REVISTIA

EJNM

EUROPEAN JOURNAL OF NATURAL SCIENCES AND MEDICINE

January – June 2022

Volume 5, Issue 1

ISSN 2601-8705 (Print)
ISSN 2601-8691 (Online)

REVISTIA

PUBLISHING AND RESEARCH

EUROPEAN JOURNAL OF

FORMAL SCIENCES AND ENGINEERING

January – June 2022

Volume 5, Issue 1

Typeset by Revistia

Address: 11, Portland Road, London, SE25 4UF, United Kingdom

Tel: +44 2080680407

Web: https://ejnm.revistia.org

Email: office@revistia.org

ISSN 2601-8705 (Print)
ISSN 2601-8691 (Online)

E-Mail: office@revistia.org

Indexed in Elsevier's Mendeley, WorldCat, RePEc & Ideas, Google Scholar, Microsoft Academics, Index Copernicus, Crossref

TABLE OF CONTENTS

Design and Development of Hybrid Converter for Marine Applications

T. Sasilatha

Dean & Professor, Department of EEE, AMET
Deemed to be University, Chennai, India

D. Lakshmi

Associate Professor, Department of EEE, AMET
Deemed to be University, Chennai, India

R. Rajasree

Assistant Professor, Department of EEE, AMET
Deemed to be University, Chennai, India

J.K. Vaijayanthimala

Research scholar, Department of EEE, AMET
Deemed to be University, Chennai, India

P. Siva

PG Research scholar, Department of EEE, AMET
Deemed to be University, Chennai, India

Abstract

Recently, there has been an increase in the growth and advancement of electric propulsion in marine electrical drives. A maximum amount of energy is utilized by ships for propulsion drives. To be aware of it and develop an optimized structure to improve the effectiveness of the propulsion system with power consumption is necessary. The proposed paper aims to develop a model and perform functional analysis as per the above understanding and requirements. The factors considered include greenhouse gas emissions, CO2 emissions, environmental aspects, and the availability of non-renewable resources, which leads to the introduction of renewable energy as a replacement method of power generation. For this work, two different renewable sources, such as solar and wind energy, were chosen. The combination of these two resources can manipulate the voltage and satisfy the load in a desirable way. For voltage improvement, a high gain converter with a minimal number of active and passive components is selected. This system adopts a storage system to meet the needs in the future. The inverter switches are controlled by the recommended control algorithm, which can balance and provide adequate power towards the drive by a feedback control loop. The

speed of propulsion in the drive is adjusted by the induction motor coupled with the propeller. The analytical study of the proposed system is carried out in MATLAB software. The simulation study revealed the effectiveness of this modern optimization technique.

Keywords: Renewable Energy, Propeller, MPPT optimization, PI controller, Buck Boost Converter

Introduction

Propulsion is used to propel the vessel ahead. This has been going on for a long time in the traditional manner. Prior to the twenty-first century, most ships used diesel engines for propulsion [1]. A few years ago, fossil fuels became a key resource for power production all over the world, resulting in pollution and a high total cost. Electric propulsion is used in order to focus on capital costs, fuel requirements, and the environment [2]. These factors draw attention to renewable energy sources such as solar, wind, hydro, tidal, geothermal, and others. Electricity generated by wind and solar has surpassed all other renewable energy sources in recent years [3-4]. This situation is playing out all across the world; China has taken the lead in installing 35 percent of the world's wind energy. Manufacturers of wind turbines throughout the world, such as Gold Wind, Vestas, Suzlon, General Electric, and Siemens Gamesa, have been attempting to build massive wind turbines with a capacity of 10–15 MW. The arrangement of the wind turbine and power electronic converters determines full-speed, semi-speed, and low-speed operation.

For wind energy conversion systems (WECS), there are two types of power electronic conversions: AC-AC and AC/DC, DC/DC, DC/DC & DC/AC. Based on the magnitude of voltage produced by the generator, it is further divided into two types: medium and low voltage converters [5]. Low-voltage conversion systems are only suited for wind turbines up to 3 MW, while medium-voltage conversion systems are acceptable for wind turbines over 3 MW. Based on solar irradiation and temperature, photovoltaic systems generate direct current (DC) power. Because each solar cell can only provide 0.5-0.6V, solar cells are coupled in series and parallel to increase power production. Due to its decreasing cost over time, environmentally beneficial character, and renewability, researchers have been more interested in the electricity generated by photovoltaic arrays [6]. The ability to achieve sufficient torque without compromising pitch angle is the most important consideration when choosing electric propulsion. There will be perfect redundancy in this type of propulsion.

Section II dealt with the overall study of the proposed method. The circuit configuration and the approach used to utilize the energy produced by those two renewable resources are described in section III. The charging and discharging of the battery interfaced with the converter is explained. The section IV reviewing peak current mode control technique, used in the feedback control loop to improve the

system performance, is visualized. The Simulink study of the proposed study and the output attained from the converter are reviewed in Section V. The effectiveness of the proposed study and its impact in the future are depicted in section VI.

II. Proposed Methodology

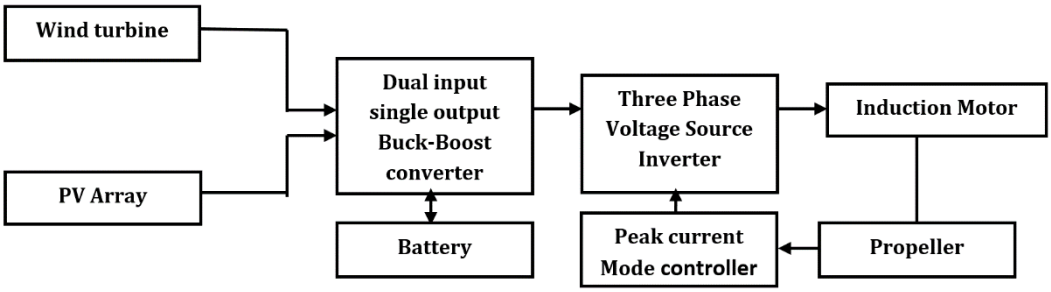


Fig.1. Block diagram of proposed system

The overall structure of the proposed methodology is shown in figure 1. The sources, such as wind turbines and PV arrays, are used in this study. When sunlight is available, the PV array generates an abundant amount of energy. Wind energy is utilized by wind turbines to supply electricity continuously. If any failure happens in any one of the generating stations, the remaining resources can manage it. The major factors which will limit voltage generation from both resources, such as solar panels, are shading, change in climatic conditions, a wide range of change in wind speed, area of the rotor, wind density, etc. Here, the role of the converter is a much needed factor. The energy gained from both sources is transmitted towards a dual input, single output buck-boost converter. Then it is stored in the battery. Whenever the generated voltage is said to be high, the converter steps down the voltage and fulfills the battery. In any condition, if the load faces voltage lag, the converter boosts the voltage received from stored energy in the battery. This is how the converter action is performed. Further, the received energy is converted into AC by a three-phase inverter to satisfy the load [7]. The shaft interlinks the induction motor with the propeller. The peak current mode controller receives feedback from the propeller and alters the switching cycle to improve the functionality of the inverter.

III. Proposed converter

The circuit configuration of the proposed converter is represented in figure 2. T_1 and T_2 switches are bidirectional conduction and bidirectional blocking (BCBB) switches in this configuration [8]. The diodes D_1 and D_2 offer freewheeling of load current. Two input sources are connected to the basic converter circuit, which consists of two switches, T_3 and T_4, as well as an inductor and capacitor, which is then connected to the load. The combination of switches T_1 and T_2 allows the converter to operate in various states. Switch T_3 enables Buck-Boost functionality, while Switch T_4 enables bidirectional operation of the DC/DC converter. The input sources, E_1 and E_2, are two

[9], whereas E1 defines the input voltage gained from the PV array, and E_2 denotes the rectified DC voltage gained from WECS. E_0 is the output voltage, and the load current is I_0. There are four different modes of operation for this converter.

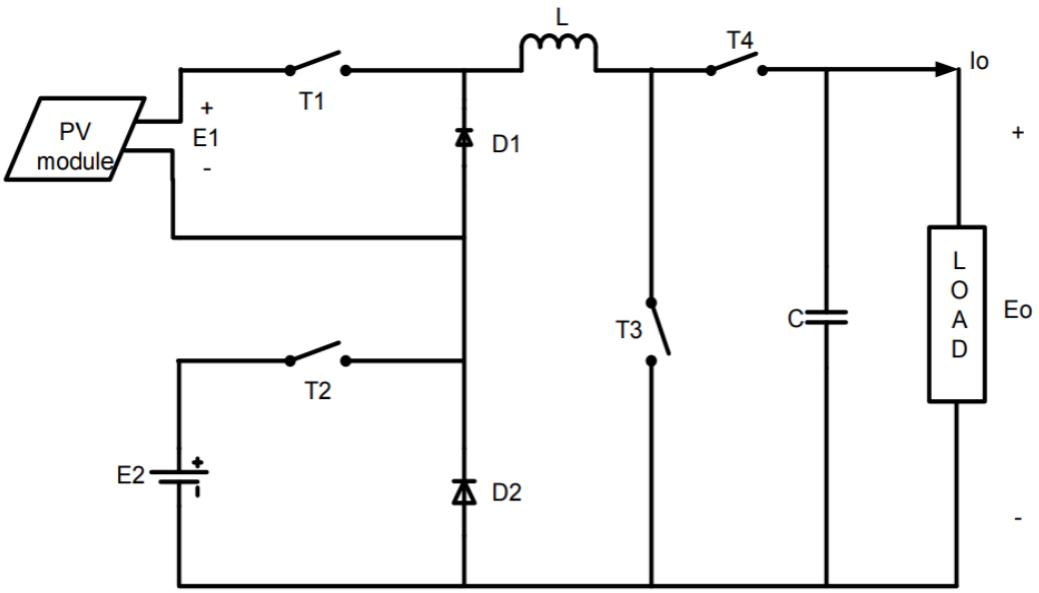


Fig.2. Dual input single output Buck-boost converter

Mode 1: In this mode, E_1 supplies the transmit current towards the converter, and the inductor L absorbs the energy supplied by the source. Both T_1 and T_3 are in conduction mode.

Mode 2: This mode utilizes the energy generated from E_2 (i.e., WECS). Again, L absorbs the energy completely when the switches T_2 and T_3 are in continuous conduction mode.

Mode 3: Both sources are activated in this mode, and the switches associated with each source, such as T_1, T_2 and T_3, are closed.

Mode 4: In the absence of both sources and switches, such as T_1, T_2, and T_3, the switch T_4 starts conducting and discharges the energy stored in L towards E_0. The capacitor C stores the charge and balances the load. By this way, the switching action is regulated. This cycle repeats, and the load requirement is managed.

