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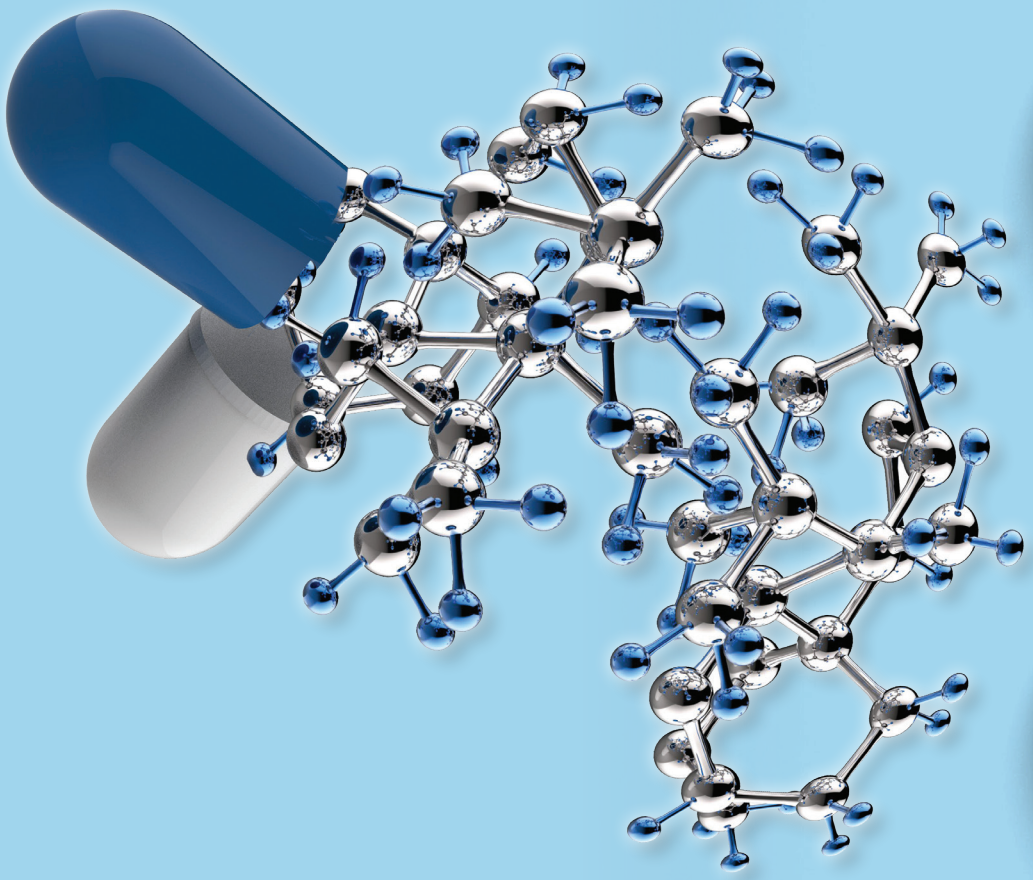

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 34, I, 2022 | 2-16 ΑΡΘΡΟ ΕΠΙΣΚΟΠΗΣΗΣ

PHARMAKEFTIKI, 34, I, 2022 | 2-16 REVIEW ARTICLE

Christian Orthodox Fasting: Potential Beneficial Effects for Human Health

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ABSTRACT

Introduction: Christian Orthodox fasting has been studied in relation to its positive health impact. The present review aims to critically summarise the current data concerning the potential beneficial effects of the dietary pattern of Christian Orthodox fasting on human health.
Methods: PubMed database was searched, in order to identify the relevant observational and clinical studies that explore the effect of Christian Orthodox fasting on health related outcomes.
Results: Positive effects concerning glucose and lipid control have been observed, whereas there is inconclusive evidence concerning blood pressure. Concerning weight control, fasters do have lower body mass, and lower caloric intake during the fasting periods. During fasting, the diet is higher in fruits and vegetables, iron and folate. However, Calcium and vitamin B2 intakes are lower, and hypovitaminosis D has been noted in monks. Additionally, monks do present better quality of life and mental health.
Conclusion: Overall, the Christian Orthodox fasting pattern may be beneficial for human health and chronic disease prevention. However, further studies are recommended on the impact of long-term religious fasting on HDL cholesterol levels, and blood pressure.

1. Introduction

Orthodox fasting takes place for a total of 180-200 days each year, as imposed by the Christian Orthodox religion. There are three main fasting periods, with different foods being prohibited during each fasting period. During the 40-day Nativity fast, fasters avoid dairy, eggs, and meat. Also, fasters abstain from fish and olive oil on Wednesdays and Fridays. During Lent, which lasts for 48 days, fasters abstain from dairy products, eggs, and meat. Additionally, fasters also abstain from olive oil on weekdays and from fish every day, except for March 25th and Palm Sunday. During the 15-day Assumption fasting fasters abstain from dairy products, eggs, and meat. Also, fasters abstain from olive oil on weekdays and from fish every day except for August 6th. Furthermore, every Wednesday and Friday fasters abstains from cheese, eggs, fish, meat, milk, and olive oil. The week following Christmas, Easter, and the Pentecost fasting prohibitions are not taking place[1, 2].

However, Orthodox Christians that do fast are like-

ly to follow various different fasting practices and fast for different periods, as well[3]. Different styles of fasting have been investigated, regarding their impact on human health. Caloric restriction, alternate day fasting, and dietary restriction, as is the Christian Orthodox fast, being the most common ones. Positive health effects have been described on chronic disease on humans and animal models when fasting[1, 2]. The Christian Orthodox fasting has been compared to the Mediterranean Diet[4], as a form of periodic vegetarianism[5]. The Mediterranean Diet, the best-studied dietary pattern in relation to cardiometabolic health exerts great health benefits.

In view of the above considerations, the present review aims to critically collect and in-depth summarise the current clinical data concerning the potential beneficial effects of Greek Orthodox Christian fasting on human health. For this purpose, PubMed database was comprehensively searched by the use of relative keywords, in order to identify the relevant observational and clinical studies.

2. Results

2.1 Cardiometabolic health status

Five studies have currently investigated the impact of Christian Orthodox fasting on glycaemia (**Table 1**). The recent cross-sectional study by Karras et al.[6] examined the effects of Christian Orthodox fasting on cardiometabolic biomarkers in 50 Athonian monks (with mean age=38.7±10.6 years). Both lipid and glucose indices, as well as homeostasis model assessment of insulin resistance (HOMA-IR) were found to be within the normal range[6]. Karras et al.[7] in a following published paper also compared the diet of 43 males form the general population that regularly fast (20-45 y of age) and 57 age-matched Athonian monks. Monks had better insulin sensitivity, as assessed by HOMA-IR[7], compared to the general public. During the Christmas fasting Sarri et al.[5] did find a significant (p 0,005) decrease on glucose levels after the fasting period. However, no changes on fasting glucose have been described in a study by Sarri et al.[8] during one-year period nor by Basilakis et al.[9] during a 40-day period. Hence, fasting might ameliorate glucose control, yet further studies are needed to draw firm conclusions.

Four studies have currently investigated the role of Christian Orthodox fasting on blood lipid control (**Table 1**). More to the point, Papadaki et al.[10] undertook a study in 10 Greek Orthodox Christian monks aged 25-65 years, with BMI > 30 kg/m2, that lived in two monasteries in Crete Island, during a fasting week, and compared them to their normal diet. Measurements were performed during Palm Sunday week (fasting) and the week following Pentecost Sunday (non-fasting). Interestingly, the blood lipid profile was better during the fasting week. Specifically, total and LDL cholesterol levels were significantly higher at the end of the non-fasting week, and a non-significant increase in HDL cholesterol was observed. During the fasting week the ratio of total to HDL cholesterol was lower, but serum triglycerides were higher. Fasting may have significantly contributed to the observed favourable biomarker profiles among this group who fast for 24.4 (±10.4) years[10]. In addition to this, considering the impact of Christian Orthodox Christian fasting on serum lipoproteins, Sarri et al.[8] found that at the end of the fasting period fasters had 12.5% lower total cholesterol and 15.9% lower LDL cholesterol than non-fasters. The LDL/HDL ratio was lower in fasters, but the change in HDL cholesterol in fasters was not statistically significant. Similar results were found when the pre- and after-fasting values of fasters were compared. However, no change was noted in non-fasters[8]. Moreover, Basilakis et al.[9], in their 40-day (fasting and non-fasting days) study on 36 nuns and monks, found a decrease in dietary and plasma cholesterol, an increase in triglycerides and a moderate drop in LDL/HDL during fasting[9]. A recent study in 60 healthy overweight Greek adults was conducted to compare the effects of Greek Orthodox fasting and time-restricted fasting on blood lipids. Energy restricted Orthodox fasting for 7 weeks resulted in significant reductions on total HDL and LDL cholesterol. Total cholesterol and HDL cholesterol were further reduced during Orthodox fasting than time-restricted fasting, despite similar anthropometric measurements[11]. Positive effects on total and LDL cholesterol

have been noted, yet the impact on HDL cholesterol is not yet clear. Subjects were not on lipid control medicines, hence studies on fasters under lipid lowering drugs may have dissimilar results, while the effect of fasting on blood lipids is not evident 6 weeks after the end of the 6 week fasting period (Lent)[12].

Two studies have investigated the effect of fasting on blood pressure (**Table 1**). Sarri et al.[13] investigated changes on blood pressure indices in fasters. Blood pressure changes of 38 devout Orthodox Christian fasters and 29 matched controls living in Crete Island for one year were investigated. Data were gathered before and at the end of the three major fasting periods of the Orthodox Christian calendar (Christmas, Easter & Assumption). Throughout the study fasters had higher mean systolic and diastolic blood pressure than controls. Non fasting period had a significant blood pressure lowering effect on fasters. Towards the end of fasting periods, fasters' prevalence of Christmas and Lent "high-normal" blood pressure was higher than that of the controls, while it was reduced during the Assumption and reached the very low levels of controls. Blood lipids were significantly associated with SBP/DBP at most measurements. In the study by Papadaki et al.[10] systolic, but not diastolic blood pressure was significantly higher while fasting. Greek Orthodox religious fasting diet did not seem to exert an observable effect on blood pressure.

2.2. Weight control

The impact of fasting on body mass and weight control has been investigated in four studies (**Table 2**). A recent study by Karras et al.[7] with 43 males form the general population that regularly fast (20-45 y of age) and 57 age-matched Athonian monks found that monks had lower BMI, and lower body fat mass[7]. Moreover, Basilakis et al.[9] undertook a 40-day (fasting and non-fasting days) prospective study on 36 nuns and monks, and found that fasting led to a decrease of weight, upper arm circumference and triceps skinfold thickness[9]. However, a non-statistically significant decrease on monks' body mass was observed by Papadaki et al.[10], while Sarri et al.[8] found that fasters had 1.5% lower BMI than non-fasters at the end of the fasting period, while a 1.4% decline of BMI was also observed in fasters after the fasting period[8]. Hence, Christian Orthodox fasting may benefit weight control.

2.3 Nutrient intakes and sufficiency status

Diet quality, as well as macronutrient and micronutrient intakes during Orthodox fasting is an important issue to consider, especially when taking into account the fact that fasting takes place 180-200 days per year. Several studies have investigated the intakes and the nutrient status of fasters (**Table 3**).

During fasting, caloric intake decreases. In this aspect, Basilakis et al. observed a decrease in caloric intake during fasting by 20%[9], while Sarri et al.[4] found that fasters presented a decrease of 180 kcal energy intake, while there was an increase of 137 kcal in the controls during fasting period. Karras et al.[6] found that Athonian monks had low energy intake during both restrictive and non-restrictive fasting days, while carbohydrate and saturated fat intakes were lower, and protein was higher during the "restrictive days"[6]. In another study by Karras et al.[7], 43 males form the general population that regularly fast (20-45 y of age) and 57 age-matched Athonian monks were enrolled. Monks had lower daily total caloric intake for both "restrictive" and "non-restrictive days" than the general public.

Considering other macronutrients' intake during fasting, monks and fasters had lower intakes of total and saturated and trans fats[4, 10], and higher intakes of dietary fibre[4, 10, 13] and lower protein intake[4].

Consumption of legumes and fish/seafood are increased during fasting, and consumption of dairy products, meat and eggs are increased significantly after the fasting week. Considering dietary components, Sarri et al.[13] found that compared to controls, fasters increased fruit and vegetable consumption during the fasting periods and decreased their sodium intake.

