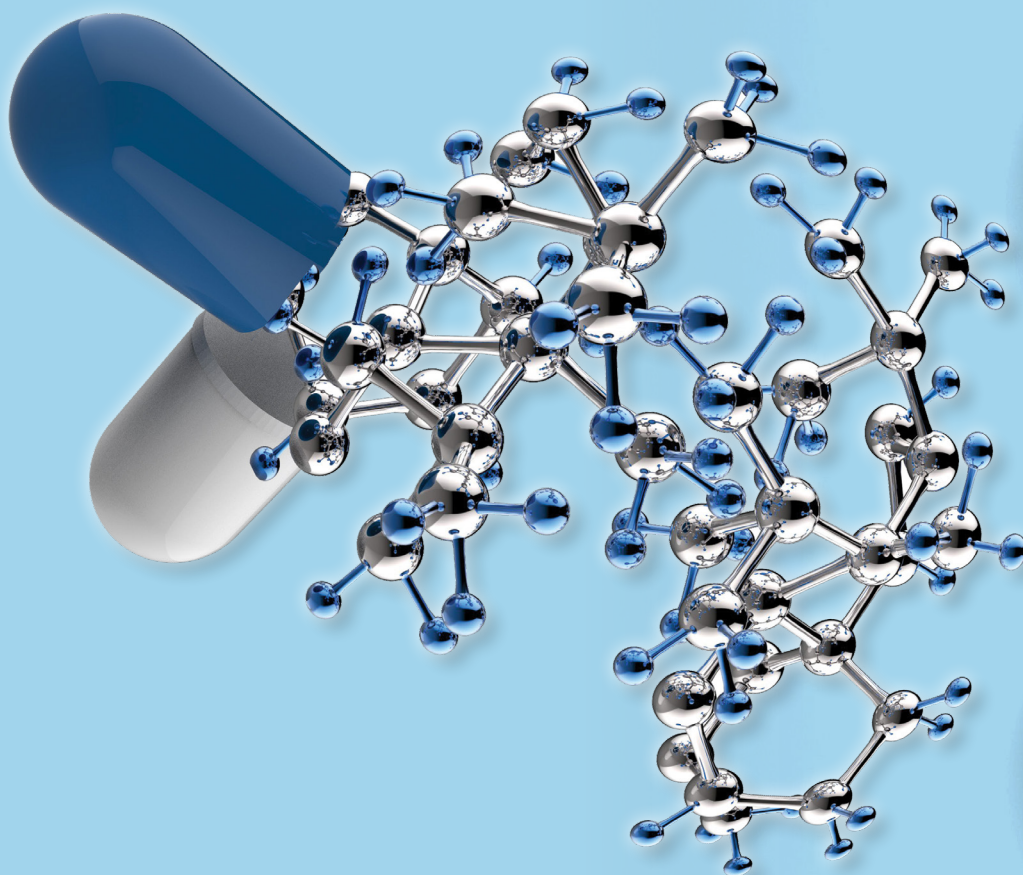


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Investigating the Influence of Personality Traits on Medication Adherence: A Cross-sectional Study

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ABSTRACT

Medical advancements have revolutionised healthcare, yet patient adherence to medication and healthcare provider instructions remains a major challenge. Non-adherence severely affects patients' prognosis, hindering their health improvement. It's crucial to understand the complex factors influencing adherence, including patient behaviour, socioeconomic conditions, and healthcare providers' guidance. This study evaluated medication adherence in 178 adult patients with chronic diseases in community pharmacies. These patients completed the questionnaires (adherence to Refills and Medications Scale; Big-Five Factor Markers). The mean value of patients' adherence to medication was 17.17. The findings showed that disease type and patients' personality traits significantly impacted adherence. Autoimmune patients and those with high conscientiousness (personality type 3) displayed better adherence. Suggested solutions for increasing adherence could include the use of daily/weekly pill cases, detailed instructions from healthcare professionals, and smart device reminders. These findings underscore the need for tailored interventions that consider individual patient characteristics to improve medication adherence and patient outcomes.

1. Introduction

In healthcare, one significant obstacle that can hinder optimal

effectiveness of the treatment is patients' non-adherence to medication treatment.¹ In chronic diseases, adherence encompasses

more than just the medication intake. It involves multiple behaviours, such as maintaining a healthy diet, engaging in regular exercise, attending medical appointments, and more.² These interconnected behaviours have a profound impact on patients' overall health and well-being. The question arises as to why patients may struggle with adhering to their prescribed medications and instructions. The term "compliance" itself implies a patient's strict obedience to the instructions and medication, often without considering their perception, understanding, and acceptance of the situation. For this reason, the term "compliance", according to the World Health Organization (WHO), has been replaced by the term "adherence", where patients are in control of their medication behaviour and interact and communicate with healthcare professionals.³ Therefore, many studies are being conducted on finding methods to assess patient's non-adherence, as it is important to identify the reasons leading to this fact and eventually find methods to improve adherence.

Unfortunately, the potential of this issue has not been fully elucidated yet and if more attention is given to patient's adherence, along with the right medication regimen, then this could potentially lead to a significant improvement in patient healthcare. This will increase patient's quality of life as well as the prevention of anything unexpected that arises during treatment. Moreover, healthcare professionals must not only prescribe medications, but also provide clear instructions on their proper usage and administration. Gil-Guillen et al. conducted a review categorizing the risk factors associated with non-adherence.⁴ The data were obtained from the fields of rheumatology, oncology, and cardiology, with the objective of identifying common factors and potential interventions to improve adherence. Therefore, apart from dispensing, a healthcare professional is also obliged to define the state of patient's health and assure the accurate medication reception, creating an understanding and trustful environment towards the healthcare professional and the treatment regimen.⁵ Proper guidance to the patient on the importance of medication and proper administration is crucial in preventing confusion and misconcep-

tions that may lead to non-adherence. This responsibility lies mainly on pharmacists and nurses who should accurately and clearly explain to the patient the dosing regimen changes, the importance of every medication, and their potential side effects.⁶ A critical factor in improving medication adherence is patient's and family members' education provided by healthcare professionals. A recent study focusing on patients with multiple sclerosis (MS) discovered that medication adherence training based on the Theory of Planned Behaviour yielded noteworthy improvements in knowledge, attitudes, norms, intention, control, and performance.⁷ In addition, comprehensive monitoring of the patient's medication regimen has been found to improve adherence and overall health status, highlighting further the importance of proper communication, and monitoring in achieving successful treatment outcomes.⁸

Properly assessing the patient's adherence to medication is crucial to identify the reasons for reduced adherence that may deteriorate the disease and find ways to improve it in order to increase the patient's quality of life.⁹ Two types of methods are available to monitor and assess adherence: direct and indirect.^{10,11} Direct methods of measuring patient's medication adherence include analysing drug levels in body fluids, monitoring biomarkers influenced by the drug, and observing drug intake. However, these methods, although precise in controlled environment, can be impractical and cost-prohibitive for daily use. Furthermore, they do not provide an assessment of adherence rates and may be influenced by underlying diseases and genetic factors. Electronic Medication Packaging Devices offer a potentially more objective and accurate approach by recording daily doses and offering audiovisual reminders.^{12,13} Indirect methods include simple questions, questionnaires, dose counting, patient reports, and repetitive prescriptions.^{11,14,15} Simple questions are subjective and unreliable, as patients may overestimate their adherence or try to convince healthcare providers that they are taking their medication correctly. Dose counting can assess whether patients receive daily doses and adherence can also be assessed through patient interviews and note-taking.¹⁶ The common aspect of these methods is subjectivity, as the answers in all cases are

based on the patient's judgment, and the assessment of the level of adherence is to a general extent, not an accurate measurement. However, the significant advantages of electronic medication packaging devices, including their simplicity, low cost, and immediate availability of results, make them a crucial tool in clinical practice.^{15,17,18}

Patient's adherence to medication is influenced by a variety of factors, including demographic characteristics, socioeconomic status, and medication-related factors.¹⁹ Socioeconomic factors, such as education, marital status, and insurance coverage, as well as demographic characteristics, like age and race, can all impact a patient's adherence to medication.^{20,21} Patients with neurological conditions, such as depression or dementia, also have a lower adherence rate due to reduced cognitive function. Adherence is also affected by treatment factors, such as polypharmacy, duration, and adverse effects, which can lead to a non-flexible dosing regimen and discontinuation of treatment.²² The healthcare system plays a crucial role in ensuring patient's adherence, with mutual trust and communication between the patient, healthcare professionals and pharmacists.^{11,20,23} Finally, in addition to the aforementioned factors, patients' personality appears to play a pivotal and primary role in determining their adherence to medication.

Personality traits are characteristics that define the way individuals interact with the world around them. These traits can provide valuable information about the best method of communicating with people and the types of jobs and tasks they are best suited for. However, personality traits can also be key indicators of other aspects of a person's life, such as innovativeness and life satisfaction. The Big Five Personality Traits theory is widely used and dominant as a theory to this day. It was proposed in 1999 by Robert McCrae and Paul Costa Jr and is based on the 35 bipolar clusters of terms related to personality traits developed by Cattell (1943) and the classic Myers-Briggs Type Index (MBTI), adding an important fifth trait of personality trait, namely neuroticism or emotional stability, which is a key domain predicting depression and anxiety disorders. The Big Five Per-

sonality Traits model includes extraversion, agreeableness, conscientiousness, openness to experience, and neuroticism. OCEAN is a common acronym.²⁴⁻²⁶

Extraversion refers to the extent to which individuals interact with the external world and experience excitement and positive emotions, while Agreeableness is the extent to which individuals value cooperation and social harmony. Conscientiousness is the extent to which individuals value planning, possess the quality of persistence, and are achievement-oriented. Openness to experience is the extent to which individuals are receptive to new ideas, emotions, and experiences. Finally, Neuroticism is the extent to which individuals experience negative emotions, such as anxiety, depression, and mood swings. Several aspects and ranges from high to low on a spectrum of characteristics distinguish each trait.²⁷

The study aims to estimate the adherence rate of patients receiving long-term medication, for chronic diseases, in private community pharmacies, evaluate their personality characteristics and study the possible correlation between demographic characteristics and personality with adherence rates. Additionally, the study aims to explore other variables, such as the cost of drugs and the behaviour of healthcare professionals, to find any correlation with medication adherence. Statistical methods were used to analyse the data.

2. Materials and Methods

A cross-sectional study was conducted to recruit patients from private community pharmacies in three Greek cities including the capital city. Adult patients who were receiving long-term medication for chronic diseases and Greek spoken were included in the study. Patients with dementia and severe mental disorders were excluded. The study was conducted in the community pharmacies after the permission of the pharmacists. The participants were recruited between May 2022 and January 2023. They were approached through the pharmacists at the local pharmacies where they are supplied their medications for chronic diseases.

Adherence to Refills and Medications Scale

(ARMS): Kripalani et al. developed the ARMS, which initially drew inspiration from the Morisky and Hill-Bone questionnaires.²⁸ The original questionnaire consists of 12 questions that assess medication adherence. Each item follows a Likert scale format, where respondents choose from options such as “none,” “some,” “most” or “all the time”. Each item was scored on a 5-point Likert scale. Permission to use the ARMS questionnaire in Greek was obtained.

50 question IPIP questionnaire: The 50-question IPIP questionnaire is designed to assess the Big Five Personality Traits - Extraversion (Type 1), Agreeableness (Type 2), Conscientiousness (Type 3), Neuroticism (Type 4), and Openness to Experience (Type 5) and their facets. It consists of a series of statements or items that respondents rate on a Likert scale, indicating the extent to which they agree or disagree with each statement.²⁹ Permission to use the questionnaire in Greek was obtained. The questions of this part aimed to determine the extent to which personality traits influenced medication adherence.

In the present study, a questionnaire-based interview, conducted in Greek, and consisted of three parts. The **first part** aimed to gather information on the sample’s demographic characteristics, such as gender, age, marital status, education level, occupation and medication profile, including the disease, the number of different types of medicines taken daily and the duration of medication. These questions were designed to determine the extent to which demographic characteristics and disease influence medication adherence.

Additionally, the participants were asked about proposals that could potentially help them adhere to their medication, such as using daily or weekly pill cases, smart device reminders, home care, detailed instructions from their doctor or pharmacist and reduced drug prices. The **second part** of the questionnaire employed the Adherence to Refills and Medications Scale (ARMS) questionnaire.²⁸ Finally, the **third part** used the “50 question IPIP questionnaire” (Big-Five Factor Markers).²⁹

Questionnaire is divided in two categories adherence and personality. The category of adherence

consists of 12 questions, while the category of personality consists of 50 questions. Cronbach alpha value is 0.86 in ARMS adherence category and 0.87 in IPIP personality category, which corresponds in high reliability of questionnaire. These indicators were consistent with others in the literature.³⁰

For statistical data-analysis, parametric and non-parametric methods such as t- test, Wilcoxon test, Chi-square test, Fisher exact test, Anova, Pearson correlation test, Ordinal logistic regression, Logistic regression were used. The reliability of the questionnaire was assessed independently, in each category (adherence, personality), with statistical criterion Cronbach alpha, which is the most common measurement of internal consistency. Statistical analysis of the data conducted with statistical package R (www.r-project.org, v3.6.2), while the level of statistical significance defined as 0.05.

The Research Ethics Committee of the University approved this study (Registration number: EI-2103). All participants understood the study purpose and process before participating in the study and filling out the informed consent. All collected data were anonymous and only members of the research team had access to them. The data collected was solely used for research purposes.

3. Results and Discussion

None of the common methods of measuring adherence, direct or indirect, are ideal, since each of these have their limitations, whereas the perfect method should be reliable, practical, of low cost, easy to use and manageable. Taking into account these considerations, the current study adopts a practical and straightforward approach through the use of questionnaires. However, these questionnaires are not self-completed, but are filled, by the pharmacist, after discussion of the patient’s missed doses, feelings, and direct measurement values, such as blood pressure and sugar, along with next prescription planning and more. The questionnaire considers not only the patient’s demographic characteristics and medication-related factors, but also their personality traits, which is considered as a critical factor. **Ta-**

Table I. Demographic characteristics and medication profile.

VARIABLE	Frequency	Proportion (%)
Demographic characteristics		
GENDER		
Man	79	44.4
Woman	99	55.6
AGE		
18-35	39	21.9
36-50	41	23.0
51-65	48	27.0
>65	50	28.1
MARITAL STATUS		
Divorced	12	6.7
In relationship	14	7.9
Married	95	53.4
Unmarried	36	20.2
Widow/er	21	11.8
EDUCATION LEVEL		
Compulsory education	24	13.5
Secondary education	45	25.3
College /technical school	25	14.0
University	60	33.7
Master/PhD	24	13.5
OCCUPATION		
State employee	17	9.6
Private employee	35	19.7
Self-employment	36	20.2
Retired	56	31.5
Unemployee/Student	26	14.6
Housekeeping	4	2.2
Other	4	2.2

Medication profile		
NO OF MEDICATION		
1	56	31.5
2	41	23.0
3	30	16.9
4 or more	51	28.7
DISORDERS		
Cardiac disorders	97	54.5
Respiratory disorders	27	15.2
Hormone disorders	83	46.6
Neurological disorders	42	23.6
Autoimmune disorders	29	16.3
Cancer	9	5.1
Chronic pain	19	10.7
other	31	17.4
YEARS ON MEDICATION		
< 2 years	42	23.6
2-5 years	48	27
> 5 years	88	49.4

ble I provides an overview of the demographic characteristics and medication profile of the sample. The dataset consists of 178 adult patients, from private community pharmacies, receiving long-term medication, for chronic diseases, with an age range of 18-92 years and a mean age of 51.8 years.

The primary objective of this study was to evaluate patients' adherence to medication. In this study, the average adherence score was 17.17. A score 12 indicates perfect adherence, while scores greater than 12 indicate lower adherence. Among the participants, 17.4% had perfect adherence, while 82.6% did not. The average value of adherence as continuous variable had mean value 17.17 (min=12, max=38). 17.4% (31 out of 178) of the sample reported perfect adherence (score=12), while the remaining 82.6% (147

out of 177) reported lower adherence (score>12).

The adherence values observed in this study align with previous research results that utilised the same measurement tool (ARMS) for assessing adherence across various diseases, including diabetes mellitus, hypertension and more. For instance, similar adherence rates were found in studies conducted in Qatar and Belgrade, which investigated medication adherence among patients with uncontrolled diabetes and those taking antiparkinsonian medication, respectively. As such, the study conducted by Jaam et al. (2018)³¹ in Qatar investigated medication adherence among patients with uncontrolled diabetes and found that 73.0% of participants did not exhibit perfect adherence. Similarly, another study by Radojević et al. (2022)³² conducted in Belgrade explored

Table II. Types of personality scores.

Variable	Mean	Median	Min	Max
Personality 1	30.70	30	11	50
Personality 2	38.75	40	21	50
Personality 3	36.29	38	14	50
Personality 4	28.81	29	11	49
Personality 5	33.20	34	14	50

Table III. Correlation of Adherence and Personality type.

Adherence	> 12	= 12
Mean Personality 2	38.73	41.23
Mean Personality 3	35.28	42.39
Mean Personality 5	32.91	36.1

Correlation	Personality 2	P-Value
Personality 3	0.27	< 0.001
Personality 5	0.34	< 0.001

Table IV. Proposals about adherence.

Proposals	Frequency	Proportion (%)	p-value
A. Daily or weekly box pills			
Total (N=178)	94	52.8	
>12(N=147)	84	57.1	0.01
=12(N=31)	10	32.3	
B. Reminder in a smart device			
Total (N=178)	91	51.1	
>12(N=147)	78	53.1	0.38
=12(N=31)	13	41.9	
C. Home care			
Total (N=178)	17	9.6	
>12(N=146)	15	10.2	0.74
=12(N=31)	2	6.5	

D. More detailed instructions from their doctor/pharmacist			
Total (N=178)	92	51.7	
>12(N=147)	76	51.7	0.07
=12(N=31)	10	32.3	
E. Price reduction			
Total (N=178)	102	57.4	
>12(N=147)	85	57.8	0.84
=12(N=31)	17	54.8	

patient adherence to antiparkinsonian medication and identified factors influencing adherence in Parkinson's disease patients. The study reported a mean adherence value of 14.9, with 74.1% of patients exhibiting low adherence and none achieving perfect adherence (value of 12). Patients with both Parkinson's disease and depression demonstrated significantly lower adherence compared to Parkinson's disease patients without depression. These findings indicate the importance of effective medication management and planning for patients and the effect of mental and psychiatric condition of the patients on their adherence. Additionally, the study suggests that individual personality traits might influence medication adherence. These adherence scores are also relevant to another recent 12-item Arabic version of ARMS with 17.93 and good consistency,³³ and slightly increased than another one in Korea with 15.53 for patients with type 2 diabetes.³⁴ ARMS seems to be a more appropriate tool for the adherence evaluation on medication, especially for the elderly and patients, institutionalised or not, with chronic multipathological conditions.^{35,36}

Jin et al.³⁷ examined how functional health literacy (FHL) affected medication adherence among elderly patients in South Korea. Their study revealed that 47.5% of participants achieved perfect adherence, indicating that factors such as education level, diseases, dosing frequency and satisfaction, with

patient counselling and medication analysis, played role in influencing adherence. Nita et al.³⁸ aimed to assess the adherence of elderly patients with chronic diseases (hypertension, diabetes, asthma, etc.), in Indonesia, using the ARMS scale. The study revealed that 88.3% of patients had low adherence. In a study conducted by Lomper et al.³⁹, a Polish version of the ARMS (ARMS-P) was developed to assess adherence levels among the hypertensive population in Warsaw. The study reported an average adherence value of 19.7. Similarly, Park et al.⁴⁰ conducted research in South Korea to examine medication adherence and related factors among elderly individuals, living alone with chronic diseases. In their study, the average adherence score was calculated to be 16.08. In Indonesia, where the prevalence of diabetes mellitus is steadily rising, a study conducted by Andanalusia et al.⁴¹ examined the adherence of patients to their antidiabetic medication. The study reported a mean adherence value of 19.52, indicating a moderate level of adherence. Only 8.3% of the sample demonstrated perfect adherence, while the remaining 91.7% exhibited imperfect adherence. Our results are in accordance with this study's results, as far as the adherence of the patients on their therapeutic pharmaceutical regimen is concerned, including most of the major chronic conditions and diseases and the overall adult age range.

In our study we also examined whether demo-

graphic characteristics such as gender, age, marital status, level of education, profession, number of medicaments, years of medication intake and assistance in taking medication had a statistically significant correlation with medication adherence. The results showed no significant association between these parameters and medication adherence. These results are in accordance with other recent studies, with no statistically significant correlation between the adherence and the age, sex, education level and years of pharmaceutical treatment.^{40,42} However, in this study a significant correlation was found between personality characteristics or the type of disease and adherence, since among the diseases, autoimmune disease patients demonstrated the greater adherence (9 out of 29) compared to those without such a disorder (p -value=0.02).