IV. Peak Current Mode Controller

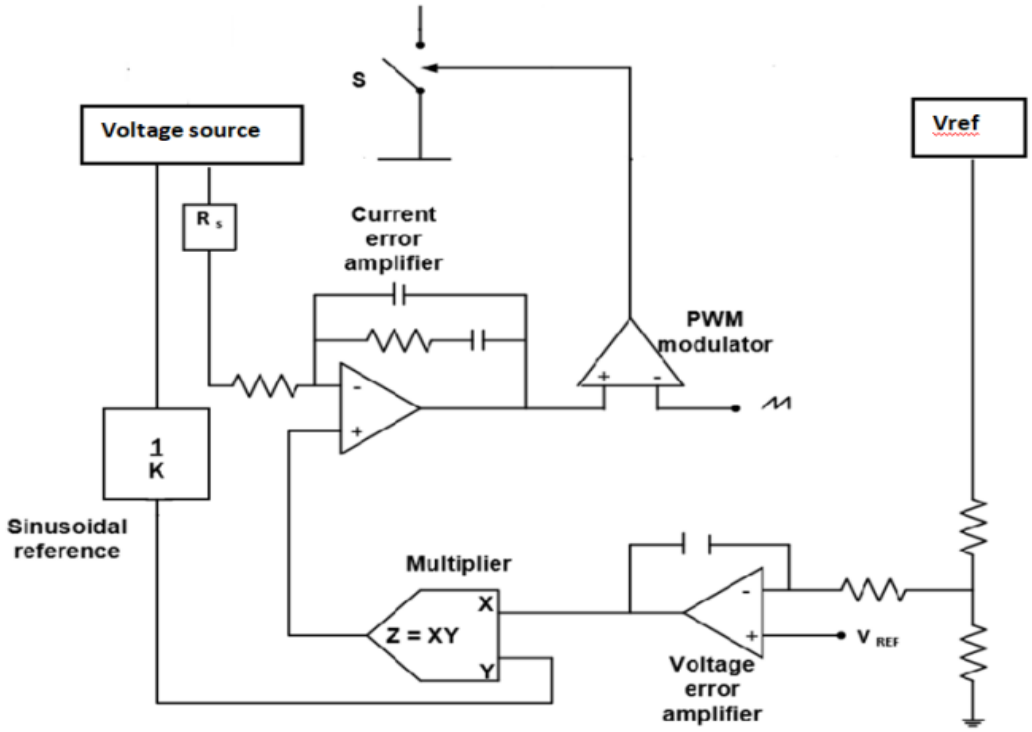


Fig.3. Closed loop control of propeller incoming voltage by suggested controller

A control technique called peak current mode control, which is shown in figure 3, is used to turn on and off the switch in a three-phase voltage source inverter. The switch is held in an on state by a clock signal with a fixed switching frequency. The switch is turned off when the sum of the switch current and the compensating ramp equals zero. Expand the voltage error amplifier with line voltage at the rectifier to obtain the reference signal. With this extension, the current reference amplitude has been established. As a result, the switching current may rise above zero, causing the switch to conduct, or it may rise above the reference current, causing the switch to turn off.

V. Experimental Analysis

The experimental analysis of the proposed study is carried out in MATLAB software. A Simulink setup is implemented by the Simulink library. The PV array's temperature and irradiance are 25 degrees Celsius and 1,000, respectively. A total of 72 cells were connected in series and parallel combinations, and the voltage gain of them should be 227V. The speed of the rotor present in WECS is 60 rad/s and it delivers a total of 150V. The outcome of both resources is represented in figures 5.1 and 5.2.

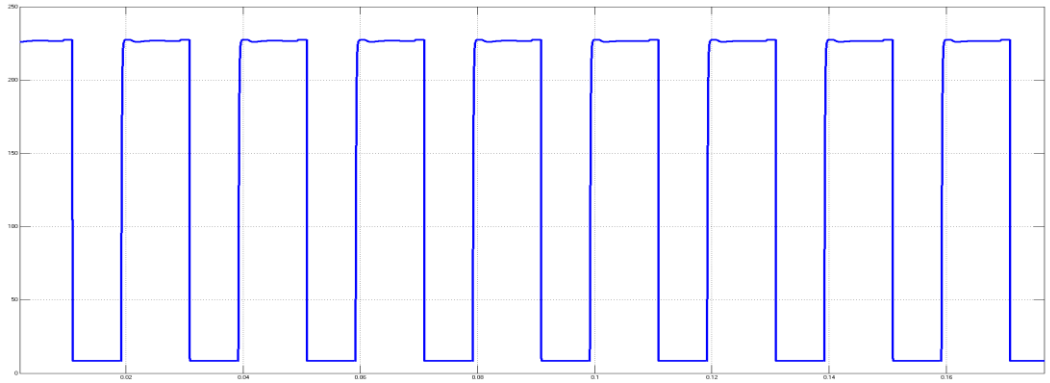


Fig.5.1. PV array's Outcome

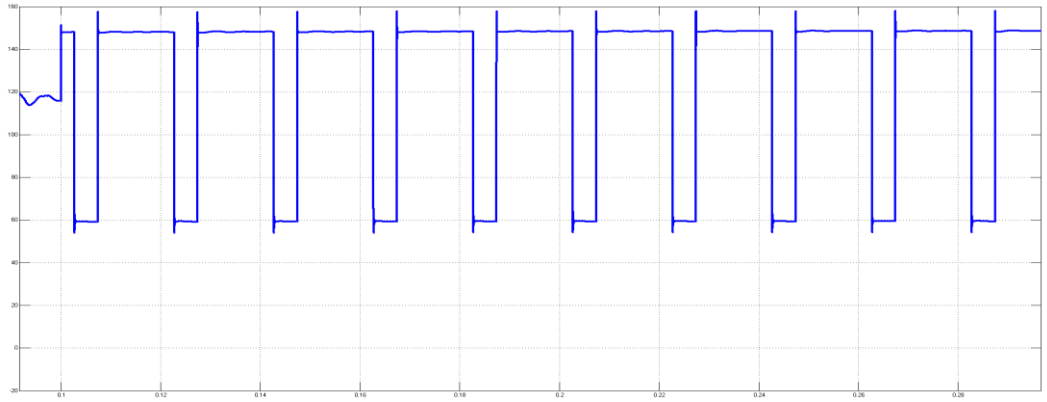


Fig.5.2. Output of WECS

The converter charges the battery and it holds 250V, which can be used in the future in case any voltage drops in resources or voltage lags across load.

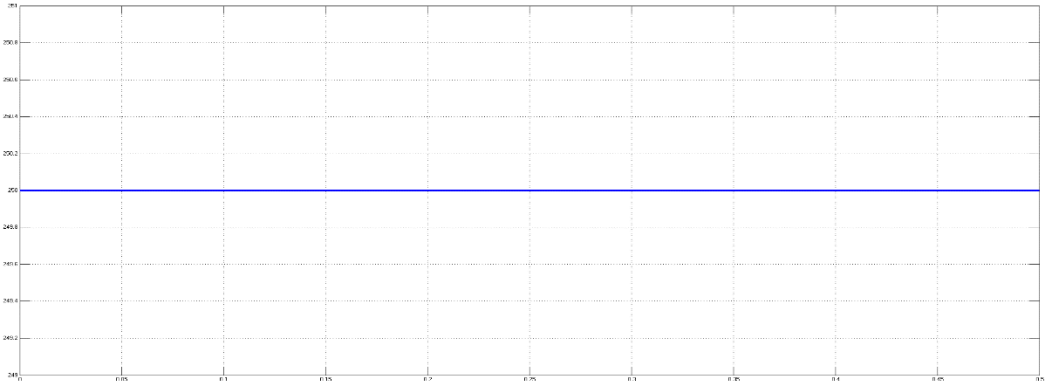


Fig.5.3. Energy stored in battery

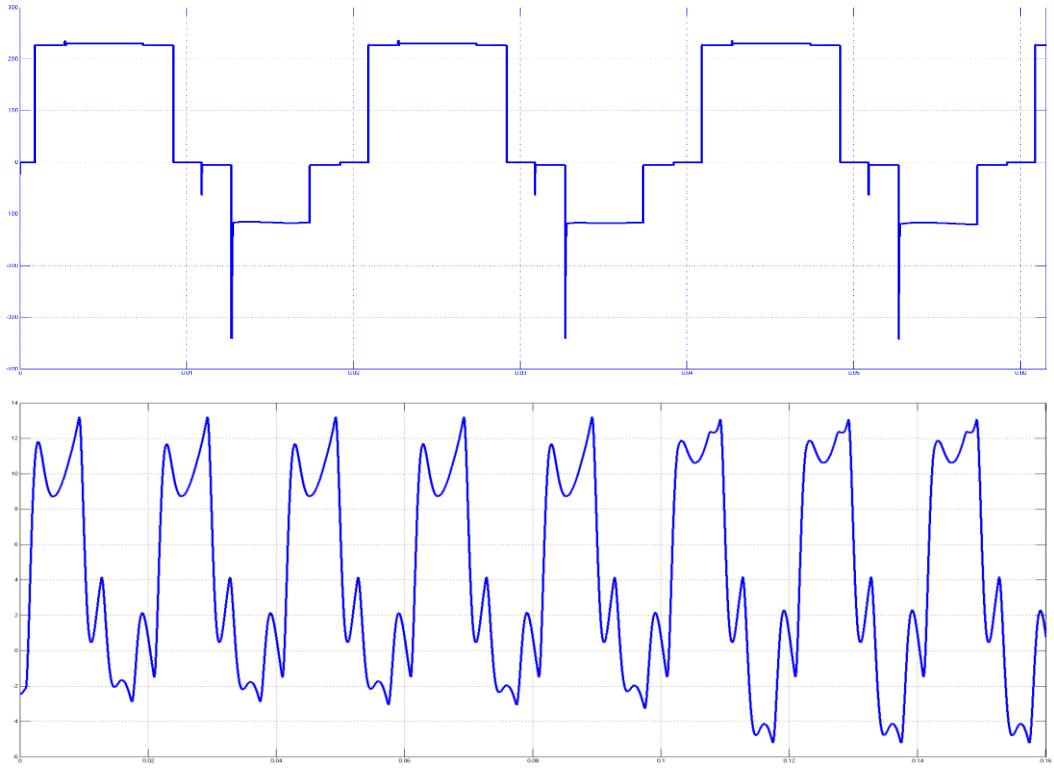


Fig.5.4. Voltage and Current across phase A

A total of 230V is gained, and the above waveform depicts the amount of voltage and current supplied by the inverter's phase A terminal.

