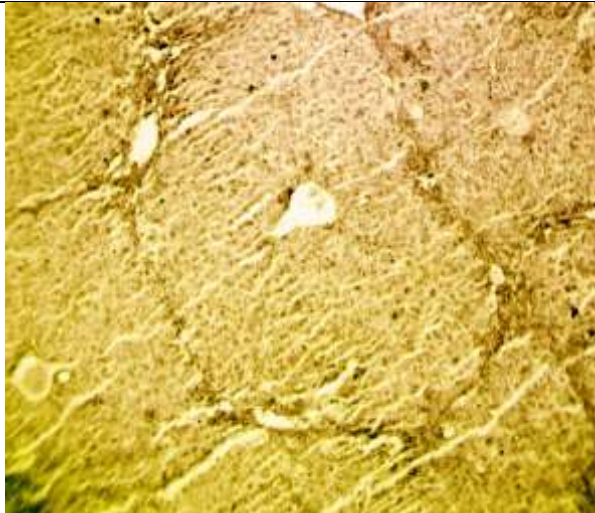
Microphoto 6.2. Liver tissue of mice of CCl<sub>4</sub> + *P. perfoliatus L.* extract group. In the same structures connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was observed



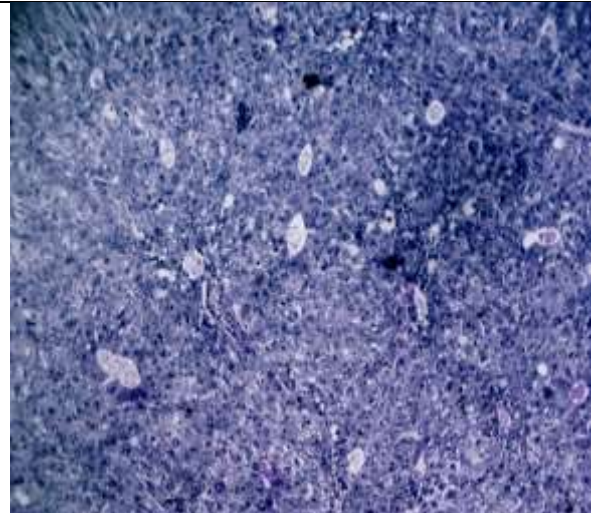
Microphoto 7.1. Liver tissue of mice of CCl<sub>4</sub> group. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



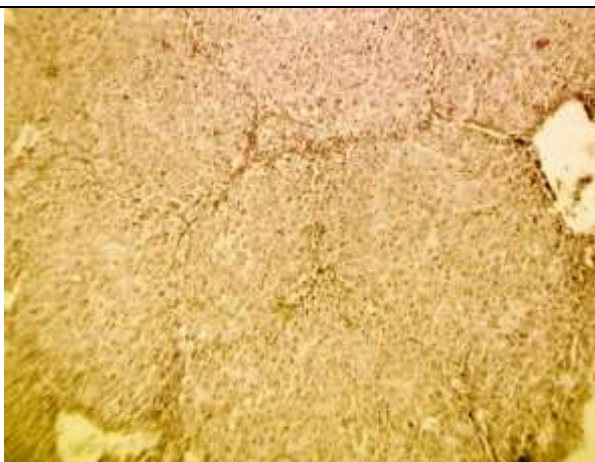
Microphoto 7.2. Liver tissue of mice of CCl<sub>4</sub> + *P. perfoliatus L.* extract group. Connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was not observed. Magnification 200x.



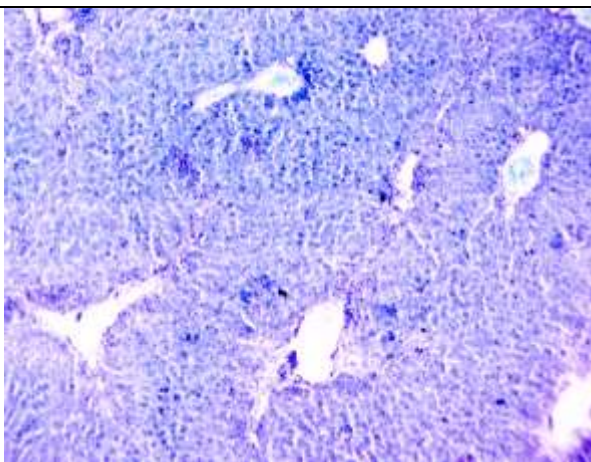
Microphoto 8.1. Liver tissue of mice of CCl<sub>4</sub> group. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



Microphoto 8.2. Liver tissue of mice of CCl<sub>4</sub> + *P. perfoliatus L.* extract group. Connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was not observed. Magnification 200x.



Microphoto 9.1. Liver tissue of mice of CCl<sub>4</sub> group. Hepatocytes of damaged mice. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



Microphoto 9.2. Liver tissue of mice of CCl<sub>4</sub> + *P. perfoliatus L.* extract group. In the same structures connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was not observed. Magnification 200x.

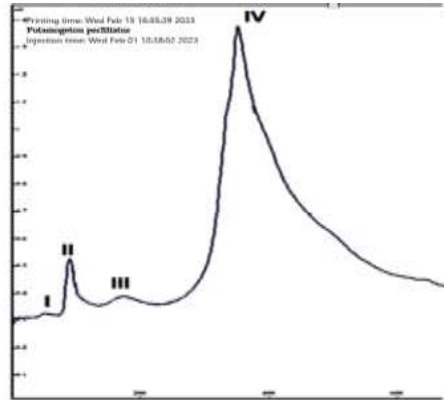
*P. perfoliatus L.* extract was subjected to gel filtration on a Sephadex G75 column, which  
DOI: 10.56580/GEOMEDI33

separated the protein solution in 4 fractions (Fig. 10). Fraction IV was of particular

interest since it was present in the highest concentration and most probably the protein

of this peak exhibited hepatoprotective properties.

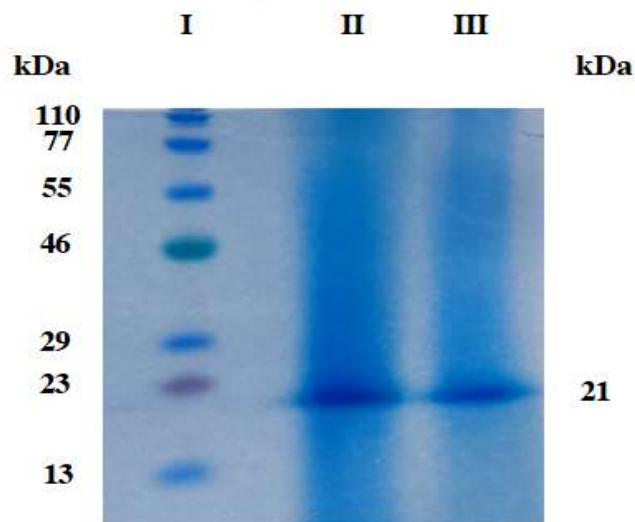
**Figure. 10. Elution profile of *P. perfoliatus* L. protein by gel-filtration on Sephadex G-75.**



In the later stages of experiments, SDS-PAGE of *P. perfoliatus* L. protein extract revealed one protein fraction with a

molecular weight of 21 kDa, which corresponds to the fourth peak of gel-filtration chromatography (Fig. 11.).

**Figure. 11. SDS-PAGE of *P. perfoliatus* L. Protein fraction with molecular weight of 21 kDa (lanes II and III). Is shown. Marker proteins (lane I).**



## Conclusions

1. Proteins of the *P. perfoliatus* L. extract retained their initial concentration for 30 days after preparation at +4°C, which allows

the use of the protein extracts in experiments for a long period of time.



2. *P. perfoliatus L.* protein extract with a concentration of 6 g/kg (0.006 mg/g) exhibited moderate hepatoprotective activity *in vivo* in CCl<sub>4</sub>-damaged mice hepatocytes.



3. *P. Perfoliatus L.* protein extract at a concentration of 6 g/kg (0.006 mg/g) had no detrimental effect on the mice hepatocytes.

4. Gel filtration of *P. perfoliatus L.* extract on Sephadex G-75 revealed four fractions, the

fourth fraction contained a high concentration of low molecular weight protein.

5. SDS-PAGE of *P. perfoliatus L.* revealed a protein with molecular weight of 21 kDa corresponding to the fourth peak of a chromatogram. With high probability, this fraction exhibited hepatoprotective properties.

## წყალმცენარე *Potamogeton perfoliatus L.*-ის ცილოვანი ექსტრაქტის ჰეპატოპროტექტული მოქმედება ტეტრაქლორმეთანით (CCl<sub>4</sub>) დაზიანებულ თაგვების ჰეპატოციტებზე

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### აბსტრაქტი

სტატიაში მოცემულია წყალმცენარე *Potamogeton perfoliatus L.*-ის ცილოვანი ექსტრაქტის ჰეპატოპროტექტული მოქმედება თაგვების ტეტრაქლორმეთანით (CCl<sub>4</sub>) დაზიანებულ ჰეპატოციტებზე, რომელიც მიკრომორფოლოგიური პრეპარატების ანალიზით გამოვლინდა. გელ-ფილტრაციული ქრომატოგრაფიის საშუალებით მიღებულია, ცილოვანი ექსტრაქტის 4 ფრაქცია. გამოთქმულია მოსაზრება, რომ მეოთხე ფრაქცია, რომელიც დაბალმოლეკულურ ცილას შეესაბამება, დიდი ალბათობით, უნდა ავლენდეს ჰეპატოპროტექტულ თვისებებს. *P. Perfoliatus L.*-ის ცილოვანი ექსტრაქტის SDS-ელექტროფორეზული ანალიზით გამოვლინდა 21 kDa მოლეკულური მასის ცილა, რომელიც შეესაბამება გელ-ფილტრაციული ქრომატოგრაფიით მიღებულ მეოთხე ფრაქციას.

**საკვანძო სიტყვები:** *Potamogeton perfoliatus L.*, ცილები, ტეტრაქლორმეთანი, CCl<sub>4</sub>, ქრომატოგრაფია, გელ-ფილტრაცია, ჰეპატოციტები, SDS-ელექტროფორეზი.

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## Original Research

## Comparative Analysis of Different Brands of Ibuprofen Available on the Georgian Pharmaceutical Market

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### Abstract

According to the World Health Organization (WHO), 1 out of 10 medical products in developing countries are substandard or falsified. Since Georgia is a developing country and is listed among the countries where substandard and falsified medical products have been discovered and reported to WHO, it is important to explore the quality of medical products available on the Georgian pharmaceutical market. As an initial study, we have chosen one of the most widely used drugs worldwide – ibuprofen, available in the pharmacy network in Tbilisi – the capital of Georgia. We have studied 10 different brands, imported and locally produced, and 2 brand-name ibuprofens (Motrin and Advil). The content of the Active Pharmaceutical Ingredient (API) was determined by a spectrophotometer, in the ultraviolet region. The obtained results showed that all brand ibuprofens contained labeled amounts of API.