Concerning the different types of personality, a score in questions answered by the participants was calculated, which correspond in the specific type of personality. Mean score for personality 1 is 30.70, for personality 2 is 38.75, for personality 3 is 36.29, for personality 4 is 28.81 and for personality 5 is 33.20 (score equal to 50 corresponds to score that represents absolutely the specific type of personality) (**Table II**).

Moreover, statistically significant correlation was found between adherence and type of personality 2 (p -value=<0.001), personality 3 (p -value<0.001) and personality 5 (p -value=0.06). Personality types 2, 3 and 5 are statistically significantly correlated (personality 2 with 3 cor =0.27, p -value<0.001, personality 2 with 5 cor =0.34, p -value<0.001). Thus a model with adherence and personality types 2,3 and 5 was run and found out that statistically significant remains only the variable of personality type 3. Consequently, the correlation between adherence, and type 2 and 5, is secondary nominal correlation. Furthermore, adherence as a continuous variable has a statistically significant negative correlation only with personality type 3 (p -value<0.001, cor =-0.39), which means that as personality score increases, adherence score decreases (thus adherence is improved) (**Table III**).

Regarding conscientiousness (personality type 3), the positive correlation with adherence could be attributed to the fact that this group of patients is capable of

understanding the importance of their treatment and the seriousness of their disease. This type may also be closely related to the health literacy of the patient, a factor that has been found to have a moderator effect on the relationship between adherence and medication concerns and illness perceptions.⁴³ Furthermore, in a secondary manner, personality types 2 and 5 gave improvement to the adherence rates, but only when conscientiousness intervened. These results are in accordance with Axelsson et al,⁴⁴ where adherence is related to conscientiousness and agreeableness and in case the former was low the adherence was decreased too, in irrelevant to agreeableness manner. However, this finding was not present in this study, since the majority of the patients had high level characteristics of personality type 3. In another study Hazrati and his colleagues⁴⁵ also found personality type 3 to be positively correlated with adherence factor; however the extraversion was found to be an independently positive factor as well.

The study has also investigated potential solutions to improve medication adherence. All participants who completed the questionnaire were asked if they would be helped from some proposals for more efficient intake of their medicines. Subsequently, the responses of the samples were divided into two subgroups (Adherence=12 and Adherence>12), and the results were analysed separately and compared (**Table IV**). Most patients consider the reduction in drug prices as the most crucial factor for their adherence. However, no difference was observed between patients with perfect and imperfect adherence in this regard. The majority of patients with imperfect adherence reported that the proposals concerning (A) daily or weekly pill cases, (B) reminders through electronic applications and (D) more detailed instructions from doctors/pharmacists would help improve adherence. These methods, which remind them to take their drugs, could be highly beneficial, particularly in cases where the number of drugs is increased, simplifying their dosage and treatment scheme.¹⁹ Moreover, the more analytical and detailed instructions from healthcare professionals to the patient is another important factor that could improve the patient's adherence, according to their beliefs. The ad-

ministrative and commercial responsibilities within their respective work units consume valuable time for healthcare professionals, limiting their ability to fulfill their scientific role. This can result in a lack of communication with patients, which in turn can contribute to increased instances of drug abuse, undertreatment, and a higher frequency of observed adverse reactions. This could be prevented by adoption of a patient-centered care character of the provision of health services, giving analytical instruction to patients, and considering potent concerns of them, building a trustful relationship with the health instructor. This lack of adherence may also rely on the heavy schedule of the professionals, and towards the reversal of such, may be the involvement of the family doctor in every general health system or the establishment of advising services as a separate and autonomous part of the pharmacist profession. Indeed, a study by Hsu et al., showed that the most important health system related factors that facilitate adherence were the overall quality of pharmacy services, the availability of multiple systems for medication refills, the existence of a person available for communication, when questions or concerns arose, offering counselling on the importance of adherence, and the providing of tools such as pill boxes and up-to-date drug lists.⁴⁶

While this research has potentially interesting findings, it is important to acknowledge some limitations. Patient recruitment was restricted to three main cities of the country, limiting the diversity of the sample. Additionally, although the sample size was adequate, it was relatively small for ensuring the safe generalizability of statistical findings. Future studies may assist towards the overcoming of these limitations.

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4. Conclusion

Patient adherence was and remains a complicated and vital factor. Although, there are quite a few strategies for its improvement, healthcare professionals are sometimes, due to many reasons, unable to follow them. In this present work, it has been shown a moderate mean adherence rate, unrelated to the demographic characteristics, and the conscientiousness as an important independent factor for the adherence improvement. Among the conditions or diseases, people with autoimmune diseases had a high level of adherence compared to others, whilst simple adherence improvement methods, like daily or weekly box pills, reminder on a smart device and more detailed instructions from health professionals, seem to be able to increase the rates of adherence, signifying the remarkable importance of support, motivation and health literacy improvement, through a multidisciplinary approach that should be carried out with the support of all those involved in the prescription and the administration of drugs. □

Authorship statement

Concept – P.T.N., C.T.; Supervision – C.T.; Data Collection and/or Processing – E.C., K.K., E.A., V.R. ; Analysis and/or Interpretation – K.K., E.A., C.T.; Literature Search – E.C., V.R.; Writing – E.C., E.A.; Critical Reviews – P.T.N., C.T.

Conflict of interest

The authors declare no conflict of interest.

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Η Ιστορία του Ελληνικού Φαρμακείου από την ίδρυση του Ελληνικού Κράτους

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ABSTRACT

Ο πρωταρχικός ρόλος του φαρμακοποιού ως επιστήμονα υγείας στην εξυπηρέτηση του κοινωνικού συνόλου αλλά και του φαρμακείου ως χώρου παροχής υπηρεσιών πρωτοβάθμιας φροντίδας υγείας, συνάθροισης και κοινωνικής συζήτησης, έχει επιβεβαιωθεί και αναγνωρισθεί από την ελληνική κοινωνία διαχρονικά. Τα παραπάνω αποτέλεσαν την αιτία για την έρευνα, τη συλλογή, την αξιολόγηση και την παρουσίαση της ιστορικής εξέλιξης του ελληνικού φαρμακείου μετά την ίδρυση του ελληνικού κράτους. Η έρευνα προσπαθεί να συγκεντρώσει και να αναλύσει με την βοήθεια της βιβλιογραφίας, την ιστορία του ελληνικού φαρμακείου ως μέρος της κοινωνικής-πολιτιστικής ιστορίας του ελληνικού κράτους και της πρωτοβάθμιας φροντίδας υγείας από την ίδρυση του ελληνικού κράτους μέχρι σήμερα. Η ιστορία της φαρμακευτικής επιστήμης και η καθημερινότητα των φαρμακείων τον 19ο, 20ο αιώνα και το πρώτο τέταρτο του 21ου αιώνα, παρουσιάζονται με χαρακτηριστικά παραδείγματα, αξιοποιώντας βιβλιογραφικές πηγές.

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1. Εισαγωγή

Στην παρούσα εργασία έγινε προσπάθεια να συγκεντρωθούν, να αναλυθούν και να διασωθούν εκείνες οι πληροφορίες που σχετίζονται με την ιστορία του ελληνικού φαρμακείου

ως μέρους της κοινωνικής-πολιτιστικής κληρονομιάς του ελληνικού κράτους και της πρωτοβάθμιας φροντίδας υγείας ιστορικά από την ίδρυση του μέχρι σήμερα. Αξίζει να σημειωθεί ότι οι φαρμακοποιοί συ-

νεισέφεραν σημαντικά στην *Επανάσταση*, τόσο σε επιστημονικό επίπεδο αλλά και σε αγωνιστικό με οικονομική υποστήριξη του αγώνα και την περίθαλψη των αγωνιστών.

Ιστορικά, γίνεται μια πρώτη καταγραφή της υπάρχουσας κατάστασης με χαρακτηριστικά παραδείγματα, αξιοποιώντας βιβλιογραφικές πηγές την ίδρυση φαρμακείων τον 19ο, 20ο αιώνα και το πρώτο τέταρτο του 21ου αιώνα στην Αθήνα, στην Θεσσαλονίκη, στο Ναύπλιο και σε νησιά με σημαντικό στην ιστορία της Ελλάδας (Ύδρα, Σύρος) όπως και ο ρόλος των γυναικών φαρμακοποιών.

Κατά τα πρώτα έτη της ίδρυσης του Ελληνικού Κράτους η ίδρυση φαρμακείων δεν απαιτούσε ιδιαίτερες προϋποθέσεις. Από το 1834 όμως εκδόθηκαν Βασιλικά Διατάγματα και περιορισμοί για την άσκηση του φαρμακευτικού επαγγέλματος και ανάλογα και με την εκάστοτε περίοδο στο φαρμακευτικό τομέα ελήφθησαν διάφορα νομοθετικά μέτρα που διαμόρφωσαν το πλαίσιο λειτουργίας τους. Χαρακτηριστικό της νομοθεσίας της λειτουργίας των φαρμακείων και της άσκησης του επαγγέλματος του φαρμακοποιού στην Ελλάδα είναι η μη κωδικοποιημένη της μορφή. Στη διάρκεια της έρευνας διαπιστώθηκε ότι οι διάφορες διατάξεις ήταν διάσπαρτες σε διάφορα νομοθετήματα από το βάθος του χρόνου μέχρι και σήμερα.

Το φαρμακείο στην Ελλάδα έχει επιδείξει πολυποικίλο και σημαντικό ρόλο με την πάροδο του χρόνου. Διαχρονικά οι Έλληνες φαρμακοποιοί πολλές φορές υποκατέστησαν την πρωτοβάθμια περίθαλψη, όπως και πρόσφατα στη διάρκεια της πανδημίας του covid 2020.

Η θέσπιση του πληθυσμιακού μέτρου για την ίδρυση φαρμακείου βοήθησε στην διασπορά των φαρμακείων σε όλη την Ελλάδα, ακόμα και στα πιο απομακρυσμένα νησιά, τα οποία ενσωματώνονταν σταδιακά στο ελληνικό κράτος.

2. Μεθοδολογία

Στην προσέγγιση του θέματος για τη συλλογή δεδομένων χρησιμοποιήθηκαν βιβλιογραφικές πηγές από την Εθνική Βιβλιοθήκη, το Υπουργείο Υγείας και Πρόνοιας, το Μορφωτικό Ίδρυμα της Εθνικής Τράπεζας της Ελλάδας, το Φαρμακευτικό Μουσείο Θεσσα-



Εικόνα 1: Εσωτερικό φαρμακείου (αρχές 20ού αιώνα) του Σταμάτιου Κρίνου (πηγή Τα Αθηναϊκά)

λονίκης, τον Πανελλήνιο Φαρμακευτικό Σύλλογο, σε συνεργασία με τους κατά τόπους συλλόγους και τις βιβλιοθήκες των ελληνικών πανεπιστημίων. Φαρμακευτικές περιοδικές εκδόσεις έδωσαν στοιχεία για την καθημερινότητα των φαρμακείων.

3. Αποτελέσματα - Συζήτηση

3.1 Τα πρώτα σημαντικά φαρμακεία της Ελλάδας

Όταν απελευθερώθηκε το **Ναύπλιο**, εγκαταστάθηκαν στην απελευθερωμένη χώρα, πολλοί επιστήμονες και ιατροί. Στην πόλη αυτή παρουσιάστηκε ο μεγαλύτερος αριθμός φαρμακείων, καθώς ήταν η Πρωτεύουσα της Επαναστατημένης Ελλάδας και όντας το πρώτο πολιτικό, στρατιωτικό και εμπορικό κέντρο της χώρας, συγκέντρωνε εκτός από τις Διοικητικές και Στρατιωτικές Αρχές και μεγαλύτερο πληθυσμό από τις άλλες πόλεις¹.

Μετά τη δολοφονία του Καποδίστρια στο Ναύπλιο και τον εμφύλιο πόλεμο οι Μεγάλες Δυνάμεις επενέβησαν και όρισαν Βασιλιά της Ελλάδας τον Όθωνα με μεταφορά της πρωτεύουσας στην **Αθήνα** το 1834, οπότε μεταφέρθηκαν εκεί και οι υπηρεσίες. Αυτό οδήγησε την ίδρυση στην Αθήνα πολλών φαρμακείων που έμειναν γνωστά μέχρι σήμερα.

Ο **Σταμάτιος Κρίνος**, ένας από τους καθηγητές του Πανεπιστημίου Αθηνών ιδρύει το 1836 φαρμακείο στην Αθήνα.² Το 1860 είναι η αφετηρία έναρξης της ιστορίας του φαρμακείου του **Γερολυμάτου**. Σημείο



Εικόνα 2: Το φαρμακείο του Νίκου Γαβριήλ Πεντζίκι, στην οδό Εγνατία, που ήταν στέκι λογοτεχνών και ζωγράφων



Εικόνα 3: Το Φαρμακείο Ζωγράφου όταν η οδός Μοναστηρίου ονομαζόταν Εγνατία

αναφοράς αποτέλεσε και το φαρμακείο του **Μπακάκου** (1917).³

3.2 Φαρμακεία στη Θεσσαλονίκη

Παρά το γεγονός της ενσωμάτωσης της Θεσσαλονίκης στο ελληνικό κράτος επίσημα το 1912, από το 1887 καταγράφεται η παρουσία Ελληνικού Φαρμακείου στην πόλη. Την ίδια χρονιά ιδρύεται φαρμακείο και φαρμακαποθήκη στη διασταύρωση των οδών Εγνατία και Αγίας Σοφίας από τον Γαβριήλ Πεντζίκη. Τη λειτουργία του φαρμακείου συνέχισε ο υιός του, Νίκος Γ. Πεντζίκης, και το φαρμακείο συνεχίζει τη λειτουργία του στις μέρες μας από άλλους φαρμακοποιούς.⁴

Από τα γνωστότερα φαρμακεία της Θεσσαλονίκης ήταν το φαρμακείο Ζωγράφου. Ο Ζωγράφος φοίτησε στην Κωνσταντινούπολη και μετά εγκαταστάθηκε στη Θεσσαλονίκη το 1891 ιδρύοντας το φαρμακείο «Ιπποκράτης» στην οδό Εγνατία. Στο φαρμακείο αυτό όπως και σε άλλα την περίοδο του αγώνα γινόταν και συναντήσεις αγωνιστών και τυχαιαν περίθαλψη τραυματίες πατριώτες.⁵

3.3 Φαρμακεία στη νησιωτική Ελλάδα

Ένα από τα παλαιότερα φαρμακεία που λειτουργούν ακόμα στην **Ύδρα** είναι το φαρμακείο που ίδρυ-

σε το 1890 ο Ευάγγελος Ραφαλιάς. Το συγκεκριμένο φαρμακείο αποτελεί μνημείο πολιτιστικής και αρχιτεκτονικής παράδοσης, αφού δεν έχουν γίνει επεμβάσεις στο κτίριο και η επίπλωση παραμένει η αρχική. Αξιοσημείωτος είναι και ο δεύτερος χώρος του φαρμακείου, όπου παλιότερα διεξάγονταν ιατρικές εξετάσεις. Επίσης, υπάρχει πλούσια συλλογή από πορσελάνινα και γυάλινα βάζα, σκεύη, εργαλεία και εκδόσεις φαρμακευτικών βιβλίων.⁶

Η ίδρυση του ελληνικού κράτους συμπίπτει με την οικονομική ανάπτυξη της Ερμούπολης στη Σύρο. Στην απογραφή του 1828 καταγράφονται 10 φαρμακοπωλεία.⁷ Το φαρμακείο του Νίκου Κωβαίου ιδρύθηκε το 1837. Μπορεί να μην είναι το πρώτο αλλά σίγουρα είναι το μακροβιότερο φαρμακείο της Σύρου, αφού λειτουργεί μέχρι και σήμερα. Σήμερα ο Νίκος Κωβαίος, που ανέλαβε το φαρμακείο το 1971, διατηρεί τον χώρο όπως ήταν τότε, μετατρέποντας το σε ένα πολύ όμορφο μουσείο διατηρώντας την ιταλική επίπλωση του 1837. Εκεί βρίσκεται και έχει διασωθεί μεγάλη συλλογή σκευών και εργαλείων, σπάνια βιβλία και εγχειρίδια φαρμακευτικής

3.4 Γυναίκες φαρμακοποιοί

Σύμφωνα με τα στοιχεία του 2021 (ΠΦΣ) σε σύνολο 11.000 φαρμακοποιών στην Επικράτεια οι γυναίκες



Εικόνα 4: Εξωτερική άποψη του φαρμακείου Ραφαλίας όπως είναι σήμερα (από τον ιστότοπο του φαρμακείου)

φαρμακοποιοί είναι περίπου 7.000

Πρώτη Ελληνίδα φαρμακοποιός είναι η Πολύμνια Παναγιωτίδου από την Ευδοκιμούπολη, που έλαβε πτυχίο φαρμακοποιού το 1899.⁸ Φοίτησε στο Αρσάκειο και πήρε αρχικά το πτυχίο της δασκάλας. Συνέχισε όμως τις σπουδές της και ήταν η πρώτη γυναίκα που γράφτηκε στην Φαρμακευτική Σχολή του Εθνικού Πανεπιστημίου των Αθηνών το 1895, που ονομαζόταν Φαρμακευτικό Σχολείο. Ήταν επίσης από τις πρώτες γυναίκες που φοιτούσαν σε ελληνικό Πανεπιστήμιο. Αποφοίτησε το 1898 και ήταν η πρώτη γυναίκα που είχε άδεια φαρμακοποιού αλλά και η πρώτη Ελληνίδα ιδιοκτήτρια φαρμακείου. Στην αρχή εργάστηκε στο φαρμακείο του νοσοκομείου Ευαγγελισμού, όταν όμως πληροφορήθηκε ότι ο μισθός της θα ήταν κατώτερος από τον αντίστοιχο του άνδρα φαρμακοποιού παραιτήθηκε. Στη συνέχεια ίδρυσε φαρμακείο στην Αθήνα. Ήταν όμως εξαιρετικά άτυχη, γιατί μόλις ένα μήνα από την έναρξη λειτουργίας του φαρμακείου της αρρώστησε από τύφο και έφυγε από τη ζωή στις 21 Ιανουαρίου 1900.⁹

Η πρώτη γυναίκα φαρμακοποιός στη Θεσσαλονίκη ήταν η Ανδρονίκη Καμέσα, που είχε φοιτήσει στο πανεπιστήμιο Αθηνών και άνοιξε φαρμακείο στην οδό Τσιμισκή το 1923, το οποίο όμως δεν λειτουργεί πλέον.

4.Συμπεράσματα

Από την έρευνα προκύπτει ότι η ιστορία των φαρμακείων στην Ελλάδα έχει στενή σύνδεση με τις ιστο-



Εικόνα 5: Το εξωτερικό του φαρμακείου Κωβαίου από τον ιστότοπο του φαρμακείου Κωβαίου

ρικές εξελίξεις της χώρας. Η ανάπτυξή τους συνδέεται με την ίδρυση του ελληνικού κράτους, την ανάπτυξη των πόλεων, και τις κοινωνικές αλλαγές, όπως η συμμετοχή των γυναικών στην επαγγελματική ζωή.

Με την ίδρυση του νέου ελληνικού κράτους, η δημιουργία φαρμακείων ήταν αναγκαία για την κάλυψη των αυξημένων αναγκών των νέων πόλεων. Η θέσπιση του πληθυσμιακού μέτρου για την ίδρυση φαρμακείου βοήθησε στην διασπορά των φαρμακείων σε όλη την Ελλάδα. Έτσι, παρά τη γεωγραφική απομόνωση, φαρμακεία εμφανίζονται και στα νησιά της Ελλάδας, όπως το φαρμακείο του Ραφαλίας στην Ύδρα, που ιδρύθηκε το 1890, και το οποίο αποτελεί μνημείο πολιτιστικής κληρονομιάς και συνεχίζει τη λειτουργία του μέχρι σήμερα.

Τα φαρμακεία στην Ελλάδα, πέρα από την προσφορά τους στην υγειονομική περίθαλψη, διαδραμάτισαν και πολιτιστικό ρόλο στην ανάπτυξη των πόλεων και της κοινωνίας, ενώ η σταδιακή ένταξη των γυναικών στον κλάδο των φαρμακοποιών αναδεικνύει την πρόοδο που σημειώθηκε στην ισότητα των φύλων στην Ελλάδα.