VI. Conclusion

Thus, Renewable energy resources (solar and Wind) are hybrid with a buck boost converter to get constant output voltage. Experimental verification of the peak control mode controller is being investigated using Matlab. This mode of operation gives satisfactory speed adjustment of the propeller which is more efficient and economical. As a result of the aforementioned factors, a propeller may be able to run at a variable speed with a low starting toque.

Acknowledgment

The first author Dr. T. Sasilatha Professor and Dean sincerely acknowledges the financial assistance received from All India Council for Technical Education, India under Research Promotion Scheme.

References

[1] T.Baldwin Immanuel, P.Rathnavel, B. Lalitha (2021). Electric Propulsion by Combination of Photovoltaic System with Induction Motor Drive. (JNxtGenTech) Vol. 1, Issue 1, June 2021

[2] R. Kumar and B. Singh, ''Single stage solar PV fed brushless DC motor driven water pump,'' IEEE J. Emerg. Sel. Topics Power Electron., vol. 5, no. 3, pp. 1337–1385, Sep. 2017, doi: 10.1109/JESTPE.2017.2699918.

[3] Zahira, R., Lakshmi, D., & Ravi, C. N. Power Quality Issues in Microgrid and its Solutions. Microgrid Technologies, 255.

[4] Dorothy, R., & Sasilatha, T. (2017). System on Chip Based RTC in Power Electronics. Bulletin of Electrical Engineering and Informatics, 6(4), 358-363.

[5] S.Satish kumar and Dr M. Sasikumar (2021) "Intelligent hybrid technique for cascaded multilevel inverter based three phase grid tie hybrid power system: a WPSNN technique" Journal of Ambient Intelligence and Humanized Computing 1868-513702 Jan 2021

[6] S.Satish kumar and Dr M. Sasikumar "An Approach of Hybrid Modulation in Fusion seven-level Cascaded Multilevel Inverter accomplishment to IM drive system"2016 Second International Conference On Science Technology Engineering and Management(ICONSTEM) 978-1-5090-1706-5, pp 384-387, 2016 IEEE.

[7] Sushmita N Shetty, Md. Abdul Raheman (2017). Modelling of Dual Input DC/DC Converter for Hybrid Energy System. 2017 2nd IEEE International Conference On Recent Trends in Electronics Information & Communication Technology (RTEICT), May 19-20, 2017, India

[8] Khaligh, A.; Jian Cao; Young-Joo Lee; , "A Multiple-Input DC–DC Converter Topology," Power Electronics, IEEE Transactions on , vol.24, no.3, pp.862-868, March 2009

[9] Ching-Jan Chen; Ching-Hsiang Cheng ; Ping-Sheng Wu ; Shinn-Shyong Wang; Unified Small-Signal Model and Compensator Design of Flyback Converter With Peak-Current Control at Variable Frequency for USB Power Delivery. IEEE Transactions on Power Electronics (Volume: 34 , Issue: 1 , Jan. 2019)

[10] R Dorothy, T Sasilatha (2017), "Smart Grid Systems Based Survey on Cyber Security Issues", Bulletin of Electrical Engineering and Informatics 6 (4), 337-342

[11] S Vidhya, T Sasilatha, "Performance analysis of black hole attack detection scheme using MD5 algorithm in WSN", 2014 International Conference on Smart Structures and Systems (ICSSS), 51-54

The Succession of Heat and Mass Driven Natural Convection Regimes Along a Vertical Impermeable Wall

Maria Neagu

Manufacturing Engineering Department,
"Dunarea de Jos" University of Galati

Abstract

This paper presents the analysis of the natural convection process that takes place near a vertical plane wall embedded in a constant temperature and linearly mass stratified fluid (the Prandtl number and the Smith number are smaller than 1.0, while the Lewis number is greater than 1.0). The wall has a constant temperature, while the flux of a certain constituent is constant at this boundary. The scale analysis and the finite differences method are used as techniques of work. The scale analysis proves the existence, at equilibrium, of heat and/or mass driven convection regimes along the wall. The finite differences method is used solve the governing equations and to verify the scale analysis results using two particular parameters sets.

Keywords: natural convection, constant mass flux, scale analysis, finite differences method

1. Introduction

The analysis of the natural convection process along a vertical plane wall is a classical problem that was taken into consideration along the past decades in applications of a specific or general character (Armfield, Patterson & Lin, 2007; Lin, Armfield & Patterson, 2008; Mongruel, Cloitre and Allain, 1996; Neagu, 2018, 2021; Patterson, Lei, Armfield & Lin, 2009; Saha, Patterson and Lei, 2010; Saha, Brown & Gu, 2012).

This research analysis refers to a vertical plane wall that have a constant temperature and that registers a constant flux of a certain constituent at it. The environment is air ($\mathrm{Pr}=0.72$ in this paper) while the Lewis number is greater than 1.0 ($\mathrm{Le}\geq 1$) and the Smith number is smaller than 1.0 ($\mathrm{Sch}<1$). The environment is mass stratified, while its temperature is constant.

The scale analysis is performed and the results are verified by solving the governing equations using the finite differences method for two particular parameters sets: $Ra = 5000$, $N = 1$, $Le = 1$, $Pr = 0{,}72$, $S_C = 0.08$ and $Ra = 5000$, $N = 5$, $Le = 2$, $Pr = 0{,}72$, $S_C = 0.04$.

2. Mathematical Formulation

Figure 1 presents the vertical plane wall and the x-y system of co-ordinates associated to it in dimensional (Fig. 1a) and in dimensionless (Fig. 1b) representations.

The temperature at the wall is T_w, while the environment has a constant temperature, T_∞. The mass flux of a certain constituent, m_w, is constant at the wall, while the environment registers a concentration of the constituent of $C_{\infty,x} = C_{\infty,0} + s_C \cdot x$.

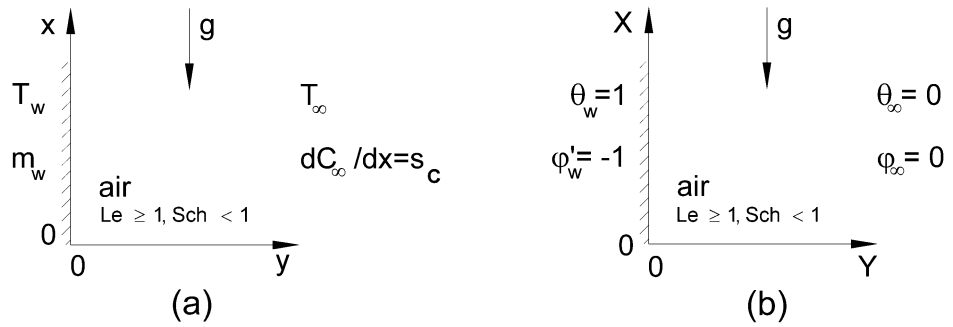


Fig. 1. (a) The dimensional problem; (b) the dimensionless problem.

2018):

$$\frac{\partial v}{\partial x} + \frac{\partial u}{\partial y} = 0; \tag{1}$$

$$\frac{\partial u}{\partial t} + u \cdot \frac{\partial u}{\partial y} + v \cdot \frac{\partial u}{\partial x} = -\frac{\partial p}{\partial y} + \nu\left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2}\right); \tag{2}$$

$$\frac{\partial v}{\partial t} + u \cdot \frac{\partial v}{\partial y} + v \cdot \frac{\partial v}{\partial x} = -\frac{\partial p}{\partial x} + \nu\left(\frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2}\right) + g\beta_t T + g\beta_c C; \tag{3}$$

$$\frac{\partial T}{\partial t}+u\cdot\frac{\partial T}{\partial y}+v\cdot\frac{\partial T}{\partial x}=\alpha\left(\frac{\partial^2 T}{\partial x^2}+\frac{\partial^2 T}{\partial y^2}\right); \tag{4}$$

$$\frac{\partial C}{\partial t}+u\cdot\frac{\partial C}{\partial y}+v\cdot\frac{\partial C}{\partial x}+v\cdot s_c=D\left(\frac{\partial^2 C}{\partial x^2}+\frac{\partial^2 C}{\partial y^2}\right), \tag{5}$$

and the boundary conditions (Neagu, 2018):

$$u=v=0,\ T=T_w,\ \frac{\partial C}{\partial y}=-\Gamma_w \text{ at } y=0; \tag{6}$$

$$v=0,\ T=T_\infty,\ C=C_{\infty,x} \text{ as } y\to\infty; \tag{7}$$

$$v=0,\ T=T_\infty,\ C=C_{\infty,0} \text{ at } x=0; \tag{8}$$

$$\frac{\partial^2 u}{\partial x^2}=\frac{\partial^2 v}{\partial x^2}=\frac{\partial^2 T}{\partial x^2}=\frac{\partial^2 C}{\partial x^2}=0 \text{ at } x=h, \tag{9}$$

define the point of start of the scale analysis.

3. Scale Analysis

This analysis follows the same pattern used before in the scientific literature (Bejan 1995; Neagu, 2018, 2021) for a better correlation, comprehension of the research characteristics and following the evolution of the natural convection regime that develops along the boundary: the initial state (section 3.1), the heat driven convection (HDC) regime (section 3.2) and the mass driven convection (MDC) regime (section 3.3).