**Keywords:** Ibuprofen, Georgia, Spectrophotometry, Generic drug, Active Pharmaceutical Ingredient.

### Introduction

Access to essential medicines still remains a public health problem. As of 2017, according to research conducted by the World Health Organization (WHO), approximately 2 billion people worldwide did not have access to essential medicine [1]. The price of medicine is significantly reduced by their generic analogs but sometimes at the expense

of quality [2]. Based on various studies, WHO states that 10% of medical products (medicines, vaccines, and diagnostic kits) in developing countries are substandard or falsified [3]. A systematic review and meta-analysis reports 13.6% of poor quality medicine [4]. According to some research, this number can be even higher [5,6].



According to WHO, which is based on Appendix 3 to Annex, World Health Assembly document A70/23, 2017, falsified and substandard medical products are defined as follows: “Falsified medical products - Medical products that deliberately/fraudulently misrepresent their identity, composition or source. Substandard medical products - Also called “out of specification”, these are authorized medical products that fail to meet either their quality standards or their specifications, or both” [3]. Anesthetics and painkillers represent 8.5% of all reported falsified and substandard medical products and share third place with lifestyle products after malaria medicine and antibiotics [3]. According to the same report Georgia is listed in the countries where substandard and falsified medical products have been discovered and reported to WHO. Therefore, it is important to explore the quality of medical products available at Georgian pharmaceutical market. To begin testing medical products at the Georgian pharmaceutical markets, we chose Ibuprofen. Ibuprofen is widely used worldwide and is known as one of the safest pain killers, fever reducer, and nonsteroidal anti-inflammatory drug, which is also used

## Materials and Methods

### Sample collections, selection of solvent, and preparing samples for measurements

10 different brands of Ibuprofen tablets, originated from different countries (including Georgia) were purchased from

for the treatment of rheumatoid arthritis. Ibuprofen has been used as a pain reliever and fever reducer during the covid pandemic, despite the initial hypothesis that ibuprofen might worsen the condition of covid patients [7] – which ultimately was rejected [8,9]. Ibuprofen comes in different forms - tablet, capsule, suspension and intravenous and is mostly available as a generic form. Tests in different countries revealed substandard and falsified medicines including Ibuprofen [10–12]. Therefore it is important that medicines be tested on a regular basis by independent parties, especially in developing countries where mostly generic drugs are available.

The aim of this work was to compare labeled amount of API to our measurements for each brand of ibuprofen tablets purchased at pharmaceutical networks in Tbilisi, the capital of Georgia.

We studied 10 different, 9 imported and 1 domestically produced ibuprofens available at the Georgian pharmaceutical network. We have also studied the brand-name ibuprofens (Motrin and Advil) ordered from the USA. Motrin was used as a standard in this study.

drug stores in Didi Dighomi district, Tbilisi in September 2020. Another two brand-name ibuprofens, Motrin and Advil were ordered from the USA. Motrin was used to plot the standard curve and Advil was tested



along with other brand ibuprofen tablets. Three tablets for each brand of Ibuprofen were randomly selected and weighed. Then each tablet was powdered and dissolved in 0.1N NaOH solution. 0.1N NaOH was selected after testing several different solutions (unpublished data). Brand names with their country of production, labeled

amount of API, and weights are given in **Table 1**. According to the label, some tablets contained 200mg of ibuprofen and some 400mg. Therefore, 200 mg tablets were dissolved in 200 ml 0.1N NaOH and 400mg tablets in 400 ml 0.1N NaOH to make 1mg/ml final concentration. 1mg/ml stock solutions were diluted as needed.

**Table 1. Ibuprofen tablets analyzed in this study.** Sample ID, Brand name, origin of production, labeled amount of ibuprofen in the tablet, origin of production, labeled amount in the tablet, weights of 3 randomly selected tablets for each brand of ibuprofen and MEAN value with Standard Deviation (SD).

Sample ID	Brand name of samples	Country of origin	Labeled amount (mg)	Tablet weight (mg). Test 1	Tablet weight (mg). Test 2	Tablet weight (mg). Test 3	MEAN ± SD
A	IBUPROFEN	Ukraine	200	523	314	317	384.7 ± 119.8
B	IBUPROFEN	Belarus	200	250	253	256	253.0 ± 3.0
C	IBUPROFEN	Russia	200	446	437	387	423.3 ± 31.8
D	INFORIN	Macedonia	400	656	646	657	653.0 ± 6.1
E	IBUPROFEN DENK 400	Germany	400	692	696	686	691.3 ± 5.0
F	IBUPROFEN NORMON	Spain	400	588	580	585	584.3 ± 4.0
G	IBUPROTEC-400	India	400	726	733	744	734.3 ± 9.1
H	MIGOFEN	Georgia	400	561	550	560	557.0 ± 6.1
I	NUROFEN	Great Britain	200	446	439	432	439.0 ± 7.0
J	IBUPROFEN-GRINDEKS	Latvia	400	675	673	676	674.7 ± 1.5
K	Advil	USA	200	471	477	477	475.0 ± 3.5
L	Motrin	USA	200	335	325	329	329.7 ± 5.0

### Spectrophotometric measurements in the UV range and data analysis

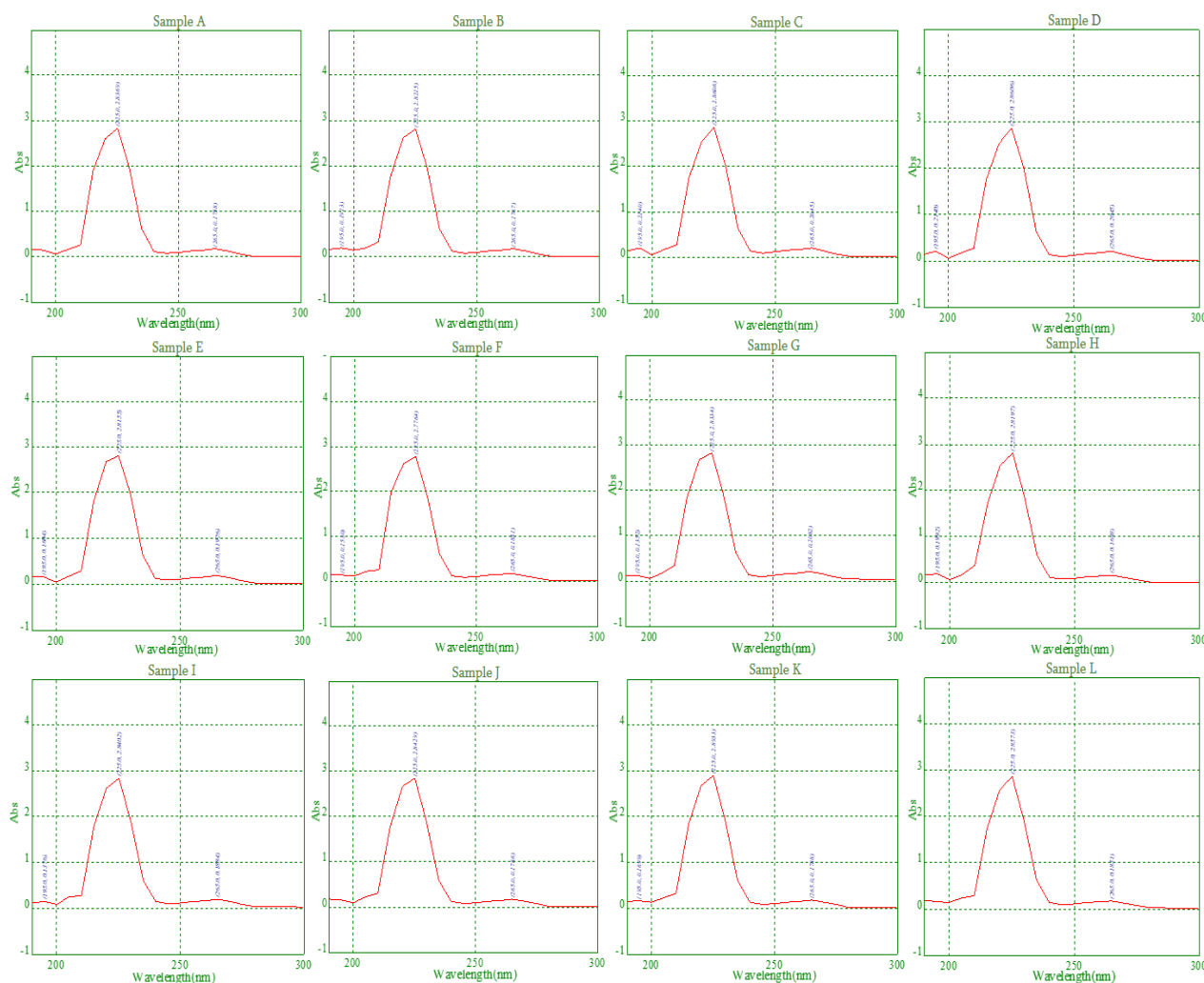
The spectrophotometric method was used to perform qualitative and quantitative tests.

Samples were scanned at Spectrophotometer (DRAWELL, UV-VIS SPECTROPHOTOMETER, MODEL: DU-8800RS) in UV range (190-300 nm; scan

interval 5nm) in 1 cm quartz cuvettes. Measurements were repeated three times for each brand of Ibuprofen. To plot standard curves (using Motrin) 6 different concentrations were used: 6.25 µg/ml; 12.5 µg/ml; 18.75 µg/ml; 25 µg/ml; 31.25 µg/ml and 37.5 µg/ml. Stock solutions for Motrin

were made the same way as other brand ibuprofens, described above. Mean values

and standard deviations of concentrations were calculated for each brand of Ibuprofen.