Considering micronutrient intakes, Sarri et al.[13] observed an increase of magnesium intake during fasting. Papadaki et al.[10] and Sarri et al.[4] have also observed higher folate intakes during fasting. As far as iron status is concerned, Sarri et al.[14] undertook

Table 1. Studies concerning the impact of Orthodox fasting on cardiometabolic factors

Cardiometabolic factor	Study population	Study period	Main Results	Author, Date	Ref number
Glucose Control	Cross-sectional study in 50 Athonian monks (mean age=38.7±10.6 years)	Restrictive and Non-Non-Restrictive fasting days	Glucose indices and HOMA-IR in normal levels	Karras et al., 2017	6
	43 males form the general population that regularly fast (20-45 y of age) and 57 age-matched Athonian monks	-	Monks had better HOMA-IR	Karras et al., 2018	7
	37strict fasters (18 males, 19 females, mean age 43.0 + or - 13.1 years), vs 48 age-and sex-matched controls (21 males, 27 females; mean age 38.6 + or - 9.6 years)	40 days	Significant decrease on glucose levels after the fasting period	Sarri et al. 2009	5
	120 Greek adults were followed longitudinally (60 fasters, 60 non-fasters)	1 year	No changes on fasting glucose on fasters	Sarri et al. 2003	8
	36 (25 women & 11 men) monks from 5 Greek monasteries	40 days	No changes on fasting glucose	Basilakis et al., 2002	9

Table 1. Studies concerning the impact of Orthodox fasting on cardiometabolic factors

(continued) **Blood Lipid Control**	10 Greek Orthodox Christian monks aged 25-65 years, with BMI > 30 kg/m^2	1 fasting week vs non-fasting week (measurements on Palm Sunday week (fasting) and the week following Pentecost Sunday (non-fasting)	End of the non-fasting week: ↑total and LDL cholesterol NS↑HDL cholesterol During the fasting week: ↓total:HDL cholesterol ↑serum triglycerides	Papadaki et al., 2008	10
	120 Greek adults were followed longitudinally (60 fasters, 60 non-fasters)	1 year	↓12.5% total cholesterol & ↓15.9% LDL cholesterol, than non-fasters. ↓LDL/HDL in fasters, NS change on HDL cholesterol in fasters Similar results were found when the pre- and after-fasting values of fasters were compared. No change was noted in non-fasters.	Sarri et al. 2003	8
	36 (25 women & 11 men) monks from 5 Greek monasteries	40 days	↓dietary and plasma cholesterol ↑triglycerides ↓ LDL/HDL during fasting	Basilakis et al., 2002	9
	37 overweight, but healthy adults followed a hypocaloric diet based on Orthodox Fasting 23 BMI matched healthy adults followed a hypocaloric, time restricted eating plan	7 weeks	Lower total cholesterol after Orthodox Fasting than time restricted fasting (178.40 ± 34.14 vs 197.09 ± 29.61 mg/dl, p 0.028) Lower HDL cholesterol after Orthodox Fasting than time restricted fasting (51.01 ± 11.66 vs 60.13 ± 15.93 mg/ dl, p 0.013)	Karras et al., 2021	11

Table 1. Studies concerning the impact of Orthodox fasting on cardiometabolic factors

(continued)	29 overweight, but healthy adults followed a hypocaloric diet based on Orthodox Fasting 16 age- and weight-matched healthy adults followed a hypocaloric, time restricted eating plan	7 weeks and 6 weeks follow up	Total cholesterol, HDL and LDL cholesterol were reduced at 7 weeks and increased at 6 weeks after Orthodox fasting cessation	Karras et al., 2021	12
Blood Pressure Control	38 devout Orthodox Christian fasters and 29 matched controls living in Crete	1 year (measurements before and at the end of the three major fasting periods)	Fasters had ↑mean SBP and SBP than controls. ↓BP during the non fasting period Blood lipids were significantly associated with SBP/DBP at most measurements.	Sarri et al., 2007	13
	10 Greek Orthodox Christian monks aged 25-65 years, with BMI > 30 kg/m^2	1 fasting week vs non-fasting week (measurements on Palm Sunday week (fasting) and the week following Pentecost Sunday (non-fasting)	↑SBP while fasting	Papadaki et al., 2008	10

a study with 35 Greek Orthodox Christian strict fasters (17 male & 18 female, with mean age 43.6+/-13.2 years) and 24 controls (11 male &13 female, with mean age 39.8+/-7.6 years) whose iron status was studied before and near the end of the Christmas fasting period (meat and dairy are prohibited). Fasters had marginally worse pre-fasting haematological indicators, yet values were well above the cut-off levels, suggesting that long-term religious fasting did not negatively affect iron status. Notably, during the fasting period, the changes in iron status measurements were more beneficial for fasters than for non-fasters. In particular, fasters increased their ferritin levels and decreased their total iron-binding capacity, especially female fasters. No one presented iron deficiency at the end of the fasting period. At the end of the fasting, dietary iron intake was significantly higher in fasters compared to non-fasters. Hence, fasting did not impact on iron status and was not associated with a significantly greater degree of iron deficiency in fasters with normal iron status[14]. This result was in accordance with the observed increase on iron intake by Papadaki et al.[10]. In another study by Sarri et al., 120 Greek Orthodox Christians from Crete were followed for 1 year. Half of the subjects fasted regularly, and half did not (non-fasters). Before and near the end of fasting-period days, measurements were performed, and there were no differences for other vita-

mins or minerals, before and after fasting, except for vitamin B2[4].

Karras et al.[6] found low vitamin D levels and high parathyroid hormone (PTH) with normal serum calcium levels in 50 Athonian monks in two of their published studies[6,7]. Both Papadaki et al.[10] and Sarri et al.[4,13] found that calcium intake decreased during fasting.

Considering the impact of fasting on antioxidant vitamins A and E status, Sarri et al. undertook a study with 37 strict fasters, mainly priests and nuns (18 males, 19 females, mean age 43.0±13.1 years), and 48 age- and sex-matched controls (21 males, 27 females; mean age 38.6±9.6 years) that were studied before and at the end of the Christmas fasting period. Both groups had good levels of vitamins A and E. Fasters had higher baseline levels of vitamins A and E than controls, however, the levels were reduced during the fasting period, while the levels increased for the controls. The changes of serum vitamin levels during the fasting period were significantly related to changes in total cholesterol, whereas vitamin E levels were also correlated with changes in LDL and total cholesterol/HDL ratio[5].

Consequently, energy intake decreases during fasting, yet diet quality is better, with higher folate, iron, and magnesium intakes, as well as higher fibre, and lower saturated and trans fatty acids intakes. Calcium and vitamins D and B2 intakes are a concern, nonetheless.

2.4 Headaches

Mitsikostas et al.[15] investigated the frequency of headache among Athonian monks, who apart from their different diet, also had a different way of life and sleep programme. 8.68% of the participant monks did suffer from headaches, which was less frequent than the general population. The prevalence of migraine was 1.78%, of tension headache 3.34%, and of mixed headaches 1.87%. Cluster headache was not traced. Ninety percent of the monks who suffered from headaches had high scores on the Hamilton Scale for anxiety and depression (scored <16). Interestingly, during the fasting periods, the frequency and the intensity of headaches was increased in the majority of the monks[15].

2.5 Lifestyle and mental health

Adherence to a healthy diet has been associated with better mental health across the lifespan[16-18]. The impact of Greek Orthodox fasting on mental health has not been thoroughly investigated, despite the impact of being religious on ethical values and mental health practices (eg. alcohol use) (**Table 4**).

A cross-sectional study examined the Health-Related Quality of Life of Orthodox Christian Athonian monks and its correlation with demographic characteristics and Sense of Coherence (SOC-13). 166 monks (mean age 45.5 ± 13.0 years) from two monasteries and one scete participated in this study. SF-12 and SOC-13 scales were completed by the monks. 83.7% of the monks lived in communal monasteries, and the mean number of years as monks was 18.4 ± 12.1. Monks had lower physical activity levels (according to the Physical Component Summary score) than the general Greek men population, believed that their physical health was worse than the general public's health, but they had better mental health status (according to the Mental Component Summary score). That, it was documented that living in Mount Athos for longer period and higher SOC score may have a protective role on the monks' mental health[19]. In another study, semi-structured personal interviews were used to investigate a stratified sample of 20 to 65 year old participants that followed the Greek Orthodox Christian lifestyle. Adoption of this lifestyle was related to healthier behaviours (such as relaxation, life satisfaction, healthful nutrition, personal hygiene, and physical activity), independent of socio-demographic factors and health status[20]. In 24 strict fasters and 27 control participants with similar depressive symptoms distribution, where adipose tissue DHA was inversely associated with depression, adherence to the adoption of Orthodox Christian diet was strongly associated with adipose DHA levels compared to controls, which may protect against chronic physical and mental diseases[21]. Recently, Spanaki et al.[22] found that middle aged and elderly people who fast have lower levels of anxiety and depression

Table 2. Studies concerning the impact of Orthodox fasting on weight control

Study population	Study period	Main Results	Author, Date	Ref number
43 males form the general population that regularly fast (20-45 y of age) and 57 age-matched Athonian monks	-	Monks had lower BMI, and lower body fat mass	Karras et al., 2018	7
36 (25 women & 11 men) monks from 5 Greek monasteries	40 days	Fasting led to a decrease of weight, upper arm circumference and triceps skinfold thickness	Basilakis et al., 2002	9
10 Greek Orthodox Christian monks aged 25-65 years, with BMI > 30 kg/m^2	1 fasting week vs non-fasting week (measurements on Palm Sunday week (fasting) and the week following Pentecost Sunday (non-fasting)	NS ↓ on monks' body mass	Papadaki et al. 2008	10
120 Greek adults were followed longitudinally (60 fasters, 60 non-fasters)	1 year	Fasters had ↓1.5% BMI than non-fasters at the end of the fasting period, while a ↓1.4% on BMI was in fasters after the fasting period	Sarri et al., 2003	8

scores, as well as better cognitive function, than those who do not fast.

3. Conclusions

Christian Orthodox fasting has been studied the last decades, with results indicating a positive overall health effect. In particular, positive effects concerning glucose and lipid control have been observed, however, there is inconclusive evidence concerning blood pressure. Especially for lipid control, certain studies have indicated a positive impact of Orthodox religious

Table 3. Studies concerning the impact on nutrient intakes and sufficiency

Nutrient	Study population	Study period	Main Results	Author, Date	Ref number
Energy	36 (25 women & 11 men) monks from 5 Greek monasteries	40 days	↓ 20% caloric intake during fasting	Basilakis et al., 2002	9
	120 Greek adults were followed longitudinally (60 fasters, 60 non-fasters)	1 year	-180 kcal/day in fasters and +137 kcal/day in controls during fasting period	Sarri et al. , 2004	4
	50 Athonian monks (mean age=38.7±10.6 years) & 43 males form the general population that regularly fast (20-45 y of age) and 57 age-matched Athonian monks	Restrictive and Non-Non-Restrictive fasting days	Athonian monks had low energy intake during both restrictive and non-restrictive fasting days	Karras et al., 2017 & Karras et al., 2018	6,7
Macronutrients and foods	50 Athonian monks (mean age=38.7±10.6 years)	Restrictive and Non-Non-Restrictive fasting days	↓carbohydrate & saturated fat intakes ↑ protein during the "restrictive days"	Karras et al., 2017	6

fasting on total and LDL cholesterol. As far as weight loss is concerned, fasters had lower body mass, and lower caloric intake during the fasting periods.

Diet quality did improve during fasting, with higher fruit and vegetable consumption, and consequently higher fibre intakes, as well as higher iron and folate intake, which have been previously associated with better mental health, as well. However, calcium and vitamin B2 intakes were lower, and hypovitaminosis D has been noted in monks.

Table 3. Studies concerning the impact on nutrient intakes and sufficiency

(continued)	10 Greek Orthodox Christian monks aged 25-65 years, with BMI > 30 kg/m^2	1 fasting week vs non-fasting week	↓intakes of total and saturated and trans fats ↑ fibre during fasting ↑Legumes and fish/seafood during fasting, ↑dairy products, meat and eggs after the fasting week	Papadaki et al. 2008	10
	120 Greek adults were followed longitudinally (60 fasters, 60 non-fasters)	1 year	Fasters (vs controls) ↓ dietary cholesterol, total fat, saturated fatty acids, trans-fatty acids & protein , and ↑ fibre at the end of the fast	Sarri et al. 2004	4
	35 Greek Orthodox Christian strict fasters (n=17 male, n=18 female; mean age 43.6+/-13.2 years) and 24 controls (n=11 male, n=13 female; mean age 39.8+/-7.6 years)	40 days Measurements before and near completion of the Christmas fasting	↑fibre intake	Sarri et al. 2005	14
	38 devout Orthodox Christian fasters and 29 matched controls living in Crete	1 year (measurements before and at the end of the three major fasting periods)	↑fruit and vegetable consumption during the fasting periods	Sarri et al 2007	13

Table 3. Studies concerning the impact on nutrient intakes and sufficiency

(continued) **Micronutrients**	38 devout Orthodox Christian fasters and 29 matched controls living in Crete	1 year (measurements before and at the end of the three major fasting periods)	↓Sodium intake ↑Magnesium intake ↑Folate intake ↓Calcium intake during fasting	Sarri et al 2007	13
	10 Greek Orthodox Christian monks aged 25-65 years, with BMI > 30 kg/m^2	1 fasting week vs non-fasting week	↑Folate intake ↑Iron intake	Papadaki et al. 2008	10
	35 Greek Orthodox Christian strict fasters (n=17 male, n=18 female; mean age 43.6+/-13.2 years) and 24 controls (n=11 male, n=13 female; mean age 39.8+/-7.6 years)	40 days Measurements before and near completion of the Christmas fasting	Longterm religious fasting does not negatively affect iron status Fasters ↑ ferritin levels and ↓ total iron-binding capacity, especially females	Sarri et al. 2005	14
	120 Greek adults were followed longitudinally (60 fasters, 60 non-fasters)	1 year	No differences for other vitamins or minerals, before and after fasting, except for vitamin B2 ↓Calcium intake during fasting	Sarri et al. 2004	4

Table 3. Studies concerning the impact on nutrient intakes and sufficiency

(continued)	50 Athonian monks (mean age=38.7±10.6 years) & 43 males form the general population that regularly fast (20-45 y of age) and 57 age-matched Athonian monks	Restrictive and Non-Non-Restrictive fasting days	↓vitamin D levels and ↑PTH with normal serum calcium levels	Karras et al. 2017 & Karras et al. 2018	6,7
	37 strict fasters, (18 males, 19 females, mean age 43.0±13.1 years), and 48 age- and sex-matched controls (21 males, 27 females; mean age 38.6±9.6 years)	40 days Measurements before and near completion of the Christmas fasting	↓Vitamin A & E levels during the fasting period for fasters These change were related to changes in total cholesterol. Vitamin E levels were correlated with changes in LDL and total cholesterol/HDL ratio	Sarri et al. 2009	5

However, considering headaches, despite the fact that monks had lower headache prevalence, the aches were increased during fasting. As far as lifestyle factors and mental health are concerned, monks did present better quality of life and mental health than the general population.