3.1. The Initial State

Because this section is similar to the results of previous analysis, only the most significant results will be mentioned here (Neagu, 2018):

the boundary layer thickness of the temperature field:

$$\delta_T\sim\alpha^{1/2}\cdot t^{1/2}; \tag{10}$$

the boundary layer thickness of the concentration field:

$$\delta_C\sim D^{1/2}\cdot t^{1/2}; \tag{11}$$

the heat driven convection regimes that exists at the beginning at each point along the wall will be replaced by a mass driven convection regime only if the equilibrium time of the regime is bigger than the transition time, t_{trz}:

$$t_{trz} \sim L^2 \cdot \left(N^2 D\right)^{-1}; \tag{12}$$

the inequality $s_C > \partial C / \partial x$, that is valid at the beginning at each point of the wall, ceases to be valid if the equilibrium time of the regime is bigger than t_s:

$$t_s \sim \frac{{s_C}^2 x^2}{{\Gamma_w}^2 D}. \tag{13}$$

3.2. The Heat Driven Convection Regime

If $Pr < 1$ and $Le \geq 1$, Saha, Patterson and Lei (2010) prove that the order of magnitude of the vertical velocity is:

$$v_T \sim \frac{g\beta_t t}{1+Pr}\Delta T. \tag{14}$$

Invoking the equilibrium between the horizontal diffusion and the vertical convection of heat, the temperature equilibrium time, boundary layer thickness and vertical velocity orders of magnitude are (Neagu, 2018):

$$\left(t_{ech,T}\right)_T \sim \frac{L^2}{\alpha}\left[\frac{X(1+Pr)}{Ra \cdot Pr}\right]^{1/2}; \tag{15}$$

$$\left(\delta_{ech,T}\right)_T \sim L \cdot \left[\frac{X(1+Pr)}{Ra \cdot Pr}\right]^{1/4}; \tag{16}$$

$$V_T \sim \left(\frac{X \cdot Ra \cdot Pr}{1+Pr}\right)^{1/2}. \tag{17}$$

a. Scale analysis of the concentration field in the HDC regime

b. In this case, the velocity order of magnitude for the concentration field is v_T.

c. Two situations appear:

d. if in the left side of equation (5) $v \cdot \partial C / \partial x \geq v \cdot s_C$, then the equilibrium between the horizontal diffusion of the constituent and the vertical convection of it gives us the equilibrium time of the concentration field:

$$\left(t_{ech,C}\right)_T \sim \frac{L^2}{D}\left[\frac{X\,Pr(1+Pr)}{Sch^2\cdot Ra}\right]^{1/2}. \quad (18)$$

Further, using equation (11), the boundary layer thickness order of magnitude becomes:

$$\left(\delta_{ech,C}\right)_T \sim L\left[\frac{X\,Pr(1+Pr)}{Sch^2\cdot Ra}\right]^{1/4}. \quad (19)$$

The equilibrium time of the concentration field, $\left(t_{ech,C}\right)_T$, is bigger than the transition time, t_{trz}, if:

$$X > X_{trz,C} = \frac{Sch^2}{N^4}\frac{Ra}{Pr(1+Pr)} \quad (20)$$

b. if, in equation (5), $v\cdot\partial C/\partial x < v\cdot s_C$, then the concentration equilibrium time and boundary layer thickness are:

$$\left(t_{ech,Sc}\right)_T \sim \frac{L^2}{D}\frac{Pr(1+Pr)}{S_C^2\cdot Sch^2\cdot Ra\cdot X}; \quad (21)$$

$$\left(\delta_{ech,Sc}\right)_T \sim L\left[\frac{Pr(1+Pr)}{Ra\cdot X\cdot S_C^2\cdot Sch^2}\right]^{1/2}. \quad (22)$$

The equilibrium time $\left(t_{ech,,Sc}\right)_T$ is compared to t_{trz} and t_s:

b1) the equilibrium time $\left(t_{ech,,Sc}\right)_T$ is bigger than the transition time, t_{trz}, if:

$$X > X_{trz,Sc} = \frac{N^2\,Pr(1+Pr)}{Sc^2 Sch^2 Ra}; \quad (23)$$

b2) the inequality $\left(t_{ech,Sc}\right)_T < t_s$ is restricted to the following domain:

$$X \geq X_{S,T} = \left[\frac{Pr(1+Pr)}{S_c^4\cdot Sch^2\cdot Ra}\right]^{1/3}. \quad (24)$$

1. The analysis of the results presented above reveals that there are only two possibilities:
2. a HDC regime along the wall if $X_{trz,Sc} < X_{S,T} < X_{trz,C}$ (figure 2 (a)) or

$$\frac{Ra\cdot S_C\cdot Sch^2}{Pr(1+Pr)\cdot N^3} \geq 1 \quad (25)$$

a HDC regime in the $\left[0, X_{trz,C}\right]$ domain and a MDC regime in the $\left[X_{trz,C}, \infty\right)$ domain (see figure 2 (b)) if $Ra \cdot S_C \cdot Sch^2 / \left[N^3 Pr(1+Pr)\right] < 1$.

3.3. The Mass Driven Convection Regime

The vertical velocity order of magnitude was derived by Lin, Armfield and Patterson (2008):

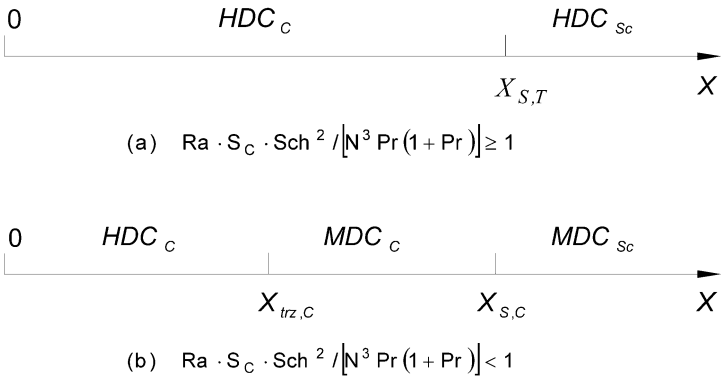


Fig. 2. The heat and mass driven natural convection regimes sequence. (a) $Ra \cdot S_C \cdot Sch^2 / \left[N^3 Pr(1+Pr)\right] \geq 1$; (b) $Ra \cdot S_C \cdot Sch^2 / \left[N^3 Pr(1+Pr)\right] < 1$.

$$v = g\beta_C \Gamma_w D^{1/2} t^{3/2} \left(1 - \sqrt{Sch}\right). \tag{26}$$

3.3.1. MDC_{Sc} Regime.

In this case, the equilibrium between the horizontal diffusion and the vertical convection of the constituent requires: $v \cdot s_c \sim D\left(\frac{\partial^2 C}{\partial y^2}\right)$ or $v \cdot s_c \sim D\frac{\Gamma_w}{D^{1/2} t^{1/2}}$. Replacing v from equation (26), the concentration equilibrium time and the boundary layer thickness are:

$$\left(t_{ech,Sc}\right)_c \sim L^2 / D / \left[Ra \cdot N \cdot S_C \cdot Le \cdot Sch\left(1 - \sqrt{Sch}\right)\right]^{1/2}; \tag{27}$$

$$\left(\delta_{ech,Sc}\right)_c \sim L / \left[Ra \cdot N \cdot S_C \cdot Le \cdot Sch\left(1 - \sqrt{Sch}\right)\right]^{1/4}. \tag{28}$$

At equilibrium, the vertical velocity order of magnitude scales as:

$$V_{Sc} \sim \left[Ra \cdot N \cdot Sch \cdot \left(1 - \sqrt{Sch}\right) / (Le \cdot Sch)^3\right]^{1/4}. \tag{29}$$

The inequality $\left(t_{ech,Sc}\right)_C < t_s$ or

$$X > X_{S,C} = 1/\left[Ra \cdot N \cdot {S_C}^5 \cdot Le \cdot Sch \cdot \left(1-\sqrt{Sch}\right)\right]^{1/4} \quad (30)$$

defines the X co-ordinate that separates the MDC_C and the MDC_{Sc} regimes in the figure 2(b).

Scale analysis of the temperature field in the MDC_{Sc} regime. The equilibrium state requires:

$\left(v_C \frac{\delta_v}{\delta_T}\right) \cdot \frac{\partial T}{\partial x} \sim \alpha \cdot \frac{\partial^2 T}{\partial y^2}$ or $\left(v_C \frac{\sqrt{Sch} \cdot \delta_c}{\delta_T}\right) \cdot \frac{\Delta T}{x} \sim \alpha \frac{\Delta T}{\delta_T^2}$. Using the equation (10) and the equation (29), the equilibrium temperature boundary layer thickness order of magnitude is:

$$\left(\delta_{ech,T}\right)_{Sc} \sim L \cdot \left(Le \cdot X \cdot S_C\right). \quad (31)$$

3.3.2. MDC_C Regime

The equilibrium between the horizontal diffusion and the vertical convection requires $v_C \cdot \frac{\partial C}{\partial x} \sim D\left(\frac{\partial^2 C}{\partial y^2}\right)$ or $v_C \cdot \frac{1}{x} \sim D\frac{1}{\delta_C^2}$. Replacing the equations (11) and (29), the equilibrium time and the concentration boundary layer thickness become:

$$\left(t_{ech,C}\right)_C \sim L^2/D/\left[Ra \cdot N \cdot Le \cdot Sch\left(1-\sqrt{Sch}\right)\right]^{2/5}; \quad (32)$$

$$\left(\delta_{ech,C}\right)_C \sim L/\left[Ra \cdot N \cdot Le \cdot Sch\left(1-\sqrt{Sch}\right)\right]^{1/5}, \quad (33)$$

while the vertical velocity scales as:

$$V_C \sim \left[Ra \cdot N \cdot Sch \cdot \left(1-\sqrt{Sch}\right)\right]^{2/5} (X/Le)^{3/5}. \quad (34)$$

Scale analysis of the temperature field in the MDC_C regime.

The equilibrium between the thermal horizontal diffusion and the vertical thermal convection requires $v_C\left(\frac{\delta_V}{\delta_T}\right) \cdot \frac{\partial T}{\partial x} \sim \alpha\left(\frac{\partial^2 T}{\partial y^2}\right)$ or $v_C \frac{\sqrt{Sch} \cdot \delta_C}{\delta_T} \cdot \frac{\Delta T}{x} \sim \alpha \frac{\Delta T}{\delta_T^2}$. The temperature boundary layer thickness scales as:

$$\left(\delta_{ech,T}\right)_C \sim L\left[\frac{X \cdot Le^4}{Ra \cdot N \cdot Sch\left(1-\sqrt{Sch}\right)}\right]^{1/5}. \quad (35)$$

The validity of the scale analysis requires the following set of conditions:

if $Ra \cdot S_C \cdot Sch^2 / \left[N^3 Pr(1+Pr)\right] \geq 1$, the imposed conditions: $(\delta_{ech,T})_T << x$, $(\delta_{ech,C})_T << x$ and $(\delta_{ech,Sc})_T << x$ at $X = X_{S,T}$, leads us to the inequality: $S_C << \sqrt{Pr}$.

if $Ra \cdot S_C \cdot Sch^2 / \left[N^3 Pr(1+Pr)\right] < 1$, then the requirements: $(\delta_{ech,T})_T << x$ for $X = X_{trz,C}$; $(\delta_{ech,C})_C << x$, $(\delta_{ech,T})_C << x$, $(\delta_{ech,Sc})_C << x$ and $(\delta_{ech,T})_{Sc} << x$ for $X = X_{S,C}$ lead us to the requirement: $S_C << 1/Le$.

Further, the scale analysis results of section 3 will be verified using the finite differences method for two particular parameters sets.