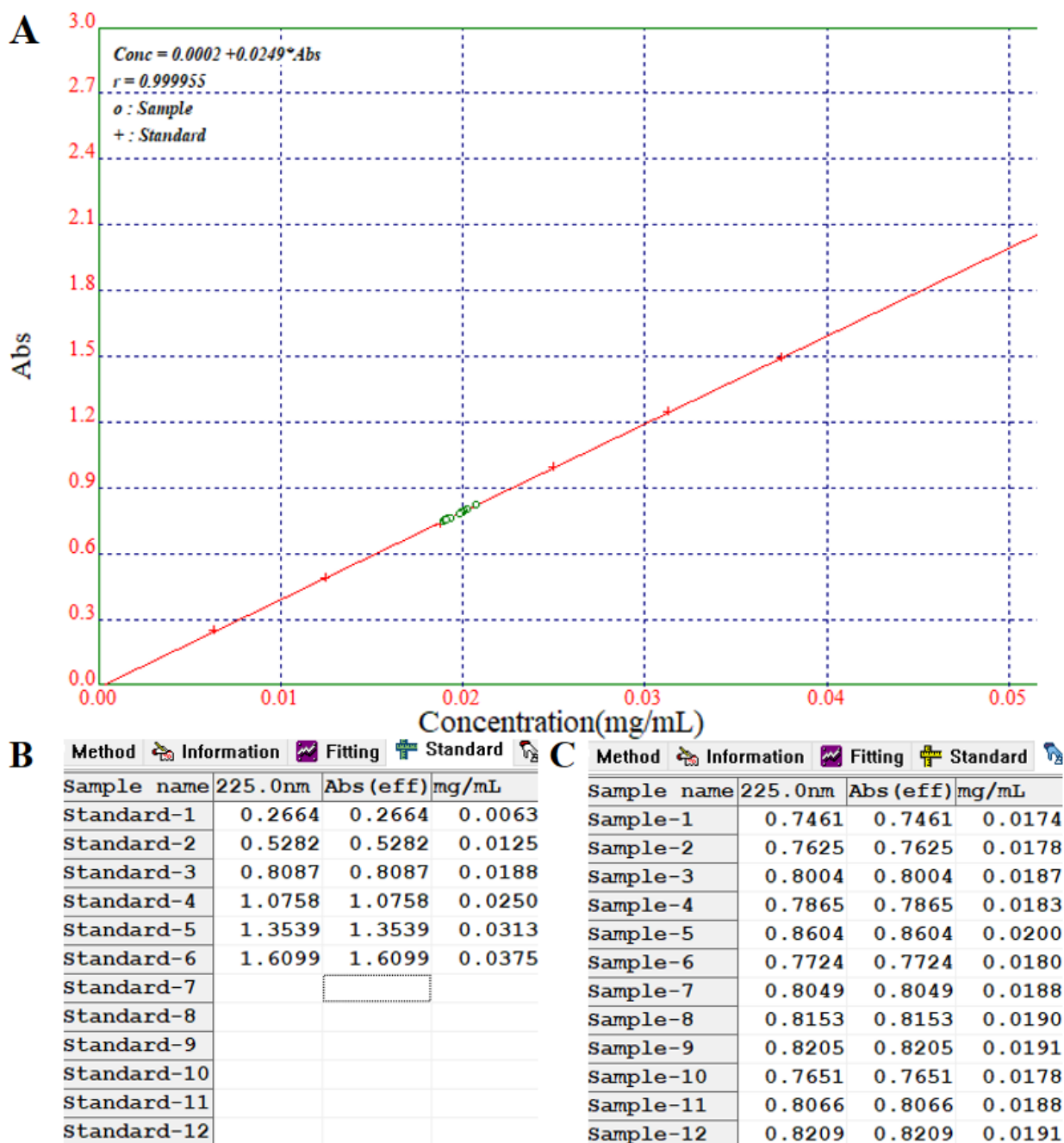


**Figure 1. Individual UV spectra for each brand Ibuprofen – samplers A-L.** Each sample was scanned in UV range 190-300 nm to identify Active Pharmaceutical Ingredient. ALL samples showed similar results.

## Results

To test if tablets contained ibuprofen as API, qualitative studies were performed. UV Spectra with maximum absorbance at 225 nm were detected, which is in concordance with other works [12–14]. All brand ibuprofens showed similar spectra in the UV range (Figure 1), indicating the presence of

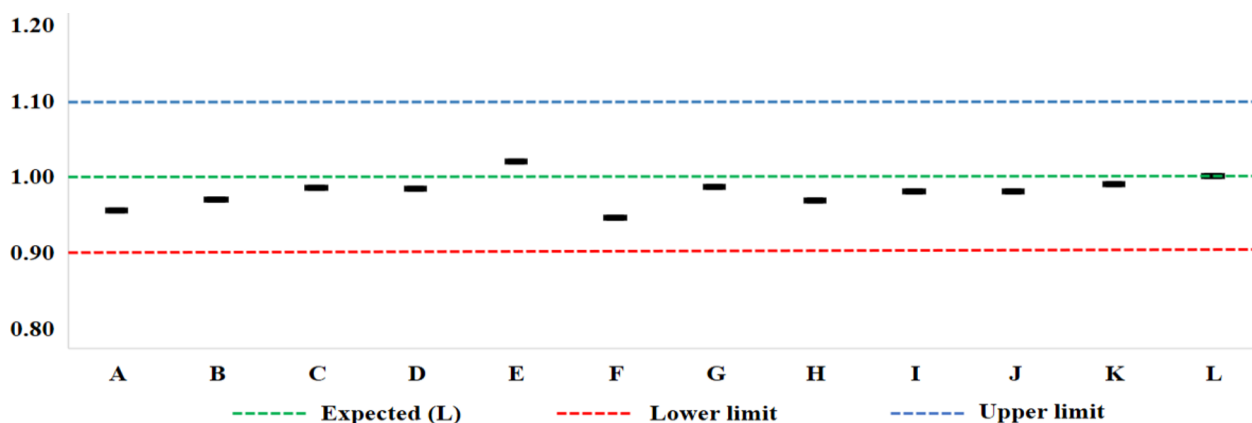
an identical substance in each of them. To perform quantitative studies we chose to use the brand name Ibuprofen - Motrin. Another brand name Ibuprofen – Advil was also tested with other brand Ibuprofens. A standard curve (including R-value) with the absorptions of Motrin and other brand Ibuprofens (mostly generics) are shown in Figure 2.



**Figure 2.** One of the representative of quantitative assay. Standard curve was plotted using Motrin (Brand name Ibuprofen) (A). Concentrations and corresponding absorptions for Motrin (B) Absorptions and concentrations for each samples tested, including Advil and Motrin itself (C). Order of the samples Sample 1-12 are in the same order A-L as shown in **Table 1**.

Similar results were generated in two other tests (not presented in the paper). To better visualize our data we have calculated the ratio of the MEAN value of concentration to

the expected value (MEAN value of concentration for Motrin) for each brand of Ibuprofen and plotted in **Figure 3**.



**Figure 3. Ratio of MEAN value of concentration to expected amount concentration (Motrin).** Mean value of none of the ibuprofens tested did not exceed  $\pm 10\%$  range of the MEAN value of standard (Motrin). All brand ibuprofens are within 10% tolerance limits of expected amount of Active Pharmaceutical Ingredient and except one (F), all tested samples were in 5% limits.

This data along with each test MEAN values of concentrations and standard deviations are shown in **Table 2**. We have applied 10% tolerance limits used in other studies [15]. From Figure 3 we can see that all brand ibuprofens are within 10% tolerance limits of

the expected amount of ibuprofen. However, it is worth mentioning that except one, all tested brand Ibuprofens were in the 5% range of the expected amount of API (**Table 2** and **Figure 3**).

**Table 2. Quantitative results for ibuprofen tablets analyzed.** 3 randomly selected tablets were analyzed on spectrophotometer in UV (SD) was calculated for each brand. Results adjusted to standard ibuprofen (Motrin) by range. MEAN value and Standard Deviation dividing MEAN value of each brand Ibuprofen by MEAN value of Motrin

Sample ID	Results of measurements ( $\mu\text{g/ml}$ )			MEAN $\pm$ SD	MEAN of Sample/MEAN of Motrin
	Test 1	Test 2	Test 3		
A	17.4	19.9	17.9	18.40 $\pm$ 1.32	0.96
B	17.8	20.2	18	18.67 $\pm$ 1.33	0.97
C	18.7	20.7	17.5	18.97 $\pm$ 1.62	0.98
D	18.3	20.1	18.4	18.93 $\pm$ 1.01	0.98
E	20	20	18.9	19.63 $\pm$ 0.64	1.02
F	18	18.9	17.7	18.20 $\pm$ 0.62	0.94
G	18.8	19.2	19	19.00 $\pm$ 0.20	0.99
H	19	19	17.9	18.63 $\pm$ 0.64	0.97
I	19.1	19.3	18.2	18.87 $\pm$ 0.59	0.98
J	17.8	19.9	18.9	18.87 $\pm$ 1.05	0.98
K	18.8	19.8	18.6	19.07 $\pm$ 0.64	0.99
L	19.1	19.1	19.6	19.27 $\pm$ 0.29	1



## Discussion

As the demand for generic drugs worldwide increases, it is important to have affordable, qualitative, and quantitative tests [16]. As we have discussed in the introduction, according to WHO, anesthetics and painkillers represent 8.5% of all falsified and substandard medical products reported to WHO [3], possibly due to the high demand for these types of medical products. The exact amount of ibuprofen sold in Georgia is unknown. However, the variety of ibuprofens found on the website of the Agency for Regulation of Medical and Pharmaceutical Activities in Georgia probably means that this product is highly demanded. Since Ibuprofen is used as a fever reducer or as an anti-inflammatory drug for consuming the medical products not controlled solely by the government agencies. It will also decrease the financial load for families who cannot afford brand-name medical products and consume generic analogs instead. We think that testing other medications at the local market has to be continued and antibiotics should be next in line since they take second place in all falsified and substandard medical products reported to WHO. In addition, according to studies conducted by the University of Edinburgh, it is estimated between 72,000 and 169,000 children may die each year from pneumonia due to antibiotics that do not meet the required standards [3].

rheumatoid arthritis, it is important to have an affordable and simple quality test for this medication. Even though government agencies are involved in the monitoring medical products in Georgia, generally, we think that the involvement of other parties, including academia, and sharing the results to the public will reassure that patients are

## Conclusions

Despite the significant price differences (prices are not reported purposely) of different brand ibuprofens produced in different countries, all of them contained labeled amounts of active pharmaceutical ingredient - Ibuprofen. The approach we have used does not require expensive equipment and long-term training of employees and therefore may be used as an affordable method to test ibuprofen on regular basis.

## Competing interests

The authors declare that they have no competing interests.

## Funding

No funding was provided for this work.

## Authors' Contributions

Conceived and designed the experiments: ZRT MV. Performed the experiments: MV TM MK. Analyzed the data: ZRT MV. Contributed reagents/material/analysis Tools: MP ZRT. Wrote the paper: ZRT

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## საქართველოს ფარმაცევტულ ბაზარზე არსებული სხვადასხვა ბრენდის იბუპროფენის შედარებითი ანალიზი

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### აბსტრაქტი

ჯანმოს მონაცემებით, განვითარებად ქვეყნებში 10 სამედიცინო პროდუქტიდან 1 უხარისხო ან ფალსიფიცირებულია. ვინაიდან საქართველო იმ ქვეყნებს შორისაა, სადაც ჯანმოს თანახმად უხარისხო და ფალსიფიცირებული სამედიცინო პროდუქტები იქნა აღმოჩენილი, ამიტომ მნიშვნელოვანია საქართველოს ფარმაცევტულ ბაზარზე არსებული სამედიცინო პროდუქტების ხარისხის რეგულარული გამოკვლევა. კვლევისთვის შევარჩიეთ თბილისის სააფთიაქო ქსელში არსებული და მსოფლიოში ერთ-ერთი ყველაზე ფართოდ გამოყენებული პრეპარატი - იბუპროფენი.

შევისწავლეთ იმპორტირებული 10 სხვადასხვა ბრენდის და ადგილობრივი წარმოების 2 ბრენდული დასახელების იბუპროფენი (Motrin და Advil). აქტიური ფარმაცევტული ინგრედიენტის (API) შემცველობას ვსაზღვრავდით ულტრაიისფერ უბანში სპექტროფოტომეტრით. მიღებულმა შედეგებმა აჩვენა, რომ ყველა ბრენდის იბუპროფენი შეიცავდა API-ს ეტიკეტირებულ რაოდენობას.