Ευχαριστίες

Πολλές ευχαριστίες στον Πανελλήνιο Φαρμακευτικό Σύλλογο, αλλά και στους κατά τόπους

Φαρμακευτικούς Συλλόγους της Ελλάδας, που συμμετείχαν ενεργά, τόσο με την απάντηση των ερωτηματολογίων όσο με τη χορήγηση στοιχείων και πληροφοριών για τα φαρμακεία-μέλη τους

Πολύ σημαντική στην έρευνα αυτή ήταν η βο-

ήθεια του έφορου του φαρμακευτικού Μουσείου κ. Νικόλτσίου Βασιλείου και του υπεύθυνου κ. Νικόλτσίου Νικολάου, για το χρόνο και το υλικό που διέθεσαν στο Φαρμακευτικό Μουσείο Θεσσαλονίκης, το οποίο είναι πηγή γνώσεων και πληροφοριών για το ελληνικό φαρμακείο. □

The history of the Greek pharmacy after the establishment of the Greek State

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ABSTRACT

The primary role of the pharmacist as a health care scientist and of the pharmacy as a place where primary health care services are given has been confirmed and recognized by the Greek society throughout time. It has been the reason for the research, collection, evaluation and presentation of pharmacy within the historical framework of the Greek State. This research tries to collect information and to analyze the history of the Greek pharmacy in Greece after the establishment of the Greek State. The history of Pharmaceutical Science, the establishment, the development and the daily life of pharmacies in the 19th, 20th and the first quarter of the 21st century are presented with characteristic examples, using bibliographical sources.

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ZnO Nanoparticles Prepared by Hydrothermal Method and their Role on Gene Expression of TA System Type II Genes in Carbapenem-resistant *Klebsiella pneumoniae*

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ABSTRACT

ZnO nanoparticles exhibit significant antibacterial activity against numerous pathogenic organisms in vitro and in vivo via penetration through the outer bacterial membrane leading to cytotoxicity or the generation of reactive oxygen species. This study examines the effects of hydrothermally produced ZnO nanoparticles on genes involved in toxin-antitoxin systems and the use of nanoparticles as antibiofilm in carbapenem-resistant *Klebsiella pneumoniae*. The result of the antibiotics sensitivity showed variable activity against *K. pneumoniae*, the isolates showed high resistance to carbapenem antibiotics including Imipenem (84%), and Meropenem (62%). The outcome demonstrated that ZnO nanoparticles have a 68% inhibitory effect on biofilm development. Using the microtiter plate method, the MIC concentration of ZnO nanoparticles as antibacterials was 19.5µg/ml. Several techniques were used to characterize ZnONPs: X-ray Diffraction Analysis (XRD), UV-visible spectroscopy, and Field emission scanning Electron microscopic (FESEM). The study's real-time PCR findings showed that after being treated with ZnO nanoparticles, the expression levels of the type II toxin-antitoxin genes (*mqsR*, *mqsA*, *mazE*, *mazF*, *relE*, *relB*, and *hipB*) had decreased.

Introduction

Antimicrobial resistance has become a global concern, emphasizing

ing the urgent need for novel and effective antimicrobial strategies¹. Zinc oxide nanoparticles (ZnONP) have emerged as a promising an-

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timicrobial agent due to their unique properties and ability to induce oxidative stress in bacterial cells^{2,3}. The present study investigates the potential of ZnONP in targeting toxin-antitoxin gene systems in *Klebsiella pneumoniae*, a significant opportunistic pathogen known for its multidrug resistance. Recent studies have highlighted the antimicrobial properties of ZnONP, demonstrating their ability to disrupt cell membranes, alter permeability, and accumulate within the cytoplasm, ultimately leading to cell death⁴. The mechanism of action is thought to involve the generation of reactive oxygen species, which can irreversibly damage cellular components, including DNA, proteins, and lipids^{1,3}.

Additionally, ZnONP has been reported to exhibit broad-spectrum antibacterial activity against various Gram-positive and Gram-negative strains, including *Klebsiella pneumoniae*^{3,5}. Toxin-antitoxin gene systems are an important mechanism bacteria employ to survive under environmental stresses, including antimicrobial exposure. These systems consist of a stable toxin and an unstable antitoxin, which work in a delicate balance to regulate cellular processes. Disruption of this balance can lead to cell death, making toxin-antitoxin systems a promising target for novel antimicrobial strategies. Bacterial chromosomes and plasmids contain a genetic component called the toxin-antitoxin system (TA). It comprises two genes that produce a labile antitoxin that counteracts the stable toxin⁶. According to antitoxin characteristics and mechanisms, TA systems have been divided into numerous kinds; type II TA systems are the most common⁷.

Usually, the antitoxin binds directly to the toxin and prevents it from doing its job by interfering with essential cellular processes such as transcription, translation, and DNA replication. In addition to being released when the antitoxins are broken down by cellular proteases under stress, the development of biofilm antibiotic tolerance, persistence, plasmid maintenance, phage resistance, and phage infectivity can also contribute to microbial pathogenicity⁸.

The transfer of genetic elements that serve as carriers of bacterial antibiotic resistance and virulence factors has often been associated with TA systems⁹.

The toxin and antitoxin in type II bacteria are proteins. Of all the bacterial TAs, type II TAs have been investigated the most, and many of them are found in different bacterial species, including the same species. According to the homology of the amino sequence of toxins, bacterial type II TA modules can be divided into 12 subgroups¹⁰. These include mqsRA¹¹, relEB¹², yefM-yoeB¹³, ω - ϵ - ζ ¹⁴, mazEF¹⁵, and yefM-yoeB¹³. In type II systems, the toxin acts as an inhibitor of essential cellular functions such as replication or protein synthesis. The primary causes of bacterial resistance in Iraq have been identified as public misconception and misuse of antibiotics¹⁶.

There is a significant rate of antibiotic misuse among the Iraqi population, ranging from 45% to 92%¹⁷. The spread of antibiotic-resistance genes from ambient bacteria to medically significant bacterial isolates is mostly facilitated by *Klebsiella pneumoniae*. Compared to the majority of bacterial isolates, *K. pneumoniae* is a rapidly developing MDR isolate that has been shown to develop antibiotic resistance at a faster rate. This is often a major worry for patients due to the increased risk of morbidity and mortality¹⁸. According to Mohammed et al. (2020)¹⁹, a significant portion of *K. pneumoniae* exhibited considerable resistance to carbapenems, aminoglycosides, and β -lactam antibiotics. Recent research has shown that hvKp isolates have carbapenem resistance²⁰.

Zinc oxide (ZnO) nanoparticles (NPs) have gained significant attention due to their unique optical, electrical, and antimicrobial properties²¹. However, they also have several limitations that restrict their applications, including cytotoxicity, ZnO NPs can be toxic to human cells, especially at high concentrations, leading to potential health risks²². Overuse in antimicrobial applications may contribute to bacterial resistance²³. ZnO NPs tend to agglomerate due to high surface energy, reducing their effectiveness in applications like catalysis and drug delivery²¹. They dissolve in acidic environments, limiting their use in certain biomedical or environmental applications. Under UV light, ZnO NPs can undergo photocorrosion, reducing their photocatalytic efficiency over time²³. Controlling size, shape, and surface properties consistently is difficult²⁴. Accumulation in or-

Table 1. Primers were used throughout the current study.

Activity	Primer target	Oligo Sequence 5'→3'	Product size(bp)
HkG ²⁹	<i>rpoB</i>	F: 5'-GTTGGCGAAATGGCGGAAAAC-3' R: 5'-ACGTCCATGTAGTCAACCTGG-3'	599
TAs ³⁰	<i>mqsR</i>	F: 5'-ACGCACACCACATACACGTT-3' R: 5'-GCCTGGGTCTGTAAACATCCT-3'	194
TAs ³⁰	<i>mqsA</i>	F: 5'-AATGTCCGGTTTGCCACCAG-3' R: 5'-GCATTCAACGAAGCCCGAAA-3'	238
TAs ^{31,32}	<i>mazE</i>	F: 5'-ATGATCCACAGTAGCGTAAAGCGT-3' R: 5'-TTACCAGACTTCCTTATCTTTCGG-3'	249
TAs ^{31,32}	<i>mazF</i>	F: 5'-ATGGTAAGCCGATACGTACCC-3' R: 5'-TGGGGCAACTGTTCTTT-3'	288
TAs ^{31,32}	<i>relE</i>	F: 5'-GACGAGCGGGCACTAAAGGAAT-3' R: 5'-TCAGAGAATGCGTTTGACCG-3'	267
TAs ^{31,32}	<i>relB</i>	F: 5'-ATGGGTAGCATTAACCTGCGT-3' R: 5'-TCAGAGTTCATCCAGCGT-3'	240
TAs ^{31,32}	<i>hipA</i>	F: 5'-AGCCCAACGCAATTGGCGAATGCA-3' R: 5'-CTGTTCTGTTGATTCTGGCGAGGC-3'	1314
TAs ^{31,32}	<i>hipB</i>	F: 5'-AGCCCAACGCAATTGGCGAATG3' R: 5'-CTGTTCTGTTGATTCTGGCGAGGC-3'	225

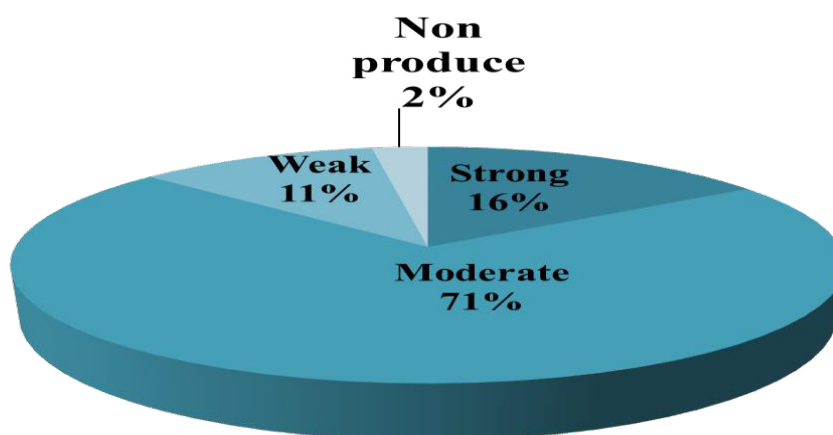
gans (e.g., liver, spleen) may cause long-term toxicity. Interactions with biological molecules (e.g., proteins, DNA) can lead to unintended side effects²¹. Alternatives like TiO₂, Ag, and SiO₂ NPs may offer better stability, lower toxicity, or enhanced performance in some applications²⁵.

The synthesis of enzymes like carbapenemases, AmpC β-lactamases, and extended-spectrum β lactamases (ESBLs)²⁶ or alterations in the outer membrane protein, permeability barrier, or target site represented by penicillin-binding protein are the main causes of *K. pneumoniae* antibiotic resistance, including beta-lactam antibiotics, which are one of the most important issues of elevated infection in hospitals²⁷. Multi-drug resistance (MDR) *K. pneumoniae* was thought to be treated with carbapenemases as a last resort. This occurred before *K. pneumoniae* initial description. Since the development of carbapenemase (KPC) in North Carolina isolates, carbapenem-resistant *K. pneumoniae* (Ckp) has been regularly found in a variety of nosocomial situations

worldwide. All β lactams and frequently other significant therapeutic drugs are ineffective against Ckp pathogens²⁸. The present study aims to investigate the efficacy of ZnO nanoparticles in targeting toxin-antitoxin gene systems in *Klebsiella pneumoniae*, to develop a novel and effective approach to combat antimicrobial resistance in this clinically significant pathogen.

Materials and Methods

Collection and identification of bacterial isolates: A total of 120 clinical isolates were collected and re-identified using selective media, biochemical tests, and the VITEK2 system (Bio-Merieux, France). The suspected isolates were collected over five months (from Dec 2023. to Oct. 2024) from different sources; urine samples, blood samples, sputum, burns, and wound swabs from several hospitals in Baghdad, including Al-Kindi Hospital, Baghdad Teaching Hospital, Al-Yarmouk Hospital (Iraq).



Biofilm formation

Figure 1. The total number of *K. pneumoniae* biofilm formation.

Detection of biofilm formation: In accordance with the protocol described by Kowalska et al. (2020)²⁹, the microtiter plate method was used to assess biofilm formation.

ELISA: Using an ELISA reader, the absorbance of each well was determined at 630 nm. The control well's OD value was subtracted. The adherence capabilities of the bacterial test isolates were categorized into four groups based on all test OD values; the isolates were categorized using method described by Babapour et al. (2016)³⁰, with the mean optical density of the negative control (contained broth only) serving as the cut-off optical density (ODc).

Antibiotic susceptibility test: The disc diffusion method, as outlined by Salih et al. (2024)³¹, was implemented for the assessment of antibiotic susceptibility of *K. pneumoniae* isolates to various antibiotics.

Preparation of zinc oxide (ZnO): All raw materials were analytical grade, ZnO nanostructure was synthesized using a simple hydrothermal method without catalysts, (0.05M) of hexahedral zinc nitrates $Zn(NO_3)_2 \cdot 6H_2O$ (Scharlab, Spain) and (0.05 M) of HexaMethelTetramine (HMT) (Hi-media, India)

were dissolved in 80 ml of deionized water, the aqua solution was stirred for ten minutes. The aqua solution was transferred into a glass autoclave and kept at 90°C for 3 hours in a box furnace. After that, the autoclave cooled down to room temperature. The obtained powder was washed several times in ethanol, and distilled water, and dried. The hydrothermal conditions promote the growth of well-defined ZnO nanostructures. After autoclaving the collected white precipitate is ZnO nanoparticles. Dry the purified ZnO nanoparticles in an oven at 60–80°C to remove residual moisture.

Antibacterial activity of ZnO nanoparticle: Two methods were used to detect the effects of these nanomaterials on the inhibition or eradication of the cells of CKP isolates.

(A) Microtiter Plate Method: By Jasim et al. (2020)³², the MIC of nanomaterials was ascertained using single 96-well microdilution plates.

(B) Well Diffusion Method: According to Yaseen et al. (2019)³³, the agar well diffusion method was used to screen for the antibacterial impact of nanomaterials.

Primer's: The effect of ZnO NPs against TA expression for genes (*mqsR*, *mqsA*, *mazE*, *mazF*, *relE*, *relB*,

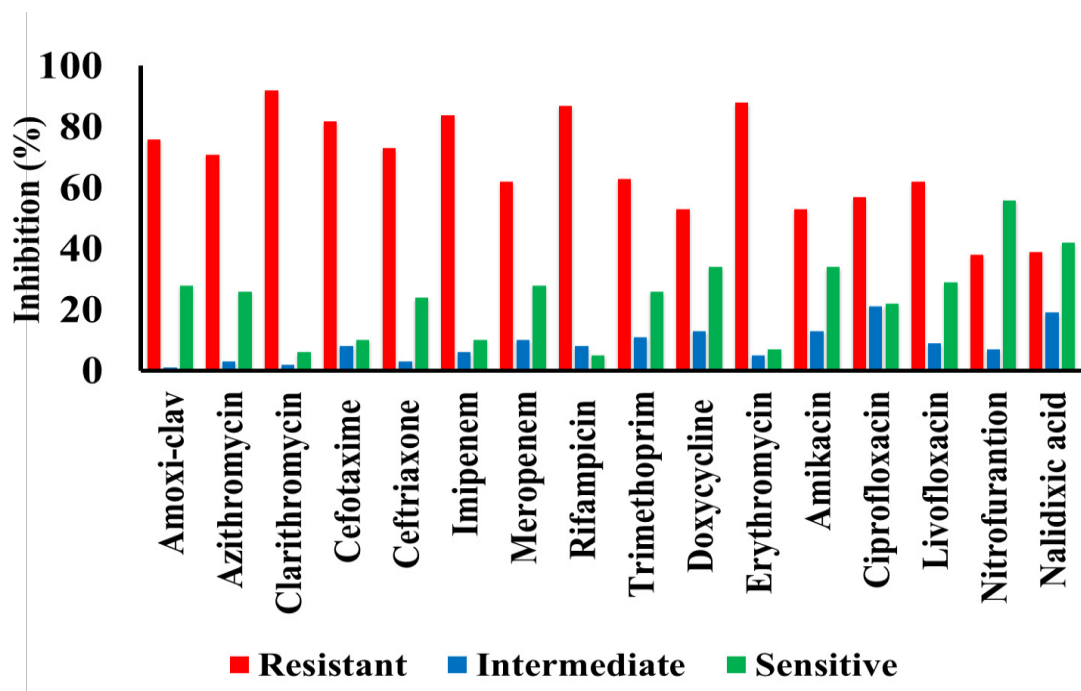


Figure 2. The percentage of antibiotic-resistant *K. pneumoniae* isolates.

and *hipB*) which is clarified in (Table 1)

Preparation of the selected isolates for RNA extraction: Three Carbapenem-resistant *K. pneumoniae* isolates with high antibiotic resistance and strong biofilm formation were chosen, and then *mqsRA*, *mazEF*, *relEB*, and *hipB* were selected. RT-qPCR techniques were done before and after being treated with (ZnO) nanoparticles. The selective isolates were grown in BHI broth (Himedia, India) tubes overnight, then treated with a sub-mic concentration of nanoparticles for 4hr, and then RNA was extracted from isolates³⁴⁻³⁶.

Quantitative reverse transcription-PCR (qRT-PCR) assays: WizPure™ qPCR master (SYBR) (Wizbiosolution/South Korea) states that the qRT-PCR setup for each sample was as follows: cDNA served as the template, two reactions were carried out for each pair of primers, and the housekeeping *rpoB* gene was targeted as an internal control to normalize mRNA levels. Using $2^{-\Delta\Delta CT}$, fold changes in mRNA expression were computed. For two hours, this reaction was

conducted in the Real Time-PCR system LM 2012. To avoid contamination, every stage of the qRT-PCR procedure was completed in a safety cabinet^{37,38}.

Results

Identification of *K. pneumoniae*: The suspicious isolates were identified using selective media such as MacConkey agar, EMB agar, and Crhromo agar; biochemical assays, and the VITEK2 system.

Biofilm formation: The microtiter plate assay was done to determine the biofilm formation by *K. pneumoniae* isolates. The results showed that (117/120) isolates could produce biofilm, including (13/120) weak, (85/120) moderate, and (19/120) strong-biofilm producing isolates, while (3/120) isolates were non-biofilm producers as in (Figure 1).

Antibiotic susceptibility test: The result of the antibiotics sensitivity test for (16) antibiotics showed variable activity against *K. pneumoniae*,

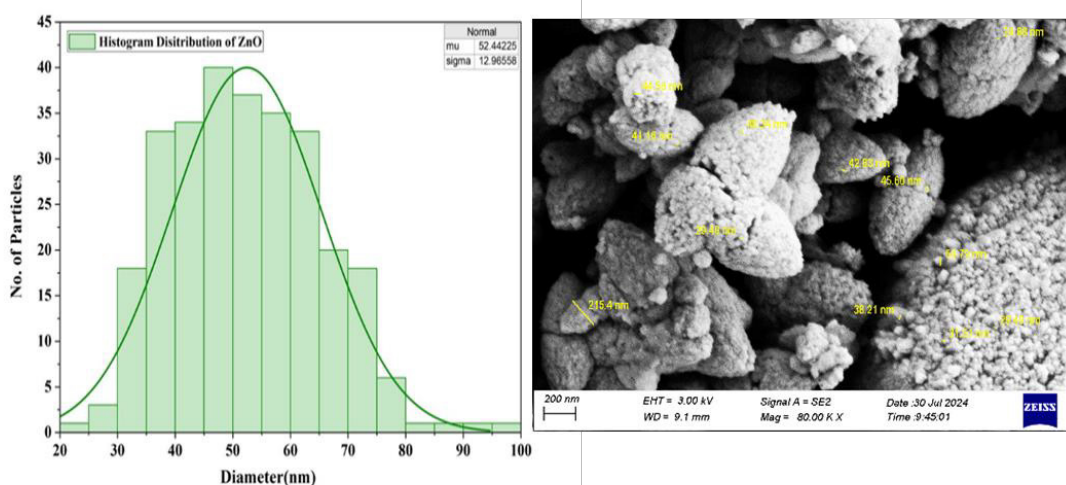


Figure 3. FE-SEM images of ZnO NPs as prepared by a hydrothermal method (Left image) histogram shows size distributions based on FE-SEM images of ZnO NPs (Right image) with scale bar 200 nm.

the isolates showed high resistance to carbapenem antibiotics including Imipenem (84%), Meropenem (62%), the resistant level of other antibiotics against *K. pneumoniae* were Clarithromycin (92%), Erythromycin (88%), Rifampicin (87%), Cefotaxime (82%), Amoxiclav (76%), Azithromycin (71%), Trimethoprim (63%), Levofloxacin (62%), Ciprofloxacin (57%), Doxycycline (53%), Amikacin (53%), Nalidixic acid (39%), Nitrofurantoin (38%) (Figure 2).