4. Numerical Modeling

The stream function formulation of the velocity field, $U = -\partial\Psi/\partial X$, $V = \partial\Psi/\partial Y$, and the vorticity definition, $\zeta = \partial V/\partial Y - \partial U/\partial X$, define the dimensionless form of the governing equations

$$\zeta = \left(\frac{\partial^2\Psi}{\partial Y^2} + \frac{\partial^2\Psi}{\partial X^2}\right); \tag{36}$$

$$\frac{\partial\zeta}{\partial\tau} + U\frac{\partial\zeta}{\partial Y} + V\frac{\partial\zeta}{\partial X} = Pr\cdot\left(\frac{\partial^2\zeta}{\partial Y^2} + \frac{\partial^2\zeta}{\partial X^2}\right) + Ra\cdot Pr\cdot\left(\frac{\partial\theta}{\partial Y} + N\frac{\partial\varphi}{\partial Y}\right); \tag{37}$$

$$\frac{\partial\theta}{\partial\tau} + U\frac{\partial\theta}{\partial Y} + V\frac{\partial\theta}{\partial X} = \frac{\partial^2\theta}{\partial Y^2} + \frac{\partial^2\theta}{\partial X^2}; \tag{38}$$

$$\frac{\partial\varphi}{\partial\tau} + U\frac{\partial\varphi}{\partial Y} + V\frac{\partial\varphi}{\partial X} + VS_C = \frac{1}{Le}\left(\frac{\partial^2\varphi}{\partial Y^2} + \frac{\partial^2\varphi}{\partial X^2}\right). \tag{39}$$

The boundary conditions take the following form:

$$\Psi = \frac{\partial\Psi}{\partial Y} = 0,\ \frac{\partial\varphi}{\partial Y} = -1,\ \theta = 1 \text{ at } Y = 0; \tag{40}$$

$$\frac{\partial\Psi}{\partial Y} = 0,\ \zeta = 0,\ \theta = \varphi = 0 \text{ as } Y = L; \tag{41}$$

$$\Psi = 0,\ \zeta = 0,\ \theta = \varphi = 0 \text{ at } X = 0; \tag{42}$$

$$\frac{\partial^2\psi}{\partial X^2} = \frac{\partial^3\psi}{\partial X^3} = \frac{\partial^2\theta}{\partial X^2} = \frac{\partial^2\varphi}{\partial X^2} = 0 \text{ at } X = H. \tag{43}$$

The conservation equations (36)–(39) with the boundary conditions (40)–(43) were solved using the finite differences method. A software was built by Neagu (2018) using a higher order hybrid scheme (Tennehill, Anderson & Pletcher, 1997) through

an iterative process: at each time step, equation (36) was solved iteratively till the relative error of Ψ, at each point of the grid, became less than 10^{-6}. The iterative process stopped when the relative errors of θ, φ and ζ became less than 10^{-6} at each grid point.

5. Results and Discussions

Two particular parameter sets are used to run the program explained above:

The $Ra = 5000, N = 1, Le = 1, Pr = 0{,}72, S_C = 0.08$ parameters set is the example chosen to exemplify the $Ra \cdot S_C \cdot Sch^2 / \left[Pr(1+Pr) \cdot N^3\right] \geq 1$ case. The results of section 2 of this analysis tell us that a HDC (heat driven convection) regime is present, at equilibrium, along the vertical wall. Equation (24) indicates that the HDC_C $(\partial C / \partial X \geq S_C)$ and the HDC_{Sc} $(\partial C / \partial X < S_C)$ regimes are separated at the $X_{S,T} = 2.32$ abscissas. A computational dimensionless domain of 0.6×6.0 was discretised uniformly using a 61×301 grid for a sufficient accuracy.

The results for this particular parameters set are present by Fig. 3. It shows the temperature (Figure 3(a)), concentration (Figure 3(b)), stream function (Figure 3(c)) and $\partial C / \partial X$ (Figure 3(d)) fields. The concentration field (Figure 3(b)) is not greater than $1/N = 1.0$ and this is an indication that HDC regime is encountered along the entire wall. The $\partial C / \partial X$ field of Fig. 3(d) exceed 0.0 ($\partial c / \partial x > s_c$) if $X \geq 1.8$ a value close to the $X_{S,T}$ value indicated by equation (24).

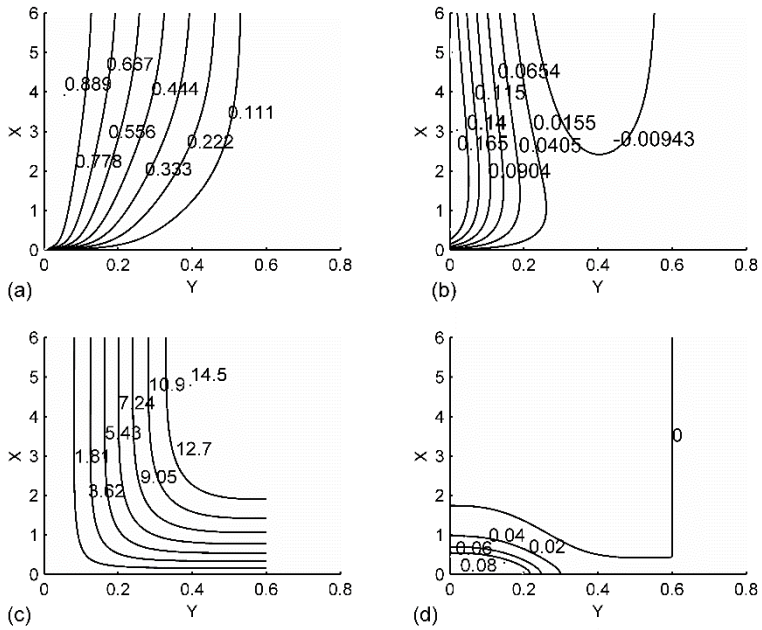


Fig. 3. The dimensionless temperature (a), concentration (b), stream function (c) and

$\partial\varphi/\partial x$ (d) fields for $Ra = 5000$, $N = 1$, $Le = 1$, $Pr = 0.72$ and $S_C = 0.08$.

In the HDC_C region, three sections present the temperature (Figure 4(a)), the concentration (Figure 4(b)) and the vertical velocity (Figure 4(c)) plots for thre abscissa: 0.5; 1.0 and 1.5. The scaled plots (Fig. 4(d)-Fig. 4(f)), realised using the equations (16), (17) and (19), collapse indicating the validity of the scale analysis.

Similarly, in the HDC_{Sc} region three abscissa: 3, 4 and 5, are chosen and their temperature (Figure 5(a)), concentration (Figure 5(b)) and vertical velocity (Figure 5(c)) plots are presented. Their scaled plots: (Figure 5(d), Figure 5(e) and Figure 5(f)), are realised using the equations (16), (17) and (22). The figures 3, 4 and 5 prove the validity of the scale analysis for the $Ra \cdot S_C \cdot Sch^2 / \left[Pr(1+Pr) \cdot N^3\right] \geq 1$ case.

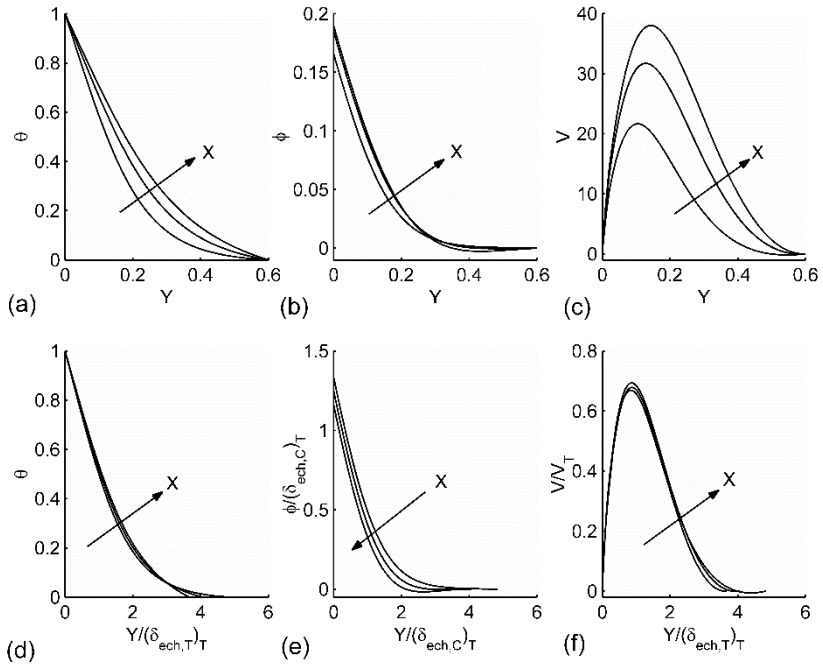


Fig. 4. The (a) θ, (b) φ and (c) V variations as a function of Y and the scaled (d) temperature, (e) concentration and (f) vertical velocity plots for the abscissa: 0.5, 1.0 and 1.5.

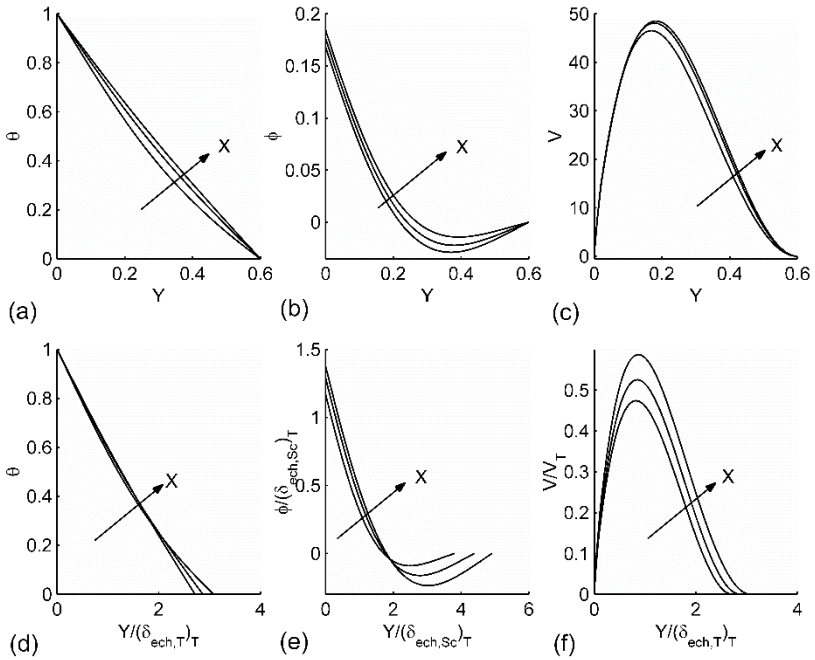


Fig. 5. The (a) θ, (b) φ and (c) V variations as a function of Y and the scaled (d) temperature, (e) concentration and (f) vertical velocity plots for the abscissa: 3, 4 and 5.