**საკვანძო სიტყვები:** იბუპროფენი, საქართველო, სპექტროფოტომეტრი, გენერიკული პრეპარატი, აქტიური ფარმაცევტული ინგრედიენტი.

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## Original Research

## Changes in Indicators of Biochemical Homeostasis in Patients With Moderate SARS-CoV-2

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### Abstract

This article demonstrates changes in biochemical homeostasis in patients with moderate SARS-CoV-2 and type 2 diabetes mellitus (T2DM) or hyperglycemia identified for the first time. The results of the study were estimated according to the indicators of a biochemical blood test in the acute phase and the convalescent phase of the SARS-CoV-2. The most significant deviations of homeostasis were observed in patients with T2DM. The development and worsening of hyperglycemia were due to the specific effect of the virus on carbohydrate metabolism mediated via ACE2 receptors located in hepatocytes and pancreatic cells. The development of hyperglycemia in SARS-CoV-2 patients was not associated with the use of drugs with hyperglycemic effects in particular glucocorticoids. The obtained data indicate the blood coagulation system's activation and the severity of this activation depended on the severity of SARS-CoV-2 and somatic complications.

**Conclusion:** Further examination is recommended to fully comprehend the complex association between SMU, AP, and WB. The study highlights the need for guidance and supervision from parents, teachers, and authority figures to prevent undesirable effects on students' WB and AP. Researchers may suggest models such as emotional intelligence to increase self-awareness, self-management, empathy, and communication. Appropriate policies and models are urgently needed to prevent serious issues related with SMU among students.

**Keywords:** SARS-CoV-2, ACE2, biochemical homeostasis, type 2 diabetes mellitus, hemostasis, pharmacotherapy.



## Introduction

The International Committee on Taxonomy of Viruses declared SARS-CoV-2 to be the name of a new virus on 11 February 2020 [1]. The disease caused by SARS-CoV-2 is more commonly called COVID-19. The development of severe bilateral pneumonia and acute respiratory distress syndrome are the most visible manifestations of it [2].

Unlike diseases caused by influenza viruses that predominantly affect the respiratory system, SARS-CoV is characterized by a defeat of the whole organism [3]. The severity of SARS-CoV-2 is largely determined by the presence of concomitant diseases, one of which is diabetes mellitus (T2DM). Disorder of carbohydrate metabolism is currently one of the most common pathologies among the global population. According to WHO, the frequency of T2DM amounts to 463 million adults [4].

The lethality of SARS-CoV-2 in patients with disorders of carbohydrate metabolism increases by two to four times [5]. Therefore, the study of the pathophysiological foundations of the aggravation of the course of the disease against the background of metabolic disorders is important.

### Purpose

To explore pathophysiological mechanisms of biochemical homeostasis in SARS-CoV-2 patients with decompensation of type 2 diabetes mellitus (T2DM) and hyperglycemia identified for the first time.

### Materials and methods

A retrospective analysis of 62 case reports of patients between 40 and 70 years with medium to severe SARS-CoV-2, complicated by community-acquired bilateral polysegmental pneumonia. Patients were separated according to the anamnesis and the results of blood biochemical analysis into three groups: 1st group (n=14): control group (CG), patients without disorders of carbohydrate metabolism; 2nd group (n=18): main group 1 (MG1), with manifested type 2 T2DM; 3rd group (n=30): main group 3 (MG3), with transient hyperglycemia. The study was performed based on the results obtained in the Voronezh State Clinical Hospital no.2 named after K.F. Fedyayevskiy. The results of the study were estimated according to the indicators of a biochemical blood test in the acute phase and the convalescent phase of the SARS-CoV-2.

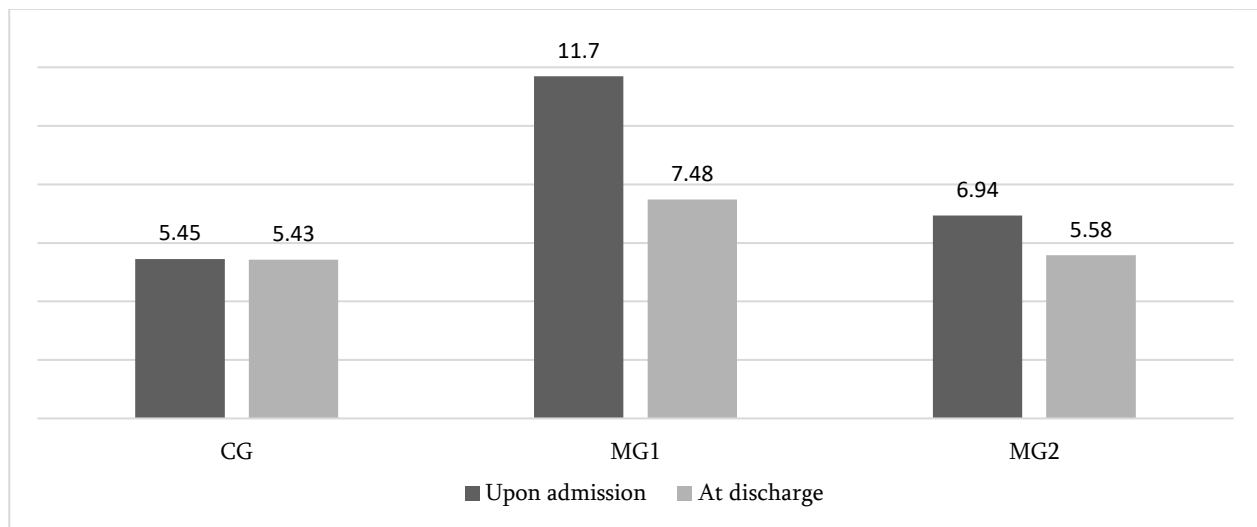
The levels of glucose, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, and amylase were analyzed. Coagulation parameters such as thrombin time, prothrombin index (PTI), fibrinogen, and hematocrit were also investigated.

### Results

It should be noted that all patients from all studied groups had moderate to severe clinical pictures of SARS-CoV-2. All patients were treated according to the clinical guidelines of the Ministry of Health of the Russian Federation.

However, in CG all indicators of blood biochemical analysis were within the normal

range, however the glucose levels were at the high end of the normal range. Glucose level amounted to  $5,05 \pm 0.4$  mM/L on average.



**Figure 1.** Blood glucose levels in patients with SARS-CoV-2 upon admission and at discharge.

The average level of glucose in MG2 was 6.94 mM/L at the time of admission to the hospital. It did not return to the normal range by the time of discharge from the

hospital and amounted to 5.6 mM/L. ALT and AST levels amounted to 67.2 IU/l and 57.4 IU/l respectively. Other indicators were not elevated in MG2.

**Table 1.** Parameters of biochemical blood test in patients with SARS-CoV-2 and carbohydrate metabolism disorders.

Indicator	Group of patients		
	CG	MG1	MG2
ALT (IU/l)	33.8	71.9	67.2
AST (IU/l)	31.4	62.5	57.5
Creatinine ( $\mu$ M/L)	75.2	85.1	68.4
Urea (mM/L)	5.6	8.24	7.16
Amylase (IU/l)	116.4	140	131.9
Total protein (g/L)	66.4	66.6	66.5
Total bilirubin ( $\mu$ M/L)	11.9	15.4	11.3



The greatest deviations of homeostasis were observed in patients with T2DM who constantly received antidiabetic drugs. The average level of glucose in MG1 amounted to 11.69 mM/L (but not less than 9 mM/L) at the time of admission to the hospital. Hyperglycemia in MG1 was sustainable. Pharmacotherapy of T2DM was changed in all patients from MG1 – they were transferred to injectable insulin with an increase of dose by 25%. Despite the increased dosage of insulin in MG1, normalization of glucose levels could not be reached. The average level of glucose upon discharge from the hospital amounted to 7.48 mM/L. ALT and AST levels increased by 84% and 90% respectively. The average level of ALT in these patients amounted to 71.9 IU/l, of AST- 62.5 IU/l. Some indicators of biochemical blood tests were within upper normal values: bilirubin 15.4 μM/L, creatinine 85.4 μM/L, urea 8.24 mM/L.

Levels of amylase (140 IU/l) and total protein (66.6 g/L) were normal. The level of urea was 1.5-2 times higher than normal in 50% of patients in MG1.

According to the research of the majority of authors, changes in the hemostatic system are one of the leading pathogenetic mechanisms in COVID-19 disease. However significant changes in coagulation indicators are detected in more severe forms of the disease [6]. A correlation between the severity of carbohydrate metabolism disorders and coagulogram variation was found in our study. The average PTI level was increased in MG1 and amounted to 103.3±2.5. In CG and MG2 PTI level was within upper normal values (100.5). Fibrinogen level was increased in all groups of patients. The greatest deviation of fibrinogen was observed in MG2 (5.5). The average level of fibrinogen was 4.1 and 4.3 in MG1 and CG respectively.

**Table 2.** Indicators of coagulogram in patients with SARS-CoV-2 and carbohydrate metabolism disorders.

Indicator	Group of patients		
	CG	MG1	MG2
Thrombin time	17.9	17.6	17.8
Fibrinogen	4.3	5.5	4.1
PTI	100.6	103.4	100.4
Hematocrit	46	43.1	41.6

Since reference values of hematocrit are different for men and women, patients were separated by gender to assess changes in hematocrit level. The greatest increase of

hematocrit was observed in women from CG (48.3). Hematocrit level was slightly increased in women from MG1 (42.7). This



parameter was within the upper normal value in women from MG2 and amounted to

## Discussion

It is known that COVID-19 causes multisystem disorders. The main element of pathogenesis is virus penetration into the cells with the involvement of functional receptor angiotensin-converting enzyme 2 (ACE2) [7]. Our study revealed that several internal organs were damaged even in patients with medium (CT2) severity of SARS-CoV-2. Both first identified hyperglycemia and decompensation of manifested T2DM type 2 indicate that pancreatic function was impaired. However, normal amylase level in the blood of patients of all groups suggests that pancreatic failure is of a functional but not a morphological nature. The development and worsening of hyperglycemia were due to the specific effect of the virus on carbohydrate metabolism: ACE2 receptors are located in hepatocytes and pancreatic cells [8]. It means that these organs are targets for SARS-CoV-2. During hyperglycemia, glycosylation of receptors occurs, which significantly increases their affinity for the virus [9, 10]. Penetration of SARS-CoV-2 into pancreatic cells may worsen beta-cell damage, causing insulin resistance [11].

Many researchers associate the development of hyperglycemia in SARS-CoV-2 patients with the use of drugs with hyperglycemic effects in particular glucocorticoids [12]. However, the increase in glucose level in our investigation was detected at the time of hospitalization before the application of glucocorticoids. It should be noted that the

40,9. Hematocrit level was within the normal range in men of all groups.

increased insulin dose did not manage to normalize the glucose level. Patients with manifested T2DM were discharged with an unachieved target level of glucose because the further increase of the amount of insulin was inadvisable on the background of SARS-CoV-2 pharmacotherapy. Thus, the development of hyperglycemia in patients with moderate COVID-19 is associated with the pathogenetic effects of the disease.

