

## Editorial Board

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## Review

### LABORATORY ASPECTS AND CLINICAL SIGNIFICANCE OF BONE TURNOVER MARKERS

7-18

**Yehudina Ye.D., Golovach I.Yu.**

**Introduction.** With an aging population, there is a marked increase in prevalence of metabolic bone diseases, especially osteoporosis. A serious complication of osteoporosis is non-traumatic bone fractures, which significantly impair quality of life and are associated with comorbid conditions and high mortality. Diseases associated with impaired bone remodeling require timely diagnosis, treatment and monitoring. The consequent public health and socioeconomic burden warrant timely diagnosis, treatment and follow-up of these disorders. Knowing the limitations of radiological techniques, biochemical markers of bone turnover measurements come handy since the changes in their levels readily reflect bone physiology. **Material and methods.** This paper presents a literature review concerning bone turnover biomarkers with the aim of providing comprehensive information on the applicability of these biomarkers for clinical use. A literature search was conducted in the PubMed, MedLine, Scopus and Embase databases from 1987–2019. Keywords used for search: bone turnover, bone formation, bone resorption, bone biomarkers, biochemical markers of bone turnover. We aimed to determine the clinical effectiveness, test accuracy, reliability, reproducibility and cost-effectiveness of bone turnover markers for monitoring the response to osteoporosis treatment, predicting bone loss and fracture risk, diagnostic of osteoporosis, Paget's disease, renal osteodystrophy, and certain oncological conditions and rheumatic diseases. **Results and discussion.** Bone turnover markers are a series of protein or protein derivative biomarkers released during bone remodeling by osteoblasts or osteoclasts. Bone biomarkers typically analyzed in high throughput automated routine laboratories are collagen degradation products, reflecting osteoclast activity and collagenous or non-collagenous proteins produced by the osteoblasts. All these markers can be quantitated well from blood samples, serum being the preferred sample of choice. Although assays for urine examination were developed for quite a few markers, blood sampling generally detours the pre-analytic issues usually involving urine sampling. The most commonly used bone resorption and bone formation markers are discussed in this article. Biochemical markers of bone resorption are mainly different fragments of type I collagen, as well as non-collagen proteins that enter the circulation from the bone matrix resorption zone. The main biochemical indicators used in clinical practice as a criterion for bone tissue resorption are pyridinoline, deoxypyridinoline, tartrate-resistant acid phosphatase, and degradation products of type I collagen - C- and N-terminal telopeptide. All the afore mentioned markers have since been superseded by the more sensitive and specific telopeptides of type I collagen, namely the C-terminal telopeptide (CTX). As compared to the bone resorption biomarkers, there is a larger repertoire of biomarkers of bone formation, reflecting osteoblast activity, namely serum bone-specific alkaline phosphatase, osteocalcin and procollagen type I N-terminal propeptide (PINP). Although produced by the osteoblasts, osteocalcin may be defined as a bone turnover marker reflecting both bone formation and bone resorption, since it is also released from the bone matrix during bone resorption. PINP is more extensively described in literature are compared to the other bone formation biomarkers. **Conclusion.** Biochemical markers of bone turnover reflect bone homeostasis, i.e., the activity of osteoblasts and osteoclasts in both physiological and pathophysiological conditions. Although quite sensitive to a multitude of exogenous and endogenous pre-analytical factors, bone markers are best used in monitoring anti-osteoporosis therapy efficacy and compliance. Combination of bone mineral density measurement by dual energy x-ray absorptiometry with biochemical markers of bone turnover levels, at least one bone resorption and one bone formation marker, may potentially improve early detection of individuals at increased risk for bone loss and eventually non-traumatic bone fracture. Furthermore, they have widespread clinical utility in osteoporosis, renal osteodystrophy, certain oncological conditions and rheumatic diseases.

**Keywords:** diagnostics, bone metabolism, bone formation, bone resorption, biomarkers of bone tissue, biochemical markers of bone metabolism.