The parameters set: $Ra = 5000$, $N = 5$, $Le = 2$, $Pr = 0{,}72$, $S_C = 0.04$, is used to exemplify the $Ra \cdot S_C \cdot Sch^{4/3} / \left[Pr(1+Pr) \cdot N^3\right] < 1$ case.

Figure 2(b) indicates that we should encounter, at equilibrium, a heat driven convection regime (HDC_C) in the $X < X_{trz,C}$ region, a MDC_C regime in the $\left[X_{trz,C} - X_{S,C}\right]$ region and a MDC_{Sc} regime in the $X > X_{S,C}$ region. Equation (20) indicates $X_{trz,C} = 3.34$, while equation (30) indicates $X_{S,C} = 7.73$. A uniform grid of 61x451 points on a domain of 0.6x9.0 was used.

The results of the numerical modeling are presented by Figure 6. The θ (Figure 6(a)), φ/(1/N) (Figure 6(b)), Ψ (Figure 6(c)) and $\partial\varphi/\partial X$ (Figure 6(d)) contour plots reveal the following aspects: at the boundary, the concentration field exceeds $1/N = 1/5$ if $X > 1.6$, while $\partial\varphi/\partial X > 0.0$ at $X > 7.5$; these values are close to the scale analysis results. Each of the figures 7, 8 and 9 presents the plots and the scaled plots for three sections performed in the three regime regions emphasized above: HDC_C, MDC_C and MDC_{Sc}.

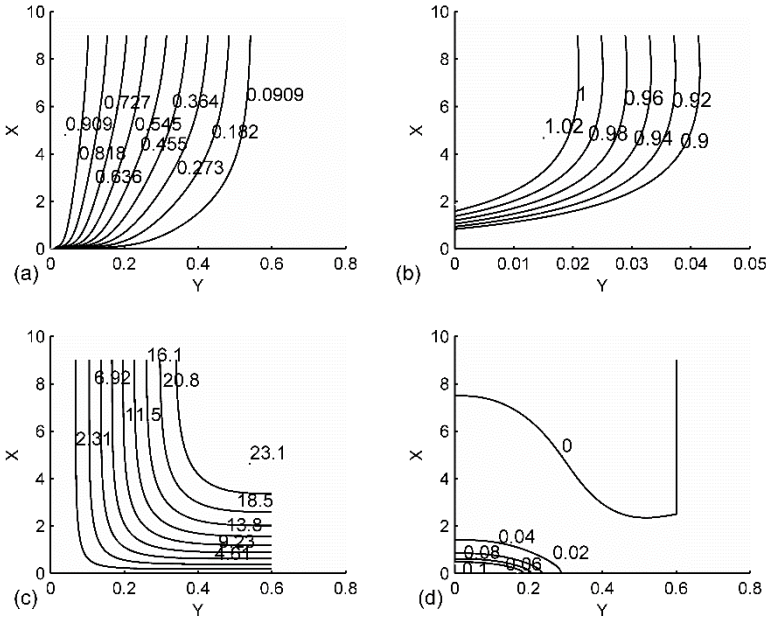


Fig. 6. The θ (a), φ/(1/N) (b), Ψ (c) and $\partial\varphi/\partial x$ (d) fields for $Ra = 5000$, $N = 5$, $Le = 1$, $Pr = 0.72$ and $S_C = 0.04$.

Figure 7(a), Figure 7(b) and Figure 7(c) presents the temperature, concentration and the vertical velocity plots for the abscissa: 0.5, 1.0 and 1.5, while Figure 7(d), Figure 7(e) and Figure 7(f) presents their scaled plots using the equations (16), (17) and (19).

Figure 8(a), Figure 8(b) and Figure 8(c) presents the temperature, concentration and the vertical velocity plots in the sections made at the abscissa: 4.0, 5.0 and 6.0, while Figures 8(d), (e) and (f) shows their scaled plots realised using the equation (33), (34), (35).

Similarly, Figures 9(a), (b) and (c) shows the temperature, concentration and vertical

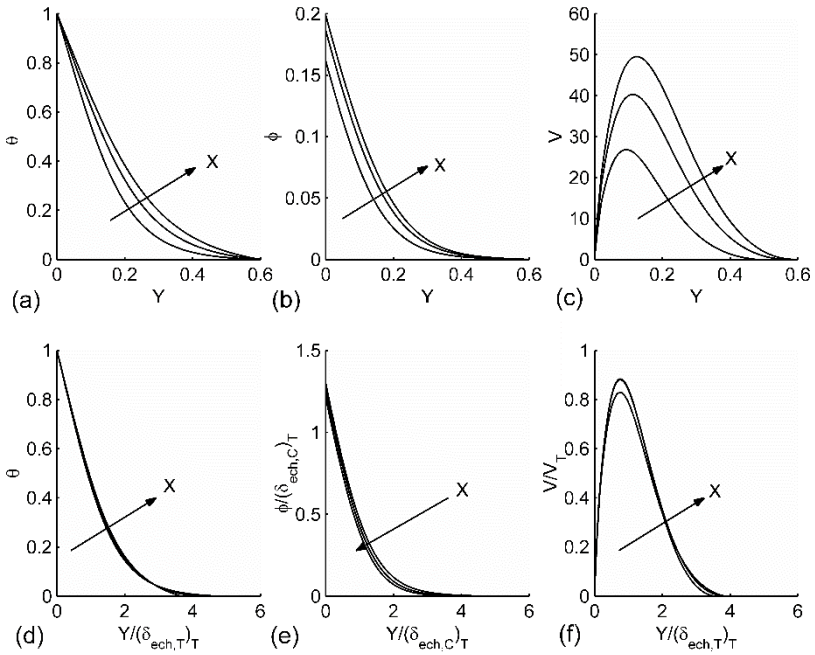


Fig. 7. The (a) θ, (b) φ and (c) V variations as a function of Y and the scaled (d) temperature, (e) concentration and (f) vertical velocity plots for the abscissa: 05, 1.0 and 1.5.

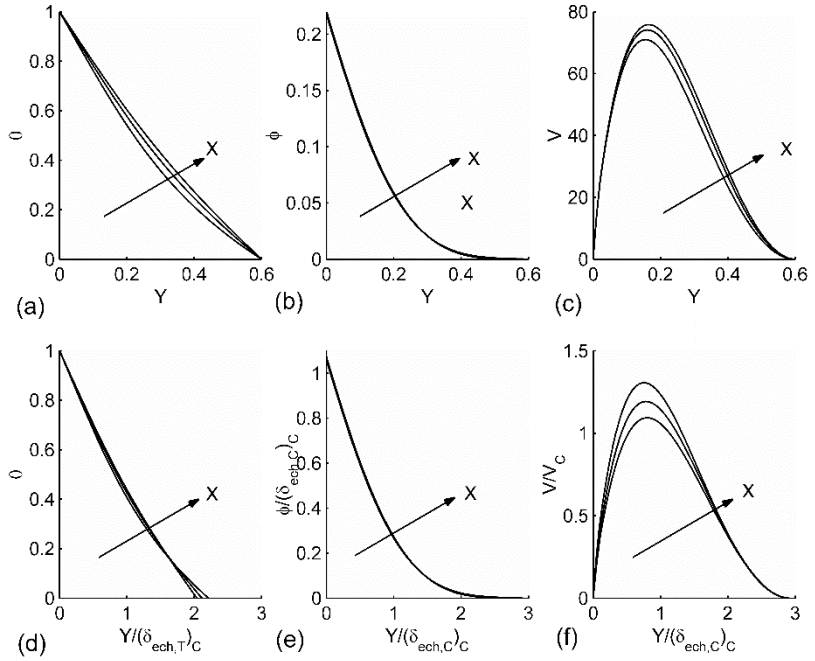


Fig. 8. The (a) θ, (b) φ and (c) V variations as a function of Y and the scaled (d) temperature, (e) concentration and (f) vertical velocity plots for the abscissa: 4.0, 5.0 and 6.0.

velocity plots for two abscissa: 8.0 and 9.0. Figure 9(d), Figure 9(e) and Figure 9(f) shows their scaled plots realised using the equations (28), (29) ad (31).

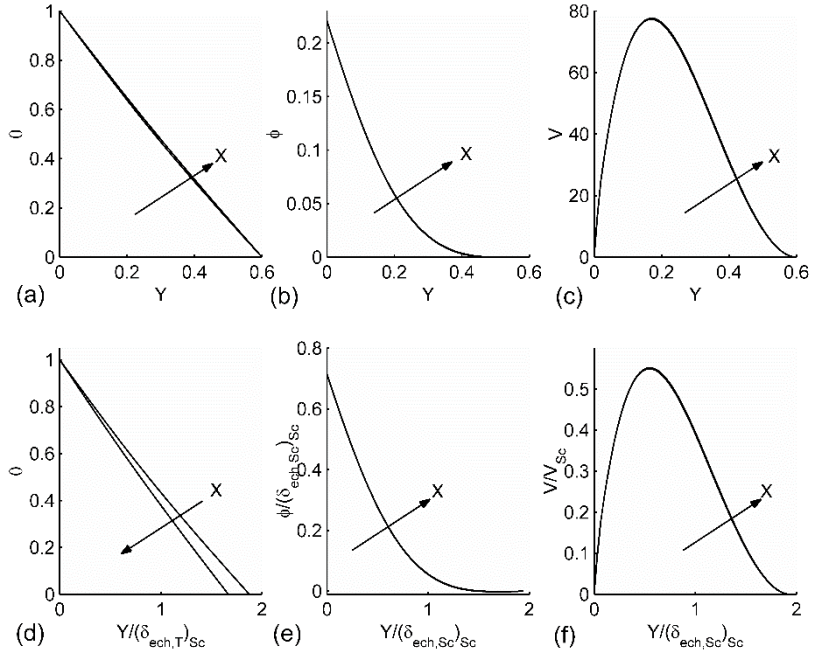


Fig. 9. The (a) θ, (b) φ and (c) V variations as a function of Y and the scaled (d) temperature, (e) concentration and (f) vertical velocity plots for the abscissa: 8.0 and 9.0.

The collapse of the scaled plots of Figures 7, 8 and 9 proves the validity of the scaled analysis results for the $\mathrm{Ra}\cdot \mathrm{S_C}\cdot \mathrm{Sch}^2 / \left|\mathrm{N}^3 \,\mathrm{Pr}(1+\mathrm{Pr})\right| < 1$ case.