A significant increase in the transaminase levels indicates cytolysis syndrome both in hepatocytes and cardiomyocytes [13]. Meanwhile, liver damage is considered to be concomitant by the majority of researchers [14]. It should be noted that in this sample of patients' failure of hepatic protein synthesis was not observed. It may be explained by the severity of the disease [15]. The systemic inflammatory reaction caused by SARS-CoV-2 is a major cause of liver damage. Another mechanism of liver damage is a cytokine storm [16]. It is known that the level of acute phase proteins increases in patients with COVID-19, and an imbalance in innate and acquired immunity occurs. This imbalance is expressed as excessive activation of cytokine synthesis by macrophages and neutrophils [17].

Hypoxia associated with viral pneumonia leads to the development of ischemic liver injury [18]. Oxygen reduction causes the accumulation of lipids, which can cause necrosis of hepatocytes. Moreover, after the mitochondrial damage cells accumulate reactive oxygen species that enhance the



production of pro-inflammatory cytokines [19].

Hyperglycemia is stimulated by the feedback mechanism by the activation of the gene transcription factor of the interferon regulatory factor-5 during the period of the cytokine storm and its binding with Uridine diphosphate N-acetylglucosamine which is formed during the metabolism of glucose [20].

Moderate COVID-19 in patients without concomitant somatic diseases does not cause

## Conclusions

The results of the study demonstrated changes in biochemical homeostasis in patients with moderate SARS-CoV-2 and type 2 diabetes mellitus (T2DM) or hyperglycemia identified for the first time. Careful study of concomitant diseases in patients is required for better identifying etiopathogenetic factors of the clinical picture of SARS-CoV-2. However, there is no doubt that pathologic premorbid changes in cell membranes are morphological preconditions for a more severe course of


renal functional disorders. We can assume that moderate COVID-19 in patients with type 2 T2DM leads to initial renal dysfunction [21]. It is confirmed by the fact that there was a significant increase in urea levels in 50% of patients from MG1.

The obtained data on the blood coagulation system's activation correspond to the literature [22, 23, 24]. The degree of severity of this activation depends on the severity of COVID-19 and somatic complications [25].

SARS-CoV-2 in patients with manifested T2DM of any type. The development and worsening of hyperglycemia were due to the specific effect of the virus on carbohydrate metabolism: ACE2 receptors are located in hepatocytes and pancreatic cells. The development of hyperglycemia in SARS-CoV-2 patients was not associated with the use of drugs with hyperglycemic effects in particular glucocorticoids. The obtained data indicate the blood coagulation system's activation and the severity of this activation depended on the severity of COVID-19 and somatic complications.



## ბიოქიმიური ჰომეოსტაზის მაჩვენებლების ცვლილება საშუალო სიმძიმის SARS-COV-2-ით დაავადებულ პაციენტებში

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### აბსტრაქტი

სტატიაში მოცემულია მეორე ტიპის შაქრიანი დიაბეტის ან პირველად გამოვლენილი ჰეპერგლიკემიის ფონზე, თანდართული SARS-COV-2-ის საშუალო სიმძიმით მიმდინარე პაციენტებში, ბიოქიმიური ჰომეოსტაზის მაჩვენებლების ცვლილება. კვლევის შედეგები მოიცავს პაციენტების სისხლის ბიოქიმიური მაჩვენებლების გამოვლენას SARS-COV-2-ის, როგორც მწვავე ისე გამოჯანმრთელების ფაზებში. ბიოქიმიური ჰომეოსტაზის მეტად მნიშვნელოვანი გადახრები აღინიშნა მეორე ტიპის შაქრიანი დიაბეტის შემთხვევაში, რაც კავშირშია ჰეპატოციტებსა და კუჭქვეშა ჯირკვლის უჯრედებში არსებულ ACE2-რეცეპტორებზე. SARS-CoV-2-ით დაავადებულ პაციენტებში ჰიპერგლიკემია არ იყო გამოწვეული პრეპარატებით, მაგალითად, გლუკოკორტიკოიდებით.

მოცემული შედეგები ადასტურებს სისხლის შემდეგბელი სისტემის აქტივაციას, რაზეც არის დამოკიდებული SARS-CoV-2-ის მძიმე მიმდინარეობა და მისგან გამოწვეული სომატური გართულებები.

**საკვანძო სიტყვები:** SARS-CoV-2, ACE2, ბიოქიმიური ჰომეოსტაზი, შაქრიანი დიაბეტის მე-2 ტიპი, ჰემოსტაზი, ფარმაკოთერაპია.

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## Original Research

## Dental Expenditures of population in Georgia

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**Abstract**

Nowadays the increasing cases of oral diseases requires to enlarge the costs of their treatment. However, in developing countries, including Georgia, where the majority of the population is low-income or unemployed, their spending on dental services is too small. Over the years, in Georgia, the share of healthcare expenses in the population's consumer expenditures varies within 10%, while the share of dental expenses in the whole consumer expenditures is about 0.5%. The share of dental expenses in healthcare expenditures is about 5%. 17.0% of respondents spend up to 100 GEL on dental services, 56.3% spend between 100 and 500 GEL, 12.0% spend between 500 and 1000 GEL, and 14.7% spend more than 1000 GEL. Expenditures for dental care vary significantly by gender, age, region, and between employed and unemployed persons. About a one third of the surveyed population cannot afford to visit a dentist for prevention or treatment due to low income.

**Keywords:** Dental expenditures, statistical survey**Introduction**

Nowadays the study of population's dental expenditures in Georgia is important. The increasing number of oral diseases, on the one hand, and the low income and unemployment of the population, on the other hand, led to low costs for dental services. The population consult the dentist not for prevention, but in many cases for the purpose of treatment. Mainly they visit to doctor for emergency dental services.

According to the statistical information of

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the World Health Organization, in 2019, per capita expenditure of dental services in Georgia amounted to 3.13 USD. According to this indicator, Georgia occupies the 127th place among the world countries. The share of dental expenses in the country's gross domestic product is 0.07% in the abovementioned period.

The survey is conducted in May 2023. One of the objectives of the research is to study dental expenses for the purposes of treatment



and prevention of oral diseases. Low incomes of the population of Georgia lead to the inability to receive proper treatment of oral

diseases. In many cases, people remain without treatment.

### Materials and methods

The purpose of the statistical survey is a detailed study of the expenses incurred by the population of Georgia on dental services. This goal is one of the component among other aims of the observation conducted by us in May, this year. 1023 people were interviewed, regardless of their gender, age, and employment.

In the research, we used the following statistical methods: sampling method, frequency distributions, bivariate analysis (cross tabulation), analysis method of statistical graphs, hypothesis testing chi-square criterion.

Data were processed in the statistical software package IBM SPSS Statistics 26

### Discussion of obtained results

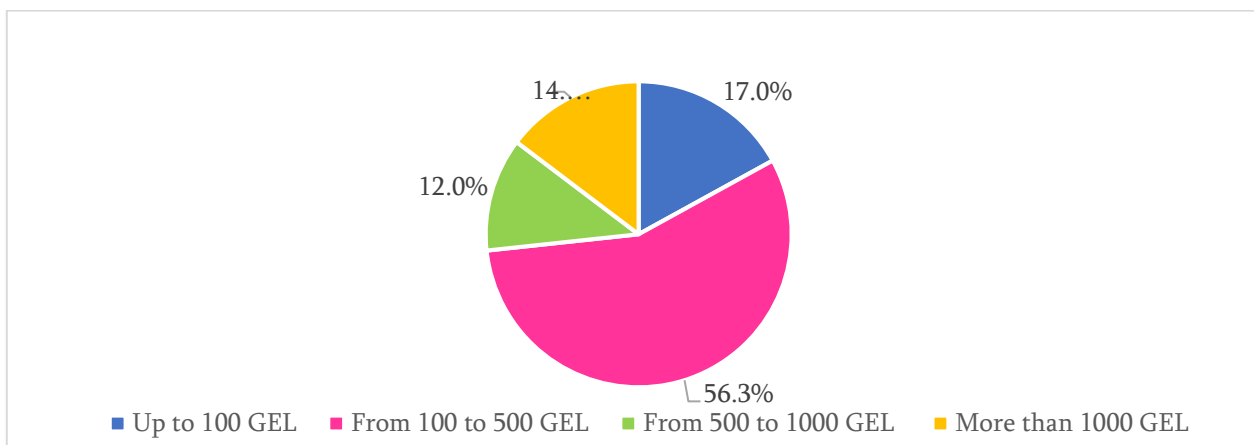
As a result of the statistical research, it was revealed that the share of expenses incurred by the population of Georgia on dental services in 2022 in the total consumer expenditures is very small and amounts to 0.57%. And the share of dental expenses in health care costs is 5.3%.

GEL, in the third group from 500 to 1000 GEL and in the fourth group more than 1000 GEL. 17.0% of respondents spend up to 100 GEL on dental services, 56.3% spend between 100 and 500 GEL, 12.0% spend between 500 and 1000 GEL, and 14.7% spend more than 1000 GEL.

To study the dental expenses in detail, we have identified the following groups. In the first group we combined expenses up to 100 GEL, in the second group from 100 to 500

The percentage distribution of the respondents by the costs incurred for dental services is presented below:

Chart 1: Dental expenditures of population, %



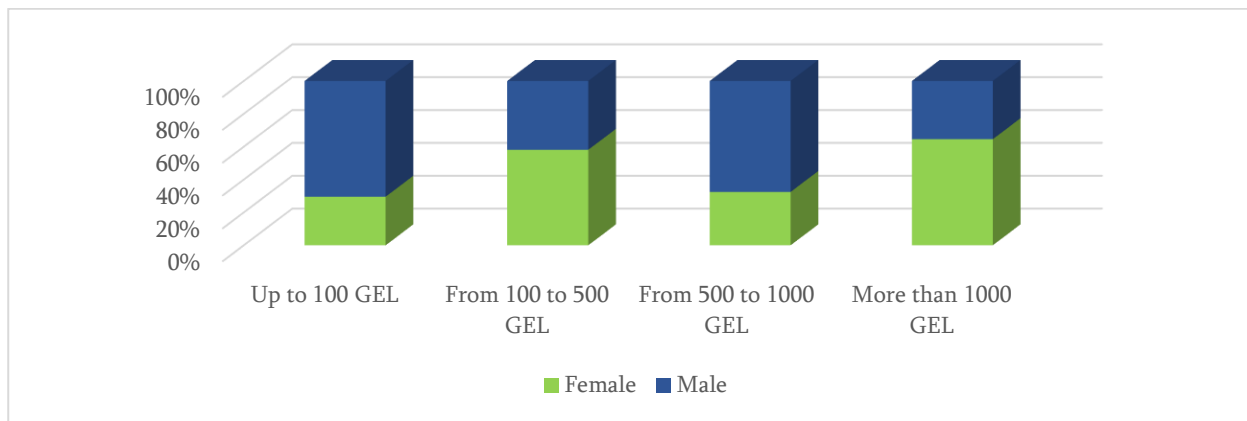
Dental expenditures are different by sex. The following frequency distribution table and

the corresponding chart confirm the abovemention.