## Experimental works

### BIOLOGICAL PROPERTIES OF CLINICAL STRAINS OF ESCHERICHIA COLI FROM DIFFERENT BIOTOPES

19-25

**Voyda J.V., Birukova S.V.**

**Introduction.** The results of laboratory diagnostics based on a limited number of phenotypic tests do not always allow to estimate with confidence the etiological significance of Escherichia (even if their serological belonging to a certain group or biovar is taken into account). The accumulated data indicate the need for an in-depth study of the intraspecific diversity of E. coli. The aim of the work is to study phenotypic properties (morphological, cultural, biochemical, hemolytic activity) and genotypic characteristics (evaluation of plasmid prevalence and determination of their molecular size) of clinical isolates of E. coli from various biotopes, and to establish the prevalence of clinically significant multidrug-resistant strains among them. **Material and methods** The morphological, cultural, biochemical properties, hemolytic activity of 677 clinical strains of E. coli isolated from different biotopes have been investigated. The material has been sown on the 5% blood agar for accounting of the hemolytic forms. The sensitivity of E. coli to the antibacterial drugs has been performed by disco-diffusion method Keurby-Bauer using standard commercial discs on medium Mueller-Hinton. The study of the plasmid spectrum has been

carried out using the alkaline method. **Results and discussion.** Coliform isolates with hemolytic phenotype colonize the intestines of persons with dysbiosis 4 times more often than in the control group (hemolytic active representatives of the control group have been found in  $(8,2 \pm 1,5)$  % of cases - according to accepted standards, the percentage that is allowed). Strains with hemolytic activity have been present in all groups of extracellular localization. Most often, this feature has been among the strains that were removed from the urine with complicated urinary system infection ( $(66,6 \pm 7,5)$  %). The antibiotic susceptibility of escherichias with hemolytic phenotype has been studied. A high prevalence of multidrug-resistant strains with hemolytic properties has been established, both among representatives of normal intestinal microflora ( $(49,2 \pm 9,8)$  %), and among uropathogenic serotypes ( $(87,2 \pm 5,4)$  %). All strains that have been removed from the respiratory tract and areas of the skin and soft tissues were characterized by multiple resistance to the studied antibiotics, which is typical for hospital strains. Plasmid profile of 35 clinical strains of *E. coli* has been studied and plasmids have been found in 88.6% of *Escherichia* strains from different biotopes, most of the isolated strains were multiplasmid. The strains had from 1 to 8 plasmids with sizes from 1 to 24 kb. It often has been found plasmid with size 19,3 among the studied multidrug-resistant escherichias. **Conclusion.** It has been set that the isolates of *E. coli* with hemolytic phenotype meet in all analyzed groups, frequency of exposure depends on the presence of festering-inflammatory process. The high sensitiveness to imipenem ( $(96,6 \pm 1,5)$  % sensitive strains), gatifloxacin ( $(92,4 \pm 2,2)$  %) and amikacin ( $(80,0 \pm 3,3)$  %) has been found out, as well as growth of resistance to the third generation cephalosporins (due to production of  $\beta$ -lactamases), other fluoroquinolones and circulation of generous amount mildly resistant strains to the derivatives of nitrofurans and nitroloxolium, which occupy a leading place in the charts of the protracted empiric therapy of patients with chronic pyelonephritis. Plasmid profiles are quite individual strain characteristic, which determines one of the phenotypic characteristics of the pathogen. The study of plasmid DNA isolated from clinical strains will make possible to determine with greater certainty the multidrug-resistant strains that cause nosocomial infections and to identify ways of spreading hospital infection in a particular hospital.

**Key words:** *Escherichia coli*, biotope, biochemical properties, hemolytic activity, antibiotics, antibiotic resistance, plasmid profile.

## ANTI-MEASLES IMMUNITY ASSESSMENT IN UKRAINIAN HEALTH WORKERS AND SCHOOL TEACHERS 26-29

Volyanskiy A. Y., Kuchma I.Y., Kuchma M.V., Klisa A.A., Stepnova Yu. B.