Previous reviews have examined the health impact of the Christian Orthodox fasting and have also reported positive effects on blood lipids[23]. A systematic review of ten studies by Koufakis et al.[24] noted a caloric restriction during fasting, accompanied by a decrease on fat intake, and an increase in carbohydrate and fiber intake. Improvements on blood lipid control have been noted throughout the studies especially on total and LDL cholesterol levels, yet with inconsistent findings on HDL cholesterol. Also, the lower dietary intakes of vitamins D and B12, and minerals especially calcium were a concern[24].

The Christian Orthodox Fasting diet pattern can be compared to a pescatarian or flexitarian diet. Pescatarians follow a vegetarian diet, yet eat fish. Flexitarians consume no processed meat, low amounts of red meat and sugar, moderate amounts of poultry, dairy, and fish, and high amounts of fruits, vegetables, legumes, and nuts[25]. Derbyshire et al.[26] reviewed 25 studies concerning the role of the adherence to a

Table 4. Studies concerning the impact of Orthodox fasting on wellbeing

Study population	Study period	Main Results	Author, Date	Ref number
166 monks (mean age 45.5 ± 13.0 years) from two monasteries and one scete	-	Monks had ↓ physical activity levels than the general Greek men population, believed that their physical health was worse than the general public, but they had ↑mental health status	(Merakou et al., 2017)	19
20 to 65 year old people who followed the Greek Orthodox Christian lifestyle	-	Adoption of this lifestyle was related to healthier behaviors independent of socio-demographic factors and health status	Chliaoutakis et al. 2002	20
24 strict fasters and 27 controls	-	No difference on depressive symptoms distribution, adipose tissue DHA was inversely associated with depression, Adherence to the Orthodox Christian diet was correlated with adipose DHA levels compared to controls, which may protect against chronic diseases	Sarri et al. 2008	21
105 fasters and 107 non-fasters	-	Lower levels of anxiety and depression scores, and better cognitive function, in people who fast vs those who do not fast	Spanaki et al., 2021	22

flexitarian diet on health, and specifically on body weight, cancer, diabetes and metabolic syndrome, and diet quality. The authors did find that such diet pattern may be related to weight loss and better metabolic health, with reduced diabetes risk and hypertension, and may possibly help patients with inflam-

matory bowel disease due to the high fibre intake[26]. Additionally, according to a recent report published on The Lancet on sustainable nutrition, it was noted that adherence to a flexitarian diet was associated with a reduction on incidence of premature mortality by 19%[25]. However, another review highlighted that adherence to a vegetarian or flexitarian diet may be associated with greater risk of eating disorders, yet the results of the existing studies are conflicting[27]. Considering vegetarian diets, Tonstad et al.[28] examined the health effects of different types of vegetarian diets, and found a protective effect against type 2 diabetes for pescatarian and semi-semi-vegetarian diets, compared to non-non-vegetarian diets[28].

Dietary guidelines around the globe do focus on a healthy, varied, inclusive diet, with emphasis on plant-based foods, that could be compared to both a flexitarian or Mediterranean Diet, as well as the Greek Orthodox /Monastic Diet[20, 29-31] in order to prevent physical and mental illness.

Overall, the guidelines and the lifestyle that accompanies the Christian Orthodox fasting may be beneficial for human health and disease prevention. Further studies are recommended on the impact of long-term religious fasting on HDL cholesterol levels, and blood pressure, while the use of this periodic vegetarianism should be further studied as a measure of medical nutrition therapy in patients with chronic diseases, such as diabetes, cardiovascular disease and hypertension, as well as depression and anxiety. □

References

1. Trepanowski J.F., Bloomer R.J., The impact of religious fasting on human health. *Nutr. J.* 9, 57, 2010 .
2. Trepanowski J.F., et al., Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings. *Nutr. J.* 10, 107, 2011.
3. Quinton R.K., Ciccazzo M. Influences on Eastern Orthodox Christian Fasting Beliefs and Practices. *Ecol. Food Nutr.* 46(5-6), 469-491, 2007.
4. Sarri K.O., et al., Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. Br. J. Nutr. 92(2), 277-84, 2004.
5. Sarri K., et al., The effect of periodic vegetarianism on serum retinol and alpha-tocopherol levels. Int. J. Vitam. Nutr. Res. 79(5-6),. 271-80, 2009.
6. Karras S.N., et al. Health benefits and consequences of the Eastern Orthodox fasting in monks of Mount Athos: a cross-sectional study. *Eur. J. Clin. Nutr.* 71(6), 743-749, 2017.
7. Karras S.N., et al., *Christian Orthodox fasting in practice: A comparative evaluation between Greek Orthodox general population fasters and Athonian monks. Nutrition.* 59,. 69-76, 2018.
8. Sarri K.O., et al. Effects of Greek Orthodox Christian Church fasting on serum lipids and obesity. *BMC Public Health.* 3, 16, 2003.
9. Basilakis A, et al. Nutritional study in Greek-Orthodox monasteries–Effect of a 40-day religious fasting. *Akt. Ernähr. Med*. 27, 250–255, 2002.
10. Papadaki A., et al. Calcium, nutrient and food intake of Greek Orthodox Christian monks during a fasting and non-fasting week. *Public Health Nutr*. 11(10), 1022-9, 2008.
11. Karras S.N., et al. Effects of orthodox religious fasting versus combined energy and time restricted eating on body weight, lipid concentrations and glycaemic profile. *Int. J. Food Sci. Nutr.* 72(1), 82-92, 2021.
12. Karras S.N., et al. Similar late effects of a 7-week orthodox religious fasting and a time restricted eating pattern on anthropometric and metabolic profiles of overweight adults. *Int. J. Food Sci. Nutr.* 72(2), 248-258, 2021.
13. Sarri K., et al. Does the periodic vegetarianism of Greek Orthodox Christians benefit blood pressure? *Prev. Med*. 44(4),. 341-8, 2007.
14. Sarri K.O., Kafatos A.G., Higgins S. Is religious

fasting related to iron status in Greek Orthodox Christians? *Br. J.Nutr*. 94(2), 198-203, 2005.
15. Mitsikostas D.D., et al. An epidemiological study of headache among the Monks of Athos (Greece). *Headache* 34(9),. 539-41, 1994.
16. Açik M., Çakiroğlu F.P. Evaluating the Relationship between Inflammatory Load of a Diet and Depression in Young Adults. *Ecol. Food Nutr.* 58(4), 366-378, 2019.
17. Adjibade M., et al. Prospective association between adherence to the Mediterranean diet and risk of depressive symptoms in the French SU.VI.MAX cohort. *Eur. J. Nutr.* 57(3), 1225-1235, 2018.
18. Ye X., et al., Mediterranean diet, healthy eating index 2005, and cognitive function in middle-aged and older Puerto Rican adults. *J. Acad. Nutr. Diet.* 113(2), 276-81.e1-3,2013.
19. Merakou K., et al. Health-related quality of life of a very special population: monks of Holy Mountain Athos, Greece. *Qual. Life Res.* 26(11), 3169-3175, 2017.
20. El Chliaoutakis J., et al. Greek Christian Orthodox Ecclesiastical lifestyle: could it become a pattern of health-related behavior? Prev. Med. 34(4, 428-35, 2002.
21. Sarri K.O., et al. Adipose DHA inversely associated with depression as measured by the Beck Depression Inventory. *Prostaglandins Leukot. Essent. Fatty Acids*. 78(2), 117-22, 2008.
22. Spanaki C., et al. The Christian Orthodox Church Fasting Diet Is Associated with Lower Levels of Depression and Anxiety and a Better Cognitive Performance in Middle Life. Nutrients. 13(2), 2021.
23. Koufakis T., et al. Orthodox religious fasting as a medical nutrition therapy for dyslipidemia: where do we stand and how far can we go? *Eur. J. Clin. Nutr.* 72(4), 474-479, 2018.
24. Koufakis T., et al. Effects of Orthodox religious fasting on human health: a systematic review. *Eur. J. Nutr.* 56(8), 2439-2455, 2017.
25. Springmann M., et al. Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detail. *The Lancet. Planetary Health*. 2(10), e451-e461, 2018.
26. Derbyshire E.J. Flexitarian Diets and Health: A Review of the Evidence-Based Literature. *Front. Nutr.* 3, 55-55, 2017..
27. Forestell C.A. Flexitarian Diet and Weight Control: Healthy or Risky Eating Behavior? *Front. Nutr.* 5, 59-59, 2018..
28. Tonstad S., et al. Type of vegetarian diet, body weight, and prevalence of type 2 diabetes. *Diabetes Care.* 32(5), 791-6, 2009.
29. Katz D.L., Meller S. Can we say what diet is best for health? *Ann. Rev. Public Health.* **35**,. 83-103,. 2014.
30. Kimokoti R.W., Millen B.E. Nutrition for the Prevention of Chronic Diseases. *Med. Clin. North Am.* 100(6), 1185-1198, 2016.
31. Sofi F., et al. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public Health Nutr.* **17**(12), 2769-82, 2014.

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 34, Ι, 2022 | 17-26 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 34, I, 2022 | 17-26 RESEARCH ARTICLE

Selection of the Carrier for Obtaining Dry Extract of *Acorus calamus* Leaves

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ABSTRACT

Abstract: Sweet flag (*Acorus calamus L.*) is a world-famous medicinal plant with valuable therapeutic properties. Numerous modern scientific researches described a wide range of therapeutic activity of the calamus and extracts from it, providing the expediency of the development of a drug based on this raw material. The main issue in our study was to determine a rational way to introduce *Acorus calamus* extract into a solid dosage form. First, literature analysis was carried out, which revealed that extracts in the dry state were the most technologically acceptable. Different methods of dry extract obtaining were theoretically studied from which the optimal one was chosen for experimental research. The optimal carrier was established through comparative studies of different excipients. According to indicators of finished product yield, drying time, hygroscopicity, and microscopic analysis it was found that the rationale was the combination of carriers Kollidon CL and Microcel 200. The experimental results based on the parameters of finished product yield, drying time, hygroscopicity, moisture absorption kinetics, flowability, bulk characteristics, as well as strength and disintegration time of the obtained tablets allowed us to conclude the best ratio of the components of the carrier for the extract of Acorus calamus leaves: Microcel 200 : Kollidon CL – 60 : 30.

1. Introduction

Acorus Calamus L. (AC), is a well-known medicinal plant (MP) widely used in modern therapeutic practice for the treatment of diseases of the nervous, hepatobiliary, and cardiovascular systems of the human body[1-4].

More than 20 drugs are registered on the pharmaceutical market of Ukraine, which have calamus in their composition. This raw material enhances the nootropic and adaptogenic effect of drugs, has anti-inflammatory and antibacterial activity. Of particular importance are medicines "Vikair" (Valenta Pharmaceutics, Russia), "Vikalin" (Monpharm, Ukraine), "Polyfitol" (DKP Pharmaceutical Factory, Ukraine), "Gastrofit" (AIM, Ukraine), "Detoxifyt" (AIM, Ukraine), and "Original Grosser Bittner® bal-

sam" (Richard Bittner AG, Austria), which are effective for the prevention and treatment of pathologies of the gastrointestinal tract[5]. It is important to note that only underground parts of the *Acorus calamus L.*, are used in the production of these drugs, although several researchers have proved the almost complete identity of the qualitative composition of biologically active substances (BAS) of rhizomes and leaves of this MP[6,7]. Neglecting the aboveground parts of the plant leads to the irrational use of its natural resource and reduction of the range of the species.

In previous research, a technology for obtaining liquid extraction of *Acorus Calamus* leaves was developed[8]. Still, since the aim of our work was to develop tablets with calamus leaf extract, the main issue in our study was to determine a rational way to introduce it into a solid dosage form.

Dry extracts are the most technologically appropriate form in comparison with liquid and thick extracts in terms of convenience for the industrial production process. Minimizing the amount of moisture allowed to achieve better storage stability and reduce the weight and volume of the intermediate product[9].

However, pure dry extracts are generally hygroscopic and finely dispersed, which in most cases does not provide the necessary technological properties of the tablet mass and stipulates the use of appropriate excipients[10].

According to the literature sources analysis, the introduction of these excipients is possible at different stages of production:

1. Introduction of the carrier after complete drying of the extract

This method is the most commonly used. But prolonged temperature exposure in obtaining a pure dry extract causes significant energy losses, increases the cost of the final product, and can negatively affect the qualitative and quantitative composition of BAS[11-13].

2. *Introduction of the carrier after the stage of evaporation (concentration) of the extract*

The method is characterized by the carrier's introduction into the thick extract, i.e. after partial removal of the extractant (evaporation). This reduces the duration of the drying stage but makes it difficult to achieve a uniform distribution of the carrier in the thick extract due to its viscous nature. At the same time, the material often sticks to the walls of the equipment, which causes significant losses both at the stage of concentration and final drying[14].

3. *Spraying the liquid or partially evaporated extract on the carrier*

Modern method, which is implemented by gradually applying the liquid extraction on a solid carrier and subsequent drying. However, the main requirement of this method is the complete adsorption and homogeneity of the distribution of BAS in the mass of the carrier, which necessitates the use of additional equipment.

4. *Spray drying*

The basic principle is based on spraying a liquid extraction with a carrier in the drying chamber at a temperature that allows to minimize drying time and to provide direct transfer of moistened materials to a free-flowing state. The disadvantages of this method are the high cost of equipment, its bulkiness, and complexity of maintenance, as well as the use of high temperatures[15-17].