Characterization of ZnO nanoparticle

FE-SEM Images of ZnO: The result in (Figure 3) shows the FE-SEM analysis images of high-density ZnO NPs prepared using a hydrothermal method with different magnifications. Image (Left), obtained using ImageJ software of at least 45 particles, shows the particles have an average diameter of 52.44 nm. The minimum diameter of ZnO particles was 24.88 nm, and the maximum was 71.79 nm. Image (Right) shows that the product mainly consists of nanoparticles assembled to a spherical shape with found aggregation of nanoparticles with different diameters.

X-ray diffraction of ZnO: The result shows the XRD patterns of ZnO nanopowder prepared by the hydrothermal method. The diffraction peaks confirm the polycrystalline structure of ZnO with the Hexagonal phase. The observed peaks match well with the reported (JCPDS 96-900-4180) data of ZnO. All diffraction peaks in the XRD patterns correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) Miller indices. The higher peak intensities of an XRD pattern are due to the high crystallinity. The XRD patterns revealed that the (101) peak intensities were sharper and higher than others, implying that the growth orientation preferred the (101) peak as in (Figure 4). Diffraction patterns from zinc metal and impurities were not observed, indicating the high purity of the as-grown sample. The average crystallite size (D) of ZnO NPs is 27.87 nm.

UV- visible of ZnO: Adopts Visible-UV Spectroscopy enables the transmission of visible light and/or transmission of ultraviolet rays during the sample to determine the presence and/or amount of a substance that absorbs light inside the sample. UV vis-spectra detected the presence of ZnO nanopar-

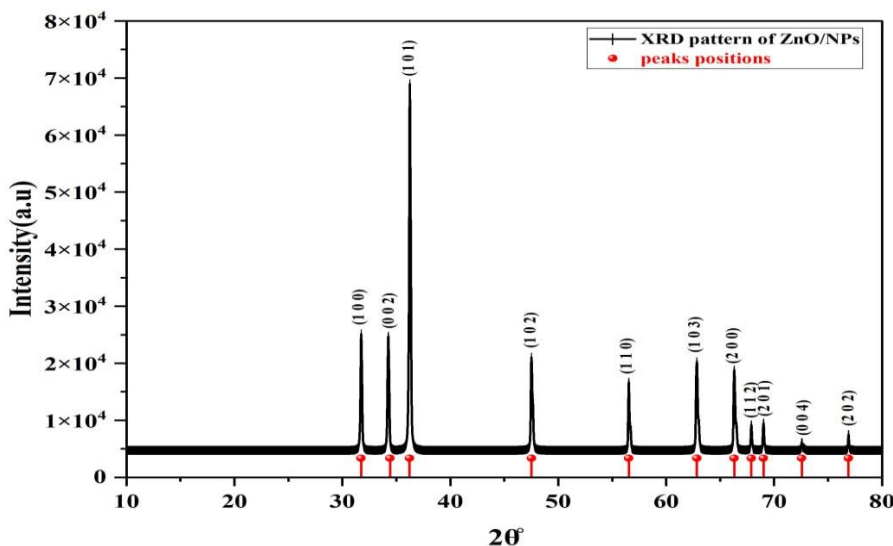


Figure 4. XRD pattern of as-prepared ZnO NPs by hydrothermal method.

ticles. The absorbance peak was reported at 366.65 nm as in (Figure5).

Effect of ZnO NPs as Antibacterial: The result of using ZnO nanoparticles as an antibacterial against *K. pneumoniae* by using microtiter plate assay using rezasurine pigment showed that MIC of ZnO was (19.5 µg/ml) as in (Figure 6A). Also, the inhibitory effect of ZnO NPs by the wells method showed good results as antibacterial the range size of the inhibition zone was (25 mm) as in (Figure 6B).

Effect of ZnO NPs as Antibiofilm: To investigate the inhibitory effects of (ZnO) nanoparticles on biofilm formation, we have chosen 19 strong biofilm *K. pneumoniae* isolates. The result showed that the inhibitory effects of (ZnO) nanoparticles as antibiofilm by microtiter plate assay were 68% positive and 32% negative as in (Figure 7).

Estimation of the effect of sub-MIC ZnO type II toxin-antitoxin system gene expression: The study's findings showed that after being treated with

ZnO nanoparticles, the expression levels of the type II toxin-antitoxin genes (*mqsR*, *mqsA*, *mazE*, *mazF*, *relE*, *relB*, and *hipB*) had decreased. as shown in (Table 2, Figure 8).

Discussion

In recent years, there has been a concerning rise in the prevalence of carbapenem-resistant *K. pneumoniae*, which poses a major threat to patient care due to the limited treatment options³⁹. One crucial factor contributing to the success of these multidrug-resistant strains is their ability to form robust biofilms, which can enhance their virulence, antibiotic tolerance, and persistence in the clinical setting⁴⁰. One study explored the impact of biofilm formation on the pathogenicity of carbapenem-resistant *K. pneumoniae*, particularly in the context of COVID-19 patients in intensive care units. The researchers found that these bacteria can cause severe infections, such as urinary tract infections, respiratory infections, and sepsis, in immunocompromised individuals⁴¹.

Carbapenem-resistant Enterobacteriaceae, includ-

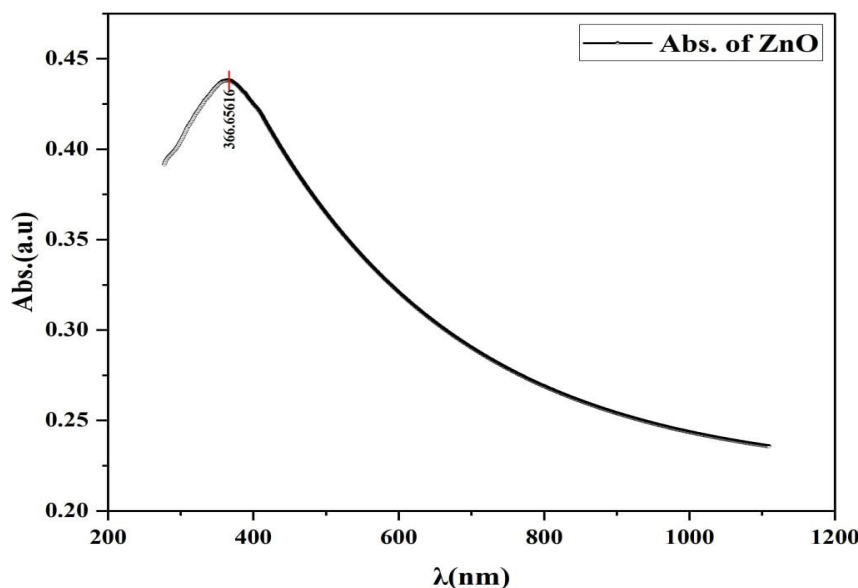


Figure 5. The UV-Vis spectrum of synthesized ZnO NPs by hydrothermal.

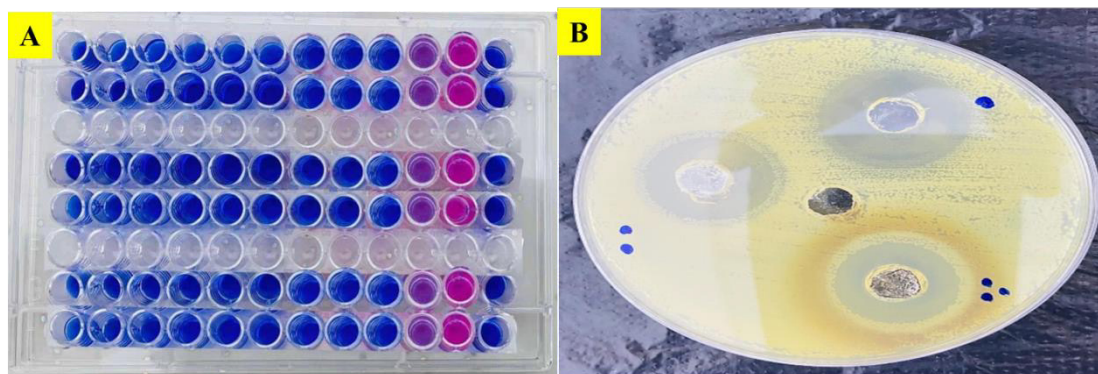


Figure 6. A-Determination of MIC of nanoparticles (ZnO) by Micro titer dilution method. B- Effect of nanoparticles against *K. pneumoniae* by well diffusion method.

ing carbapenemase-producing *K. pneumoniae*, have been responsible for nosocomial outbreaks globally and have become endemic in several countries⁴². These organisms possess potent resistance mechanisms that render them resistant to most, if not all, available antibiotic treatments, including the once “last line of defence” carbapenems and polymyxins³⁹. The rapid spread and high mortality associated with

these infections have led to substantial morbidity, mortality, and healthcare costs, with estimates suggesting attributable mortality as high as 44%, particularly in the setting of bacteremia⁴².

The results of the study conducted by Mahmoud (2020) showed that ZnO nanoparticles prepared by green synthesis examined by (FE-SEM) have a range size diameter between (29-55 nm), and

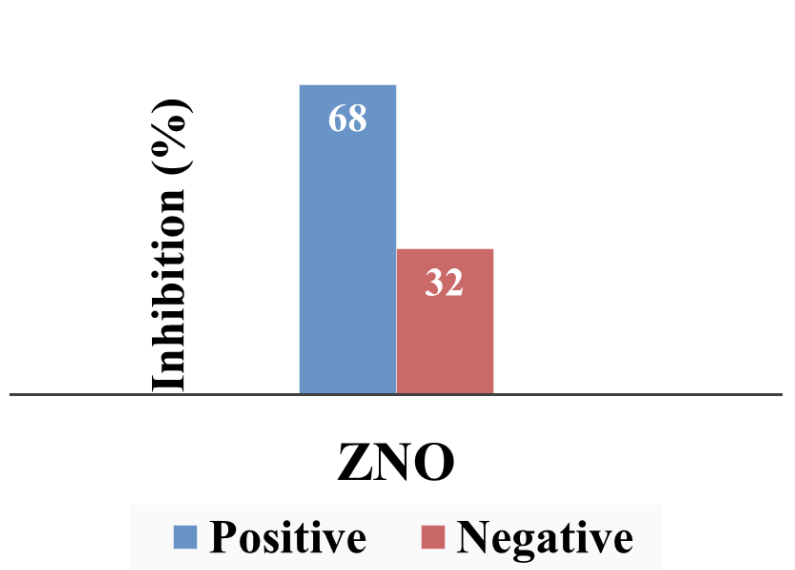


Figure 7. The effect of ZnO nanoparticle as antibiofilm against *K. pneumoniae*.

Table 2. CT, Δ CT, $\Delta\Delta$ CT values, fold change ($2^{-\Delta\Delta$ CT), and log2 fold change of type II toxin-antitoxin system genes (*relE*, *relB*, *mqsR*, *mqsA*, *mazE*, *mazF*, *hipB*) after sub-MIC ZnO treatments.

Colour of bands	Genes	Ct		Δ Ct		$\Delta\Delta$ Ct	Fold change	Log 2 - $\Delta\Delta$ CT
		before treatment	after treatment ZnO	before treatment	after treatment ZnO	after treatment ZnO		
	<i>mqsR</i>	22.9	30.44	0.78	7.32	6.54	0.0107	-1.9687
	<i>mqsA</i>	22.89	31.57	-0.23	8.45	8.68	0.0024	-2.612
	<i>mazE</i>	22.98	32.60	-0.14	9.48	9.62	0.0012	-2.8959
	<i>mazF</i>	23.17	32.98	0.05	9.86	9.81	0.0011	-2.9531
	<i>relE</i>	23.2	32.14	0.08	9.02	8.94	0.0020	-2.6912
	<i>relB</i>	23.54	34.00	0.42	10.88	10.46	0.0007	-3.1487
	<i>hipB</i>	23.25	31.86	0.13	8.74	8.61	0.0025	-2.5918

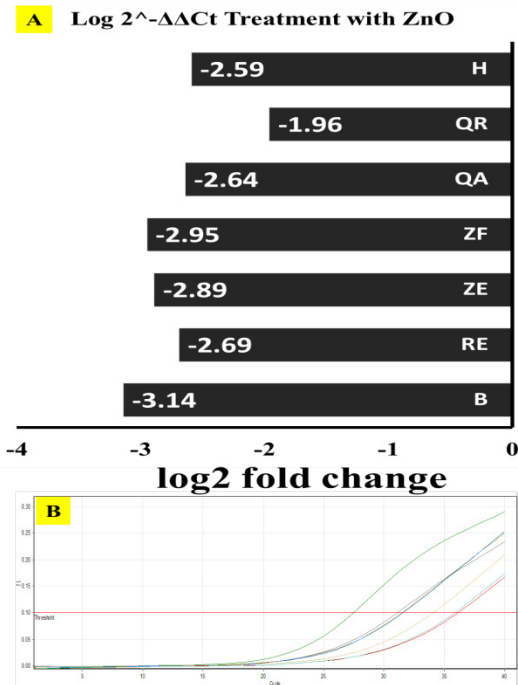


Figure 8. (A) *log2 fold change gene expression of type II toxin-antitoxin system genes (relE, relB, mqsR,mqsA, mazE,mazF, hipB) after sub-MIC ZnO treatments.* (B) *Standard curve RT-PCR amplification of TAs genes plot by qRT-PCR. The colours represented the different TAs genes of K. pneumoniae after sub-MIC ZnO treatments.*

the nanoparticles were irregular or polymorphic shapes³⁸. A local study shows that the ZnO prepared by the hydrothermal method has a pure wurtze hexagonal structure as compared with (JCPDS) cards⁴⁴. Also, no peaks of metallic Zn are observed Which means that the O₂ ratio in the gas mixture is sufficient for the complete oxidation of Zn independently and no additional thermal annealing in the oxidation atmosphere is needed, also no other peaks appear for the organic material used in a paste made. The highly blue-shifted maximum absorption occurring at about 366.65 nm confirms the formation of the nanoscale ZnO component since the maximum absorption for the bulk ZnO occurs at around 385 nm⁴⁵. The unique physicochemical properties of ZnO nanoparticles, including their high surface-to-volume ratio and reactive oxygen species generation, have been identified as key factors contributing to their antibacterial efficacy. Nu-

merous studies have demonstrated the potent antibacterial activity of ZnO nanoparticles against both Gram-positive and Gram-negative bacteria⁴⁶. The proposed mechanisms of action include disruption of the bacterial cell membrane, interference with cellular processes, and generation of oxidative stress leading to cell death⁴⁷. The findings indicated that isolates with significant biofilm formation were highly prevalent in wound and UTI infections. Using catheters and the necrotic tissue of wounds serve as a substrate for bacterial attachment, which may be the cause⁴⁷. Furthermore, the MDR isolates developed biofilms at a significantly higher rate. The findings were consistent with a local investigation by Abdelraheem and Mohamed (2021), that found that isolates from wound and burn swabs that produced biofilms had more antibiotic resistance than those that did not ⁴⁸. According to the study conducted by Hassan (2024) findings, tem-

perature (30°C, 37°C, and 44°C) and pH (5, 7, 9, and 11) had a substantial differential impact on the gene expression levels of *hipB* anti-toxin, *mqsR* toxin, and *relE* toxin⁴⁹. The activity of enzymes, which are essential for gene expression and other cellular functions, can be affected by temperature and pH. These factors can trigger the SOS stress response and alarmone (p)ppGpp, which controls genes like TA system genes or enzymes for growth and stress survival⁵⁰. The *mqsRA* TA system has been shown in recent studies to exhibit reduced expression of the *mqsA* antitoxin under stress⁵¹. And increased expression of the *mqsR* gene, which indicates a 3.67-fold rise in the *relE* gene⁵².

Conclusion

We investigate from the above that ZnO nanoparticles have activity against (Ckp) isolates in addition to having antibiofilm activity, it has also been proven that ZnO NPs affect gene expression of toxin-antitoxin type II, whether it increases or decreases depending on the type of targeted gene. This is considered a promising solution to eliminate the problem of bacterial resistance to antibiotics by disrupting the toxin-antitoxin system and allowing the toxin to kill bacteria or cause significant damage to them, it reduces their virulence and prevents them from being able to cause infection. □

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The Influence of Hydration Temperature and Lipid Weight in C-4-hydroxy-phenylcalix[4]pyrogallolarene Liposome Preparation as Skin Brightening

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ABSTRACT

The C-4-hydroxy-phenylcalix[4]pyrogallolarene is a compound proven to inhibit tyrosinase enzyme activity. This activity makes C-4-hydroxy-phenylcalix[4]pyrogallolarene as a potential skin-brightening agent. However, the poor water solubility of this compound limits its application as a skin brightener. Therefore, this study formulated C-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes to address this issue. The liposomes were prepared using the thin-layer lipid film hydration method with L- α -phosphatidylcholine as the lipid base. The resulting C-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes were found in spherical forms, and they exhibited multilamellar vesicle characteristics. This study also examined the influence of hydration temperature and L- α -phosphatidylcholine concentration on liposome size and polydispersity index (PDI). ANOVA analysis using Design Expert-13 indicated that the factors of hydration temperature, L- α -phosphatidylcholine weight, and their interaction all influenced the size and PDI of the resulting C-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes.

1. Introduction

Melanin production is a complex process involving the crucial role of tyrosinase.¹ The overactivity of tyrosinase is often implicated in the overproduction of melanin, resulting in hyperpigmentation². Hyperpigmentation is characterized by dark spots and uneven skin tone.³ This condition can affect a person's appearance. A strategy to address hyperpigmentation is to inhibit tyrosinase activity. Various natural and synthetic inhibitors, such as kojic acid, arbutin, and hydroquinone, have been extensively studied for their ability to reduce melanin production by directly inhibiting tyrosinase activity.⁴ Furthermore, the quest for new and more effective tyrosinase inhibitors continues, with ongoing research focusing on both synthetic derivatives and natural compounds that can provide safe and effective solutions for hyperpigmentation.^{5,6}

The synthetic compound of calixpyrogallolarene derivative, specifically *C*-4-hydroxy-phenylcalix[4]pyrogallolarene, as shown in Figure 1, has garnered significant attention in the field of medicinal chemistry, particularly for its role as a tyrosinase inhibitor.⁷ The mechanism by which *C*-4-hydroxy-phenylcalix[4]pyrogallolarene acts as a tyrosinase inhibitor can be attributed to its phenolic structure. The presence of hydroxyl groups in calixpyrogallolarene structure enhances its ability to form hydrogen bonds with tyrosinase, thereby inhibiting its activity. This interaction is crucial as it can lead to a decrease in melanin production, making it as a potential candidate for cosmetic applications aimed at skin brightening.⁸ Moreover, the antioxidant properties of *C*-4-hydroxy-phenylcalix[4]pyrogallolarene further support its role in inhibiting tyrosinase, as oxidative stress has been linked to increased tyrosinase activity and subsequent melanin production.⁷ These properties make *C*-4-hydroxy-phenylcalix[4]pyrogallolarene more effective as a tyrosinase inhibitor compared to kojic acid, which is already used in cosmetic formulations.⁹ While *C*-4-hydroxy-phenylcalix[4]pyrogallolarene presents intriguing properties as a tyrosinase inhibitor, its limitation in solubility restricts its practical applications in cosmetics. Addressing these challenges, we formulated liposomes

with *C*-4-hydroxy-phenylcalix[4]pyrogallolarene in the present work.

Liposomes are lipid-based vesicles consisting of phospholipid bilayers that encapsulate an aqueous core, allowing for the simultaneous delivery of both hydrophilic and lipophilic drugs.¹⁰⁻¹¹ The structural versatility of liposomes is one of their most significant advantages. They can be engineered to vary in size, lamellar structure, and lipid composition, which influences their pharmacokinetic properties and drug release profiles.¹²⁻¹⁴ This adaptability allows for the optimization of liposomal formulations to achieve desired therapeutic outcomes.^{11,15} This is particularly relevant for calixpyrogallolarene, which may face solubility challenges in conventional formulations. By utilizing liposomes as a delivery vehicle, the bioavailability of *C*-4-hydroxy-phenylcalix[4]pyrogallolarene can be significantly enhanced. The ability to tailor the liposomal formulation to the specific physicochemical properties of *C*-4-hydroxy-phenylcalix[4]pyrogallolarene can lead to improved therapeutic outcomes as a tyrosinase inhibitor.