**Introduction** Adults suffer from measles harder than children and more frequently have complications. Medics and school teachers are at increased risk of measles disease. In the case of measles, medics and teachers can infect a large number of people in contact with them with possible serious consequences. The study of anti-measles humoral immunity in doctors and teachers of Ukraine during the measles epidemic in the country is to identify and vaccinate susceptible individuals. **Materials and methods** In February-March 2019, the levels of specific anti-measles IgG in blood serum of 981 medics and 308 teachers aged 18 to 85 were analyzed. The concentration of anti-measles IgG was determined by ELISA using Ridascreen enzyme-linked immunosorbent assays produced by r-Biopharm (Germany) using the Lisa Scan EM enzyme-linked immunosorbent analyzer (Czech Republic). In accordance with the instructions measles IgG levels of 150 mIU/ml or less were considered a negative result, from 151 to 200 mIU/ml inclusive - a dubious result, above 200 mIU/ml - a positive result. Statistical processing of the obtained data was carried out using non-parametric statistics methods with the Atte Stat 12.0.5 statistical software package integrated into Microsoft Excel 2013. The average values of IgG concentration by age groups are equal to sample medians, since sample distributions do not fit the normal distribution criteria according to Kolmogorov-Smirnov confidence criterion estimates. **Results and discussion.** It was shown that the largest number of measles-susceptible medics and teachers was observed in the age group of 18 to 27 years (7.5% did not have a protective level of specific anti-measles IgG and 5.6% showed a dubious level of protection). In the age groups from 28 to 37 years old and from 38 to 47 years old, a rather large number of measles-susceptible individuals were also detected (5.3% and 4.6% with no protective level of anti-measles IgG and 5.0% and 3.7 % - with a dubious level of protection, respectively). The minimum number of medics and teachers unprotected from measles was determined in the group aged 48 to 57 years (0.6% of negative and 0.3% of doubtful results), and among the group over 57 years of age, there were no individuals with a lack of protective levels of antibodies to measles virus. The possible reasons for the predominance of teachers and health workers unprotected from measles in the age groups from 18 to 47 years and the absence of unprotected people over 57 years of age are discussed. **Conclusion** The authors of the article concludes that there is no need for vaccination against measles in medics and teachers over 57 years of age and recommends vaccination of representatives of other age groups only after determining of the anti-measles immunity level, since more than 90% of the tested population have protective IgG levels.

**Key words:** vaccination, measles, specific anti-measles IgG, health workers, school teachers

## DYNAMICS OF IMMUNE STATUS INDICATORS IN PATIENTS WITH RELAPSING-REMITTING MULTIPLE SCLEROSIS BEFORE AND AFTER TREATMENT OF BETFER-1A DRUG 30-34

Vdovichenko N.I., Kolyada T. I., Tupotilov O. V., Negreba T. V., Kolyada O. M.

The article presents the results of examination of 58 patients with relapsing-remitting multiple sclerosis (RRMS) treated IFN- $\beta$ . Depending on the effectiveness of treatment, patients were divided into two groups: responders, included 37 persons (64%), in this group patients did not relapse during the year from the beginning of treatment, the degree of EDSS remained unchanged or decreased, the group EDSS before the start of therapy was  $2.2 \pm 1.4$  points. After therapy, EDSS was  $2.1 \pm 1.4$  points. - non-responders, this group included 21 individuals, the patient was included in the group if he had at least one relapse during the year and / or an increase in the degree of disability on the EDSS was found to be 1 point or more. The group EDSS before the start of therapy was  $2.5 \pm 1.5$  points. After therapy, EDSS was  $3.0 \pm 1.5$  points. The immunological characteristics of the patients are presented, depending on the clinical efficacy of the treatment; the presence of disease-associated polymorphic variants of HLA-DR has been determined. After the course of therapy with IFN- $\beta$ , in patients with RRMS, regardless of the clinical efficacy of treatment, the relative number of T-lymphocytes and natural killer cells remained significantly reduced. In the group of responders levels of circulating immune complexes and IgG levels did not differ from control after treatment, in contrast to the levels of lymphocytotoxic autoantibodies and complement activity, which remained elevated relative to the control. After treatment in patients with low efficacy of IFN- $\beta$  therapy (nonresponders), the relative number of CD19+ cells, as well as the levels of IgG, circulating immune complexes and lymphocytotoxic autoantibodies remained elevated relative to the control group. The presence of the haplotype HLA-DRB1\*1501-DQB1\*0602 (one of its specific markers is allele G SNP rs9271366) is established to determine the association between the disease-associated polymorphism of HLA-DR and the effectiveness of IFN- $\beta$  therapy. The presence of the minor disease-associated G allele was determined in 11 responders (29.7%) (heterozygous), another 26 patients (70.3%) were homozygous for major allele A. In the group of non-responders, 9 persons (42, 9%) were heterozygous, 12 (57.1%) were homozygous for major allele A.