5. *Introduction of the carrier after obtaining a liquid extraction with subsequent evaporation and drying*

The basic technological principle is that the required amount of solid carrier is introduced into the filtered liquid extraction, after which the obtained suspension is evaporated during mixing under vacuum and temperature to (23±3)% of moisture content. Then the resulting mixture is sent for final drying. The advantage of this method is, first of all, a significant reduction in evaporation and drying time (2-2.5 times compared to the drying of the extract without carrier) because the carrier, in this case, acts as a

"drying agent" – its distribution in the extract causes uniform heating of the material. Moreover, extractant evaporation is much easier from the surface of the solid carrier. Also important is the fact that as the extractant is removed, the carrier instantly absorbs the extractives and protects BAS from physical factors. In addition, the absence of a period in which the extractives are without a carrier averts the absorption of moisture out of the environment[18].

Given the above, and considering the advantages of method 5 in the research of the selection of rational carrier it was decided to select this last-mentioned method for obtaing dry extract of *Acorus Calamus*.

2. Materials and methods

2.1. Research materials

To obtain a dry extract of *Acorus calamus* leaves, a liquid extraction produced with 70% ethyl alcohol by remaceration using ultrasound and surfactant (0.1% polysorbate 80) was used. The extraction was performed in a hermetically sealed Ultrasonic extraction reactor PEX 1 (R.E.U.S., Contes, France); ultrasound frequency – 35 kHz. Process duration – 3-fold remaceration for 45 minutes; process temperature – (65±5)°C. The obtained extractions were stored at a temperature (8±2)°C for 2 days and filtered through an ashless paper filter under vacuum[8,19].

The following excipients were selected as carriers: Neusilin US2 (Fuji Chemical Industries Co., Ltd), Fujicalin SG (Fuji Chemical Industries Co., Ltd), Microcel 200 (Blanver Farmoquimica Ltd), GalenIQ 721 (Beneo GmbH), Kleptose (Roquette Frères), Aerosil A-380 (Evonik Industries), Kollidon CL (BASF), Syloid 244FP (WR Grace and Company).

2.2. Obtaining dry extract samples

2.2.1. Obtaining pure dry extract

The liquid extraction was concentrated on a LabTech EV311 plus rotary vacuum evaporator (Labtech S.R.L., Italy) under the following conditions: temperature – (55±5)°C, rotation speed – 100-150 rpm, vacuum depth – (900±10)mBar; moisture content – (25±2)%. Final drying to residual moisture (3.0±0.5)% was performed in a vacuum oven DZF-6050 (Zhengzhou Keda Machinery and Instrument Equipment Co., Ltd., China) at a temperature of (55±5)°C. Thereafter, the dry extract was ground in a mortar and separated through a sieve with 1.0 mm of pore diameter.

2.2.2. Obtaining dry extract with a carrier

To obtain the samples of an extract with carriers, the excipients selected for the study were introduced into the liquid extraction. The resulting suspension was evaporated in a rotary vacuum evaporator LabTech EV311 plus (Labtech SRL, Italy) and dried in a vacuum oven DZF-6050 (Zhengzhou Keda Machinery and Instrument Equipment Co., Ltd., China) under conditions similar to the pure dry extract obtaining. After drying, the obtained samples were also ground in a mortar and separated through a sieve with 1.0 mm of pore diameter.

2.3. Research methods

2.3.1. Determination of moisture content

Moisture content and dry residue (DR) were determined according to the methods specified in European Pharmacopoeia (Ph. Eur.) chapter 2.5.12 and 2.8.16[20]. DR shows the amount of solid phase (g) in 1 ml of extraction.

2.3.2. Microscopic analysis

Performed using a laboratory microscope Konus Academy Microscope with camera DLT-Cam Basic 2 MP (Italy).

2.3.3. Evaluation of Physico-chemical characteristics

During the process of obtaining the dry extract with the carrier, some samples were characterized by the formation of strong interactions with the walls of

the flask, which significantly affected the yield of the finished product and the duration of the drying process. To take this factor into account, the obtained samples of dry extract in pure form and with carrier were examined through these indicators.

The yield of the finished product (%) was calculated by comparing the theoretically calculated mass of the mixture "dry extract/carrier" with the practically obtained value.

The theoretical mass of the mixture was calculated using the formula:

$$m_{\text{т}} = (V * DR) + \left(m_c * \frac{(100-w_c)}{100}\right),$$

where V – the volume of the liquid extraction, ml;
DR – dry residue, g/ml;
mc – the weight of the carrier, g;
wc – moisture content of the carrier, %.

The duration of drying was defined as the period (h) from the beginning of the process until optimum moisture content for the extract is obtained.

These values were calculated at the stage of evaporation in rotary evaporator LabTech EV311 plus (Labtech S.R.L., Italy) for 600 ml of extraction in a flask with a capacity of 1000 ml.

2.3.4. Determination of hygroscopicity and kinetics of moisture absorption

Determination of moisture absorption kinetics of extract samples was performed in a desiccator according to the method specified in Ph. Eur. 5.11[20], under the following conditions: temperature – (25±1)℃, relative humidity – (80±2)%; test time – 24 h (during the first 5 h samples were tested every 1 h). Hygroscopicity in extreme conditions was evaluated at relative humidity (100±2)% during 24 h of observation.

2.3.5. Evaluation of technological properties

The technological properties of dry extract samples and tablets obtained from it were evaluated by the requirements of Ph. Eur[20].

The conclusion about the flowability of the dry extract samples was made according to the values of the Carr index:

$$C = 100 \times \frac{V_0 - V_f}{V_0},$$

where V0 – bulk volume, ml;
Vf – volume after shrinkage, ml.

The strength of the tablets was determined by the resistance to crushing using Tablet hardness tester Monsanto type (Bexco, Belgium).

The disintegration time of the tablets has been tested using the device for determining the disintegration of tablets and capsules "PTZ AUTO" (Pharma Test, Germany).

2.4. Statistical Analysis

The results are presented as mean ± standard deviation. Statistical analysis was performed using Student's t-test. A value of $p < 0.05$ was taken as the level of significance.

3. Results and discussions

At the first stage of our research, it was necessary to investigate the effect of selected excipients on the drying process of *Acorus calamus* leaves extract. The excipients were introduced into the liquid extraction in the concentration of 50% regarding the DR. The extract was then evaporated and dried. The tests were performed in comparison with the pure dry extract. The results are presented in **Table 1.**

According to the results, samples № 4, 7, and 8 were characterized by a low level of product yield. During drying, a strong interaction was observed between the walls of the flask and the powder mass, which as a result could not be removed. The samples also required a significant drying time. In our opinion, the significant drying time when using these carriers was due to their solubility in the liquid phase of extraction, which caused an increase in the amount of adsorption-bound and osmotically retained moisture. Substances of this nature are prone to the formation of viscous elastic masses, which retain moisture in the surface layers of the material

Table 1. Characterization of excipients and their effect on the extract drying process

Sample №	Carrier	Mean particle size of the carrier μm	Solubility of the carrier in the extractant	The yield of the finished product, %	Drying time, h
1	Without carrier			39.8 ± 0.1	4.5 ± 0.5
2	Microcel 200	180	N/S	90.7 ± 0.1	2.5 ± 0.4
3	Kollidon CL	130	N/S	95.9 ± 0.2	2.2 ± 0.3
4	Fujicalin SG	120	S/S	54.4 ± 0.7	3.5 ± 0.5
5	Syloid 244FP	3	N/S	79.2 ± 0.4	3.1 ± 0.5
6	Aerosil A-380	10	N/S	79.1 ± 0.4	3.3 ± 0.5
7	Kleptose	3	S	44.7 ± 0.9	4.4 ± 0.5
8	GalenIQ 721	180	S	50.0 ± 0.5	4.1 ± 0.5
9	Neusilin US2	90	N/S	94.8 ± 0.1	2.1 ± 0.3

Note: n = 3, p < 0,05; N/S – not soluble; S/S – somewhat soluble; S – soluble.

and prevent the drying process[21]. Given this, the use of these carriers was impractical from a technological perspective.

Sample № 9 had a high level of product yield and a short drying time. However, during the production of the extract, a change in color to yellow-green was observed, which probably indicated the reaction of flavonoids with aluminum with the formation of a multichromatic complex. This fact indicated a high probability of extract BAS deactivation and made it impossible to use Neusilin US2 (as well as all excipients containing calcium, magnesium, or aluminum) as a carrier for calamus leaf extract[22].

Samples № 2, 3, 5, 6 were the most optimal in terms of product yield and drying time. The complete insolubility of the carrier in the liquid phase of the extraction contributed to the preservation of high dispersion of particles and the significant contact surface, which caused a significant reduction in drying time[23,24]. Therefore, these samples were selected for further research.

To assess the degree and type of interaction of the extract with the carrier, microscopic analysis was performed at the next stage. The results are presented in **Figure 1.**

As can be seen in **Fig. 1**, all test samples were represented by agglomerates of extract particles with a form factor approaching 1.

The obtained samples with Kollidon CL and Microcel 200 were bulky strong agglomerates formed due to the high force of particles' mutual adhesion. They were characterized by almost equal size from 1.0 to 1.5 μm (± 0.1) and heterogeneous surfaces.

Samples of Aerosil A-380 and Syloid 244FP differed significantly in particle size, ranging from 0.1 to 5.0 μm (± 0.1), and were characterized by a rough surface and uneven distribution in the field of view.

An important aspect was also to study the hygroscopicity of selected samples. It is known that increased moisture content of a tablet mass can cause its possible adhesion to a press tool, worsening of flowability, and, accordingly, deterioration of the dosage uniformity, while excessively dry material can provide an unsatisfactory ability to compress. The tests were performed at 100% relative humidity for 24 h. The results are presented in **Table 2.**

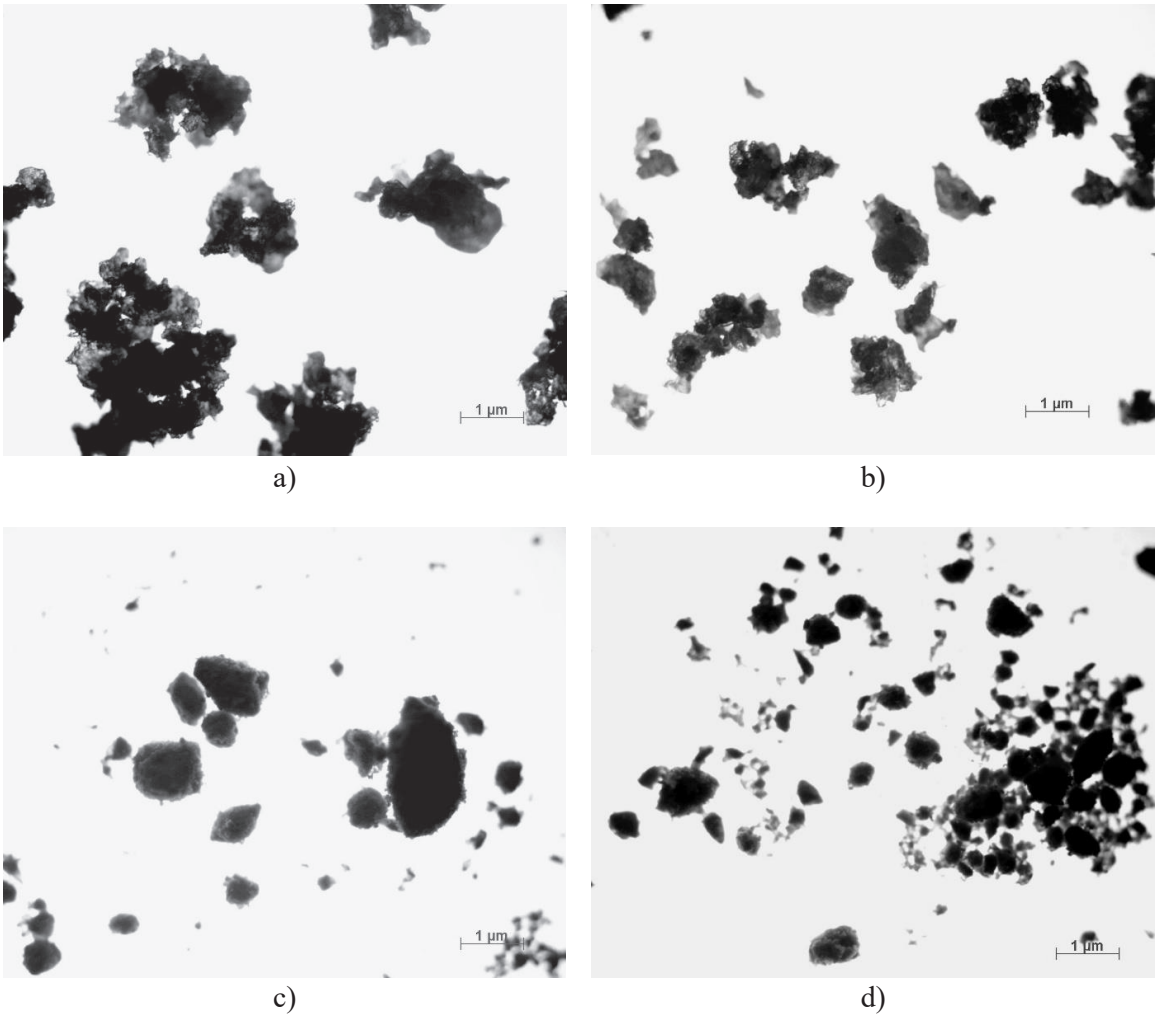

Fig. 1. *Microscopic analysis of extract samples with different carriers (x70): a) Kollidon CL; b) Microcel 200; c) Aerosil A-380; d) Syloid 244FP*

Table 2. Study of selected samples hygroscopicity

Carrier	Increase in mass, %
Microcel 200	18.58 ± 0.12
Kollidon CL	25.28 ± 0.20
Syloid 244FP	23.12 ± 0.19
Aerosil A-380	20.32 ± 0.15

Note. n = 3, $p < 0.05$.