In this research, we employed L- α -phosphatidylcholine as lipid-based. This lipid-based agent serves as a highly effective liposome carrier due to its unique physicochemical properties and biocompatibility, and it can be tailored for specific applications. It can enhance the stability and integrity of the liposomal bilayer, maximizing therapeutic efficacy and minimizing the risk of adverse reactions when used in drug delivery applications.¹⁶⁻¹⁸ These advantages are important for maintaining the therapeutic payload during storage and transit through the biological environment. The application of liposomes formulated with L- α -phosphatidylcholine has been successfully used to improve the topical delivery of ascorbic acid, enhancing its penetration into the skin and thereby maximizing its antioxidant effects.¹⁹ The spherical *C*-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes are observed, and they exhibit multilamellar vesicle (MLV) characteristics. In this study, the effects of hydration temperature and L- α -phosphatidylcholine weight on the size and polydispersity index (PDI) were examined using Design Expert-13 with ANOVA statistical methods. The influence of each factor and their interaction on the

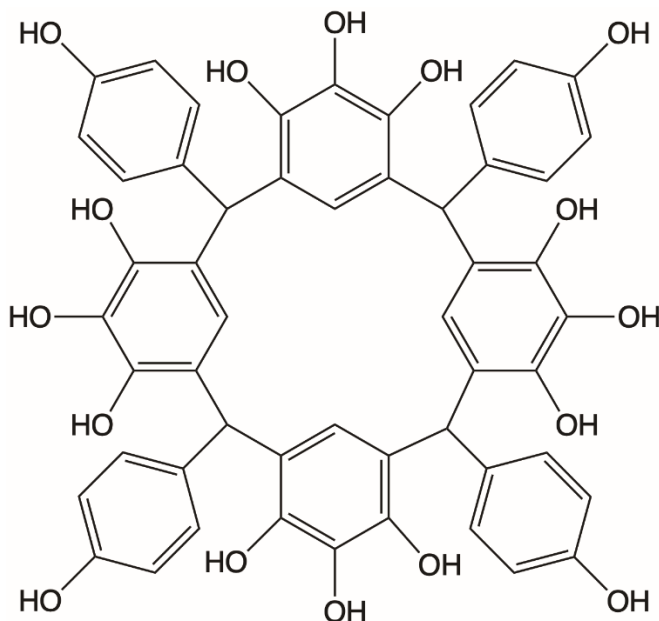


Figure 1. Structure of *C*-4-hydroxy-phenylcalix[4]pyrogallolarene

size and PDI of the *C*-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes was discussed.

2. Material and Methods

2.1. Material

The L- α -phosphatidylcholine (soy lecithin) (Merck), chloroform (Merck), *C*-4-hydroxy-fenilcalix[4]pyrogallolarene (provided by Department of Chemistry, Universitas Gadjah Mada), and deionized water generated by a Thermo reverse osmosis water purification system with a resistance of 18.2 M Ω .cm were employed in this experiment.

2.2. Methods

2.2.1. Liposome Preparation Procedure

Liposome preparation was conducted using the

thin layer lipid film hydration method.²⁰ A two-factor, two-level factorial design was employed to investigate the influence of L- α -phosphatidylcholine weight (0.5 and 1.5 g) and hydration temperature (60 and 80°C) on liposome characteristics. The experimental design was generated using Design Expert-13 software trial version (Stat-Ease, MN, USA), resulting in four experimental runs.

Liposomes were prepared by dissolving L- α -phosphatidylcholine in chloroform in a round-bottom flask, with concentrations of either 0.5 or 1.5 g/50 mL as per the experimental design. The organic solvents were removed using a rotary evaporator (Buchi Rotavapor R-300, Flawil, Switzerland) to form a thin lipid film, which was then dried in a desiccator overnight to remove any residual solvent. The lipid film was hydrated with deionized water, which contains 0.01 g *C*-4-hydroxy-phenylcalix[4]pyrogallolarene at either 60 or 80 °C according to the experimental design. The mixture was then stirred

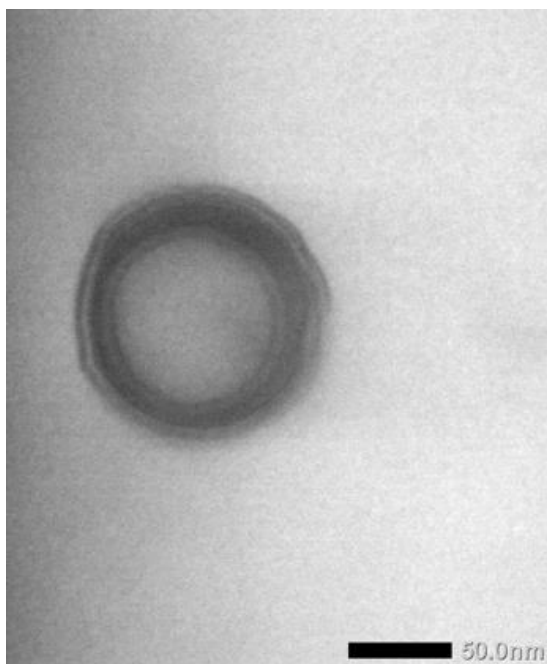


Figure 2. TEM micrograph of the C-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes

for 3 hours. The resulting dispersion was refrigerated for 24 hours. Morphological liposomes were analyzed using transmission electron microscopy (TEM). Characterization of the liposomes focused on size and polydispersity index (PDI) measurements, which were performed using scattering light intensity (Horiba Scientific SZ-100, Japan). The measurement was conducted at 25 °C, with a scattering angle of 90°. Data analysis was performed using Design Expert-13 software. The effects of soy lecithin concentration and hydration temperature on liposome size and PDI were evaluated using analysis of variance (ANOVA). Main influences and interaction plots were generated to visualize the impact of each factor on the responses. The statistical significance of the factors and their interaction was determined at a 95% confidence level ($p < 0.05$).

2.2.2. TEM Analysis

The morphological features of the liposomes were observed using TEM. For TEM sample preparation,

a drop of the liposome suspension was placed on a carbon-coated copper grid and allowed to adsorb for 5 minutes. Excess liquid was removed using filter paper. The sample was then negatively stained with 2% (w/v) uranyl acetate solution for 2 minutes. The excess stain was removed, and the grid was air-dried at room hydration temperature. TEM images were captured using a JEOL JEM-1400 operating at an acceleration voltage of 100 kV. Multiple fields were viewed, and representative images were captured to analyze the shape, lamellarity, and overall structure of the liposomes.

1.1.3. Particle Size Analysis

Liposome physical properties were characterized using a Horiba Scientific SZ-100, Japan. The PDI, zeta potential, and Z-average (intensity-weighted mean hydrodynamic diameter) were measured for each liposome formulation. Samples were diluted with filtered deionized water to achieve an optimal concentration for analysis. Measurements were performed

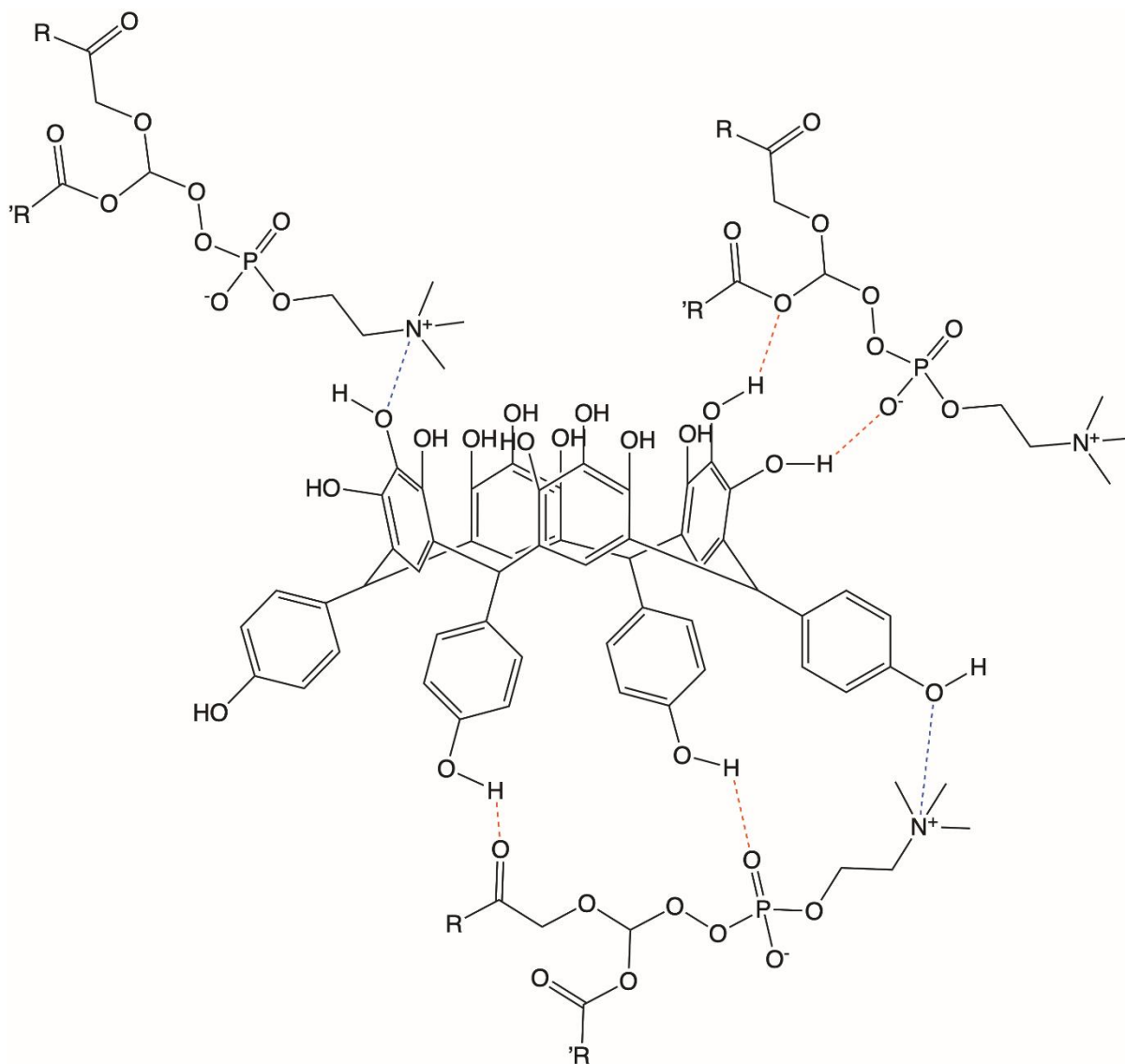


Figure 3. Hydrogen bonds and electrostatic interactions between C-4-hydroxyphenylcalix[4]pyrogallolarene and L- α -phosphatidylcholine. Hydrogen bond and electrostatic interaction are shown as red and blue dotted lines, respectively.

at 25 °C after a 2-minute equilibration period. The PDI, which indicates the width of the particle size distribution, was obtained through cumulant analysis of the dynamic light scattering data. Zeta potential was determined using laser Doppler micro-elec-

trophoresis, providing information on the surface charge and stability of the liposomes. The Z-average size was calculated from the intensity of scattered light, offering a measure of the overall mean diameter of the liposome population. All measurements

Table 1. ANOVA results for liposome size factorial model

Source	Sum of Squares	df	Mean Square
Model	14209.63	3	4736.54
A-Lipid Weight	1668.72	1	1668.72
B- Hydration Temperature	7577.70	1	7577.70
AB	4963.20	1	4963.20
Pure Error	0.0000	0	
Cor Total	14209.63	3	

were repeated on three independently prepared batches to ensure reproducibility.

3. Results and Discussion

The characterization of the *C*-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes was performed using TEM, as shown in Figure 2. The TEM analysis results indicated that the liposomes were spherical in shape and exhibited multilamellar vesicle (MLV) characteristics. Spherical liposomes demonstrated the lowest energy configuration, rendering them as the most stable form. This stability was particularly advantageous in the encapsulation of drugs, where achieving a minimum energy state was crucial for preserving liposome integrity during drug delivery.²¹ Moreover, the spherical geometry promotes uniform stress distribution across the liposome surface, minimizing the risk of rupture and ensuring controlled and consistent drug release.²² From the chemistry point of view, the spherical morphology of liposome could be due to hydrogen bonds and electrostatic interactions between *C*-4-hydroxy-phenylcalix[4]pyrogallolarene and L- α -phosphatidylcholine, as proposed in Figure 3.

MLVs offer several advantages that make them highly suitable for drug delivery applications. Their increased hydrophobic volume compared to unilamellar vesicles (ULVs) allows for the efficient encapsulation of hydrophobic drugs, potentially enhancing both delivery and therapeutic efficacy.²³ Additionally, the multilamellar structure imparts greater stiffness, which can improve cellular uptake by enhancing the

interaction between MLVs and cellular membranes. The stiffness increases with each additional lipid bilayer, further optimizing this interaction.²³ Moreover, MLVs demonstrated high encapsulation efficiency for a wide range of drug molecules, including small molecules, peptides, and proteins, within their internal aqueous compartments, facilitating sustained drug release, particularly in non-vascular administration routes.²⁴

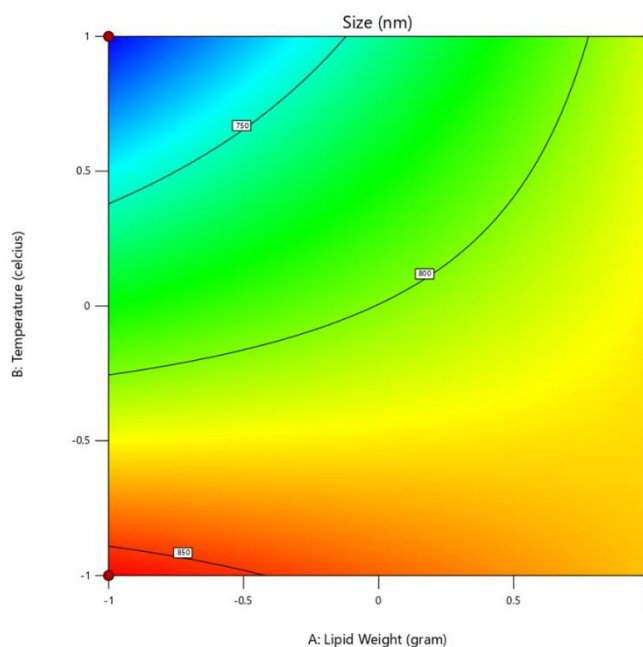
3.1. Liposome Size

We used a two-level factorial design to determine the influence of hydration temperature and lipid weight on the size and PDI of the *C*-4-hydroxy-phenylcalix[4]pyrogallolarene liposomes. The ANOVA results indicate that neither the model nor the interaction between lipid weight and hydration temperature significantly influenced the size of the produced liposomes ($p > 0.05$), as shown in Table 1. Despite the lack of statistical significance, the derived regression equation $Y = 800.28 + 20.42X_1 - 43.53X_2 + 35.23X_1X_2$ provides insight into potential trends affecting liposome size. In this equation, Y represents liposome size, X_1 denotes lipid weight, and X_2 signifies hydration temperature, while the term X_1X_2 captures the interaction between lipid weight and hydration temperature.

The equation suggests that lipid weight has a more substantial impact on increasing liposome size compared to hydration temperature. Specifically, lipid weight exhibits a positive correlation with liposome size, indicating that an increase in lipid weight tends

Table 2. ANOVA results for liposome PDI factorial model.

Source	Sum of Squares	df	Mean Square
Model	0.0144	3	0.0048
A-Lipid Weight	0.0006	1	0.0006
B-Hydration Temperature	0.0005	1	0.0005
AB	0.0132	1	0.0132
Pure Error	0.0000	0	
Cor Total	0.0144	3	

**Figure 4.** Contour plot of lipid weight and hydration temperature Influence on liposome size

to result in larger liposomes. Conversely, hydration temperature shows an inverse relationship, where higher hydration temperatures are associated with smaller liposome sizes. Additionally, the interaction term implies that the effect of hydration temperature on liposome size varies depending on the lipid weight. At lower lipid weights, an increase in hydration temperature leads to a reduction in liposome size. In contrast, at higher lipid weights, an increase in hydration temperature contributes to an increase in liposome size.

Figure 4 clearly demonstrates the influence of lipid weight and hydration temperature on the sizes of liposomes. With increasing lipid weight, the size of the liposome increases due to the larger sizes (red and orange regions) with higher lipid weights. An increase in hydration temperature results in a decrease in the size of the liposomes, as observed from the change in color from warm to cooler colors in the plot. This inverse relationship would imply that the lipid weight is conditioning liposome growth in a hydration temperature-dependent manner, which

is bound to reduce size, likely because of its effect on the formation of the lipid bilayer. In non-linear statistics, two variables interact non-additively if their combined action on the dependent variable is something more than an additive relationship. For instance, the size reduction is greatly enhanced by hydration temperature at lower lipid weights, while at higher lipid weights, the increase in hydration temperature has less effect on the resulting size. This would imply that there is a need to carefully optimize both lipid weight and hydration temperature for size control of liposomes in formulations.

Our findings showed a difference in liposome size between the light scattering assay (particle size analyzer) and TEM results. This is consistent with research conducted by Filipov et al., 2023,²⁵ which found that DLS typically reports larger sizes than TEM due to the inclusion of the hydration shell and potential particle agglomeration in solution. In contrast, TEM requires drying the sample, which can cause shrinkage or morphological changes, potentially leading to smaller size estimations.

1.2. PDI Results of Liposome

The analysis suggests that lipid weight and hydration temperature may have an influence on the PDI of the liposomes, as shown in Table 2. However, ANOVA results indicate that the effects of both variables, as well as their interaction, do not reach statistical significance ($p > 0.05$). Despite this, the fitted model, described by the equation $PDI = 0.4055 - 0.0125X_1 - 0.0115X_2 - 0.0575X_1X_2$, offers some insight into potential patterns. In this model, PDI represents the polydispersity index, X_1 corresponds to lipid weight, and X_2 represents hydration temperature, with X_1X_2 representing the interaction between these factors.

According to the model, lipid weight has a notable negative effect on PDI, suggesting that increasing lipid weight results in a reduction in PDI, which implies improving uniformity in liposome size distribution. Hydration temperature, in contrast, has a more modest impact, with higher hydration temperatures slightly decreasing PDI, pointing to a minor improvement in size distribution. The interaction between li-

pid weight and hydration temperature suggests that the combined increase of these two variables could further decrease PDI. Although the ANOVA results did not reach statistical significance, the model hints at a potential relationship between lipid weight, hydration temperature, size, and PDI.

The contour plot of the level of lipid weight (A) and hydration temperature (B) on the PDI of liposomes reflects the size distribution of liposomes, as shown in Figure 5. PDI values between 0.34 and 0.44 are obtained, through which the color gradient and contour lines represent an interaction of both factors in the size distribution of liposomes. The lipid weight drastically affects PDI, which generally goes up along with relatively higher lipid weights, corresponding to a broader size distribution. This is mainly observed in the red and orange areas, where lipid weight is highest, corresponding to approximately 1 g. On the other hand, hydration temperature has a subtle effect: lower hydration temperatures develop liposomes with higher PDI values. At the same time, PDI decreases as the hydration temperatures rise, which results in a more homogeneous size distribution of liposomes. The relationship between lipid weight and hydration temperature is non-linear; the lowest values for PDI are achieved for low weight of lipid and high hydration temperature, which means that this combination provides a better homogenizing effect on size. However, the high lipid weight and low hydration temperature cause high PDI values, which means the size of the liposome formed was found to be highly heterogeneous in these conditions. In other words, both lipid weight and hydration temperature were significant in their interaction effects on PDI, which defines the homogeneity of liposomal size.

3. Conclusions

This study emphasizes the influence of lipid weight and hydration temperature for stable C-4-hydroxy-phenylcalix[4]pyrogallolarene liposome size and PDI. Even though the effects were not statistically significant, lipid weight increased liposome size, and higher hydration temperatures reduced it. The interaction between these factors influenced both

size and PDI, with lower lipid weight and higher hydration temperatures resulting in more uniform liposomes. These findings offer insights for improving liposomal formulations, especially for compounds with solubility challenges, and suggest further investigation into other formulation factors.

4. Acknowledgements

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Study of the Leadership Potential of Pharmacy Students

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ABSTRACT

The article is devoted to studying the leadership potential indicators of pharmacy students as one of the key professional competencies. According to the results of psychological testing of respondents, the fundamental components of social and psychological factors that characterize their leadership potential have been analyzed, the subjective characteristics and style of their leadership were determined. It was found that the level of leadership abilities of the subjects directly affects the nature and degree of manifestation of indicators that characterize leadership. It was found that a high level of key characteristics of transformational and transactional leadership influence is most inherent in respondents with high and medium leadership abilities.

Introduction.

Professional activity with a social orientation referring to the type of "person-to-person" in psychology, requires a high professional level of the specialists, but also demands specific personal qualities, among which leadership skills are of major

importance.