**Keywords:** multiple sclerosis, IFN- $\beta$ , immune status, disease-associated gene polymorphism

## IMBALANCE OF IgG SUBCLASSES IN PRESCHOOL CHILDREN WITH A DIFFERENT TYPE OF VACCINE RESPONSE TO MMR 35-38

Smilianska M.

**Introduction.** The only way to protect children against measles, mumps and rubella viruses (MMR) is vaccination. However, among vaccinated individuals there are always people who react poorly to vaccine preparations until they are completely insensitive due to their individual genetic or phenotypic characteristics. It is not always possible to achieve a full protective effect of vaccination. A comprehensive study of humoral immunity factors, such as total IgG, their subclasses, virus-specific antibodies, can predict the nature of the formation of a specific immune response to a vaccine, it is important when choosing a tactic for vaccination of preschool children. The aim of the work was

to determine the subclasses of IgG (1, 2, 3, 4), their relationship and relationship with the type of vaccine immune response to the MMR vaccine in children of preschool age. **Material & methods.** The content of serum IgG and subclasses was studied in children 6–7 years old with no specific response or a very strong vaccine response to one or more components of the MMR vaccine and it was 23% ARI, or 32 children and 31% HRI, or 44 children, respectively. The control group consisted of 19 children of the appropriate age without herpes virus load and with a normal vaccination response to the MMR. Antibodies of the IgG class to the measles virus were determined using an enzyme immunoassay test system manufactured by IBL international GMBH - Meales virus IgG ELISA (Germany); rubella virus was determined using the enzyme immunoassay test system Rubella IgG-ELISA manufactured by Xema Co.Ltd. (Kiev, Ukraine); to the mumps virus was determined using an enzyme immunoassay test system manufactured by R-Biopharm AC (Germany) - RADASCREEN® Mumps Virus IgG (K5521). To determine the content in the serum of IgG subclasses (IgG1, IgG2, IgG3, IgG4), ELISA was used using the IgG subclasses test systems manufactured by Vector Best. The determination of the antigens of the Herpesviridae family was carried out by an immunofluorescence method using specific monoclonal mouse antibodies of Santa Cruz Biotechnology, Inc. (USA). **Results.** An imbalance of IgG subclasses in preschool children with a different type of specific response to the CCP vaccine has been established. Thus, in the group with hyperreactive response, the level of IgG4 was significantly elevated, and in the group of reactive children, an increased level of IgG1 and a decrease in IgG3 were determined. The imbalance of IgG subclasses in preschool children can be associated with herpes viral load and the presence of individual members of the Herpesvirus family, which together leads to the development of a different type of immune response to the CCP vaccine. The determination of total IgG and subclasses, specific antibodies, can allow to predict the nature of the formation of a specific immune response to the vaccine, it is important when choosing the tactics of vaccination of children of preschool age. **Conclusion.** Quantitative determination of serum IgG in parallel with other classes of immunoglobulins is one of the mandatory tests in the study of the human immune status. Namely, an IgG imbalance of one or several subclasses can cause a partial imbalance in the system of regulation of the humoral immune response in children of preschool age (for example, a decrease in the level of cytokine production, or specific antibodies).

**Keywords:** gamma-globulins, immunoglobulins, imbalance, vaccination

## BURNET ROOTS EXTRACT DRY: STUDY OF PHARMACO-TECHNOLOGICAL AND ANTIBACTERIAL PROPERTIES

39-43

Shulga L. I., Bezkravna K. S., Soldatov D. P., Osolodchenko T. P.