**Table 1. Dental expenditures by sex, Percent**

		Expenditures				Total
		Up to 100 GEL	From 100 to 500 GEL	From 500 to 1000 GEL	More than 1000 GEL	
Sex	Female	11.5%	62.2%	8.8%	17.5%	100.0%
	Male	27.4%	44.8%	18.3%	9.60%	100.0%
Total		17.0%	56.3%	12.0%	14.7%	100.0%

**Chart 2: Dental expenditures by sex, Percent**



The results of the chi-square criterion used to test the statistical significance of differences by gender are as follows:

**Table 2. Chi-Square Tests**

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	48.084 <sup>a</sup>	3	0.000
Likelihood Ratio	46.972	3	0.000
Linear-by-Linear Association	9.06	1	0.003

According to the table, the significance level  $\alpha$  is equal to 0.000, which is less than the preselected significance level of 0.05. Thus, we can conclude that there is a significant statistical difference for dental expenditures by gender.

Regarding dental care expenditures among the employed and unemployed persons, the expenditures of employed persons are much higher than those among the unemployed persons. The corresponding table and chart are presented below:

**Table 3. Dental expenditures by employed and unemployed persons, Percent**

	Expenditures				Total
	Up to 100 GEL	From 100 to 500 GEL	From 500 to 1000 GEL	More than 1000 GEL	
Employed	15.0%	52.0%	13.0%	20.0%	100.0%
Unemployed	32.0%	61.0%	6.0%	1.0%	100.0%

**Chart 3. Dental expenditures by employed and unemployed persons, Percent**



To test whether there is a statistically significant difference in dental care expenditures between the employed and the

unemployed persons, we used the chi-square test of hypotheses. The test result is given in the table:

**Table 4. Chi-Square Tests**

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	35.786a	3	0.000
Likelihood Ratio	49.313	3	0.000
Linear-by-Linear Association	35.571	1	0.000

According to the table, the significance level  $\alpha$  is equal to 0.000, which is lower than the preselected significance level of 0.05. Thus, we can conclude, that there is a significant statistical difference in dental care costs between the employed and the unemployed persons.

According to the results of the research, there are significant difference in dental expenses by regions. The summary table of the chi-square criterion for hypothesis testing is presented below:

**Table 5. Chi-Square Tests**

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	415.173a	24	0.000
Likelihood Ratio	376.368	24	0.000
Linear-by-Linear Association	4.823	1	0.028

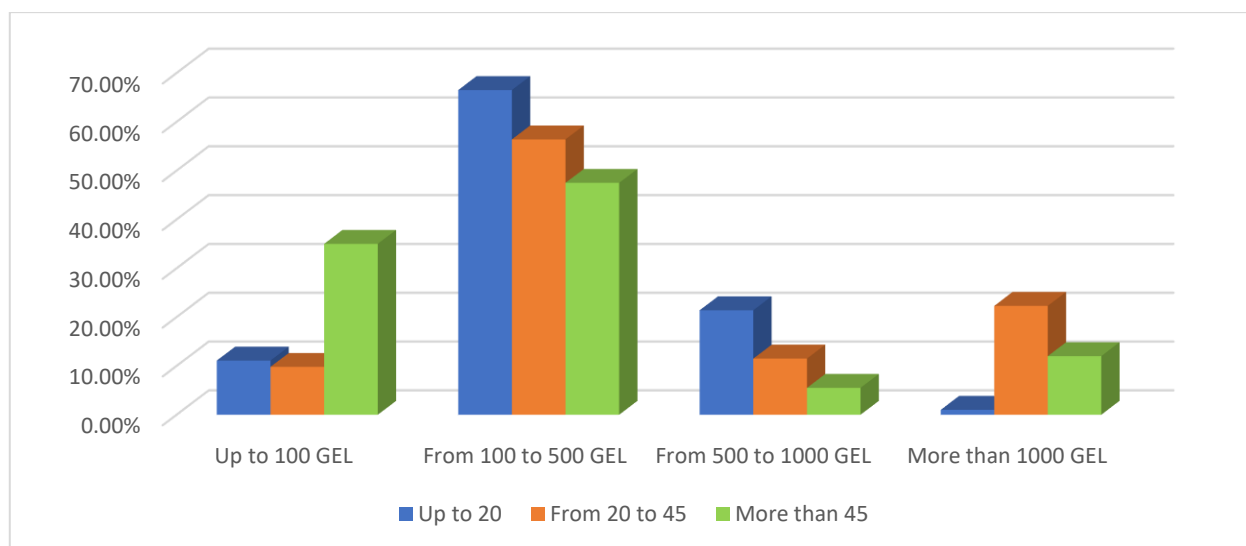
The significance level equals to 0.000. It is also less than 0.05 in this case. Thus, we can conclude that there is a significant statistical difference in dental costs by regions.

There are also significant differences in dental expenditures between different age groups. The cross-tabulation table and chart for dental expenses by age groups are presented below:

**Table 6. Dental expenditures by age groups, Percent**

		Expenditures				Total
		Up to 100 GEL	From 100 to 500 GEL	From 500 to 1000 GEL	More than 1000 GEL	
Age	Up to 20	11.1%	66.5%	21.4%	1.0%	100.0%
	From 20 to 45	9.8%	56.4%	11.5%	22.3%	100.0%
	More than 45	35.0%	47.5%	5.5%	12.0%	100.0%

**Chart 4. Dental expenditures by age groups, Percent**





The result of the chi-square criterion used to test the statistical significance of the

difference in dental care expenses according to age groups is presented in the table below:

**Table 7. Chi-Square Tests**

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	103.657	6	0.000
Likelihood Ratio	110.807	6	0.000
Linear-by-Linear Association	5.012	1	0.025

The significance level equals to 0.000. It is also less than 0.05 in this case. Thus, we can conclude that there is a significant statistical difference in dental costs by age groups.

Based on our research, there are also some interesting results. About a one third of the respondents (31.4%) consult a doctor for

preventive purposes. Dentist’s consultation is mainly due to urgent need. However, some population cannot afford to visit a dentist even in case of urgent need due to very low income. 30.0% of respondents do not consult a doctor in case of oral diseases, the main reason of which is lack of money for treatment.

**Conclusions**

Dental expenditures are quite small in the conditions of wide spread of oral diseases in Georgia. The share of dental care expenses in the structure of consumer expenses of the population is about 0.5%. And the share of dental expenditures in healthcare costs is about 5%.

17.0% of respondents spend up to 100 GEL on dental services, 56.3% spend between 100 and 500 GEL, 12.0% spend between 500 and 1000 GEL, and 14.7% spend more than 1000 GEL.

Expenditures for dental care vary significantly by gender, age, region, and the employed and unemployed persons.

About a third of the surveyed population

cannot afford to visit a dentist for prevention or treatment due to low income.

The final recommendations can be formulated as follows:

- Appropriate policies should be implemented in the direction to increase the income of the population, which increases the expenses on dental services and every resident will have the opportunity to improve their oral health;
- Appropriate policies should be implemented in the direction to increase the number of employees, which is a source of increasing income;
- Appropriate policies should be implemented in the direction of reducing and eliminating the causes of oral diseases.



## მოსახლეობის მიერ სტომატოლოგიურ მომსახურებაზე გაწეული ხარჯები საქართველოში

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### აბსტრაქტი

სტომატოლოგიურ დაავადებათა მზარდი რაოდენობა დღევანდელ პერიოდში მათი მკურნალობის ხარჯების ზრდას მოითხოვს. თუმცა განვითარებად ქვეყნებში, მათ შორის საქართველოში, სადაც მოსახლეობის უმრავლესობა დაბალშემოსავლიანია ან უმუშევარი, სტომატოლოგიურ მომსახურებაზე გაწეული ხარჯები ძალიან მცირეა. წლების განმავლობაში მოსახლეობის სამომხმარებლო ხარჯების სტრუქტურაში ჯანმრთელობის დაცვის ხარჯების წილი 10 %-ის ფარგლებში მერყეობს, სტომატოლოგიური მომსახურების ხარჯების წილი კი დაახლოებით 0,5 %-ია. რაც შეეხება ამ უკანასკნელის წილს ჯანდაცვის ხარჯებში, ის დაახლოებით 5 %-ს შეადგენს.

გამოკითხულთა 17.0 % -ის დანახარჯები სტომატოლოგიურ მომსახურებაზე 100 ლარამდეა, 56.3 %-ის ხარჯები 100-დან 500 ლარამდეა, 12.0 %-ის ხარჯები 500-დან 1000 ლარამდე, ხოლო 14.7 %-ის დანახარჯები 1000 ლარზე მეტს შედგენს.

სტომატოლოგიურ მომსახურებაზე გაწეული ხარჯები მნიშვნელოვნად განსხვავდება სქესის, ასაკის, რეგიონულ ჭრილში, ასევე დასაქმებულთა და უმუშევრების მიხედვით.

გამოკითხული მოსახლეობის დაახლოებით მესამედი დაბალი შემოსავლის გამო ვერ ახერხებს სტომატოლოგიურ ვიზიტს პროფილაქტიკისა თუ მკურნალობის მიზნით.

**საკვანძო სიტყვები:** სტომატოლოგიური მომსახურების ხარჯები, სტატისტიკური გამოკვლევა.

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## Original Research

## Effects of different drying, extraction methods, and solvent polarity on the antioxidant properties of *Paeonia daurica* subsp. *mlokosewitschii*

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### Abstract

The study presents the effect of drying methods (microwave-drying and freeze-drying), different solvents (80% methanol, 99% methanol, 80% ethanol), and different extraction methods on the antioxidant activity of *Paeonia daurica* subsp. *mlokosewitschii* leaves, estimated based on DPPH free radical scavenging activity. The highest antioxidant activity was revealed in the freeze-dried leaves. The drying method significantly influenced the antioxidant activity: the thermal microwave-drying resulted in lower antioxidant potential. The solvent polarity also played a significant role in the determination of the antioxidant activity of *Paeonia daurica* subsp. *mlokosewitschii* leaves: the lowest IC<sub>50</sub> values (specific concentration of the sample required for 50% inhibition) were revealed for freeze-dried plants extracted with 80% methanol, followed by IC<sub>50</sub> values obtained for the extraction with 80% ethanol, and the highest IC<sub>50</sub> values were revealed for extracts of microwave-dried plants extracted with 99% methanol. The subsequent drying of freeze-dried plant extracts had no significant effect on the antioxidant activity of the extract. The subsequent drying of the methanol extract from microwave-dried plants at room temperature or 40°C significantly reduced IC<sub>50</sub> value, however, the results were comparable with those, obtained for the methanol extracts of freeze-dried plants without subsequent drying of the extract. Thus, the optimal method of drying and extraction of *Paeonia daurica* subsp.



*mlokosewitschii* leaves for preserving the antioxidant activities was established: freeze-drying of leaves followed by 24 h extraction with 80% methanol.

**Keywords:** *Paeonia daurica* subsp. *mlokosewitschii*, antioxidant activity, DPPH assay, solvents, drying methods.

## Introduction

Research on anti-oxidants has become quite popular over the years as these substances are now considered potential therapeutic candidates to prevent free radical-induced damage to the human body [1]. Now synthetic antioxidants such as butylated hydroxytoluene are widely used, however, their negative effects, such as liver damage and a higher probability of carcinogenesis were demonstrated [2]. Therefore, the search for natural sources of antioxidants is increasingly important and medicinal plants are considered an easily available and potent source of antioxidants as they contain a mixture of different chemical compounds that may act individually or in synergy to cure disease and improve health.