According to the obtained data (**Table 2**), the sample of the extract with Microcel 200 provided the best stability to environmental moisture.

Based on the results of microscopic analysis, hygroscopicity, and the effect of carriers on the drying process, it has been decided to use a combination of carriers – Kollidon CL and Microcel 200 – because the 1st best influenced the drying process and the yield of finished products, while the 2nd provided stability to the environmental humidity. Another important factor was the potential of these excipients for tableting, given that Microcel 200 improves the compressibility of tablets while Kollidon CL promotes their rapid disintegration, acting as a disintegrant[10,25].

After determining the rational carrier, it was necessary to establish the optimal quantitative ratio of these components to reduce the drying time and maximize product yield.

The first step was to determine the rational quantity of Kollidon CL in the carrier. The concentration limits of Kollidon CL – 20-40% regarding the DR of extract were selected based on literature sources data[10,24,25]. The results of the correlation study between the finished product yield and the quantity of Kollidon CL are given in **Table 3.**

Table 3. Correlation between finished product yield and quantity of Kollidon CL

The concentration of Kollidon CL regarding the DR of extract	Finished product yield, %
20%	87.48 ± 0.04
25%	91.19 ± 0.05
30%	94.85 ± 0.07
35%	95.02 ± 0.09
40%	95.19 ± 0.10

Note. n = 3, $p < 0.05$.

By the obtained results (**Table 3**), an increase of Kollidon CL quantity provided the product yield growth when obtaining a dry extract of *Acorus calamus* leaves. Given that, an insignificant statistical difference in values was observed in the concentration range of Kollidon CL 30-40%, the concentration of 30% regarding the DR of the extract was chosen for further studies.

Determination of the rational quantity of Microcel 200 in the carrier was performed based on the parameters of moisture absorption, flowability, bulk characteristics, as well as strength and disintegration time of the obtained tablets. The concentration of Microcel 200 (20-80%) was selected based on literature data and recommendations specified in the manufacturer's brochure [10,26].

Determination of moisture absorption kinetics was performed during 24 h of observation at temperature (25±1)°C and relative humidity (80±2)%. The results are shown in **Figure. 2.**

According to the data shown in **Fig. 2**, the stability to the humidity of the environment has been improving with increasing of Microcel 200 content, which indicated the moisture-regulating potential of this excipient. This was also confirmed by the comparison of moisture absorption rates of the extract samples with 80 % of the test substances: in 24 hours the weight gain with Microcel 200 was 3.69% in contrast to the sample with Kollidon CL, which gained 8.46%.

The results of the study of bulk characteristics, flowability, and tableting potential of the selected samples are given in **Table 4.**

Table 4. Pharmaco-technological characteristics of selected samples

Microcel 200/Kollidon CL content regarding the DR of extract	Hardness, N	Disintegration time, sec	Carr index, %	Flowability characteristics
20/30	25.27 ± 0.52	2380 ± 21	31.44 ± 0.55	Poor
30/30	31.81 ± 0.61	1806 ± 15	27.78 ± 0.48	Poor
40/30	38.67 ± 0.59	1556 ± 11	22.11 ± 0.46	Passable
50/30	50.56 ± 0.74	1450 ± 12	17.34 ± 0.35	Fair
60/30	68.89 ± 0.91	1560 ± 13	12.46 ± 0.30	Good
70/30	73.11 ± 0.98	2090 ± 19	10.08 ± 0.22	Excellent

Note. n = 3, $p < 0.05$.

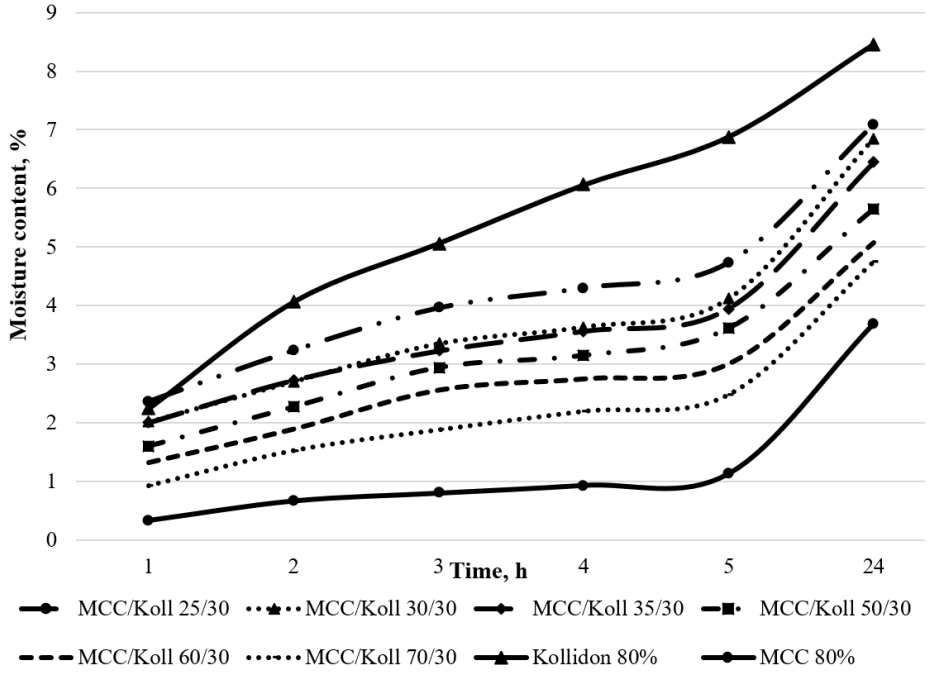

Fig. 2. *Moisture absorption kinetics of selected samples*

The obtained results (**Table 4**) demonstrated the positive effect of Microcel 200 on flowability and strength of tablets: the best results were observed when using this excipient in concentrations 60% and 70%. Therefore, the obtained data allowed us to conclude the best ratio of the components of the carrier for the extract of *Acorus calamus* leaves: Microcel 200 : Kollidon CL – 60 : 30. However, no combination of these excipients provided the required (according to Ph. Eur.) tablet disintegration time indicating the need for further research in this direction.

4. Conclusion

Based on the analysis of literature sources, a rational method for obtaining a dry extract of *Acorus calamus* leaves was found. It relies on introducing the carrier into the liquid extract with subsequent evaporation and drying under vacuum, which, in turn, will allow obtaining finished products with minimal losses in pharmaceutical companies' extraction preparations.

Several researches estimated that introduction of excipients such as Neusilin US2, Microcel 200, Aerosil A-380, Kollidon CL, Syloid 244FP before evaporation and drying of the extraction can significantly decrease the time of drying and reduce the process temperature, as well as increase the yield the finished product. It was found that the solubility of the carriers in the extract liquid phase affected the level of losses and the duration of the drying process.

The experimental research of samples of an extract with the carrier on indicators of drying time, yield of finithe shed product, and hygroscopicity, the optimum carrier providing necessary technological properties of *Acorus Calamus* leaves extract – a mixture of Microcel 200 and Kollidon CL – was found.

The results of the studies of flowability, bulk characteristics, as well as the potential for compression and disintegration of tablet based off the extract with the carrier, allowed us to establish a rational ratio of the carrier components, which was 60% of Microcel 200 and 30% of Kollidon CL regarding the DR of extract.

Further research will be aimed at improving the

technological characteristics of the tableting mixture to obtain quality tablets.

The obtained results are part of the Ph.D. thesis "Development of the composition and technology of tablets based on *Acorus calamus* leaves".

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References

1. Korang L.A., Derimedvid L.V. Antiexudative properties of liquid alcohol-water extract of calamus leaves (*Acorus calamus L.*). *Pharm. Drug Toxicol.* 13, 263–9, 2019. doi: https://doi.org/10.33250/13.04.263
2. Derimedvid L.V., Korang L.A., Kalko K.O., Vereitinova V.P. Influence of azalea leaf extract (*Acorus calamus L.*) on cholestasis and biliary function of the liver in experimental hepatitis. *Pharm. Drug Toxicol.* 14, 114–21, 2020. doi: https://doi.org/10.33250/14.02.114
3. Derimedvid L.V., Korang L.A. Antioxidant and anticytolytic properties of acorus leaf extract. *Pharm. Drug Toxicol.* 13, 393–8, 2019. doi: https://doi.org/10.33250/13.06.393
4. Derimedved L.V., Korang L.A., Yudkevich T.K. Timoleptic properties of dealcoholized extract of calamus leaves (*Acorus calamus L.*). *Nor. J. Dev. Int. Sci.* 40, 44-9, 2020.
5. Compendium. URL: https://compendium.com.ua (Retrieved 7 July 2021).
6. Yaremenko M.S., Gontova T.M. Comparative analysis of the amino acid composition of leaves and rhizomes of azalea. Industrial Pharmacy: Stages of formation and future: a collection of scientific papers: NUPh Publishing House, 137–140, 2017.
7. Derymedvid L., Korang L., Shakina L. Comparative cytotoxic analysis of extracts obtained from leaves and roots of sweet flag (*Acorus calamus L.*) on rat bone marrow cells in vitro. *ScienceRise: Pharm. Sci.* 1, 17-22, 2020. doi: https://doi.org/10.15587/2519-4852.2020.196405.
8. Andryushayev O., Ruban O., Maslii Y., Rusak I. Intensification of the extraction process of phenolic compounds from *Acorus calamus* leaves. *ScienceRise: Pharm. Sci.* 4, 3-10, 2021. doi: http://doi.org/10.15587/2519-4852.2021.238329.
9. Proestos C. The Benefits of Plant Extracts for Human Health. *Foods*. 9, 1653, 2020. doi: 10.3390/foods9111653.
10. Ruban O., Alkhalaf M., Gerbina N. Selection of a filler for tablets manufactured with direct compression method containing dry ginger extract. *EUREKA: Health Sci.* 3, 26-34, 2019. doi: 10.21303/2504-5679.2019.00904.
11. Rustiani E., Miranti M., Rahmahuda N. Formulation of tablet from papaya and bay leaf extract with variation of concentration polyvinylpyrrolidone as a binder. *Asian J. Pharm. Clin. Res.* 10, 169, 2017. doi: 10.22159/ajpcr.2017.v10s5.23124
12. Oh E., Kim U., Lee B., Moon C. Multivariate Statistical Optimization of Tablet Formulations Incorporating High Doses of a Dry Herbal Extract. *Pharmaceutics*. 11, 79, 2019. doi: 10.3390/pharmaceutics11020079.
13. Development and Standardization of Capsular Pharmaceutical Dosage form from Dry Extract of Ferula Tenuisecta. *Indian J. Forensic Med. Toxicol.* 14, 7540-47, 2020. doi: 10.37506/ijfmt.v14i4.12840
14. Qusaj Y., Leng A., Alshihabi F., Krasniqi B., Vandamme T. Development Strategies for Herbal Products Reducing the Influence of Natural Variance in Dry Mass on Tableting Properties and Tablet Characteristics. *Pharmaceutics*. 4, 501-16, 2012. doi: 10.3390/pharmaceutics4040501.

15. Gallo L. Valeriana officinalis Dry Plant Extract for Direct Compression: Preparation and Characterization. *Sci. Pharm.* 80, 1013-26, 2012. doi: 10.3797/scipharm.1206-05.
16. Santana C., Medeiros F., Correia L., Diniz P., Véras G., Medeiros A. Dissolution and uniformity of content of tablets developed with extract of Ximenia americana L. *PLoS One*. 13, e0197323, 2018. doi: 10.1371/journal.pone.0197323.
17. Nhan N., Vu N., Thanh L., Phuong T., Bach L., Toan T. Instant tea from Condonopsis javanica L. root extract via spray drying. *Foods Raw Mater.* 8, 385-91, 2020. doi: 10.21603/2308-4057-2020-2-385-391
18. Cianchino V., Jofre M., Peralta C. A new design of silybum marianum tablets by direct compression. *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci*. 5, 572, 2019. doi: 10.26479/2019.0502.42.
19. Yaremenko M., Gontova T., Boryak L., Mala O., Andryushayev O. Determination of optimal extraction conditions of phenolic compounds from *Acorus calamus* leaves. *EUREKA: Health Sci.* 3, 63-70, 2020. doi: https://doi.org/10.21303/2504-5679.2020.001317.
20. Council of Europe. European Pharmacopoeia. Strasbourg, 2016.
21. Kowalski S. Thermomechanics of drying processes. Milton Keynes, UK: Lightning Source UK Ltd., 2011.
22. Zhang L., Liu R., Gung B., Tindall S., Gonzalez J., Halvorson J., Hagerman A. Polyphenol–Aluminum Complex Formation: Implications for Aluminum Tolerance in Plants. *J. Agric. Food Chem.* 64, 3025-33, 2016. doi: 10.1021/acs.jafc.6b00331.
23. Rahmati E., Sharifian F., Fattahi M. Process optimization of spray-dried Moldavian balm (Dracocephalum moldavica L.) extract powder. *Food Sci. Nutr.* 8, 6580-91, 2020. doi: 10.1002/fsn3.1949.
24. Coelho A., Lima Neto J., Moura A., Morais I., Cunha F., Melo-Neto B. et al. Pharmaceutical development of tablets containing a spray-dried optimized extract from Lippia origanoides H. B. K.: influence of excipients and toxicological assessment. *Braz. J. Pharm. Sci.* 54, e17226, 2018. doi: 10.1590/s2175-97902018000217226.
25. Smekhova I.E., Vainshtein V.A., Ladutko Y.M., Druzhininskaya O.V., Tureckova N.N. Disintegrants and their influence on the dissolution of substances of biopharmaceutical classification system classes. *Drug Dev. Reg.* 4, 62-72, 2018.
26. Barchuk O.Z., Groshovyi T.A., Zaliska O.M., Shalata V.Ja., Revyatsky I.Yu. Development of optimal composition and technology of tablets based on the exstract of goat's rue herb, extract of bilberry leaves and ταυρινε by direct compression method. *Pharm. Rev.* 4, 30-40, 2017. doi: 10.11603/2312-0967.2017.4.8352.