**Introduction.** The basis for timely development of new drug products, in particular the herbal ones, for therapy of the digestive system diseases is an increase in the total number of gastroenterological patients. The surveys of the domestic pharmaceutical market as for the offer of the herbal drug products used for treatment of various diseases of the digestive system have found a limited range of the drugs based on the substances obtained from medicinal herbs and expediency of search for promising herbal sources. Based on the experience of the traditional medicine, Greater Burnet, the herbal raw material of which is used for production of the extract dry, has been selected for further examination. Whereas Burnet roots extract dry is anticipated to underlie the production of the solid dosage forms, examination of its technological properties is justified. The scientific sources portray that the subterranean organs of Greater Burnet exert the bactericidal effect against microorganisms of intestinal, dysentery and paratyphoid groups, as well as antifungal activity of the tincture of Burnet against 10 archival and clinical strains of *Candida* fungi, which is a reason for microbiological screening of the obtained extract. **The object of this paper** is to study the technological parameters of Burnet roots extract dry and to specify its antibacterial effect. **Materials and methods.** The study object is Burnet roots extract dry. The following methods are used: optical microscopy method for specification of crystallographic characteristics, weight method upon surveying of the extract's moisture absorption. The pharmaco-technological properties (fluidity, bulk density, tapped density, natural slope angle) have been studied; compressibility parameters and Gausner coefficient have been calculated under the methods of the State Pharmacopoeia of Ukraine. The antibacterial properties have been examined at the facilities of the Biochemistry and Biotechnology Laboratory of the State Institution "Mechnikov Institute of Microbiology and Immunology of NAMS of Ukraine" through the method of diffusion in agar in the "wells" modification on the reference testing cultures of microorganisms: *P. vulgaris* ATCC 4636, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *C. albicans* ATCC 885-653 with the use of the drug product "Chlorophyllipt", alcohol solution 10 mg/ml ("Galychfarm" JSC, "Kyivmedpreparat" JSC, Ukraine) as the comparative drug. The optical density of the microbial suspension of the said microorganisms has been in line with the scale McFarland 0.5 units. The obtained data have been processed statistically in Excel program. **Results & discussion.** The found anisodiametric shape of the particles of Burnet extract can anticipate a reduction of fluidity. The values of the extract's loss on drying are  $3.77 \pm 0.10$  %. The results of determining the moisture absorption of the extract samples under the conditions of relative air humidity of 75% and 100% prove its hygroscopicity, which, upon creation of a solid dosage form, requires adjustment through processing of the modern auxiliary substances with the moisture-absorbing properties. The technological parameters of Burnet roots extract dry are characterized; reasonableness of selection of the modern auxiliary substances with the aim to improve such technological parameter as fluidity is proven in the paper. The natural slope angle makes  $34.20 \pm 0.58$  degrees, being within the satisfactory range acceptance for the production. The values of Gausner coefficient and compressibility degree show a good fluidity of the extract, however they are not peculiar to its properties. Sensitivity of all the examined testing strains of microorganisms to Burnet extract, including *P. vulgaris* and *P. aeruginosa*, is proven; at the same time the comparative drug: "Chlorophyllipt" has shown no antimicrobial effect against them. **Conclusions.** The pharmaceutical and technological properties of Burnet roots extract dry have been examined; subject to the results our emphasis is given to a thorough approach for selection of auxiliary substances in development of the tablet composition based on it. The antibacterial effect of Burnet extract in respect to the testing strains of microorganisms is determined and microbiological researches as for specification of a potential antihelicobacter effect are scheduled.

**Key words:** Greater Burnet, extract dry, pharmaco-technological properties, antibacterial properties, gastroenterology

## SYNTHESIS OF DYNAMIC RIBOFLAVIN DERIVATIVES AND THE STUDY OF THEIR ABILITY TO UREASE PHOTOINACTIVATION

44-49

Martynov A.V., Bomko T.V., Farber B.S., Nosalskaya T.N., Kleyn I.

**Introduction.** In one patents demonstrated the riboflavin ability to inactivate vaccine strains of viruses and bacteria, as well as cancer cells during the production of vaccines. DNA and RNA within living organisms have specific aptamers or flavin places where Riboflavin and its derivatives are able to selectively join. Upon further blue light irradiation 440–450 nm or ultraviolet light at 280–350 nm such complexes between nucleic acids and riboflavin observed selective nucleolysis nucleic acids in the field of riboflavin attachment. Research aim was to synthesize a series of riboflavin derivatives, including dynamic derivative having greater photodynamic activity (higher sensitivity to light) in the visible region spectrum at lower concentrations. **Materials and methods.** For gradient HPLC analysis were used: acetonitrile and lithium perchlorate, perchloric acid in a kit for chromatograph BD2003. For fixing the UV / spectrum of the synthesized compounds was used spectrophotometer Gene-quant - 1300. To analyze riboflavin succinyl-dynamic systems was used HPLC- system Milichrome A-02. To study the ability of riboflavin derivatives for urease's photo-inactivation reagents were used: test-system Urease-U and spectrophotometer Gene-quant – 1300 for fixing end of reaction. In addition, we used a set of chromatography conditions for analysis of riboflavin derivatives: gradient separation of acetonitrile (from 0% to 100%) / 0.05 M lithium perchlorate buffer + 0.01 M perchloric acid at 40 °C, and fraction detection in UV region. As a comparison, we used substance riboflavin (I). **Results and discussion.** The main difference of (I) from dynamic riboflavin (IV) is much higher water solubility (up to 2%) for (IV). To compare the efficiency photoinactivation by riboflavin (I) and dynamic riboflavin (IV) was studied photoinactivation efficiency of urease as a model of microbial toxin and its transformation into a toxoid.