An example of the above type of profession is the activity of modern pharmacy professionals who must feel like leaders and demonstrate leadership skills, which would allow them to serve as an example for other health care providers and effectively manage both themselves

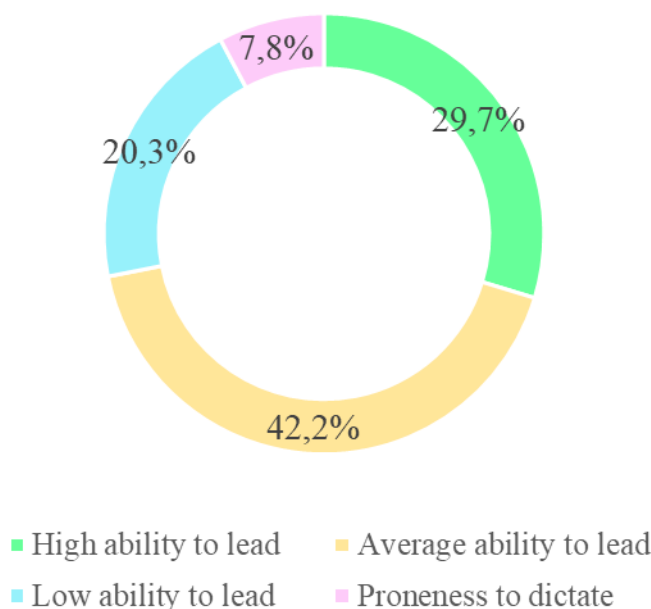


Figure 1. Indicators of respondents' ability to lead

and others. Leadership skills act as the essential competence in the activities of modern pharmacists, which contributes to the development of a high level of self-awareness, self-control, and social sensitivity in them and determines their ability to effectively manage interpersonal relations, which ultimately influences the effectiveness of the organization's activities^{1,2}.

The essence of modern leadership lies in the direct and natural ability of the leader to create a friendly atmosphere within the team, fruitful adjustment of the emotional part of the team functioning, as well as the ability to effectively manage the negative moods of the team members.

Thus, modern leadership is an important socio-personal competence, since it contains both a component of social influence and a personal resource that allows a person to take on the role of a leader.

Accordingly, the issue of leadership skills of modern pharmacy professionals is one of the priority areas of research, which will improve both the training quality of the professionals themselves and significantly increase the effectiveness of the aid provided

by them. The relevance of socio-psychological research on leadership issues is related to the insufficient level of development of this topic on one hand, and on the other hand to the increasing demands of practice for a thorough analysis of problems and prospects of this issue^{5,6}. At the same time, the problem of pharmacy specialists' leadership qualities has not been considered separately. The common goals and objectives of the medical and pharmaceutical communities increases the need to study leadership in the pharmaceutical industry, whose specialists, together with doctors, should now be ready to take on a leader's mission to ensure the well-being of a patient and society as a whole.

Leadership research is particularly urgent for future pharmaceutical professionals who are in the process of preparing to embark on professional duties, as the expression of their leadership qualities will influence the success of their future careers.

The purpose of the present study was to establish the leadership potential of pharmaceutical education applicants in Ukraine by establishing indicators of their leadership potential and subjective leadership characteristics.

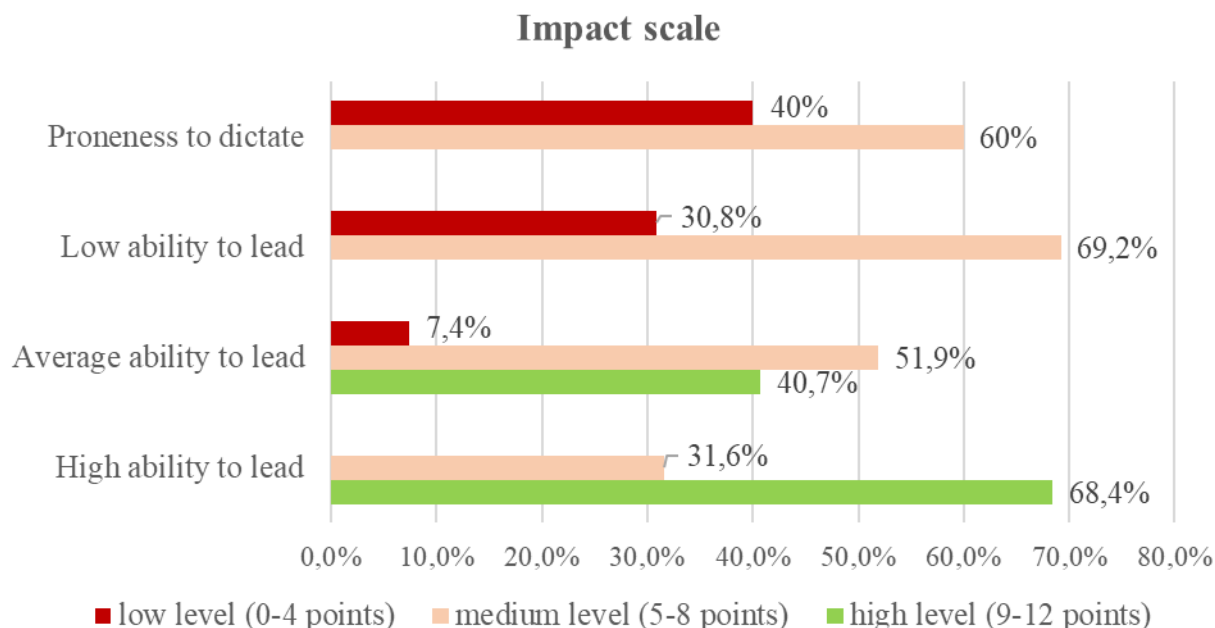


Figure 2. Ratings of respondents with different LA on the "Impact" scale

Materials and Methods.

The study performed testing of pharmacy students according to psychological methods that determine leadership abilities and subjective indicators of leadership. In the course of the study, analytical, structural and logical approaches, methods of statistical processing of the obtained data, structural indicators, grouping of the data obtained and comparison analysis were used.

Results and Discussion.

Based on generalized results of theoretical and empirical studies of outstanding domestic and foreign scientists devoted to the study of the leadership phenomenon, we outlined the key criteria for selecting the diagnostic tools for assessing the leadership qualities of the subjects, in particular:

- the suitability of psychological questionnaires for diagnosing the leadership potential of specialists in the "person-to-person" labor system, which directly includes future pharmaceutical specialists;

- compliance with the existing psychometric criteria and optimal parameters concerning the procedure for researching leadership potential and selecting relevant diagnostic tools;
- compliance with the basic principles and rules of psychological measurement and systematic interpretation of the data obtained;
- the possibility of algorithmization of methodological tools for further transfer to the automated survey and processing mode;
- promptness of the diagnostic procedure;
- the possibility of using both direct and remote psychological survey.

Based on the results of the analysis of literary sources devoted to the study of the problems of key aspects of leadership, we concluded that the manifestation of leadership qualities largely depends on the leadership abilities (LA), that a person develops from childhood in the case of effective socialization. That is why at the determinative stage of the experimental study, we used the Ie. Zharikov and Ie. Krushelnysky psychological method of interviewing respondents, enabling measuring and evaluating their leadership abilities⁷.

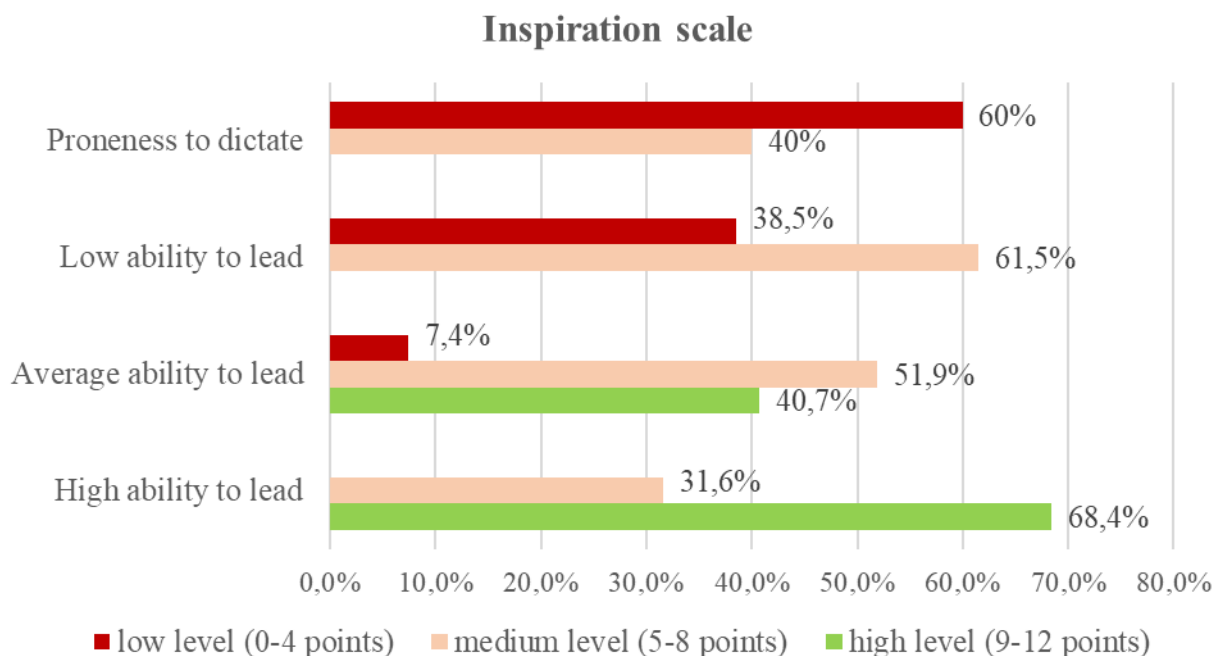


Figure 3. Ratings of respondents with different LA on the Inspiration scale

In the course of testing according to this psychological technique, respondents answered 50 statements of the questionnaire. The interpretation of the obtained values was carried out according to the “key” of the methodology.

According to the authors of the above methodology, the ability of a person to be a leader is largely due to the development of one’s organizational and communicative skills. Such signs are the following key subjective manifestations of a person: optimism; determination, that is the ability to make independent and timely decisions in critical situations; initiative; perseverance; ability to take reasonable risks; patience; readiness for long-term performance of daily and monotonous tasks; independence, which is manifested in the willingness and ability to work without excessive care; mental stability; high adaptability; ability to self-criticism, which allows assessing both own successes and failures adequately; pickiness not only to others but also to oneself; criticality, which enables to see weaknesses in attractive proposals; reliability; endurance, which allows efficient work even in conditions of overload; readiness

to solve complex problems by original and atypical methods; psychological flexibility, which determines the ability of a person to change the style of behavior depending on a particular situation.

In addition, we also identified the subjective characteristics and style of leadership (transformational and transactional)⁸ by testing respondents using the multidisciplinary leadership questionnaire by B. Bass and B. Avolio.

According to the authors of this psychological technique, leadership is an important socio-personal competence of a person, which determines the effectiveness of its implementation both in professional and public life, since it contains in its structure both a component of social influence and a personal resource that allows a person to take a leadership role.

In the course of the survey according to this methodology, respondents were asked 21 questions of this psychological questionnaire with the ability to choose the highest priority answer out of five proposed. The test results were evaluated according to the proposed key of the methodology, which allowed obtaining indicators of leadership manifestations of

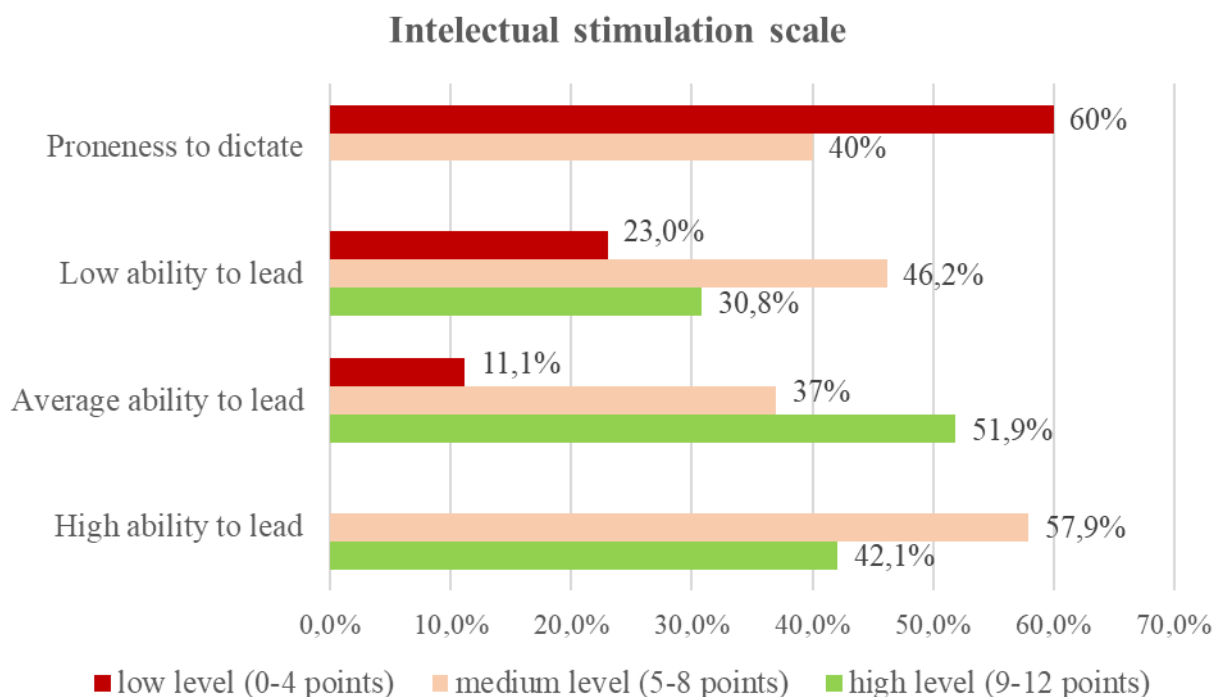


Figure 4. Ratings of respondents with different LA on the Intellectual Stimulation scale

the subjects on the following scales: impact; ability to inspire; intellectual stimulation; individual approach; motivation; management; and granting independence.

It should be noted that this psychological questionnaire contains statements that belong to both the transformational and transactional characteristics of a leader's personality. At the same time, transformational leadership not only can influence the behavior of the leader himself but also can change the behavior of his followers by transforming their values, thoughts, and thinking, which in general leads to an understanding of the overall results and contributes to the formation of partnerships built on unconditional respect and authority⁹.

Thus, according to the transformational aspect of influence, the leader initiates a certain transformation that occurs both in the organization and in the personality structure of its individual representative. According to this questionnaire, such transformational qualities are a set of characteristics and concepts that are characterized by such method

scales as: "Influence", "Ability to inspire", "Intellectual stimulation" and "Individual approach".

The study of future pharmacy professionals' transactional leadership influence was carried out using the obtained values according to such scales of the methodology as "Motivation", "Management" and "Granting independence".

The survey involved 64 graduate students of the National University of Pharmacy (Kharkiv, Ukraine), most of whom were women (53 people – 82.8%), the remaining 11 were men (17.2%), the average age of participants in this group was 22 years.

The representativeness of our sample is confirmed by the Student's t-distribution. Considering that the total number of applicants for higher pharmaceutical education of graduate courses at the National University of Pharmacy is 120 people, respectively, the calculated level of significance " α " was- 0.05, which corresponds to the reliability level of 95%.

Test results according to Ie. Zharikov-Ye. Krushelnytskyi method allowed establishing that only 19 respondents (29.7%) have a high propensity for

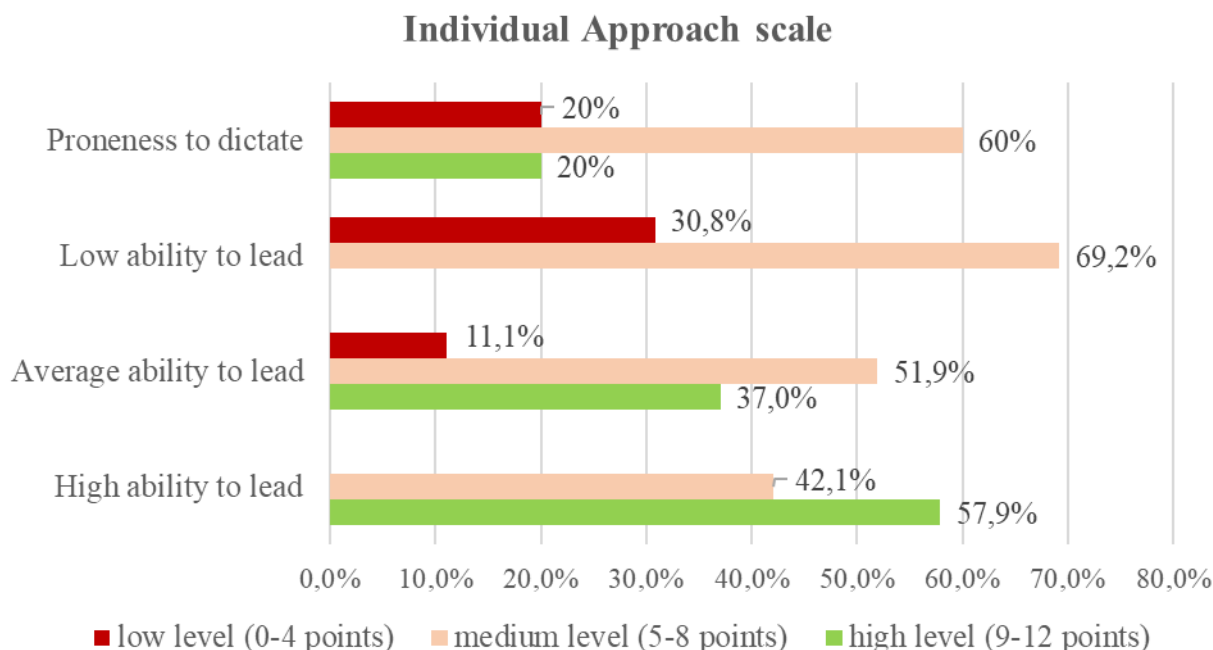


Figure 5. Ratings of respondents with different LA on the scale "Individual approach"

leadership. Such future specialists can adequately analyze both their behavior and actions, and assess the actions of others. In the general context, this allows them developing an appropriate purposeful, and constructive model of their behavior, which facilitates the effective coordination of the directions and actions of others.

The average level of LA is inherent in 27 respondents (42.2%). Typically, the characteristic features of the leadership of such respondents are such key features as: sufficient mental resistance to adverse life situations, constructive self-criticism, fair personal autonomy and reliability. At the same time, it should be noted that the above indicators may change in some way in case of less favorable situations.

13 respondents (20.3%) have a low level of LA. As a rule, such respondents are characterized by difficult adaptation to new living and working conditions and a low level of self-criticism. In addition, they are characterized by a quick rejection of a certain goal in the event of significant obstacles in its implementation, as well as insufficient willpower. Instead, 5 applicants (7.8%) have a tendency to dictate, which

indicates ineffective ways of building constructive relationships in interpersonal communications.

The results of the above-mentioned psychological testing are graphically presented in Figure 1.

Thus, this psychological testing allowed us to analyze the subjects' susceptibility to leadership behavior and the degree of expression of this indicator.

The results of further testing of respondents on the B. Bass-B. Avolio multidisciplinary leadership questionnaire made it possible to determine the subjective signs of leadership of respondents with various levels of leadership abilities and their leadership style.

The results obtained showed that only respondents with a high level of LA – 13 people (68.4%) and average values of LA– 11 respondents (40.7%) have high values on the Influence scale. On the part of others, these respondents are able to build trust in themselves, which contributes to the formation of high authority and further helps them to convey their ideas and be an appropriate example of a person to whom they listen and look up.