*Paeonia daurica* subsp. *mlokosewitschii* is an endemic species of Georgian flora of narrow local distribution: it occurs only in Shida (Inner) Kakheti, Shiraki (Kashebi), and Dagestan (headstream of the Andis-Koisu River [3]. For the first time, the species was discovered in 1897 by Polish naturalist Ludwik Młokosiewicz [4]. The use of species of the genus *Paeonia* as traditional herbal remedies has been reported for dysmenorrhea, amenorrhea, epilepsy, spasms, and gastritis [5]. According to

numerous phytochemical the treatment of hematemeses, blood stasis, investigations, 451 compounds, such as monoterpenoid glucosides, flavonoids, tannins, stilbenes, triterpenoids, steroids, and phenols have been isolated from *Paeonia* plants [6]. Studies of the pharmacological activities of these compounds have revealed their antioxidant, anti-inflammatory, antibacterial, antitumor, antiviral, and neuroprotective properties [7]. However, due to the narrow local distribution of *Paeonia daurica* subsp. *mlokosewitschii*, the phytochemical composition and medicinal properties of the species were not extensively investigated.

Different drying and extraction methods can alter the antioxidant activity in a sample and, subsequently, the possibility of its further use in the pharmaceutical industry [8]. This study aimed to determine the optimal drying and extraction method for *Paeonia daurica* subsp. *mlokosewitschii* leaves, preserving higher antioxidant activity, based on DPPH assay.

## Materials and methods

### Plant material

Leaves of *Paeonia daurica* subsp. *mlokosewitschii* were collected in July 2020 in the National Botanical Garden of Georgia



(Tbilisi). Samples were placed in paper bags and transported to the laboratory within 1 h. Once in the laboratory, healthy leaves with uniform color, dimensions, and texture were detached from the plants. Samples of fresh plant material were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

### **Drying processes**

Leaves were dried using two different drying methods, i.e., freeze-drying and microwave-drying. For each drying method, 2 g of fresh leaves was used. Leaves were freeze-dried using a DW-10N freeze dryer in a vacuum flask of 500 mL at 10 Pa and a final condenser temperature of  $-55^{\circ}\text{C}$  until the plant material reached a constant weight, determined by measuring the dry weight (DW) of leaves. The drying process took 6 hours. Using microwave-drying, leaves were dried in a microwave oven (Beko; 800 W) until the plant material reached a constant DW. The drying process took 3 min.

### **Sample extraction**

Extraction efficiencies of different solvents, namely, 80% methanol, 99% methanol, and 80% ethanol were tested. Leaves dried by different methods were extracted in a ratio of 1:10 with solvent, followed by continuous stirring for 24 h at room temperature using an orbital shaker at 270 rpm. Extracts were centrifuged at 5000g for 15 min and stored at  $-80^{\circ}\text{C}$  for further analysis.

For evaluation of the efficiency of different extraction methods, the supernatant was either used directly for the assay or dried at room temperature (in the hood) or at  $40^{\circ}\text{C}$  (in the drying cabinet).

### **DPPH free radical scavenging activity assay**

The free radical scavenging activity was measured using by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier [9].

The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol. The working solution was obtained by diluting the DPPH solution with methanol to attain an absorbance of about  $0.98\pm 0.02$  at 517 nm using the spectrophotometer. A 3 ml aliquot of the working solution was mixed with 100  $\mu\text{l}$  of the sample at various concentrations (10 - 500  $\mu\text{g/ml}$ ). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was taken at 517 nm. A typical blank contained 3 ml of the working solution and the appropriate volume of the corresponding solvent and was incubated under the same conditions. Ascorbic acid was used as standard. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Scavenging effect (\%)} = \left[ \frac{(\text{control absorbance} - \text{sample absorbance})}{(\text{control absorbance})} \right] \times 100$$

The concentrations of the sample required for 50 % inhibition ( $\text{IC}_{50}$ ) were calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the  $\text{IC}_{50}$  value, the higher the antioxidant activity of the samples.  $\text{IC}_{50}$  was calculated by plotting the concentration of extract versus inhibition of DPPH (%) and data were fit with a straight line (linear



regression). IC<sub>50</sub> value was estimated using the fitted line, i.e.,

$$Y = a * X + b,$$

$$IC_{50} = (50 - b)/a$$

### Statistical analysis

All the procedures for extraction and antioxidant studies were repeated in triplicate. The results were expressed as means ± standard deviation of three parallel replicates. All data of the DPPH assay were analyzed statistically by one-way analysis of variance (ANOVA) using Microsoft Excel. A *p-value* of less than 0.05 was considered statistically significant.

### Results and discussion

The DPPH method was introduced nearly 50 years ago by Blois [10] and now the method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant capacity. DPPH is a stable free radical, which accepts an electron or hydrogen radical to become a stable, diamagnetic molecule, which has an absorbance in its oxidized form around 515-520 nm [11]. DPPH produces a purple solution in methanol and the solution becomes yellow when it reacts with antioxidant molecules which results in the scavenging of the radical by hydrogen donation, this change in the color can be monitored by the decrease in absorbance [12]. DPPH assay is a rapid and efficient method used for the evaluation of the free radical scavenging activity of medicinal plants [13]. For quantitative assessment of free radical scavenging activity by the DPPH

assay, usually, parameter IC<sub>50</sub> is determined. IC<sub>50</sub> is the concentration of substrate that causes a 50% loss of the DPPH activity (manifested as the loss of color) and it is used for the interpretation of the results obtained by the DPPH method.

The goal of this study was the determination the optimal drying method and extractant for the preservation of the antioxidant activity in *P. daurica* subsp. *mlokosewitschii* plant material. The ability to retain antioxidants was estimated based on DPPH free radical scavenging activity and the percentage of DPPH free radical scavenging activity was calculated and compared with ascorbic acid as a standard (Fig. 1).

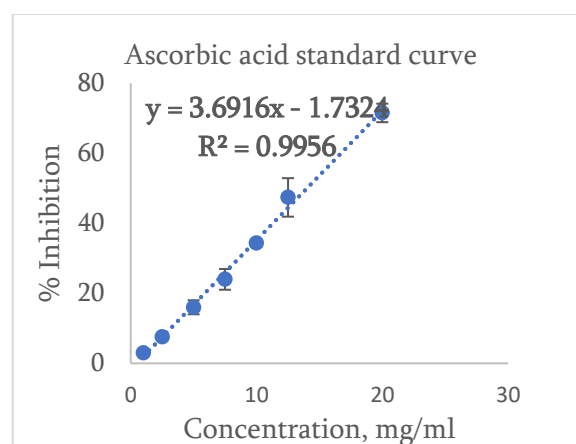


FIGURE 1. Ascorbic acid standard curve. Values are mean ± SD from three independent experiments.

### Effect of different drying methods on DPPH free radical scavenging activity of extracts

The classical way for the start of isolation of natural compounds and the simplest and inexpensive methods to preserve the medicinal properties of plants is drying. However, it is well known that drying



temperature may affect the metabolic profile and antioxidant properties of the corresponding extracts [14]. Temperature and duration of treatment are the determining factors for the selection of the most efficient drying method to preserve antioxidant activity in plant materials [15]. Indeed, specific variations of temperature in the different drying methods may protect against the degradation of these components, leading to the maintenance or enhancement of the product quality of the analyzed plant material. Traditionally, drying at high temperatures, such as sun-drying, oven-drying, and microwave-drying are the most widely used and inexpensive drying methods. However, exposure of plant material to high temperatures for a prolonged period can affect the content of heat-labile bioactive compounds [16]. Previous studies have indicated that freeze-drying may avoid the loss of valuable chemical components when compared to other conventional methods [17].

In this study, we compared the effect of microwave-drying and freeze-drying on the antioxidant activity analyzed by DPPH assay in *P. daurica* subsp. *mlokosewitschii* leaves. The results of the study indicated that the extracts regardless of drying methods demonstrated a dose-dependent scavenging activity by reducing DPPH radical (Figs. 2 and 3). The antioxidant activity was higher in freeze-dried leaves than in microwave-dried leaves (Figs. 2 and 3).

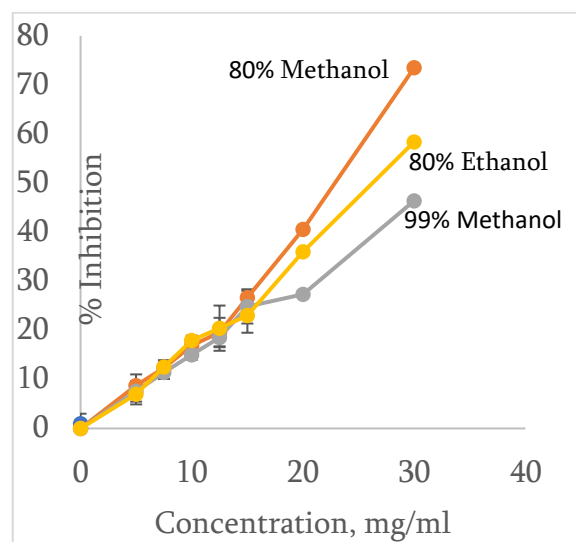


FIGURE 2. The radical scavenging activity, represented by the percentage of inhibition, of extracts of freeze-dried *P. daurica* subsp. *mlokosewitschii* leaves obtained using different solvents. Values are mean  $\pm$  SD from three independent experiments.

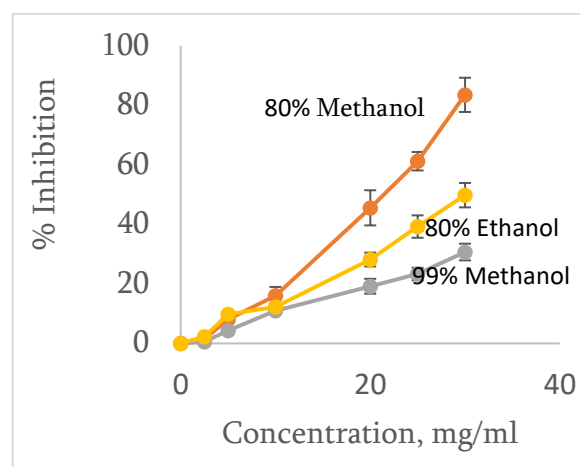


FIGURE 3. The radical scavenging activity, represented by the percentage of inhibition, of extracts of microwave-dried *P. daurica* subsp. *mlokosewitschii* leaves obtained using different solvents. Values are mean  $\pm$  SD from three independent experiments.

By plotting the graph of extract concentrations against the scavenging



activity, IC<sub>50</sub> values were calculated. Among treatments, freeze-drying and extraction with 80% methanol revealed the highest radical scavenging activity (the lowest IC<sub>50</sub> = 17.99 µg/ml), while microwave- drying and extraction with 99% methanol Showed the lowest scavenging activity (IC<sub>50</sub> = 58.29

µg/ml, Table 1). Interestingly, the IC<sub>50</sub> for microwave-dried leaves extracted with 80% ethanol was higher, than the corresponding value for plants dried by freeze-drying. However, both values were still higher than the IC<sub>50</sub> obtained for freeze-dried leaves extracted with 80% methanol.