The photoinactivators effectivity criterion is the ability to inactivate urease by disrupt performance of the active center of the enzyme. In our case - to slow down or completely block the urease ability to catalyze the hydrolysis of urea. Full succinylated derivative (V) acts in minimum effective concentration  $0,8 \pm 0.1 \mu\text{g/mL}$ . Dynamic derivative (IV) with an immediate showed activity at a concentration  $0.2 \pm 0.05 \mu\text{g/ml}$  and after 24 hours also not changed. The starting riboflavin (I) was very active, regardless to storage time and they active concentration in solution was  $1.2 \pm 0.2 \mu\text{g/mL}$ . The effective concentration statistically differed between compounds (I), (IV) and (V) at  $p \leq 0.05$ . **Conclusion.** The first compound obtained is combinatorial - maleylated / succinylated riboflavin (IV). The synthesized compound (IV) and (V) retain photodynamic activity. The minimum effective concentration of the compound (IV) with an immediate application amounted to  $0.2 \pm 0.05 \mu\text{g/mL}$ , whereas for the (V) this value was  $0.8 \pm 0.1 \mu\text{g/mL}$ . Active dynamic structure (IV) and even exceed the original riboflavin activity at immediate use after dissolution, it is still unstable and gradually hydrolyzed. The most stable activity possessed source riboflavin (I), its active concentration is not dependent on the storage time of the aqueous solution and was  $1.2 \pm 0.2 \mu\text{g/mL}$ .

**Keywords:** riboflavin, photodynamic, vaccine, urease, dynamic combinatorial derivatives

## STUDY OF THE INFLUENCE OF PHARMACEUTICAL FACTORS ON THE OPTIMIZATION OF THE RELEASE OF BIOLOGICALLY ACTIVE SUBSTANCES UPON RECEIPT THE WATER EXTRACTS FROM GYNECOLOGICAL PLANT MEDICAL COLLECTION

50-53

Konovalenko I. S., Polovko N. P.

**Introduction.** Infusions are water extracts that do not require either sophisticated equipment or expensive or scarce extractants. From a biopharmaceutical point of view, aqueous extracts provide good availability of drug substances. Compared with individual medicinal substances, they have a milder and simultaneous complex effect on the body. These circumstances are one of the reasons that these dosage forms, which appeared even before Galen, still retained their significance. In the formulation of pharmacies, infusions and decoctions can be prepared independently, and can also be part of potions. Therefore, the study of water extraction technology is important for the practical activities of the pharmacist. **Materials and methods.** As objects of the study a medical plant collection was used, which included clover inflorescence, yarrow grass, linden flowers, thyme grass, which were selected for the pharmaceutical development of the collection for non-hormonal therapy of menopausal syndrome. Purified water was used as extractant. The completeness of extraction was determined by the quantitative content of extractives and flavonoids content, expressed as rutin. In order to study the effect of various pharmaceutical factors on the release of extractives from medicinal plant materials, three collection fractions were studied that were identical in composition but different in degree of grinding components, which were obtained by sifting through sieves No. 1, 2, 3. Medicinal raw materials were ground by grass cutter. The particle size of the first fraction was 1–3 mm, the second fraction was 3–4 mm, and the third was 4–6 mm. Based on the obtained results, a fraction of 1–3 mm was selected for further studies to substantiate the extraction parameters. **Results & Discussion.** To optimize the extraction conditions, the level of extractives from medicinal plant materials with a grinding degree of 1–3 mm was studied under the above extraction conditions. As a result of the study, the influence of the degree of grinding of raw materials on the level of extractive substances obtained from aqueous extracts of plant collection was studied. It was experimentally established that an increase in the time of infusion in a water bath from 5 to 15 minutes leads to an increase in the content of extracts as well as biologically active substances in the infusion. Further heating in a water bath does not increase the yield of extractives and flavonoids, and a color change may indicate the destruction of a number of biologically active substances under the influence of temperature. It is advisable to increase the infusion time before cooling for 30–45 minutes, since further cooling does not increase the content of active substances and leads to precipitation. **Conclusion.** 1. We studied the factors affecting the optimization of the release of biologically active substances in the preparation of aqueous extracts from medicinal plant collection. 2. We investigated various extraction regimes of the drug collection and found that the optimal mode of extraction of biologically active substances from the plant collection is to insist on a water bath for 15 minutes, followed by cooling at room temperature for 30–45 minutes.