6. Conclusions

The natural convection process developed in the boundary layer of a vertical plane wall continues to be the subject of new discoveries. If the temperature and the heat flux of a certain constituent are constant at the wall, then a constant temperature and a linearly mass stratified environment ($\mathrm{Pr} < 1$, $\mathrm{Le} \geq 1$, $\mathrm{Sch} < 1$) imposes special features on the natural convection process. Depending on the process parameters, we can encounter:

a heat driven convection regime along the wall if $\mathrm{Ra}\cdot \mathrm{S_C}\cdot \mathrm{Sch}^2 / \left|\mathrm{N}^3 \,\mathrm{Pr}(1+\mathrm{Pr})\right| \geq 1$;

a succession of heat and mass driven convection regimes if

$\mathrm{Ra}\cdot \mathrm{S_C}\cdot \mathrm{Sch}^2 / \left[\mathrm{N}^3\,\mathrm{Pr}(1+\mathrm{Pr})\right] < 1$.

This result is similar to the results found by previous scientific works:

for $\mathrm{Pr} < 1$, $\mathrm{Le} \geq 1$, $\mathrm{Sch} \geq 1$ case the coefficient that separates the two possibilities (HDC and HDC-MDC) is $\mathrm{Ra}\cdot \mathrm{S_C}\cdot \mathrm{Sch}^{4/3} / \left[\mathrm{N}^3\,\mathrm{Pr}(1+\mathrm{Pr})\right]$, (Neagu, 2021);

for $\mathrm{Pr} \geq 1$, $\mathrm{Le} \geq 1$ case, the coefficient is $\mathrm{Ra}\cdot \gamma^2 \cdot \mathrm{S_C}\cdot \mathrm{Le}^{4/3} / \mathrm{N}^3$, where $\gamma = 1 - \left(1+\mathrm{Pr}^{1/2}\right)^{-1}$, (Neagu, 2018).

These results open the gate for further scientific paths:

a closer analysis of the way in which the non-dimensionalisation process is realised for these particular cases.

a closer analysis of the implications that these results could have on the stability management of the natural convection process.

References

[1] Armfield, S. W., Patterson, J. C. and Lin, W. (2007). Scaling investigation of the natural convection boundary layer on an evenly heated plate, International Journal of Heat and Mass Transfer, 50, 1592-1602

[2] Bejan, A. (1995). Convection Heat Transfer (2nd ed.). New York, U.S.A.: John Wiley & Sons

[3] Lin, W, Armfield, S. W., Patterson, J. C. (2008). Unsteady natural convection boundary layer flow of a linearly-stratified fluid with Pr<1 on an evenly heated semi-infinite vertical plate, International Journal of Heat and Mass Transfer, 51, 327–343

[4] Mongruel, A., Cloitre, M. and Allain, C. (1996). Scaling of boundary-layer flows driven by double-diffusive convection, International Journal of Heat and Mass Transfer, 39, 3899-3910

[5] Neagu, M. (2018). Natural convection near a vertical wall of constant mass flux and temperature situated in a mass stratified fluid, IOP Conference Series: Materials Science and Engineering 444 0822020, 1–14, doi: 10.1088/1757-899X/444/8/0822020

[6] Neagu, M. (2021). Heat/mass driven natural convection of air near a boundary of constant mass flux and temperature, Journal of Multidisciplinary Engineeering Science and Technology, 8(11), 14727-14735, Retrieved from http://www.jmest.org/vol-8-issue-11-november-2021/

[7] Patterson, J. C., Lei, C., Armfield, S. W. and Lin, W. (2009). Scaling of unsteady natural convection boundary layers with a non-instantaneous initiation, International Journal of Thermal Sciences, 48, 1843-1852

[8] Saha, S. C., Patterson, J. C. and Lei, C. (2010). Natural convection boundary–layer adjacent to an inclined flat plate subject to sudden and ramp heating, International Journal of Thermal Sciences, 49, 1600-1612

[9] Saha, S. C., Brown, R. J. and Gu, Y. T. (2012). Scaling of the Prandtl number of the natural convection boundary layer of an inclined flat plate under uniform surface heat flux, International Journal of Heat and Mass Transfer, 55, 2394-2401

[10] Tannehill, J. C., Anderson, D. A. & Pletcher, R. H. (1997). Computational Fluid Mechanics and Heat Transfer (2nd ed.). Washington, U.S.A.: Taylor&Francis

Appendix

Nomenclature

C dimensional concentration of the dissolved species
D diffusion coefficient of the species
g gravitational acceleration
k thermal conductivity of the fluid
h height of the computational domain
H dimensionless height of the computational domain
HDC heat driven convection regime
L characteristic length
Le Lewis number, (α / D)
m_w constant mass flux at the wall
MDC mass driven convection regime
N buoyancy ratio, $[\beta_c (m_w L / D)] / [\beta_t (T_w - T_{\infty,0})]$
p the environment pressure
Pr Prandtl number, ν / α
Ra Rayleigh number, $[g\beta_t (T_w - T_{\infty,0}) L^3 / \alpha\nu]$
s_C environment mass stratification parameter, $(dC_{\infty,x} / dx)$
S_C non-dimensional mass stratification parameter, $[s_C / (m_w L / D)]$
Sch Smith number, ν / D
t dimensional time
t_s time when the $v \cdot \partial C / \partial x$ term becomes dominant in the left hand side of Eq. (5)
t_{trz} dimensional time when the transition to a mass driven convection regime takes place
T dimensional temperature
T_w constant temperature at the wall
u,v dimensional velocity components
U,V dimensionless velocity components, uL/α , vL/α
x,y dimensional Cartesian coordinates
X,Y non-dimensional Cartesian coordinates, x/L , y/L
$(\ )_C$ value defined in a mass driven convection regime where $\partial C / \partial x \geq s_C$

$( )_{Sc}$ value defined in a mass driven convection regime where $\partial C/\partial x < s_C$

$( )_T$ value defined in a heat driven convection regime

Greek symbols

α thermal diffusivity

β_T the coefficients of volumetric expansion with temperature, $(-1/\rho)(\partial\rho/\partial T)_P$

β_C the coefficients of volumetric expansion with concentration, $(-1/\rho)(\partial\rho/\partial C)_P$

Γ_w dimensional concentration gradient at the wall, (m_w/D)

δ boundary-layer thickness

δ_C boundary-layer thickness of the concentration field

φ non-dimensional concentration of the dissolved species, $[(C-C_{\infty,x})/(m_w L/D)]$

ν kinematic viscosity

θ non-dimensional temperature, $[(T-T_\infty)/(T_w-T_\infty)]$

ρ fluid density

τ non-dimensional time, $\tau = t\alpha/L^2$

ψ dimensional stream function

Ψ non-dimensional stream function, ψ/α

ζ dimensionless vorticity

Subscripts

0 reference value

∞ condition at infinity

T related to the temperature field

ech equilibrium state

Sc the $V \cdot S_C$ term is dominant in the left side of Eq. (5)

C the $V \cdot \partial\varphi/\partial X$ term is dominant in the left hand side of Eq. (5)

v Related to the velocity field

x evaluated at abscissa x

Antioxidant and antibacterial potential of *Ulva lactuca* species from Romanian Black Sea Coast

Emin Cadar

Ovidius University of Constanta, Faculty of Pharmacy, Capitan Aviator Al. Serbanescu Street, No. 6, Campus, Corp C, Constanta, 900470, Romania

Ticuta Negreanu-Pirjol

Ovidius University of Constanta, Faculty of Pharmacy, Capitan Aviator Al. Serbanescu Street, No. 6, Campus, Corp C, Constanta, 900470, Romania

Bogdan-Stefan Negreanu-Pirjol

Ovidius University of Constanta, Faculty of Pharmacy, Capitan Aviator Al. Serbanescu Street, No. 6, Campus, Corp C, Constanta, 900470, Romania

Abstract

Green algae biomass represents an important natural resource found in marine environments which could offer new applications in the pharmaceutical and cosmeceutical industries. The paper aim was to characterize some fluid extracts of *Ulva lactuca* L. green macroalgae species, in the Ulvaceae family, from Romanian Black Sea Coast, through physical, chemical and biological methods. For biomedical applications it is important to study how the difference between the marine environment specific conditions, from which they are harvested, can influence the chemical composition of macroalgae. Another important aspect which could affect the bioactive green macroalgae extracts composition, is the influence of applied extraction method. In this way, the less distructive cold maceration method in different solvent concentrations was chosen, according with literature data. The extracts have been studied in order to determine total polyphenols content, total antioxidant capacity by photochemiluminescence method and antibacterial activity. The results emphasized an antioxidant and antibacterial activity of the studied macroalgae extracts, which can be improved in mixture with other bioactive natural substances, in order to obtain new pharmaceutical preparations for topical applications. The valuable obtained results offer the possibility to propose this natural resource for next studies as biomaterial in tissue engineering, wound dressing and drug delivery systems. We consider that *Ulva lactuca* L. green macroalgae species of Romanian Black Sea Coast, could be an important source of marine bioactive compounds with various uses in the biomedical field.

Keywords: *Ulva lactuca* L., bioactive compounds, antioxidant capacity, antibacterial activity

Introduction

Marine algae serve as a large part of the phytoplankton found in various marine environments of the world. Macroalgae represent, macroscopic algae with increased growth rate, which can be found in various areas and have relatively low use of fresh water [1]. Interest in algal biotechnology has been on the rise in recent times, especially on the production of various macroalgae species, used in the food, medical, cosmetic and pharmaceutical industries. Of significant importance is the increased global production of seaweeds in the last few decades [2]. In data literature, there are multiple studies conducted on *Ulva lactuca* L., which grows in the waters of seas and oceans [3], [4].

(a)

(b)

(b)

Fig. 1 (a, b). *Ulva lactuca* L. green macroalgae species

Ulva lactuca L., popular known as *sea lettuce*, see Fig. 1, represents a green macroalgae which belongs to the Chlorophyta Phylum, as described by Linnaeus in the 17th century [5]. It can be found connected, sessile or floating, in various marine environments and it is widespread on the Romanian Black Sea shore. *Ulva lactuca* L. presents polymorphism which led to the assumption, that different species exist depending on the environment. Linnaeus was the first one to discover that *Ulva lactuca* L. presents different phenotypes with a tubular form tail. Taxonomists from the 19th century believed that green algae with tubular tail represent a completely different genus called *Enteromorpha*. In time though, different studies of genetic analysis showed that diverse phenotypes were not linked to genetic variations, so there is no real evidence to support the existence of species other than *Ulva lactuca* L. [6]. Literature still describes distinct *Ulva* species, but reproduction among these species has been demonstrated and therefore they can only be described from now on as *Ulva lactuca* L. variants.