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ΦΑΡΜΑΚΕΥΤΙΚΗ, 34, I, 2022 | 27-37 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 34, I, 2022 | 27-37 RESEARCH ARTICLE

Investigation of Three-component Reaction of 1,3-Dipolar Cycloaddition of Isatin, α-Amino Acids and Dipolerophiles Based on Maleic Acid and Study of Antimicrobial and Antiradical Activity of Synthesized Compounds

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ABSTRACT

A new series of symmetrical derivatives of hexamethylene(ethylene, oxalylamine, m-phenylene) - N,N'-bis(spiroindole-3,3'-pyrrolo[3,4-c] pyrrole-2a',5a'-dihydro-2,2',6'(1H,1'H,5'H)-trione) was syn-thesised by means of 1,3-dipolar cycloaddition of in situ generated azomethynilides to dipolarophiles containing bis-maleimide fragment. The IR and ^{1}H NMR spectroscopic characterization of newly synthesised compounds is reported. The synthesized compounds were tested for antibacterial activity in two stages. Among the substances selected in the first stage, the three most promising substances were identified, which showed significant antibacterial activity with an MBC of 50 mg/ml. When studying the interaction of the synthesized substances with DPPH, one compound was found, which showed an average level of antiradical activity. The structure-action relations of the synthesized compounds was discussed.

1. Introduction

The pharmacological activity of synthetic drugs depends on the pharmacophore fragments contained in their molecules. A number of pharmacophores are responsible for certain types of biological activity and due to this, researches pay their attention to introducing such fragments into new molecules. In recent years, the number of publications devoted to spiro-pyrrole-2-oxindoles has significantly increased in scientific literature. One of the best approaches for the synthesis of these derivatives is 1,3-dipolar cycloaddition of azomethynilides to electron deficient alkenes. This reaction is an easy way to either mono- and *bis*-derivatives of spiro-pyrrole-2-oxindoles. Application of different starting materials allows to obtain structural complexity of *bis*-derivatives of spiro-conjugated pyrrolo-2-oxindoles, linked togeth-

er by a polymethylene, an aroma-tic or hetero-aromatic linkage. The method of joining symmetric fragments of spiro-conjugated pyrrolo-2-oxindoles in the reactions of 1,3-dipolar cycloaddition depends on the structure of dipolarophile. The nucleus of spiro-pyrrole-2-oxindoles underlies many natural alkaloids. They exhibit a wide range of biological activity, including immunomodulatory, analgesic, anti-inflammatory, antimicrobial and cytostatic. Alkaloids with bis-2-oxindo-line moiety: *gelaganimines A* and *B* and alkaloid with *bis*-spiro-2-oxindoline nucleus: gelanidine C were isolated from the plant *Gelsemium elegans*. These natural compounds exhibit cytotoxic, anti-microbial and anti-inflammatory activity. In addition, rigidly spatially organized spiro-2-oxindole molecules are complementary to three-dimensional binding sites of essential biological targets (enzymes, receptors, ion channels) and are therefore of interest in the search for new biologically active substances[3]. Among synthetic derivatives of spiropyrroloxyindoles and their *bis*-analogues were found substances with antibacterial, antifungal, antivirus[14], anti-inflammatory[12, 14], anti-allergic, antitumor, , anticonvulsant activity[14]. Thus, in the present work we described the synthesis of symmetric derivatives of *bis*-spiropirolo-2-oxindole via three-component one-pot interaction of isatin with α-amino acids and maleic acid dipolarophiles. We also aimed to investigate antimicrobial and antiradical activity of the products obtained as well as to determine "structure-activity" relationships.

2. Materials and Methods

The starting isatin **1** and α-amino acids **2** were obtained from commercial sources and used without further purification. Dipolarophiles used, namely N,N'-hexamethylene-*bis*-maleimide **3**, N,N'-ethy-lene-*bis*-maleimide **4**, N,N'-*bis*-maleimidoxylalamine **5**, *m*-phenylene-*bis*-maleimidine **6** were pre-pared according to the known method.

2.1 *The general method for synthesis of hexamethylene(ethylene, oxalylamine, m-phenylene)-N,N'-bis(spiroindole-3,3'-pyrrolo[3,4-c]pyrrole-2a,5a'-dihydro-2,2',6'(1H,1'H,5'H)-triones) (7a-c, 8a-c, 9, 10a-c)*

The mixture of isatin **1** (2 mmol), appropriate α-amino acid **2** (2 mmol) and the dipolarophile **3**-**6** (1 mmol) was dissolved in a mixture of *i*-PrOH-H2O (3:1) and refluxed for 30 min - 10 h (for compound **7c** – 30 min; for compound **8b** – 1 h; for compounds **7a**, **7b**, **8c** – 2 h; for compounds **8a**, **9** – 4 h; for compound **10b** – 6 h; for compound **10c** – 8 h; for compound **10a** – 10 h). The resulting precipitate was filtered off, washed with mixture of *i*-PrOH-H2O (1:1) and recrystallized from *i*-PrOH-H2O (1:1) mixture.

2.2 *Characterizations*

^{1}H NMR spectra were recorded on a Varian WXR (400 MHz) spectrometer in DMSO-d6, using TMS, as an internal standard. Elemental analysis was performed on the analyzer Carlo Erba CHNS-O EA 1108. Infrared spectra were recorded on the Bruker Tensor 27 FT-IR device in the range 400-4000 cm-1 in KBr pellets. Electron impact mass spectra were recorded on a Varian 1200 L (Varian Inc., Palo Alto, CA) instrument at 70 eV. The progress of the reactions and purity of the obtained compounds were monitored by TLC on Silufol UV-254 plates (acetone-heptane 4:1 or H2O–MeOH 1:9).

2.3 *Antimicrobial activity study*

The microbiological experiment was carried out in accordance with WHO and the Ministry of Public Health of Ukraine recommendations. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 885-653 were used as test strains of microorganisms.

The antimicrobial activity of the synthesized substances was studied in vitro by the method of diffusion into agar in the modification of wells. The research was conducted in two stages. In the first stage, the antimicrobial activity of the drugs was studied in comparison with cefalexin and fluconazole (for the

Candida albicans culture). 1 mg of each substance was dissolved in 1 ml of DMSO. From the resulting suspension, 0.3 ml was collected and transferred to wells on Petri dishes with microbial cultures. The level of antimicrobial activity of substances was established by the diameter of the zone of growth retardation of microorganisms around the well compared with the control. The second stage of the research was the study of the antimicrobial activity of the substances relative to Staphylococcus aureus ATCC 25923 and *Candida albicans* ATCC 885-653 by the method of double serial dilutions in a liquid nutrient medium. The results were taken into account when determining the presence or absence of growth of microorganisms. To determine the minimum bactericidal / fungicidal concentration (MBC/ MFC), cultures were carried out on agar or broth from samples from the minimum inhibitory concentration (MIC) and incubated in a thermostat for 24 hours at 370C with meat-peptone agar and 250C for 48 hours with Saburo agar (*Candida albicans*).

2.4 *Antiradical activity study*

The study of antiradical activity was carried out *in vitro*. The method is based on the interaction of the studied substances with 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable free radical; its methanol solution is colored purple (λmax = 517 nm). DPPH interacts with a substance capable of binding free radicals, while the resulting product is colored yellow and no longer absorbs at this wavelength. Method: Two milliliters of 2 mM or 0.2 mM solution of studied compound in DMSO was mixed with 2 mL of 0.1 mM DPPH methanolic solution. Obtained mixture was incubated at the room temperature for 30 min, and optical density (Ad) was measured. The optical density of sample that was obtained by mixing of 2 mL of DMSO with 2 mL of 0.1 mM DPPH methanolic solution was determined simultaneously (ADPPH). Antiradical activity (ARA) was calculated by the next formula: ARA% = (ADPPH-Ad)/ADPPH × 100%. In the case of a negative meaning, ARA in % was estimated like 0. Weighing of reagents and synthesized compounds was conducted on electronic scales «ANG 200C» (Axis, Gdansk, Poland), and the optical density was measured by a spectrophotometer Spekol-1500 Analytik jena (Germany).

Mathematical calculations were performed using the STATISTICA 10 StatSoft Inc. system and Excel spreadsheet processor of MS Office 2019 Professional Plus.

3. Results and Discussion

A series of new *bis*-derivatives of spiroindole-3,3'-pyrrolo[3,4-c]pyrrole **7**-**10** were synthesized in high yields *via* three-component domino-interaction of isatin **1** with α-amino acids **2a-d** and dipolarophiles **3-6** (**Figure 1**). It is worth noting that the reaction required a strict maintenance of isatin/amino acid/dipolarophile (2:2:1) ratio. Otherwise, mono-derivatives were formed or it was difficult to isolate the product. The choice of the appropriate solvent was of great importance for the efficiency of the synthesis. Optimal conditions for synthesis were found by trial and error (**Table 1**). It was found that boiling the reagents in a mixture of isopropanol-water (3:1) gives the best result (mild reaction conditions, efficiency, high yields). Water was added to improve the solubility of amino acids. Isopropyl alcohol/water mixture (3:1) was chosen as the optimal solvent for the three-component interaction. It should be noted that in the case of ethanol or MeOH/H_2O (3:1) (widely used in the synthesis of similar derivatives[12,]) mixture used as a solvent reaction led to increase of reaction times and lower yields as well as purity of the target products. The products **7**a-c, **8**a-c, **9**, **10**a-c can be easily recrystallized from isopropanol-water mixture (1:1) and constitute amorphous powders of white or pale-yellow color.

Literature data evidence that the reaction mechanism involves *in situ* formation of unstable azomethynilides followed by their 1,3-dipolar cycloaddition to double bond of the symmetric dipolarophile.

The structure of the compounds **7**a-c, **8**a-c, **9**, **10**a-c was confirmed by ^{1}H NMR (**Table 2**), IR-spectroscopy (**Table 3**), mass-spectrometry and elemental analysis.

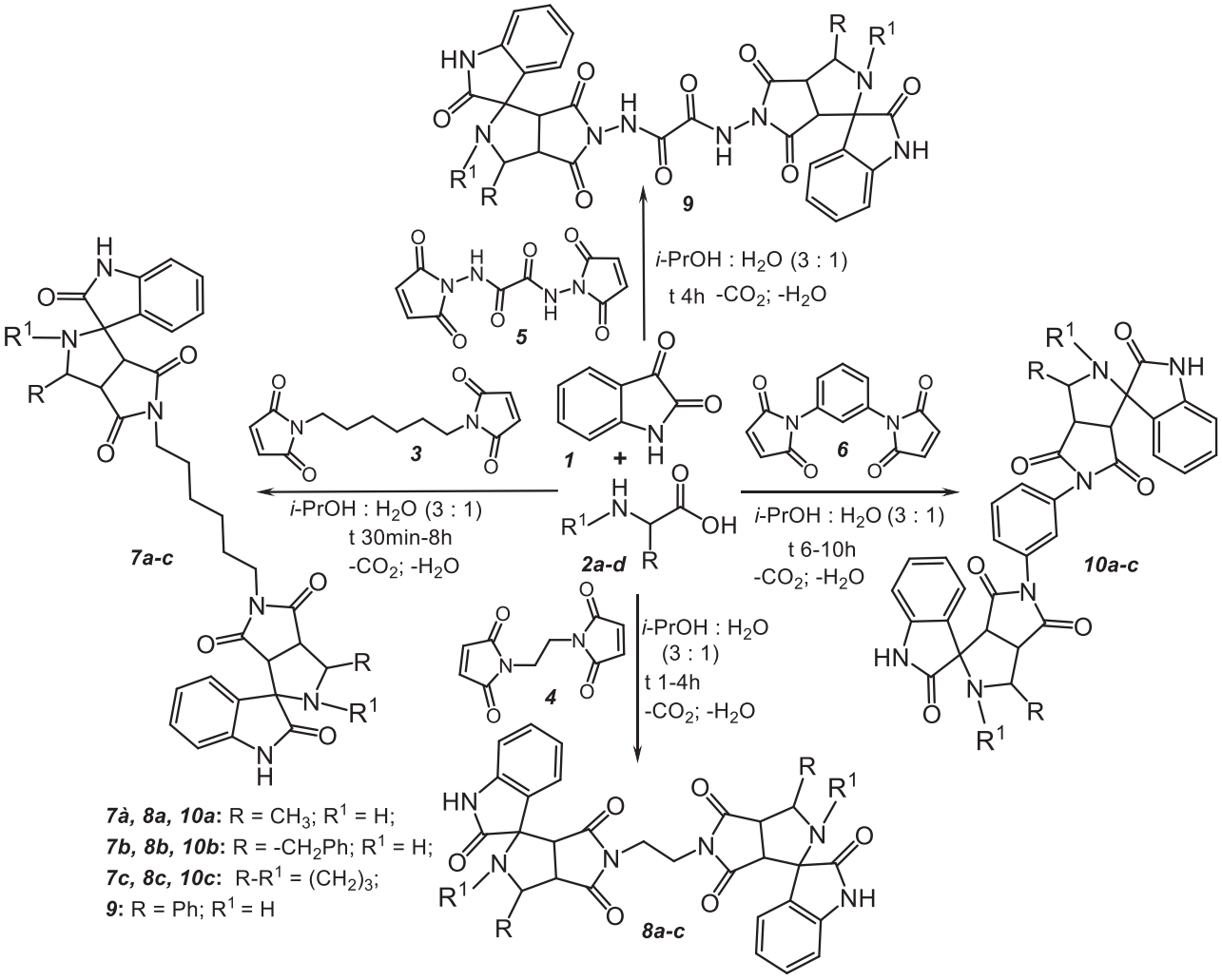

Figure 1*. Synthesis of hexamethylene(ethylene, oxalylamine, m-phenylene)-N,N'-bis(spiroindole-3, 3'-pyrrolo[3,4-c]pyrrole-2a',5a'-dihydro-2,2',6'(1H,1'H,5'H)-trione) derivatives 7a-c, 8a-c, 9, 10a-c*