**Keywords:** water extraction, extractives, medical plant material, gynecological drug.

## PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTILISTERIAL ATTRIBUTES OF DIFFERENT AQUEOUS AND ETHANOLIC LEAF EXTRACTS

54-60

Daniel O. Ebakota, Onilude A. Abiodun, Obayagbona Omoregbe Nosa

**Introduction.** *Listeria monocytogenes* represents the *Listeria* species most commonly associated with disease in humans. The majority (99%) of the infections caused by *L. monocytogenes* are food-borne being ingestion of contaminated food especially contaminated ready-to-eat food products that do not undergo subsequent reheating. This organism has great economical implications in the food industry due to recalls of contaminated food products and temporary shutdown of many food processing plants. There has been lots of interest recently in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases. The revival of interest in the use of African medicinal plants by many developing countries and the World Health Organization (WHO) has led to intensified efforts to explore the numerous plants with medicinal importance. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*. Knowledge of the chemical constituents of plants and their anti-*Listeria* ability is desirable because such information will be value for synthesis of complex chemical substances. The aim of this work was to study the phytochemical qualitative profiles and *in vitro* antilisterial attributes of aqueous and ethanolic leaf extracts from several plants against earlier documented Ready To Eat (RTE) associated multi antibiotic resistant *L. monocytogenes* strains. **Materials and Methods.** The examined aqueous and ethanolic leaf extracts were derived from *Psidium guajava* (guava), *Zingiber officinale* (Ginger), *Dacryodes edulis* (African pear), *Citrus aurantifolia* (Lime), *Funtumia elastica* (silkrubber), *Vernonia amygdalina* (Bitter leaf), *Cassia alata* and *Moringa oleifera* (horseradish tree). The respective *L. monocytogenes* strains utilized were; LMSN 70, LMEW 94 and LMMP 104. *In vitro* assay of aqueous and ethanol extracts of plants was assayed by agar well diffusion assay. **Results and discussion.** Alkaloids, saponins, steroids, terpenoids, cardiac glycoside, reducing sugar, phenolics, resins, flavonoids, and tannins were detected in *Z. officinale* ethanolic leaf extract whilst cardiac glycoside was absent in ethanolic leaf extracts of *D. edulis*, *C. aurantifolia* and *F. elastica*. Alkaloids, saponins, steroids, terpenoids, cardiac glycoside, reducing sugar, phenolics, resins, flavonoids, and tannins were detected in crude aqueous leaf extracts of *Z. officinale* and *M. oleifera*. *F. elastica* ethanolic leaf extract displayed the highest antilisterial potential at the least concentration; 100mg/ml whilst amongst the aqueous extracts, *V. amygdalina* leaf extract exhibited maximal antilisterial activities comparable with antilisterial inhibitory growth zones elicited by the respective ethanolic extracts with the exception of *M. oleifera* extracts. **Conclusion.** Further studies aimed at the fractionation of the respective crude extracts especially *F. elastica* and exposing the respective multi antibiotic resistant food borne *L. monocytogenes* strains to these fractionated leaf extracts should be conducted.