Average values on the scale are characteristic of

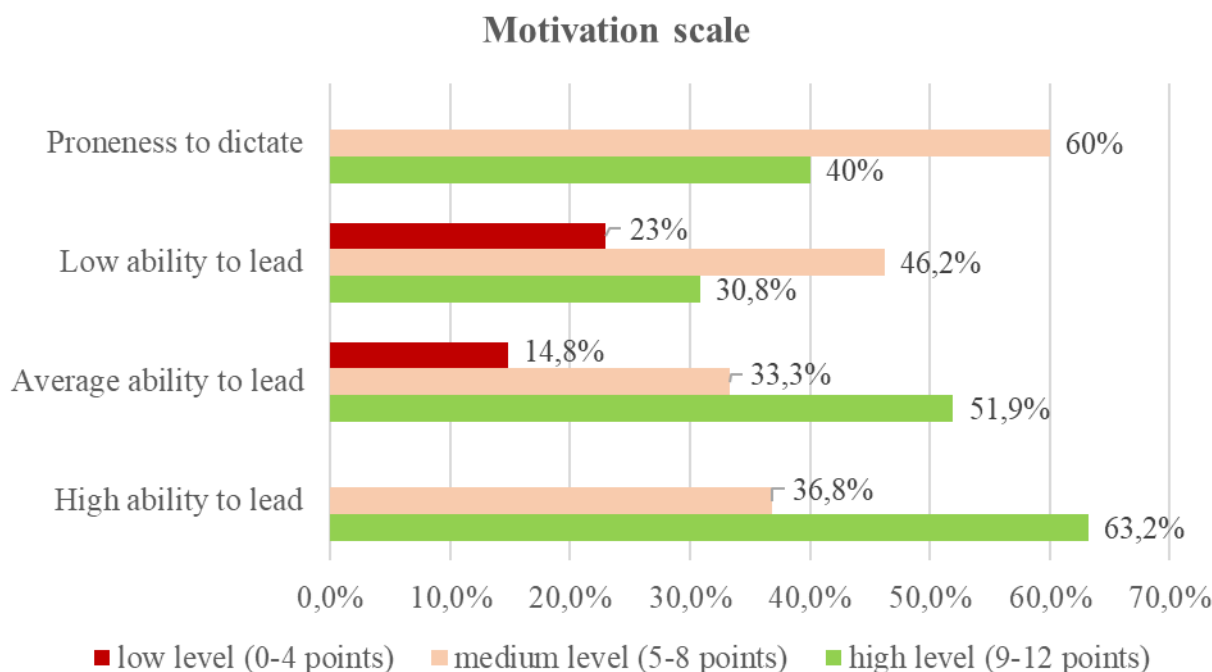


Figure 6. Ratings of respondents with different LA on the Motivation scale

more than a third of respondents with high LA – 6 respondents (31.6%), 14 respondents with medium LA (51.9%), 9 respondents with low LA (69.2%), as well as 3 respondents who are somewhat prone to dictate (60%).

Low values on the “Impact” scale among the subjects are inherent in the following groups of subjects: 2 respondents with an average level of LA (7.4%), 4 subjects with low levels of LA (30.8%), as well as 2 subjects who are prone to dictation (40%) – Figure 2.

Indicators on the “Inspiration” scale allow us to assess the ability of respondents to broadcast relevant symbols and images of stimulation to others to help the latter independently take appropriate steps to overcome obstacles to achieving the goal.

The analysis of the obtained indicators on this scale found that the majority of respondents with a high level of LA have a greater ability to inspire – 13 respondents (68.4%). In addition, 11 respondents with medium LA (40.7%) have high scores on the Inspiration scale. At the same time, there were no high values on this scale among respondents with low LA

and respondents prone to dictatorial manifestations.

The low level of the ability to inspire is more characteristic of the respondents with a tendency to dictate – 3 respondents (60%), more than a third of the respondents with low LA– 5 respondents (38.5%) and 2 respondents with average leadership abilities. All other subjects have average values on this scale (Figure 3).

Values on the Intellectual Stimulation scale allow highlighting the ability of respondents to encourage others to use a creative approach when achieving the goal. A person with high scores on this scale is able to create the most optimal collective environment that will allow its members to show their best abilities and carry out effective self-realization. In the vast majority, this ability is mainly inherent in the subjects with high and medium LA values – 8 respondents (42.1%) and 14 respondents (51.9%), respectively. Also, 4 respondents (30.8%) with low LA (33.3%) have somewhat high values on this scale.

Low values on this scale are characteristic of 3 subjects with average LA indicators (11.1%), 3 respondents with an average LA (23%), as well as 3

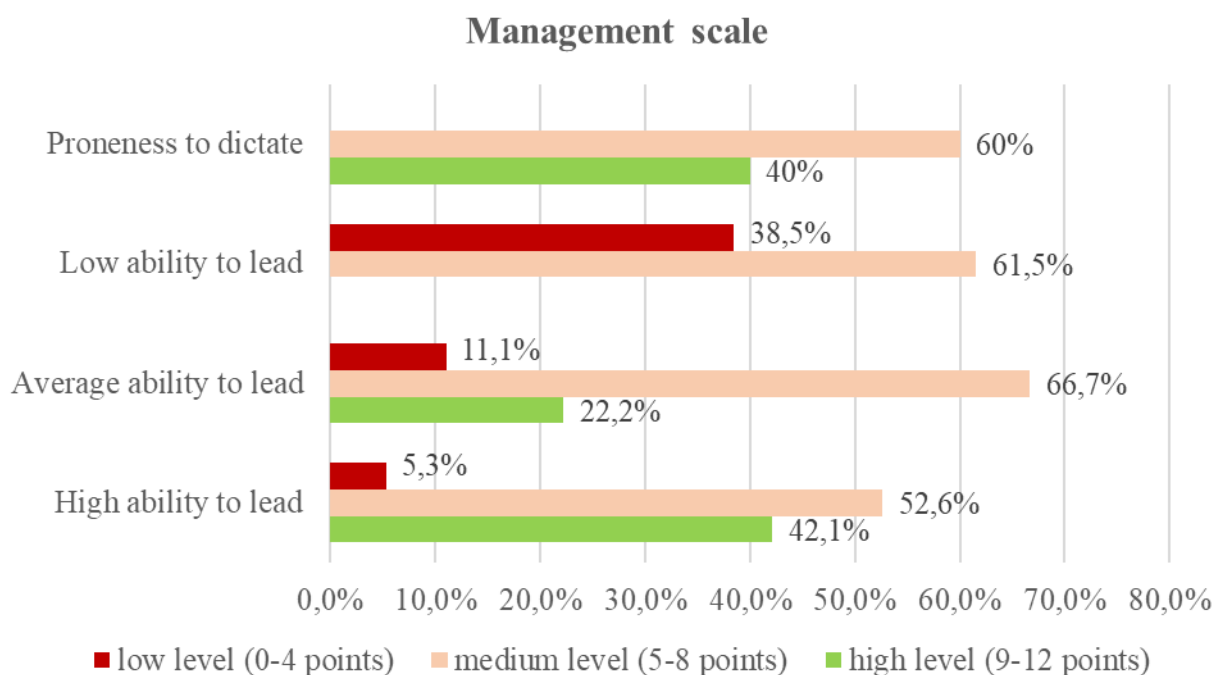


Figure 7. Ratings of respondents with different LA on the "Management" scale

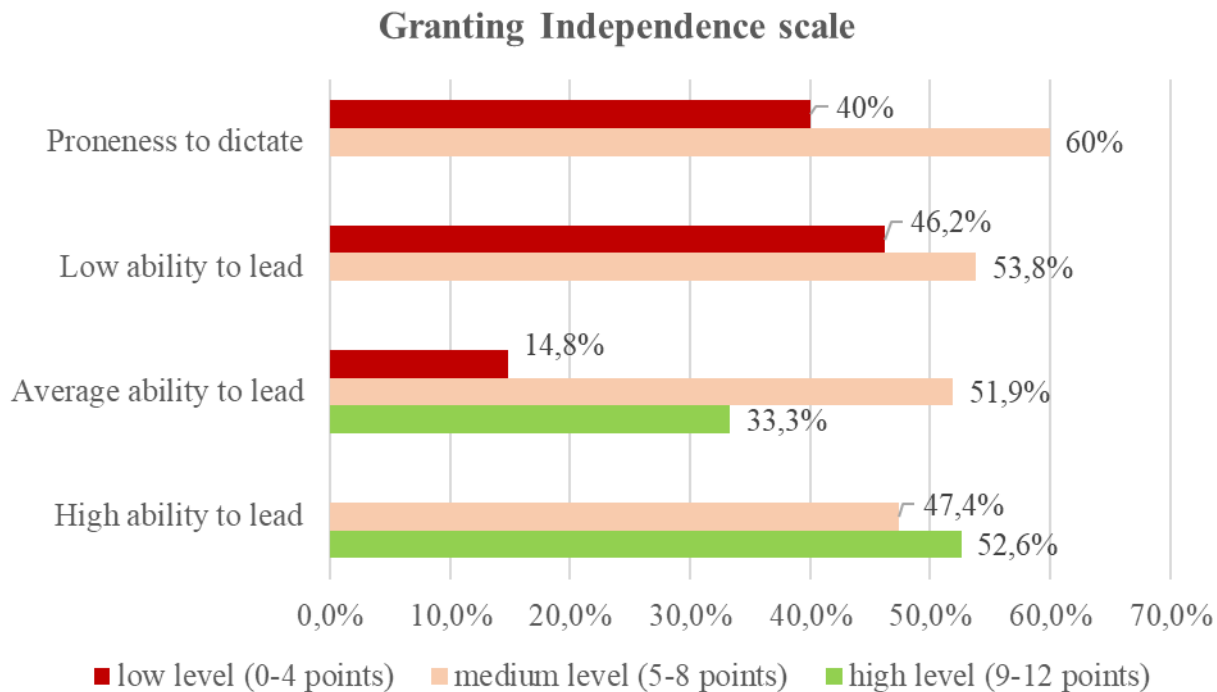


Figure 8. Ratings of respondents with different leadership abilities on the scale "Granting independence"

subjects with dictatorial manifestations (60%).

All other respondents have average scores on this scale (Figure 4).

The results on the Individual Approach scale highlight the ability of respondents to broadcast a subjective approach to others. To a greater extent, this ability is inherent in respondents with high and medium values of leadership potential – 11 applicants with high LA (57.9%) and 10 with medium LA (37%).

Low scores on this scale are inherent to some extent to respondents with average values of LA (11.1%) – 3 representatives and low LA – 4 subjects (30.8%), as well as 1 respondent (20%) with dictatorial directions of his leadership.

Other representatives of all study groups have average values for an individual approach to others (Figure 5).

Transactional leadership influence is based on the use of a number of rewards and punishments for others.

Thus, it was found that high scores on the “Motivation” scale are characteristic of all respondents, without exception, to varying degrees, namely 12 respondents (63.2%) with high LA, 14 respondents (51.9%) with medium LA, almost a third of the respondents with low LA indices – 4 respondents (30.8%) and 40% of the respondents with a tendency to dictate – 2 respondents. They are able to clearly outline the tasks set and specify the expected results.

A small number of respondents with medium and low values of LA have low ratings on the Motivation scale – 4 and 3 respondents, respectively (14.8% and 23%).

All other respondents with different LA values have average values on this scale (Figure 6).

The values obtained on the “Management” scale allow us to assess the degree of influence of the respondents’ personalities concerning their management of processes in the course of achieving a goal. It was found that this ability is more characteristic of the subjects with high rates of LA– 8 applicants (42.1%), 6 respondents with medium LA (22.2% of the total number of such subjects), as well as 2 respondents (40%) who are prone to dictation.

A small number of respondents is characterized by an insufficient degree of control of processes and phenomena along the way – to a greater extent these are respondents with low LA, namely, 5 such respondents (38.5%), 3 respondents with medium LA (11.1%), as well as 1 respondent with high LA (5.3% of the total number of such specialists). At the same time, the average values on the scale are inherent in the majority of subjects - Figure 7.

Ultimately, the Granting Independence scores measure the ability of respondents to effectively organize teamwork to achieve a jointly defined goal.

Thus, the results obtained on the scale indicate that, first of all, future specialists with high and medium LA indices can do this – 10 and 9 respondents, respectively (52.6% and 33.3%).

At the same time, low capacity for independence and delegation of authority is inherent in 4 respondents with medium LA (14.8%), almost half of the group of respondents with low levels of LA – 6 representatives (46.2%), as well as 2 respondents with dictate abilities (40% of such representatives).

All other respondents are characterized by average values on the scale (Figure 8).

The above leadership qualities require high professional and emotional competence of specialists, which helps them manage themselves, provide emotional support to others, and improves the quality of interpersonal communications in general.

Thus, the results of the above psychological testing allow us to demonstrate the direct impact of the respondents’ leadership abilities on the nature and degree of manifestation of indicators that generally characterize leadership. At the same time, respondents with high leadership abilities have a significantly higher capacity for both transformational and transactional leadership.

Conclusions.

According to the results of psychological testing, the level of leadership abilities of applicants for pharmaceutical education was established. It was found that high indicators that characterize transformational and transactional leadership influence are

more characteristic of respondents with high and medium leadership abilities.

It was found that most respondents with medium and low leadership abilities are highly desirable to improve their leadership competencies and characteristics.

Respondents whose leadership style tends to dictatorship should rethink and transform their leader-

ship potential, which, undoubtedly, is very high, but at the same time, not always effective in achieving their goals. Such respondents need to learn to trust others more and give them more independence. They should also understand that only teamwork, grounded on mutual respect, trust and partnership, is the key to the rapid and constructive achievement of the goals.

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Investigation of the Correlation between the Antioxidant, Antimicrobial Activity and the Content of Phenolic Compounds of St. John's Wort (*Hypericum perforatum* L.) Liquid Extracts

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ABSTRACT

Infection diseases is a worldwide important problem for medicine and pharmacy. The purpose of work was study the total content of some biologically active substances (BAS), determine antimicrobial, anti-fungi and antioxidant activity of obtained St. John's Wort extracts, and study a correlation analysis between the content of natural compounds and antimicrobial/antifungal and antioxidant activity. Results demonstrate the highest amount of polyphenols, flavonoids, anthraquinone derivatives and organic acids were 1.37 ± 0.02 , 0.66 ± 0.01 , 0.05 ± 0.005 and $0.66 \pm 0.005\%$ in 60% EtOH extract, respectively. The hydroxycinnamic acids was dominated in 40% extract (0.72 ± 0.01). The most potent antioxidant property possessed 60% EtOH extract of John's Wort herb. The high correlation was found between the total polyphenols, flavonoids and antioxidant/antimicrobial/antifungal effects against all Gram-positive, Gram-negative bacterial strains and the fungus *C. albicans*. These findings have showed the great potential of St. John's wort in the development and creation of new medicines with antimicrobial, antioxidant and antifungal effects that are not inferior to, and even superior to, the effects of synthetic analogues.

1. Introduction

Nowadays, the problem of bacterial infection is still relevant. According to recent statistical studies, it has been found that every year 13.7 million people per year die from bacterial infections in the world. The mortality rate for all ages was 99.6 deaths per 100.000 population. Of the pathogens studied, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* accounted for 54.9% of the 7.7 million deaths, with *S. aureus* being associated with more than 1.1 million deaths. *S. aureus* was the leading bacterial cause of death in 135 countries and was associated with the largest number of deaths among people over 15 years of age (940.000)¹. In addition, this problem is compounded by the emergence of resistance in bacteria to widely used antibiotics, which makes treatment more complex, time-consuming and expensive². Thus, the search for new antimicrobial natural compounds is perspective for today.

Thousands of natural compounds were shown antimicrobial effects against Gram-negative, Gram-positive strains³. Natural compounds have a number of advantages over synthetic compounds: high efficiency, minimal side effects and low cost of the production method. Also, natural compounds, especially derivatives of phenolic compounds, have a high level of antioxidant effect, which is quite important in infectious diseases⁴.

The *Hypericum Taurin* ex L. is a genus with 508 species worldwide. The most widespread species is *Hypericum perforatum* L. that belong to *Hypericaceae* family. *H. perforatum* is an herbaceous perennial plants that origin to Europe, Asia and Africa⁵. *H. perforatum* contains derivatives of anthraquinone, flavonoids, prenylated phloroglucinols, hydroxycinnamic acids, volatile compounds and organic acids⁶. The main constituents of *H. perforatum* represented by hyperforin (2-4,3%), hypericin (0.1-0.15%), hyperoside (0.4-0.8%), rutin (0.8-1.6%) and catechins (0.5-0.9%)⁷. Due to rich chemical composition the *H. perforatum* herb is applied in folk medicine for centuries. The *H. perforatum* herb has a wide range of application in medicine: inflammation of bronchs,

stomach ulcers, diabetes mellitus, wound healing, colds, obesity and depression⁸.

There are a lot of scientific researches about determination a level antioxidant activity of *H. perforatum* herb extracts^{9, 10}. However, there is no date about assessing antioxidant\antimicrobial\anti-fungi activity and its correlation with content of BAS by potentiometric method. So, the aim of the study was to determine the total content of polyphenols, flavonoids, hydroxycinnamic and organic acids, anthraquinone derivatives, moreover study antimicrobial and anti-fungi activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and fungi *Candida albicans*. In addition, it was aimed to study a correlation analysis between the content of BAS in *H. perforatum* herb extracts and antimicrobial/antifungal and antioxidant activity.

2. Materials and methods

2.1 Plant material

H. perforatum herb was the object of the study, which were collected in the places of its cultivation. The material was collected in 2022 during the flowering period in the vicinity of the village of Ternova, Kharkiv region.

2.2 Equipment

The pH meter HANNA 2550 (Germany) with a combined platinum electrode EZDO 50 PO (Taiwan) was applied for potentiometric measurements. Quantitative analysis of biological active compounds was carried out on UV-spectrophotometer UV – 1000 (China) with matched 1 cm quartz cells. Weighing was carried out using digital analytical balance AN100 (AXIS, Poland) with d = 0.0001 g.

2.3 Extraction procedure

A six samples of 10.0 g (exact mass) of St. John's Wort herb had the size of particles 1-2 mm. The extraction was conducted with distilled water, 20%,

40%, 60%, 96% EtOH at 80° C within 1 hour with a condenser, ratio raw material/solvent – 1/20. The extraction technique was completed twice to provide totally extract all BAS, then the filtrates were joint and evaporated by vacuum rotary to ratio of extract to raw material 1:2. The six extracts of 96, 60, 40, 20% EtOH and aqueous were obtained. The green tea (*Camellia sinensis* L.) extract was obtained by the mentioned above method with 60% EtOH.

2.4 Quantitative analysis

The total content of phenolic compounds was measured by the Folin-Ciocaltau assay, the absorbance was measured at 760 nm. The calibration curve ($Y = 0.1055X + 0.1745$, $R^2 = 0.9951$). was plotted with interval concentrations 1.0 – 5.0 µg/ml, the calibration equation

The total phenolic compounds content was expressed as gallic acid according to equation 1.¹¹.

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V} \quad (\text{Eq.1})$$

where, C_x – concentration of gallic acid according to the calibration curve, $C \times 10^{-6}$, g/ml; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The sum of anthraquinone derivatives was determined by a molecular absorption analysis, the absorbance was measured at 591 nm. The total anthraquinone derivatives content was expressed as hypericin in extract according to equation 2.¹².

$$X(\%) = \frac{C_x \times K_{dil}}{718 \times V} \quad (2)$$

where, A – absorbance of analyzed solution; 718 – specific adsorption coefficient of hypericin; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The total flavonoids were determined using assay of complex formation with AlCl_3 , the absorbance was measured at 417 nm. The total flavonoids content was expressed as rutin [13] calculated according to equation 3.

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{A_s \times V} \quad (\text{Eq.3})$$

where, A – absorbance of analyzed solution; A_{st} – absorbance of standard solution of rutin; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The total hydroxycinnamic acids derivatives content was measured by assay of complex formation with NaNO_2 - Na_2MoO_4 , the absorbance was measured at 525 nm¹⁴. The total content of hydroxycinnamic acids derivatives was expressed as chlorogenic acid, was calculated according to equation (4)

$$X(\%) = \frac{C_x \times K_{dil}}{188 \times V} \quad (\text{Eq.4})$$

where, A – absorbance of analyzed solution; 188 – specific adsorption coefficient of chlorogenic acid; V – volume of extract, ml; K_{dil} – coefficient of dilution.

The total organic acids content was determined by acid-base titration with the fixation end-point by potentiometric method. The total content of organic acids was expressed as citric acid^{15, 16}, according to equation 5.

$$X(\%) = \frac{(V_{equiv} - V_x) \times 0.0032 \times K_{dil} \times K \times 100}{V} \quad (\text{Eq.5})$$

where, 0.0032 – the amount of citric acid, which is equivalent to 1 ml of sodium hydroxide solution (0.05 mol/L), g; V_{equiv} is the volume of sodium hydroxide solution (0.05 mol/L), which was used for titration, ml; V_x – the volume of sodium hydroxide solution (0.05 mol/L), which was spent for titration in a blank experiment, ml; V – volume of extract, ml; K_{dil} – coefficient of dilution; K is correction coefficient for 0.05 mol/L sodium hydroxide solution.

2.5 Antioxidant activity assay

Antioxidant activity of extract was evaluated by potentiometric method^{17,18}. It was calculated according to equation 6. and expressed as mmol-equiv./m_{dry res.}:

$$AOA = \frac{C_{\alpha} - \alpha \times C_{red}}{1 + \alpha} \times K_{dil} \times 10^{-3} \times \frac{m_1}{m_2} \quad (\text{Eq.6})$$

where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E_{ethanol})nF/2.3RT}$, C_{ox} – con-

centration of $K_3[Fe(CN)_6]$, mol/L; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/L; $E_{ethanol} = 0.0546 \cdot C_{\%} - 0.0091$; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; $F = 96485.33$ C/mol – Faraday constant; $n = 1$ – number of electrons in electrode reaction; $R = 8.314$ J/molK – universal gas constant; $T = 298$ K; K_{dil} – coefficient of dilution; m_1 – mass of dry residue; m_2 – mass of dry residue in 1.0 mL of extract.