Table 1. Optimization of solvent selection on the example of synthesis of compounds 7a, 8*b*, 7*c*

Solvent	Heating time, h			Yield, %		
	7a	**8*b***	**7*c***	**7a**	**8*b***	**7*c***
Propanol-2 - water (3:1)	2	1	0.5	75	90	81
Methanol-water (3:1)	2	2	1	60	82	64
Ethanol-water (3:1)	4	2	2	62	80	73
Acetonitrile-water (3:1)	5	3	2	60	74	68
1,4-Dioxane water (3:1)	5	4	3	55	50	55
Tetrahydrofuran	8	4	4	42	44	40

Table 2. Reaction times, yields, melting points and spectral data for hexamethylene(ethylene, oxalylamine, m-phenylene)-N,N'-bis(spiroindole-3,3'-pyrrolo[3,4-c]pyrrole-2a',5a'-dihydro-2,2', 6'-(1H,1'H,5'H)-triones) 7a-c, 8a-c, 9, 10a-c

Compound	Reaction times, yields, melting points and spectral data
1	**2**
7*a* R=CH_3, R^1=H $C_{34}H_{36}N_6O_6$	time 2 h; 75% yield; 260-262°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 1.13(d, 6H, 2×5'-CH_3, *J*=6.41), 1.32(m, 4H, CH_2CH_2), 1.52(m, 4H, CH_2CH_2), 3.17-3.24(d, 2H, 2×2'a-CH, *J*=7.9), 3.29-3.47(m, 6H, 2×5'a-CH, 2×1'-NCH_2), 3.65(d, 2H, 2×2'-NH, *J*=4.88), 4.19-4.38(m, 2H, 2×5'-CH), 6.67-6.95(m, 6H, ArH), 7.08-7.27(m, 2H, ArH), 10.33(c, 2H, 2×1-NH); EI-MS[M]$^+$ 624
7*b* R=Bn, R^1=H $C_{46}H_{44}N_6O_6$	time 2 h; 92% yield; 246-248°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 10.27(s, 2H), 7.33-7.18(m, 8H), 7.13(t, *J*=7.1, 4H), 6.84(m, 4H), 6.73(d, *J*=7.7, 2H), 4.33 (m, 2H), 3.54(d, *J*=3.7, 2H), 3.48-3.36(m, 6H), 3.25(d, *J*=7.6, 2H), 3.20 (dd, *J*=13.8, 5.5, 2H), 2.58(dd, *J*=14.0, 8.2, 4H), 1.57(m, 4H), 1.37(m, 4H) EI-MS[M]$^+$ 776
7*c* R-R^1=$(CH_2)_3$ $C_{38}H_{40}N_6O_6$	time 30 min; 81% yield; 150-152°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 1.29(m, 4H, CH_2CH_2), 1.49(m, 4H, CH_2CH_2), 2.27(m, 4H, 2×1'-NCH_2), 3.26-3.41 (m, 14H, 2×2'a-CH, 2×$CH_2CH_2CH_2$), 3.43-3.60(m, 4H, 2×5'a-CH), 4.20 (q, 2H, 2×5'-CH, *J*=7.02), 6.73-6.96(m, 6H, ArH), 7.11-7.31(m, 2H, ArH), 10.52(s, 2H, 2×1-NH) EI-MS[M]$^+$ 676
8a R=CH_3, R^1=H $C_{30}H_{28}N_{60}6$	time 4 h; 88% yield; 238-240°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 10.32(c, 2H); 7.15(c, 2H); 6.88(c, 4H); 6.74(c, 2H); 4.19(c, 2H); 3.60(c, 4H); 3.50(c, 2H); 3.25-3.11(м, 4H); 1.13(c, 6H) EI-MS[M]$^+$ 568
8*b* R=Bn, R^1=H $C_{42}H_{36}N_{60}6$	time 1 h; 90% yield; 302-304°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 10.32(s, 2H); 7.09-7.29(m, 12H); 6.92-6.99(m, 2H); 6.83-6.89(m, 2H); 6.74(d, *J*=7.63, 2H); 4.24-4.34(m, 2H); 3.59-3.76(m, 4H); 3.36-3.49(m, 4H); 3.27(d, *J*=7.93, 2H); 3.17-3.24(m, 2H); 2.57-2.67(m, 2H) EI-MS[M]$^+$ 720
8*c* R-R^1=$(CH_2)_3$ $C_{34}H_{32}N_6O_6$	time 2 h; 94% yield; 270-272°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 10.48(br. s, 2H); 7.20(br. s, 2H); 6.87(d, *J*=3.97, 4H); 6.78(d, *J*=7.63, 2H); 4.16(d, *J*=7.63, 2H); 3.49-3.65(m, 6H); 3.32-3.38(m, 2H); 2.32(d, *J*=6.71, 2H); 2.21(br. s, 2H); 1.78(br. s, 8H) EI-MS[M]$^+$ 620
9 R=Ph, R^1=H $C_{40}H_{30}N_8O_8$	time 4 h; 83% yield; 265-266°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 11.65(s, 2H), 10.34(s, 2H), 7.48-7.36(m, 4H), 7.24(m, 10H), 6.89(t, 2H), 6.79(d, *J*=7.7, 2H), 5.50(dd, *J*=7.7, 2.7, 2H), 4.14(s, 2H), 3.96-3.80(m, 2H), 3.57(m, 2H). EI-MS [M]$^+$ 750
10a R=CH_3, R^1=H $C_{34}H_{28}N_6O_6$	time 10h; 90% yield; 240-242°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 10.39(s, 2H, NH), 7.66(t, *J*=8.1, 1H), 7.47-7.31(m, 3H), 7.16(t, *J*=7.4, 2H), 7.03-6.95(m, 2H),6.84(t, *J*=7.5, 2H), 6.78(d, *J*=7.6, 2H), 4.31(d, *J*=6.6, 2H), 3.80(d, *J*=6.3, 2H),3.54(t, *J*=6.6, 2H),3.43(d, *J*=7.5, 2H),1.25(d, *J*=6.4, 6H) EI-MS [M]$^+$ 616
10*b* R=Bn, R^1=H $C_{46}H_{36}N_6O_6$	time 6h; 81% yield; 220-222°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 10.31(s, 2H), 7.71(t, *J*=7.9, 1H), 7.47(m, 3H), 7.40-7.30(m, 4H), 7.26(t, *J*=7.4, 4H), 7.15 (dd, *J*=12.5, 6.9, 4H), 7.07(d, *J*=7.4, 2H), 6.86(dd, *J*=16.1, 8.1, 2H), 6.77(d, *J*=7.6, 2H), 4.46-4.34(m, 2H), 3.80(d, *J*=6.8, 2H), 3.67(t, *J*=7.3, 2H), 3.51-3.43(m, 2H), 3.28(m, 2H), 2.78(dd, *J*=12.3, 8.5, 2H) EI-MS[M]$^+$ 768
10*c* R-R^1=$(CH_2)_3$ $C_{38}H_{32}N_6O_6$	time 8h; 73% yield; 330-332°C; **^{1}H NMR, δ, ppm (*J*, Hz)** 10.58 (c, 2H, NH), 7.73-7.53 (м, 1H), 7.46-7.14 (м, 3H), 7.08-6.71 (м, 8H), 4.29 (д, *J*=6.8, 2H), 3.78-3.64 (м, 4H), 2.50 (м, 2H), 2.36 (м, 2H), 1.95 (м, 8H) EI-MS[M]$^+$ 668

Table 3. Characteristic bands in IR-spectra of hexamethylene(ethylene)-*N,N'-bis*(spiroindole-3, 3'-pyrrolo[3,4-*c*]pyrrole-2a',5a'-dihydro-2,2',6' (1*H*,1'*H*,5'*H*)-triones) 7a-c, 8a-c, 9, 10a-c

Cmp	IR, cm^{-1}				
	ν NH	ν CH(Ar)	ν CH(Alk)	ν C=O	ν C=C(Ar)
7*a*	3340, 3232	3087	2957, 2935	1693	1619, 1473
7*b*	3325	3062, 3029	2940, 2862	1702	1620, 1453
7*c*	3384	3087	2934, 2857	1697	1619, 1472
8*a*	3269, 3177	3030	2933, 2870	1703	1624, 1473
8*b*	3250, 3185	3061, 3028	2955, 2895	1707	1620, 1471
8*c*	3265, 3207	3096	2962, 2879	1713	1620, 1473
9	3330	3030	2962	1735	1618, 1471
10*a*	3327	3026	2962, 2879	1713	1619, 1601, 1493, 1471
10*b*	3382	3030	2950	1713	1619, 1601, 1492, 1471
10c	3402	3030	2961	1714	1619, 1492, 1471

^{1}H NMR spectra of compounds **7*a-c*** are characterized by the following features. NH-signals of 2-oxindole moiety appear as singlets and can be found in the range of 10.38-10.86 ppm. Methylene protons of pyrrolo[3,4-c]pyrrole molecular framework give rise to doublet at 3.40-3.50 ppm for H-2a', triplet at 3.50-3.60 ppm for H-5a' or H-7a' for compound **7*c*** and multiplet at 4.00-4.40 at for H-5'. Protons H-2a', H-5a' and H-5' are most likely to have *cis*-orientation owing to their *J*-coupling values of 7-8 Hz.

^{1}H NMR spectra of compounds **8*a-c*** comprise a set of ABCD spin system signals for 2-ox-indole fragment which appear as two doublets and two triplets. These spectra are also characterized by the presence of signals of ethylene linkage protons in the range of 7,00-7,50 ppm. Downfielded protons of 2-oxindole core NH-group can be observed at around 10,30 ppm. Signals for NH protons of pyrrolo[3,4-c]pyrrole moiety for compounds **8*a*, 8*b*** are in the range of 3,50-3,80 ppm and are absent in the case of compound **8**c.

^{1}H NMR spectra of compounds **10*a-c*** include the following characteristic signals. *M*-Pheny-lene fragment gives two signals, specifically triplet at 7.65 ppm and multiplet in the range of 7.40 ppm; NH-protons of the oxindole moiety and the pyrrole fragment appear at around 10.40 ppm and 3.40 ppm correspondingly; ABCD spin system signals for 2-oxindole fragment can be found at 6.80-7.20 ppm. Peak relating to NH group of pyrrole moiety is not observed in 1H NMR spectrum of compound **10*c***.

Compounds **7**a-c, **8**a-c, **9**, **10**a-c were tested for antimicrobial activity against both Gram-positive and Gram-negative bacteria strains as well as against fungal species of *Candida albicans* (**Table 4**).

Table 4. Antimicrobial activity data for hexamethylene(ethylene, oxalylamine, *m*-phenylene)-*N,N'-bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-2a',5a'-dihydro-2,2',6'-(1*H*,1'*H*,5'*H*)-triones) 7a-c, 8a-c, 9, 10a-c

Compound	Diameter of the growth inhibition zones, mm (the mean for three experiments)				
	Gram-positive bacteria		Gram-negative bacteria		Fungi
	B. subtilis	*S. aureus*/ **MBC,** μg/ml	*E. coli*	*P. aeruginosa*	*C. albicans*/ **MFC,** μg/ml
1	**2**	**3**	**4**	**5**	**6**
7a	27±0.15	22±0.56/50	18±0.65	growth	26±0.32/*12.5*
7b	27±0.38	18±0.17/growth	15±0.56	growth	28±0.27/growth
7c	25±0.19	20±0.51/growth	17±0.50	growth	19±0.18/growth
8a	24±0.21	24±1.15/*50*	20±0.57	21±0.19	23±0.34/*12.5*
8b	19±0.19	19±0.58/growth	20±0.58	22±0.35	23±0.87/growth
8c	21±0.17	15±0.11/growth	19±1.10	22±0.42	22±0.90/growth
9	17±0.52	growth/growth	18±0.53	growth	growth/growth
10a	26±0.18	31±0.45/*50*	15±0.61	growth	35±0.45/growth
10b	25±0.22	27±0.58/growth	20±0.45	growth	25±0.35/growth
10c	26±0.53	27±0.58/growth	21±0.55	growth	28±0.60/growth
Control	growth	growth	growth	growth	growth
Cefalexinum	32±0.37	32±0.55/*12.5*	35±0.24	36±0.35	n/t
Fluconazole	n/t	n/t	n/t	n/t	16±0.34/*3.125*

growth - the growth of microorganisms continues even with test compound or without it, as in the control

Diameters of the growth inhibition zones for tested compounds against *C. albicans* were higher compared to those of the reference drug and minimal fungicidal concentration for compounds **7a**, **8a** was 12.5 mg/mL. Compound **10a** showed high level of antibacterial activity against *S. aureus* which was comparable to those of reference drug. It was found that compounds **8**a-c containing pharmacophores linked by ethylene moiety are active against *P. aeruginosa*, at the same time other tested compounds turned out to be inactive against this strain. The worst antimicrobial activ-

ity showed compound **9**, comprising oxalylamine linkage. According to the results of microbiological screening, the three most active compounds were found in a number of test substances: hexamethylene-*N,N'-bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-metyl-2a',5a'-dihydro-2,2',6' (1*H*,1'*H*,5'*H*)-trione) **7*a***, ethylene-*N,N'-bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-metyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **8*a***, *m*-phenylene-*N,N'-bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-5'-metyl-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) **10*a***. All three compounds have a methyl radical in position 5'. In reference the authors reported a similar regularity. It was also found that antimicrobial activity of the tested compounds significantly depends on binding fragment between two the same pharmacophores as well as 5'-substituent in *bis*-derivatives of spiroindole-3,3'-pyrrolo[3,4-***c***]pyrrole.