**Keywords:** Antilisterial, Agar well diffusion, Aqueous extract, Ethanolic extract, Phytochemicals, RTE borne *L. monocytogenes*

## SYNTHESIS AND CHARACTERIZATIONS OF ABSORBENT DRESSING TURMERIC EXTRACT CURCUMIN CHITOSAN-ALGINATE HYDROGEN AND ZNO NANO FOR MEDIATE AND HIGH EXUDATION

61-69

Astuti Amin, Wahyu Hendrarti, Jenny Wunas

This study carried out the test of the effectiveness of Hydrogel formula of turmeric extract - chitosan and ZnO nano for wound in *Oryctolagus cuniculus*, New Zealand rabbits, using Sodium alginate as a hydrogel agent. It was done to obtain a physically stable formula

made from turmeric - chitosan and ZnO nano Hydrogel extracts to heal a wound. The stable hydrogels were tested further in vivo with three New Zealand rabbits and divided into three treatment groups, are stable Hydrogel preparations, positive controls, and negative controls. The results showed that stable hydrogel of chitosan turmeric extract and ZnO nano were effective to heal the wound as marked by a reduction in wound diameter faster than wound diameter in negative control rabbits. Further in the Anova test with a Complete Randomized Design (CRD) data obtained F count value of 596 > F table 1% 10.92. Indeed, there is a very significant difference between the three treatment groups. Additionally, the result of Tukey's HSD (honestly significant difference) test obtained that from the three treatment groups has a significantly different effect with negative control and not significantly different from positive control; preparations that have been known to have a healing effect on wound.

**Keywords:** Absorbent Dressing, Alginate, Chitosan, Curcumin, ZnO Nano, Healing.

## **CHOICE OF THE PRESERVATIVE IN THE COMPOSITION OF VAGINAL GEL WITH RESVERATROL AND HYALURONIC ACID FOR TREATMENT OF UROGENITAL SYMPTOMS IN THE CLIMAX PERIOD**

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**Aim.** Microbial contamination of a product is possible in the process of production, storage and use, which can shorten the shelf life and adversely affect the safety profile of the drug. Therefore, an important criterion for evaluating the quality of a medicinal product is microbiological purity. The aim of the study was to determine and compare the antimicrobial effectiveness of preservatives in the composition of the vaginal gel with resveratrol and hyaluronic acid. **Materials and methods.** The objects of the study were samples of vaginal gel with various antimicrobial preservatives, which have no irritant effect on the mucous membranes, and are safe for the human body. Preservative selection studies have been conducted using the antimicrobial preservative efficacy evaluation technique described in SPU 2.0 (Vol. 1, Sec.5.1.3, p. 773). The principle of the method is that to the samples of the finished dosage form with various preservatives, which are in the primary packaging, introduce a certain number of test microorganisms and store these samples at a certain temperature (from 20 to 25 °C) in a dark place. Immediately after inoculation and at specified intervals (2, 7, 14 and 28 days), samples (usually 1.0 g) are taken from the inoculated samples and the number of viable microorganisms is determined. All studies were performed in aseptic conditions, using a laminar box (Biological Safety Cabinet AC2-4E1 "Esco", Indonesia). *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 885-653, *Aspergillus brasiliensis* ATCC 16404 were used as test microorganisms for inoculation of gel samples. **Results.** The research on the choice of antimicrobial preservative in the composition of vaginal gel for the treatment of urogenital symptoms, has been conducted, according to which it can be concluded that samples of gels with preservatives Cosgard 0.5%, Leucidal 2.0% and EuxylPE 9010 0.7% meet the criterion "A" according to the requirements of SPU for non-sterile medicines and are promising for further research on the development of composition and optimal technology of semisolid dosage form of vaginal gel. According to the research, the antimicrobial effectiveness of 0.5% Cosgard preservative gel was higher than the 2.0% Leucidal preservative gel samples and 0.7% Euxyl PE 9010, which will contribute to the quality of the developed gel during its storage. On the basis of the conducted researches the expediency of preservative Cosgard at a concentration of 0.5% introduction into the composition of vaginal gel with resveratrol and hyaluronic acid has been established. **Conclusions.** It has been established that the optimal antimicrobial preservative in the composition of vaginal gel is the preservatives Cosgard in concentration 0.5%. The conducted studies are promising for further research on the development of composition and optimal technology of semisolid dosage forms.

**Key words.** Vaginal gel, antimicrobial preservative, resveratrol, hyaluronic acid.