**Table 1.** IC<sub>50</sub> (DPPH assay) of extracts of freeze-dried and microwave-dried *P. daurica* subsp. *mlokosewitschii* leaves. Values are mean ± SD from three independent experiments. \*p < 0.005, ns-nonsignificant, compared to 80% methanol extract.

Sample	Freeze-drying	Microwave-drying
Ascorbic acid	13.05 ±0.23	13.05 ±0.23
Methanol 80%	17.99 ± 1.00	21.30 ± 1.08
Ethanol 80%	24.81 ± 0.49*	20.31 ± 1.13 <sup>ns</sup>
Methanol 99%	32.71 ±2.13*	58.29±1.11*

In the next experiment, we investigated the effect of drying the extract and re-extraction of the pellet with the corresponding solvent (80% methanol, 99% methanol, or 80% ethanol, Table 2), since some studies recommend this method for DPPH assay [18]. Two methods of drying the extract: at room temperature (25 °C, drying in the hood) and drying at 40 °C (drying in the drying cabinet) were used to assess the effect of the temperature on the antioxidant content and DPPH free radical scavenging activity. Interestingly, the lowest IC<sub>50</sub> value was obtained for extracts obtained by freeze-drying and dried at 40 °C, and extracts obtained by microwave-drying and dried at room temperature, extracted with 80% methanol (Table 2). The drying of the extracts did not affect significantly IC<sub>50</sub> value for freeze-dried plants: a significant

difference between values was not revealed for IC<sub>50</sub> values of freeze-dried leaves extracted with 80% methanol (Table 1) and freeze-dried leaves extracted with 80% methanol followed by subsequent drying of the extract at the room temperature or 40 °C (Table 2). Interestingly, the opposite pattern was observed for leaves of microwave-dried plants: the drying of 80% methanol extracts at room temperature or 40 °C significantly reduced IC<sub>50</sub> value (Table 2) in comparison with the IC<sub>50</sub> obtained for 80% methanol extract (Table 1).



**Table 2.** IC<sub>50</sub> (DPPH assay) of extracts of *P. daurica* subsp. *mlokosewitschii* leaves subsequently dried at various temperatures. Values are mean ± SD from three independent experiments. \*p < 0.005, ns-nonsignificant, compared to 80% methanol extract, obtained by either freeze or microwave-drying.

Sample	Freeze-drying		Microwave-drying	
	Room temperature	40 °C	Room temperature	40 °C
Ascorbic acid	13.05 ± 0.23			
Methanol 80%	18.43 ± 0.87	17.46 ± 0.66 <sup>ns</sup>	16.67 ± 0.81	18.31 ± 1.21*
Ethanol 80%	24.50 ± 0.64*	23.29 ± 1.28*	20.01 ± 0.52*	24.25 ± 2.49*
Methanol 99%	27.02 ± 1.69*	21.31 ± 0.45*	31.17 ± 1.64*	32.98 ± 0.86*

Our results are consistent with the results of the study [19], demonstrating that low-temperature drying, such as freeze-drying and oven drying at 40 °C, produced products with higher antioxidant potential (total flavonoid content, total phenolic content, total antioxidant capacity, and radical scavenging activity) than drying at high temperatures. Also, the results of [20] showed that based on the antioxidant properties of maize waste, freeze-drying was more efficient than microwave-drying and spray-drying techniques. The highest recovery of maize waste phenolic compounds in this study was obtained using freeze-drying. Freeze-drying has high efficiency in moisture removal and maintains antioxidant compounds in plants [21]. During the freeze-drying process, ice crystals develop inside the tissue matrix and the removal of moisture content causes the tissue to become more brittle [22], which, in turn, causes the rupture of the cell structure, leading to the higher extraction efficiency of antioxidant compounds [23].

#### Effect of extraction solvents on DPPH free radical scavenging activity of extracts

In addition to the plant drying methodology, extraction methodology is also important in the antioxidant assay. The yield of the extract depends on the polarity of the solvent used for the extraction [24]. Obviously, the solubility of natural compounds and the choice of solvents can determine the yield of compounds. Now, polar solvents, such as methanol, ethanol, and acetone are major solvents used to extract some flavonols, alkaloids, polyphenols, and saponins [25].

The results of our experiments demonstrate that both, the polarity and the concentration of the solvent affect DPPH scavenging activity in *P. daurica* subsp. *mlokosewitschii* extracts. The highest DPPH scavenging activity was revealed in freeze-dried leaves, extracted with 80% methanol (Table 1 and Figs. 2 and 3). Interestingly, for microwave-dried leaves the DPPH scavenging activity was similar in extracts obtained using both 80% methanol and 80% ethanol, however,



the use of 99% methanol decreased DPPH scavenging activity more than twice (Table 1). The same trend was observed for the extraction of freeze-dried leaves with different solvents, but differences between DPPH scavenging activities in different extracts were not so pronounced.

Similarly, to the first experiment, in the experiment with extract drying the lower IC<sub>50</sub> values were revealed for freeze- and microwave-dried plants extracted with 80% methanol, followed by IC<sub>50</sub> values obtained for the extraction with 80% ethanol, and the highest IC<sub>50</sub> values were revealed for extracts of microwave-dried plants extracted with 99% methanol, regardless of the method of drying the extract (both at the room temperature or 40 °C, Table 2).

One-way analysis of variance (ANOVA) of the results obtained for the methanol and ethanol extracts showed that DPPH scavenging activity of *P. daurica* subsp. *mlokosewitschii* leaves were significantly influenced ( $p < 0.05$ ) by increasing the polarity of extracting solvent.

The polarity-dependent increase in extraction yield, antioxidant activity, reducing properties, and free radical scavenging activity of bean varieties was demonstrated [26]. The study of the effects of solvent type (ethanol, methanol, acetone, and water) and methanol concentration on extraction of total phenolic compound, total flavonoid compounds, and antioxidant capacity *Anacamptis collina* revealed that 95% methanol was the best extractant [27]. Such findings can be explained by the high

affinity of antioxidant compounds towards more polar solvents. The results suggest the suitability of polar solvents for the extraction of antioxidant compounds from plant materials, particularly *P. daurica* subsp. *mlokosewitschii* leaves. Also, the obtained results suggest that phytochemical compounds extracted in polar solvents will be pharmaceutically more important due to comparatively higher values of antioxidant activity.

## Conclusions

The results of the study demonstrated that drying methods (microwave-drying and freeze-drying), the polarity and the concentration of the solvent, and different extraction methods significantly affect the antioxidant activity of *Paeonia daurica* subsp. *mlokosewitschii* leaves. The highest antioxidant activity was revealed in the freeze-dried leaves, while the thermal drying resulted in lower antioxidant potential. The solvent polarity also played significant roles in the determination of antioxidant activity: the lowest IC<sub>50</sub> values were revealed for freeze-dried plants extracted with 80% methanol, followed by IC<sub>50</sub> values obtained for the extraction with 80% ethanol and the highest IC<sub>50</sub> values were revealed for extracts of microwave-dried plants extracted with 99% methanol. The subsequent drying of the methanol extract of microwave-dried plants both at room temperature or 40 °C reduced IC<sub>50</sub> value, however, this method is time-consuming and the IC<sub>50</sub> values were





comparable with those, obtained for the methanol extracts of freeze-dried plants without subsequent drying of the extract. As the result of the study the optimal method of drying and extraction of *Paeonia daurica* subsp. *mlokosewitschii* leaves for preserving the antioxidant activities was established:

freeze-drying of leaves followed by 24 h extraction with 80% methanol.

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## შრობის, ექსტრაქციის სხვადასხვა მეთოდების და საექსტრაქციო ხსნარების პოლარულობის გავლენა მცენარე *Paeonia daurica* subsp. *mlokosewitschii*-ის ანტიოქსიდანტურ აქტიურობაზე

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### აბსტრაქტი

სტატიაში წარმოდგენილია შრობის მეთოდების (მოკლექტალღოვანი შრობა და ლიოფილიზაცია) გავლენა მცენარე *Paeonia daurica* subsp. *mlokosewitschii*-ის ფოთლების ექსტრაქტის ანტიოქსიდანტურ აქტიურობაზე. ანტიოქსიდანტური აქტიურობა შეფასდა ექსტრაქტის უნარით, შებოჭოს 2.2 დიფენილ-1-პიკრილჰიდრაზინის (DPPH) რადიკალები.

ყველაზე დიდი აქტიურობა გამოავლინა ლიოფილიზირებული ფოთლების ექსტრაქტმა. ექსტრაგირების პროცესში მნიშვნელოვანი აღმოჩნდა გამოყენებული ხსნარების პოლარულობა, რამაც განსაზღვრა მცენარის ფოთლების ექსტრაქტის ანტიოქსიდანტური აქტიურობა. IC<sub>50</sub>-ის (ექსტრაქტის 50%-იანი ინჰიბირებისათვის აუცილებელი კონცენტრაცია) ყველაზე დაბალი მაჩვენებელი გამოვლინდა 80%-იანი მეთანოლის თანაობისას მომზადებულ ლიოფილიზირებულ ფოთლების ექსტრაქტში. მასთან შედარებით IC<sub>50</sub>-ის მნიშვნელობა მეტი აღმოჩნდა 80%-იანი ეთანოლის პირობებში მიღებულ ექსტრაქტში, ხოლო IC<sub>50</sub>-ის ყველაზე მაღალი მაჩვენებელი გამოავლინა მიკროტალღურ ღუმელში გამშრალმა მცენარის ფოთლებმა, ექსტრაქციის პროცესში გამოყენებული იყო 99%-იანი მეთანოლი.

მცენარის ექსტრაქტის შემდგომმა ლიოფილიზაციამ, არსებითი გავლენა არ იქონია ექსტრაქტის ანტიოქსიდანტურ აქტიურობაზე.



მცენარის მეთანოლიანი ექსტრაქტის შემდგომმა შრობამ მიკროტალღურ ღუმელში 40°C-ს ტემპერატურაზე დააქვეითა IC<sub>50</sub>-ის მნიშვნელობა. აღნიშნული შედეგები შედარებული იყო ლიოფილიზირებული ფოთლების მეთანოლიანი ექსტრაქტის მონაცემებთან, ექსტრაქტის მომდევნო შრობის გარეშე.

ამრიგად, დადგენილია *Paeonia daurica* subsp. *mlokosewitschii* -ის ფოთლების შრობის ოპტიმალური მეთოდი, რომელიც განაპირობებს მისი ანტიოქსიდანტური აქტიურობის შენარჩუნებას, რაც გულისხმობს ფოთლების ლიოფილიზაციას და 24 საათის განმავლობაში ექსტრაქტის მომზადებას 80%-იანი ეთანოლის გამოყენებით.

**საკვანძო სიტყვები:** *Paeonia daurica* subsp. *mlokosewitschii*, ანტიოქსიდანტური აქტიურობა, DPPH მეთოდი, ხსნარები, შრობის მეთოდები.

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