The study of antiradical activity was carried out *in vitro*. The method is based on the interaction of the studied substances with 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable free radical; its methanol solution is colored purple (λmax = 517 nm). DPPH interacts with a substance capable of binding free radicals, while the resulting product is colored yellow and no longer absorbs at this wavelength.

Studies in vitro on the one hand are a powerful prediction tool for drug search and SAR analysis. On the other hand, they provide humane treatment of animals in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes[33].

One of these methods is the determination of antiradical activity which correlates with other types of activity (in particular antioxidant, anti-inflammatory, anticonvulsant). This is due to the fact that many pathological processes are accompanied by the overproduction of reactive oxygen forms with subsequent damage to lipids, amino acids, nucleic acids and some functional proteins[33]. A range of spirocondensed 2-oxindoles displays a high level of antiradical activity relative to the reference drug ascorbic acid[34]. Taking into account this fact, it was of interest to study the antiradical effect of the studied compounds (**Table 5**).

Table 5. Antiradical activity data for hexamethylene(ethylene, oxalylamine, *m*-pheny-lene)-*N,N'-bis*(spiroindole-3,3'-pyrrolo[3,4-*c*]pyrrole-2a',5a'-dihydro-2,2',6'-(1*H*,1'*H*, 5'*H*)-triones) 7a-c, 8a-c, 9, 10a-c

Compound	Antiradical activity, %	
	1×10^{-3}M	1×10^{-4}M
Ascorbic acid	63.8±1.37	16.9±0.83
7a	3.8±1.10	1.7±0.43
7b	4.3±0.02	2.6±0.01
7c	5.1±0.71	1.4±0.64
8a	3.4±1.05	1.3±0.25
8b	5.2±0.23	1.8±0.65
8c	5.9±1.04	1.8±0.48
9	42.8±1.10	13.3±1.65
10a	6.0±0.82	4.1±1.51
10b	5.0±1.10	3.1±1.05
10c	5.7±0.62	2.1±0.48

The experiment showed that the synthesized compounds **7a-c**, **8a-c**, **10a-c** do not show antiradical activity. Only compound **9** shows a reasonable level of activity relative to the reference drug – ascorbic acid. It should be noted that the lack of antiradical activity for most compounds in this model (in the experiment with DPPH) does not mean their lack of antioxidant properties, as was shown in reference.

4. Conclusion

Series of symmetrical derivatives of hexamethylene(ethylene, oxalylamine, *m*-phenylene)-*N,N*'-*bis*(spiroindole-3,3'-pyrrolo[3,4-*c*] pyrrole-2a',5a'-dihydro-2,2',6'(1*H*,1'*H*,5'*H*)-trione) was synthesized for the first time by means of 1,3-dipolar cycloaddition of *in situ* generated azomethynilides to dipolarophiles containing *bis*-maleimide fragment. In this regard 1,3-cycloaddition reactions involving *N,N'*-hexamethylene-*bis*-meleimide, *N,N'*-ethylene-*bis*-meleimide, *N,N'-bis*-maleimido-xalylamine and *m*-phenylene-*bis*-maleimide as dipolarophiles were studied for the first time. This fact allowed us to obtain new *bis*-derivatives of spiroindol-3,3'-pyrrolo[3,4-c]pyrrole in which two spiroheterocyclic fragments are linked by different bridges, such as ethylene, oxalylamine and phenylene moieties. Antimicrobial screening of compounds synthesized revealed three the most promising substances which showed significant activity level against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans*. Antiradical screening of synthesized compounds revealed the most promising substance, which showed a reasonable level of activity. □

Conflict of Interest *The authors declare that they have no conflict of interest.*

References

1. Kolesnik S.V., Altuhov A.A., Akhmedov E.Y., Tarjanik E.L. Synthesis, properties and biological activity of the amides of 2-(benzx)ylamino) (2-oxoindolin-3-ylidene)acetic acid. *Azerbaijan Pharmaceut. and Pharmacother.* J. 1, 14-18, 2014.
2. Singh G.S., Desta Z.Y. Isatins As Privileged Molecules in Design and Synthesis of Spiro-Fused Cyclic Frameworks. *Chem. Rev.* 112, 6104-6155, 2012.
3. Shvets A.A., Kurbatov S.V., Sintez bis-β,β'-spiropirrolidinoksindolov, soderzhashchikh rodaninovyy fragment. *Khimiya geterotsiklicheskikh soyedineniy.* 48, 859-866, 2012.
4. Hu M.K., Wu L.J., Hsiao G., Yen M.H. Homodimeric tacrine congeners as acetylcholinesterase inhibitors. *J. Med. Chem.* 45, 2277-2282, 2002.
5. Kang T.H., Matsumoto K., Tohda M. Pteropodine and isopteropodine positively modulate the function of rat muscarinic M1 and 5-HT2 receptors expressed in Xenopus oocyte. *Eur. J. Pharmacol.* 444, 39-45, 2002.
6. Cui C.B., Kakeya H., Osada H. Novel mammalian cell cycle inhibitors, spirotryprostatins A fnd B, produced by Aspergillus fumigatus, which inhibit mammalian cell cycle at G2/M phase. *Tetrahedron*. 52, 12651-12666, 1996.
7. Song F., Liu X., Guo H., Ren B., Chen C., Piggott A.M., Capon R.J. Brevianamides with Antitubercular Potential from a Marine-Derived Isolate of Aspergillus versicolor. *Org. letters.* 14, 4770-4773, 2012.
8. Qu J., Fang L., Ren X.D., Liu Y., Yu S.S., Li L., Ma S.G. Bisindole Alkaloids with Neural Anti-inflammatory Activity from Gelsemium elegans. *J. Nat. Prod.* 76, 2203-2209, 2013.
9. Zhang W., Huang X.J., Zhang S.Y., Zhang D.M., Jiang R.W., Hu J.Y., Ye W.C. Geleganidines A–C, Unusual Monoterpenoid Indole Alkaloids from Gelsemium elegans. *J. Nat. Prod*. 78, 2036-2044, 2015.
10. Dudley A.C., Shih S.C., Cliffe A.R., Hida K., Klagsbrun M. Attenuated p53 activation in tu-

mour-associated stromal cells accompanies decreased sensitivity to etoposide and vincristine. *J. Cancer.* 99, 118-125, 2008.
11. Kim S.Y., Roh H.J., Seo D.Y., Ryu J.Y., Lee J., Kim J.N. Base-catalyzed one-pot synthesis of dispiro-1,3-dioxolane bisoxindoles from N-methylisatin and methyl propiolate. *J. Tetrahedron Lett.* 58, 914-918, 2017.
12. Bharitkar Y.P., Das M., Kumari N., Kumari M.P., Hazra A., Bhayye S.S., Mondal N.B. Synthesis of Bis-pyrrolizidine-Fused Dispiro-oxindole Analogues of Curcumin via One-Pot Azomethine Ylide Cycloaddition: Experimental and Computational Approach toward Regio- and Diastereoselection. *Org. Letters.* 17, 4440-4443, 2015.
13. Jein M., Khanna P., Saxena A., Bhagat S., Olsen C.E., Jain S.C. Synthesis of Some Novel bis-spiro[indole-pyrazolynil-thiazolidine]-2,4'-diones. *Synth. Commun.* 36, 1863-1872, 2006.
14. Ramu P., Augustine Arul Prasad T., Scholastica M.V., Arul S.A. Synthesis, characterization and biological activity of novel spiroheterocycles from isatin derivatives. *Der Pharma Chem.* 6, 30-36, 2014.
15. Tsukamoto S., Umaoka H., Yoshikawa K., Ikeda T., Hirota H. Notoamide O, a Structurally Unprecedented Prenylated Indole Alkaloid, and Notoamides P-R from a Marine-Derived Fungus, Aspergillus sp. *J. Nat. Prod.* 73, 1438-1440, 2010.
16. Kato S., Yoshino T., Shibasaki M., Kanai M., Matsunaga S. Catalytic Asymmetric Synthesis of Spirooxindoles by a Mannich-Type Reaction of Isothiocyanato Oxindoles. *Angew. Chem. Int. Ed*. 51, 7007-7010, 2012.
17. Zhao K., Zhi Y., Li X., Puttreddy R., Rissanen K., Enders D., Asymmetric synthesis of 3,3'-pyrrolidinyl-dispirooxindoles via a one-pot organocatalytic Mannich/deprotection/aza-Michael sequence. *Chem. Commun.* 52, 2249–2252, 2016.
18. Bian Z., Marvin C.C., Martin S.F. Enantioselective Total Synthesis of (−)-Citrinadin A and Revision of Its Stereochemical Structure. *J. American Chem. Society.* 135, 10886-10889, 2013.
19. Kong L.M., Feng T., Wang Y.Y., Li X.Y., Ye Z.N., An T., Qing C., Luo X.D., Li Y. Bisleuconothine A, a bisindole alkaloid, inhibits colorectal cancer cell in vitro and in vivo targeting Wnt signaling. *Oncotarget*. 7, 10203-10214, 2016.
20. Lievina R.M. (1961). Metody polucheniia khimicheskikh reaktivov i preparatov, Moscow, 85.
21. Balouiri M., Sadiki S. Methods for in vitro evaluating antimicrobial activity. *J. Pharm Anal.* 6, 71-79, 2016.
22. Patel J.B., Cockerill F.R., Bradford P.A., Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Document M100-S25. Clinical and Laboratory Standards Institute. 35(3), 2015.
23. Volianskyi Y.L., Hrytsenko I.S., Shyrobokov V.P., Vyvchennia spetsyfichnoi aktyvnosti protymikrobnykh likarskykh zasobiv. *Kyiv: DFTs MOZ Ukrainy* 38, 2004.
24. Derzhavna farmakopeia Ukrainy, 1 vyd. (2001) Kharkiv: Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv.
25. Kedare S.B., Singh R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci Technol.* 48, 412-422, 2011.
26. Szabo M., Iditoiu C., Chambre D., Lupea A. Improved DPPH determination for antioxidant activity spectrophotometric assay. *Chem. Papers*. 61, 214-216, 2007.
27. Azizian J., Asadi A., Jadidi K. One-pot highly diastereoselective synthesis of new 2-substituted 8-(spiro-3'-indolino-2'-one)-pyrrolo[3,4-a]-pyrrolizine-1,3-diones mediated by azomethine ylide induced by microwave irradiation. *Synth. Commun.* 31, 2727-2733, 2001.
28. Pavlovska T.L., Redkin R.G., Lipson V.V., Atamanuk D.V., Molecular diversity of spirooxindoles. Synthesis and biological activity. *Mol. Divers.* 20, 299-344, 2016.
29. Syumka Y.I., Red'kin R.G., Shemchuk L.A., Hlebova K.V., Filimonova N.I. Synthesis and antimicrobial activity of hexamethylene-N-maleinimidospiroindole-3,3'-pyrrolo[3,4-c]pyrrole derivatives. *J. Org. Pharm. Chem.* 15, 56-62, 2017.

30. Golik N.Y., Komissarenko A.N., Kolesnik S.V., Ahmedov E.Y., Osolodchenko T.P., Menkus Y.V. Antimicrobial activity of the N-R-Alkylamintes derivatives. *Azerbaijan Pharmaceut. and Pharmacother*. J. 2, 21-24, 2015.
31. Directive 2010/63/eu of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* (2010) L 276, 33-79.
32. Directive 2010/63/eu of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* (2010) L 276, 33-79.
33. Voskoboynik O.Y., Kolomoets O.S., Nosulenko I.S., Berest G.G., Bilyi A.K., Karpenko O.V., Belenichev I.F., Kovalenko S.I. Synthesis, Modification, and Anticonvulsant Activity of 3'-R1-Spiro[indoline-3,6'-[1,2,4]triazino[2,3-c]quinazolin]-2,2'(7'H)-diones Derivatives. *J. Heterocycl. Chem.* 56, 1605-1612, 2019.
34. Korobko D., Hadjipavlou-litina D., Logoyda L. Antioxidant and anti-inflammatory properties of a series of new 7,8-disubstituted theophylline containing a pyrazole ring. *Asian J. Pharm. Clin.Resh.* 11, 448-450, 2018.
35. Kazunin M.S., Voskoboynik O.Y., Nosulenko I.S., Berest G.G., Sergeieva T., Okovytyy S., Karpenko O.V., Priimenko B.O. and Kovalenko S.I. Synthesis, Tautomerism, and Antiradical Activity of Novel Pteridinetrione Derivatives. *J. Heterocycl. Chem.* 55, 1033-1041, 2018.

ΕΚΔΗΛΩΣΕΙΣ - MEETINGS

• APRIL 18-20, 2022, TOKYO, JAPAN
INTERNATIONAL MEET ON PHARMACEUTICS AND DRUG DELIVERY SYSTEMS (PHARMAMEET2022)
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5TH RUSSIAN CONFERENCE ON MEDICINAL CHEMISTRY
Info: https://medchem21.com/index.php/en/home-english/ medchemvolga21@gmail.com

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12TH JOINT MEETING ON MEDICINAL CHEMISTRY 2022
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