The standardized green tea leaf 60% extract was used as the reference drug.

2.6 Test organisms

Museum strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Proteus vulgaris* NTCS 4636, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885/653 were used in accordance with the recommendations for the assessment of antimicrobial activity of drugs.

2.7 Antimicrobial activity assay

In our study, we used 1% solution of extract, the solvent of which were 60% ethanol. The method of diffusion of the drug into agar carried out using the method of “wells”¹⁹. Gentamycin, and fluconazole were used as reference drugs for assessing antimicrobial and anti-fungal activity.

The standardized green tea leaf 60% EtOH extract was used as the reference drug.

2.6 Test organisms

Museum strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Proteus vulgaris* NTCS 4636, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 885/653 were used in accordance with the recommendations for the assessment of antimicrobial activity of drugs.

2.7 Antimicrobial activity assay

The method of diffusion of the drug into agar car-

ried out using the method of “wells”¹⁹. Preparation of microorganisms suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to scale of McFarland) with using of equipment of Densi-La-Meter (Czech, wavelength 540 nm). Suspensions were prepared according to equipment and information list. Colony forming unit was 107 microorganisms at 1 ml of growth medium and determined by standard of McFarland). On solidified agar, using a pipette under sterile conditions in Petri dishes made 1 ml of a suspension of microorganisms. After uniform distribution of microorganisms over the entire surface of the agar, the plates were incubated at room temperature for 15-20 minutes. Next, wells with a diameter of 6 mm were made in the cups, into which solutions of the test substances were introduced. The samples incubated at 37° C for 16-24 hours. After incubation, the plates were placed upside down on a dark matte surface so that light fell on them at an angle of 45° (accounting in reflected light). The diameter of the growth retardation zones measured using a caliper. Gentamycin, and fluconazole were used as reference drugs for assessing antimicrobial and anti-fungal activity.

Table 1. Interpretation criteria for microbial sensitivity

Microbial sensitivity	Diameter of the growth retention zone, mm
High sensitivity	>25
Sensitive	15-25
Low sensitivity	10-15
Not sensitivity	<10

2.7 Correlation analysis

Pearson's (r) correlation coefficient was used to analyze the correlation between antioxidant activity (AOA) and the amount of phenolic, catechin, flavonoid, hydroxycinnamic acids derivatives and organic acids. The correlation coefficient takes a value in the range of -1 to +1. Correlation is very high if it is within the range from 0.90 to 1.00; from 0.70 to 0.90

is a high correlation; from 0.50 to 0.70 is a moderate correlation; from 0.30 to 0.50 is a low correlation; from 0.00 to 0.30 negligible correlation¹³

2.8 Statistical analysis

For all the experiments, two samples were analyzed and all the assays were carried out in 5 times. The results were expressed as mean values with confident interval. The MS EXCEL 7.0 and STATISTIKA 6.0 were used to provide statistical analysis.

3. Results

3.1 Qualitative analysis of BAS

According to obtained results shown in Table 1, the 60% EtOH extract ($1.37 \pm 0.02\%$) had the most significant amount of polyphenols, followed by 96% EtOH extract ($1.10 \pm 0.02\%$), whereas the lowest one – aqueous extract ($0.40 \pm 0.02\%$).

Table 2 demonstrates that the most significant content of flavonoids was found in 60% EtOH extract ($0.66 \pm 0.01\%$), whereas in the aqueous extract ($0.02 \pm 0.002\%$) was the lowest one. The percentage of flavonoids out of total of polyphenols was 54, 48, 17, 6 and 5% for 96%, 60%, 40%, 20% EtOH and aqueous extracts, respectively. The highest percentage of flavonoids was in 96% EtOH extract, whereas the lowest in aqueous extract.

The content of hydroxycinnamic acids increasing in the following order aqueous extract ($0.15 \pm 0.005\%$) > 20% EtOH extract ($0.26 \pm 0.005\%$) > 96% EtOH extract ($0.34 \pm 0.01\%$) > 60% EtOH extract ($0.54 \pm 0.01\%$) > 40% EtOH extract ($0.72 \pm 0.01\%$). The percentage of hydroxycinnamic acids out of total of polyphenols was 31, 39, 71, 49 and 38% for 96%, 60%, 40%, 20% EtOH and aqueous extracts, respectively. The highest percentage of hydroxycinnamic acids was in 40% extract, whereas the lowest in 96% EtOH extract. (Table 2)

The content of anthraquinone derivatives was found only in 96 and 60% extracts. The total content of anthraquinone derivatives of 96% extract was 40% lower than 60% extract. The percentage

of anthraquinone derivatives was 2 and 4% out of polyphenols for 96 and 60% extract, respectively. (Table 2)

The highest amount of organic acids was determined in 60% EtOH extract ($0.66 \pm 0.005\%$), followed by 96% EtOH extract ($0.39 \pm 0.005\%$), whereas the lowest one in 40% extract ($0.28 \pm 0.005\%$). The total content of organic acids was lower 65%, 52%, 71%, 34% than content polyphenols in 96%, 60%, 40%, 20% extracts, respectively. (Table 2)

3.2 Antioxidant activity

A potentiometric method for determining antioxidant activity was used to evaluate the effect of the obtained extracts of *H. perforatum* herb. Table 3 shows that the level of antioxidant activity increases in the following order: 20% EtOH extract ($50.00 \pm 1.00 \text{ mmol-equiv./m}_{\text{dry res.}}$) > aqueous extract ($55.20 \pm 1.10 \text{ mmol-equiv./m}_{\text{dry res.}}$) > 40% EtOH extract ($59.59 \pm 1.19 \text{ mmol-equiv./m}_{\text{dry res.}}$) > 96% EtOH extract ($61.68 \pm 1.23 \text{ mmol-equiv./m}_{\text{dry res.}}$) > 60% EtOH extract ($70.71 \pm 1.41 \text{ mmol-equiv./m}_{\text{dry res.}}$). In light of the data obtained, it can be established that the 60% EtOH extract has the highest level of antioxidant activity. In light of the data obtained, it can be established that the 60% extract has the highest level of antioxidant activity. According to the modern classification of antioxidant activity, which was previously developed by us²⁰, it was found that all extracts obtained have a high level of antioxidant activity. Moreover, a comparative analysis of the “strength” of antioxidant activity was carried out with the gold standard 60% EtOH extract of *C. sinensis* leaf. The *C. sinensis* leaf extract was obtained by the same technological method as *H. perforatum* herb extract. The obtained extracts were significantly inferior in antioxidant effect to *C. sinensis* leaf extract. Further, a 0.06 mol/L solutions (in terms of the amount of polyphenols expressed as gallic acid) of extracts of *H. perforatum* herb and *C. sinensis* leaf were prepared. As a result of the study, it was found that when compared at the same concentrations, the aqueous extract had the highest antioxidant effect, and the least - 60% EtOH extract. (Table 4)

Table 2. The sum of phenolic compounds, flavonoids, anthraquinone, hydroxycinnamic acids and organic acids in *H. perforatum* herb liquid extracts

Sample	Total phenolic compounds, % ± SD	Total anthraquinone, % ± SD	Total flavonoid, % ± SD	Total hydroxycinnamic acids, % ± SD	Total of organic acids, % ± SD
96% EtOH extract	1.10 ± 0.02	0.02 ± 0.005	0.59 ± 0.01	0.34 ± 0.01	0.39 ± 0.01
60% EtOH extract	1.37 ± 0.02	0.05 ± 0.005	0.66 ± 0.01	0.54 ± 0.01	0.66 ± 0.01
40% EtOH extract	0.98 ± 0.02	—	0.17 ± 0.001	0.72 ± 0.01	0.28 ± 0.01
20% EtOH extract	0.53 ± 0.02	—	0.03 ± 0.002	0.26 ± 0.01	0.35 ± 0.01
aqueous extract	0.40 ± 0.02	—	0.02 ± 0.002	0.15 ± 0.01	0.47 ± 0.01

Table 3. The level of antioxidant activity of *H. perforatum* herb liquid extracts

Sample	Antioxidant activity, mmol-equiv./m _{dry res.} ± SD	Conditional term of antioxidant level
96% EtOH extract	61.68±1.23	High level
60% EtOH extract	70.71±1.41	High level
40% EtOH extract	59.59±1.19	High level
20% EtOH extract	50.00±1.00	High level
aqueous extract	55.20±1.10	High level
<i>C. sinensis</i> leaf extract	548.79 ± 10.98	Very high level

**Note: SD – standard deviation, n=3, p<0.05*

Table 4. Comparing the value of antioxidant activity of *H. perforatum* herb liquid extracts with *C. sinensis* leaf 60% extract at the concentration 0.06 mol/L expressed in the total phenolic compounds

Sample	Concentration, mol/L	Antioxidant activity, mmol-equiv./m _{dry res.} ± SD
96% EtOH extract	0.06 ^a	56.07±1.12
60% EtOH extract		51.61±1.03
40% EtOH extract		60.00±1.20
20% EtOH extract		100.00±1.02
aqueous extract		138.00±2.76
<i>C. sinensis</i> leaf extract		54.36±1.09
Epigallocatechin-3-O-gallate		61.20 ± 1.22

**Note: SD – standard deviation, n=3, p<0.05, a – molar concentration of H. perforatum herb liquid extracts and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid*

3.3 Antimicrobial activity

In this research work, the antimicrobial activity of the obtained *H. perforatum* herb extracts was investigated against the following strains of *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, as well as a strain of the fungus *C. albicans*. According to the obtained results, all extracts obtained from the *H. perforatum* herb had an effective antimicrobial effect. (Table 5)

S. aureus was most sensitive to the 60% EtOH extract (23.0 ± 0.3 mm) and least sensitive to the aqueous extract (15.0 ± 0.6 mm). When comparing the results of the gentamicin standard and the 60% EtOH extract, it was found that the 60% EtOH extract was 10% better at inhibiting the growth of the *S. aureus* strain of bacteria. According to the results presented in Table 4, it was found that *B. subtilis*, as well as *S. aureus*, was highly sensitive to the 60% EtOH extract (23.0 ± 0.3 mm), followed by 96% EtOH extract (22.0 ± 0.4 mm), and the aqueous extract inhibited the growth of the bacterial strain the least (17.0 ± 0.4 mm). The most resistant strains of bacteria to the action of *H. perforatum* herb extracts was *P. vulgaris*. A 60% EtOH extract of *H. perforatum* herb inhibited the growth of *P. vulgaris* by 20% better than the reference standard gentamicin. *E. coli* and *P. aeruginosa* were most sensitive to the action of 60% extract, in second place – 96% EtOH extract; the investigated bacterial strains were the most resistant to an aqueous extract. (Table 5)

When studying antifungal activity against *C. albicans*, the results showed that 96 and 60% EtOH extracts of *H. perforatum* herb most actively inhibited the growth of the fungus, and the fungus was the least sensitive to the action of 20% EtOH and an aqueous extract. When compared with the fluconazole standard, it was found that the 96 and 60% EtOH extracts inhibited fungal growth 10% better than fluconazole. (Table 5)

3.4 Correlation results

The dependence of antioxidant, antimicrobial and antifungal activity on the content of different groups

of BAS was studied using the method of linear regression. Table 6 shows that the correlation between the antioxidant effect and the content of polyphenols was very high, in the case of flavonoids it was high, in the case of hydroxycinnamic acids it was moderate, and the lowest correlation value was observed for organic acids.

According to the research results presented in Table 6 it was found that there is a high correlation between phenolic compounds and inhibition of the growth of *S. aureus*, in the case of flavonoids, hydroxycinnamic acids and antioxidant activity - moderate, and in the case of organic acids - there is no correlation.

The antibacterial effect against *B. subtilis* is very highly dependent on the content of polyphenols and flavonoids, in turn, the antioxidant effect and the content of hydroxycinnamic acids have a moderate effect, and in the case of organic acids there is no dependence.

The study showed that there is a significant correlation between phenolic compounds and inhibition of *E. coli* growth, while in the case of flavonoids, hydroxycinnamic acids and antioxidant activity there is a moderate correlation, and in turn, organic acids do not affect the growth inhibition of *E. coli*. (Table 6)

When studying the relationship between inhibition of growth of *P. vulgaris* and the content of different groups of BAS, it was found that there is a very high dependence of antimicrobial activity on the amount of polyphenols, in turn, with the amount of flavonoids and hydroxycinnamic acids there was a high correlation, and in the case of organic acids - a correlation was not found. The correlation between antimicrobial and antioxidant effects was also studied, and according to the results, it was found that this relationship is of a moderate level. (Table 6)

The correlation between the growth inhibition of *P. aeruginosa* and the sum of polyphenols and flavonoids is high, with the sum of hydroxycinnamic acids and antioxidant activity it is moderate, and in the case of organic acids it is absent. (Table 6)

A significant high correlation between inhibition of the growth of *C. albicans* and the content of polyphenols and flavonoids, in turn, with the sum

Table 5. The value of antimicrobial and anti-fungi activity of *H. perforatum* herb liquid extracts

Sample	Concentration, mM	Diameter of the growth retardation zone, mm ± SD					
		Gramm-positive		Gramm-negative			Fungi
		<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>P. vulgaris</i> ATCC 4636	<i>Paeruginosa</i> ATCC 27853	<i>C.albicans</i> ATCC 653/885
96% EtOH extract	0.018 ^a	22.0 ±0.3	22.0 ±0.4	19.0 ±0.4	16.0 ±0.5	19.0 ±0.4	18.0 ±0.4
60% EtOH extract	0.024 ^a	23.0 ±0.3	23.0 ±0.3	20.0 ±0.4	17.0 ±0.4	20.0 ±0.4	18.0 ±0.4
40% EtOH extract	0.018 ^a	21.0 ±0.3	21.0 ±0.3	19.0 ±0.3	16.0 ±0.5	18.0 ±0.4	17.0 ±0.4
20% EtOH extract	0.009 ^a	20.0 ±0.4	20.0 ±0.4	18.0 ±0.3	15.0 ±0.6	18.0 ±0.3	16.0 ±0.5
aqueous extract	0.006 ^a	15.0 ±0.6	17.0 ±0.4	14.0 ±0.6	12.0 ±0.6	15.0 ±0.5	15.0 ±0.5
Gentamycin	0.003	22.0 ± 0.3	24.0 ± 0.2	25.3 ± 0.3	25.0 ± 0.2	25.6 ± 0.6	12.0 ± 0.4
Fluconazole	0.003	18.0 ± 0.4	12.0 ± 0.6	14.3 ± 0.3	12.3 ± 0.3	10.0 ± 0.5	20.0 ± 0.4
96% EtOH		16.0 ± 0.4	16.0 ± 0.4	13.0 ± 0.5	13.0 ± 0.5	12.0 ± 0.5	12.0 ± 0.5

**Note: SD – standard deviation, n=3, a – molar concentration of extracts was calculated as total phenolic compounds expressed as gallic acid*

of hydroxycinnamic acids and antioxidant activity there is a moderate dependence, and in the case of organic compounds there was no correlation at all. (Table 6)

4. Discussion

4.1 Qualitative analysis

Tuhujac M. *et al.*²¹ reported about aqueous, 70% and 96% EtOH extracts of *H. perforatum* herb. According to their results, the content of total polyphenols and flavonoids in aqueous extract was 0.43 and 0.09%, in the 70% extract 0.63 and 0.13%, whereas in the 96% EtOH extract 0.50 and 0.11%, respectively. Compared with our research the highest content of flavonoids and polyphenols was found in 60%

EtOH extract. The extraction of BAS directly depends on the solvent polarity and chemical properties of compounds. In *H. perforatum* herb present flavonoid glycosides (rutin, hyperoside etc.), according to their chemical property they less soluble in polar solvent (water) and better in medium polar solvent. In arecent study Silva B.A. *et al.*²² investigated the content of hydroxycinnamic acids and anthraquinone derivatives in the methanolic extract of *H. perforatum* herb. The result of research showed that the total of hydroxycinnamic acids was 0.80%, whereas the total anthraquinone content was 0,04%, while we obtained 0.72% of hydroxycinnamic acids in the 40% extract, and 0.05% of anthraquinone in the 60% EtOH extract. The difference in the content of phenolic compounds, in our opinion, is associated with different brewing times, leaves/extractant ratio

Table 6. Correlation coefficients (R – Pearson’s coefficient) between antioxidant/antimicrobial activity and total phenolic compounds, flavonoids, hydroxycinnamic acids, organic acids content

	Total phenolic compounds content	Total flavonoids content	Total hydroxycinnamic acids content	Total organic acids content	Antioxidant activity
Antioxidant activity	0.9110	0.8816	0.5495	0.6799	—
Antibacterial activity against <i>S. aureus</i>	0.8780	0.7620	0.6580	0.1432	0.6192
Antibacterial activity against <i>B. subtilis</i>	0.9317	0.8395	0.6501	0.2534	0.7178
Antibacterial activity against <i>E. coli</i>	0.8506	0.6901	0.7205	0.0806	0.5775
Antibacterial activity against <i>P. vulgaris</i>	0.8838	0.7262	0.7347	0.1338	0.6329
Antibacterial activity against <i>P. aeruginosa</i>	0.8742	0.8002	0.5689	0.2750	0.6481
Antifungal activity against <i>C. albicans</i>	0.9580	0.9148	0.6075	0.7793	0.2500

used, species, climate, and geographical position.

4.2 Antioxidant activity

The potentiometric assay was chosen for evaluation antioxidant activity for several reasons, as it is highly sensitive, cheap and moreover accurate and precise. To compare the antioxidant effect, we used the obtained green tea leaf extract. The results showed that green tea extract inactivates free radicals significantly better than *H. perforatum* herb extracts. The 60% EtOH extract was found to have the highest level of antioxidant activity than other *H. perforatum* extracts. After this, we decided to compare the antioxidant effect of extracts at the same concentration of phenolic compounds, as a result, it was shown that green tea extract works at the same

level as 60% EtOH extract of *H. perforatum* herb. In addition, it was found that the order of levels of antioxidant activity of the extracts changed dramatically. At different concentrations of phenolic compounds, the 60% EtOH extract had the highest level of antioxidant activity, and when compared at the same concentration, the aqueous extract was the best.

Antimicrobial activity

The studied *H. perforatum* herb extract showed antimicrobial and antifungal activity against the strains of *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *B. subtilis* and *C. albicans*. According to the obtained data, at first glance it can be considered that the antimicrobial and antifungal activity of *H. perforatum* herb

extracts is significantly inferior to the action of gentamicin and fluconazole, because their concentration of solutions was significantly lower than the content of polyphenols in the extract. However, we would like to note that gentamicin has serious toxicity to the auditory nerve, kidneys and liver, which can lead to serious complications of the disease²³. Comparing the antifungal effects of fluconazole and *H. perforatum* herb extract, it was found that they inhibited the growth of the fungal strain at the same level, while the concentration of fluconazole was also lower, like gentamicin. We can declare that fluconazole is a leader as anti-fungi medicine, but at the same time it weakly inhibits the growth of gram-negative and gram-positive bacteria, but to *H. perforatum* herb extracts both strains of bacteria and fungus are sensitive. Thus, *H. perforatum* herb extracts is a combined pharmaceutical that affects different mechanisms of vital activity of bacteria and fungi, thereby having a wide spectrum of action against different strains of bacteria and fungi, and at the same time not possessing serious toxicity.

4.3 Correlation analysis

Audrone *et al.* reported about to study the correlation between antioxidant and antimicrobial activity and the content of polyphenols and flavonoids in extracts of raspberry shoots, leaves, seeds and fruits. It was found that there was a significant correlation between the content of BAS and the antioxidant effect, but in the case of antimicrobial activity there was no

correlation ($R = 0.3$). According to our data, it was shown that there is a high correlation between the sum of polyphenols, flavonoids and antioxidant/antimicrobial/antifungal effects against all Gram-positive, Gram-negative bacterial strains and the fungus *C. albicans*. The lowest correlations were observed in the case of organic acids. Therefore, polyphenols and flavonoids play a major role in antioxidant, antimicrobial and antifungal activities.

5. Conclusions

In the research, it has been determined the content of BAS, antioxidant, antimicrobial and antifungal activity of the obtained extracts of St. John's wort. The dominant content of the sum of polyphenols, flavonoids, anthraquinones and organic acids was observed. The 60% extract has a high level of antioxidant activity, and actively inhibits the growth of all studied Gram-positive, Gram-negative strains of bacteria and the fungus *C. albicans* in the range from 15 to 23 mm (diameter of growth inhibition). We have shown that there is a high correlation between the content of polyphenols, flavonoids and antioxidant\antimicrobial\antifungal action, in the case of hydroxycinnamic acids it is moderate, and in turn, for organic acids there is no correlation at all. These findings show the great potential of St. John's wort in the development and creation of new medicines with antimicrobial, antioxidant and antifungal effects that are not inferior to, and even superior to, the effects of synthetic analogues.

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