An increased number of studies enphasize marine habitats and other factors such as water temperature or marine pollutants can influence the chemical composition of marine algae. The study of macroalgae increased in interest due to their role of bioindicators [7], [8]. They represent markers that can highlight negative changes in marine ecosystems due to the accumulation of different pollutant agents in their thallus [9]. *Ulva lactuca* L. predominantly invades and blooms near beaches, where it can affect tourism and damage marine ecosystems. The acidic vapors and biodegradation can induce animal deaths and possibly human deaths, due to *Ulva lactuca* L. large quantities degradation [10]. *Ulva lactuca* L. blooms have been reported all over the world, but the biggest event in the world so far was the green tide from the Yellow Sea for ten consecutive years which covers approximately 10% of the whole Yellow Sea surface [11]. In Romania, the South-east coast of the Black Sea, near Constanta and Mangalia cities reported the biggest *Ulva lactuca* L. blooms.

The Romanian Black Sea coast offers great possibilities for harvesting *Ulva lactuca* L., which can be found in significant numbers near the shore and at low depths. *Ulva lactuca* L. is an edible green seaweed with no threats to human health, traditionally consumed in various Asian countries but also approved in Europe [12].

Ulva lactuca L. consists of various natural compounds that can be utilized in many areas like pharmaceutical, cosmetical, chemical, energy or food sector. Seaweed composition includes soluble and insoluble dietary fibers together with minerals, polysaccharides, vitamins, chlorophylls, proteins. The integral use of the plant can represent a more profitable approach in order to utilize all the different constituents that *Ulva lactuca* L. possesses [13], [14].

In the last thirty years, medicinal plants practice has become a major trend worldwide, as the analysis of algae, plant extracts and natural products has shown that green algae represent a potential source of antibacterial activity. Traditional compounds face crises due to the rapidly growing resistance of bacteria, e.g. *Staphylococcus aureus*. Unlike chemicals, herbs are considered non-toxic due to their natural origin and long-term use as folk medicine. In this paper we present the results obtained regarding the antioxidant and antibacterial activity evaluation of this common green macroalgae species present along Romanian Black Sea Coast.

Materials and methods

The analyzed samples consist of green algae species *Ulva lactuca* L., class Ulvophyceae, order Ulvales, family Ulvaceae. The vegetal material was obtained from Romanian Black Sea shore, from four different zones: Vadu, Constanta (the highest macrophyte seaweed area), Mamaia and Navodari, Constanta County, Romania, in the August - October 2021 period. The marine algae collected samples were processed by washing thoroughly with tap water and drying the harvested vegetal material, at room temperature, on metal sieves, 25 ± 2 ˚C, for 24 hours, followed by drying at 40 ˚C in oven with natural convection and grinded in an electric grinder to a fine powder.

Analysis methods were used in accordance to phytochemical, physico-chemical and pharmacognostical studies in literature data. Studies and experiments were made in the laboratories of Ovidius University of Constanta, Faculty of Pharmacy.

The chemical composition was explored in order to identify and classify the classes of compounds that support the physical-chemical and biological characteristics of the samples in comparison with the literature data [3].

Three types of extracts were obtained on using distinct solvents and different reactions for identifying the presence of important active principles of pharmaceutical interest, as Table 1.

For chemical compounds identification of the three extracts, they were analysed separately, using methods fit for the physical and chemical properties for each group of active principles [3].

Table 1. Type of methods and specific reactions used in farmacognostic study [3]

Type of extractive solution	Reaction/Method utilised
Etheric extractive solution (A)	Fluorescent UV (λ=365nm) and Lieberman-Burchard
Alcoholic extractive solution (B)	Fehling reaction, Borntrager reaction and UV method (λ = 365 nm), Iron Chlorure reaction, Liebermann Bourchard reaction
Water extractive solution (C)	Fehling reaction, reaction with H_2SO_4 conc. and tymol, Foaming reaction and reaction with $FeCl_3$

Total phenolic content determination and pigments chemical composition

Total phenolic content determination (TPC): Total phenols present in *Ulva lactuca* L.

samples were determined UV-Vis spectrophotometrically, applying Folin-Ciocalteu reagent as stated by Singleton et al. [15]. Absorbance was determined at 760 nm in comparison to the calibration curve of gallic acid.

T*otal chlorophylls determination* (T-Ch): We homogenized three grams of *Ulva lactuca* samples in acetone (30 mL, 80%) and incubated over the night, to protect it from sunlight, at 4 °C in order to obtain a complete extraction followed by centrifugation at 10,000 xg for 5 min. Total chlorophyll (T-Chl), chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) found inside the supernatant were measured UV-Vis spectrophotometrically at 664 nm wavelength, in accordance to Lichtenthaler and Buschmann method [16].

Total carotenoids determination (TCAR): Total carotenoids were determined UV-Vis spectrophotometrically at 450 nm wavelength, in accordance to AOAC [17]. *β*-carotene was utilised as a standard.

Antioxidant activity

Algae fluid extracts were obtained using two different methods, cold maceration and Soxhlet method, see Fig. 2.

Sample preparation - The vegetal product has been made into fluid extracts using as solvents, ethyl alcohol 50% and 70% concentration, using two common extraction methods:

Cold maceration for variable concentrations of vegetal product, 2.5 g, 5.0 g, respectively 10 g in 100 mL, both ethyl alcohol 50% and 70% concentration, for 12 days at darkness, room temperature, periodic stirring.

Soxhlet extraction for three hours refluxing for variable concentrations of vegetal product, 2.5 g, 5.0 g, respectively 10 g in 150 mL ethyl alcohol 70% concentration.

All the hydroalcoholic extracts obtained were filtered at normal pressure through quantitative Whatman filter paper and present different colors, from pale green to green-brown and dark green.

For determining *total antioxidant capacity (TEAC),* 10 μL volume of algae extracts were taken for analysis. Each determination lasted 120s. Analyses employed the photo-chemiluminescence method by ACL (Antioxidative Capacity in Lipid Soluble Substances) procedure Analytik Jena, using Photochem apparatus Analytik Jena AG, Germany, see Fig. 2. Triplicate samples of hydroalcoholic extracts were quantified by comparison with the standard substance Trolox®, Hoffman-LaRoche's trade name (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). For calibration, the standard kit of reagents, Analytik Jena, Germany, was used: R1 (dilution solvent), R2 (buffer reagent), R3 (photosensitive reagent), R4 (reagent sized). For the calibration curve standard solutions containing 0.5, 1.0, 2.0, 3.0 nmol Trolox were measured (suitable for 10 μL R4), as Trolox calibration curve, Fig. 3.

Fig. 2. *Ulva lactuca* L. hydroalcoholic extracts samples obtained by cold maceration and Soxhlet extraction and Photochem apparatus Analytik Jena AG, Germany

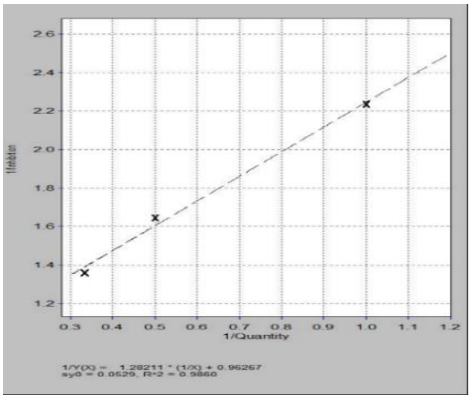


Fig. 3. Trolox standard calibration curve

Antibacterial activity

In order to demonstrate the antibacterial activities, we measured areas of inhibition for the different microorganisms that were classified under standardized conditions: from the gram positive bacteria we had *Staphylococcus aureus* (MTCC 737) and *Staphylococcus epidermidis* (MTCC 3615). We also had the gram negative bacteria - *Escherichia coli* (MTCC 1687) and *Pseudomonas aeruginosa* (MTCC 424). Ampicillin was used as reference substance, the standard drug.

Solvents chloroform, *n*-hexane and ethyl alcohol – water (1:1) were used to extract the material previously powdered (300 g), using the maceration process for 72 hours, ocassionally stirring from time to time.

Each of the three extracts were concentrated after it was brought together with the filtrate and we kept them in the desiccator for future experiments. The *n*-hexane extract, chloroform extract and ethanol: water (1:1) extract were concentrated after combining the filtrate and kept in desiccator for further investigation.

Results and Discussions

The marine algae samples were first treated with a non-polar solvent such as benzen or dyethil ether, then with a medium polarity solvent such as methyl alcohol or ethyl alcohol and finally with water. Each extract has been afterwards analysed in order to identify the presence of active principles of pharmaceutical interest. The extracts were analysed individually using the specific methods for each group of active principles.

Table 2. Active principles identified in green algae *Ulva lactuca* L.

Table 2

Type of extractive solution	Class of active principles
Etheric extractive solution (A)	steroles
	triterpenes
	cumarines
Alcoholic extractive solution (B)	catehic tannins
	reducing compounds
	antracenozides
	cumarines
Water extractive solution (C)	reducing compounds
	saponosides
	catehic tannins

highlights the active principles identified in green algae *Ulva lactuca* L. It is interesting that in all three extractive solutions studied different classes of bioactive compounds have been identified that are responsible for the antioxidant and antimicrobial activity that we studied in the paper.

Total phenolic content determination and pigments chemical composition

The antioxidant capacity and antibacterial potential of the marine algae samples were highlighted by identifying and determining different natural compounds and their active principles, recognized in literature data for having the potential to give antioxidant and antibacterial activity. In the above table we showed our results for the determination of total phenolic contents, total chlorophylls (*a +b*) and total carotenoids. Table 3 presents the results obtained for the compositions of the studied marine algae. There is a good consistency with the literature data of other researchers who have worked on green seaweed. Phenolic compounds can be defined by their aromatic rings and their hydroxyl groups. A phenolic compound which contains an aromatic ring with one or two groups of hydroxyls are named simple phenols, for example benzoic acid or hydroxycinnamic acid which can be found in marine algae structure. Polyphenols represent a large family comprised by multiple phenol units [18]. From this method we can conclude that the most stable extracts and the highest inhibition are the *Ulva lactuca* L. extracts made with the Soxhlet extraction, from which it can highlight *Ulva lactuca* L. extract 10 g/100 mL ethanol 70%, with an increased antioxidant activity. From the cold maceration, the best results were

obtained from *Ulva lactuca* L. extract 5 g/100 mL ethanol 50% and *Ulva lactuca* L. extract 2.5 g/100 mL ethanol 70%.

Table 3. Total phenolic content, total chlorophylls and total carotenoids contents from extracts of green macroalgae *Ulva lactuca* L. from the Romanian Black Sea shore

Sample	Total phenolic TPC (mg. GAE/100g d.w.)	Total chlorophylls T-Chl (a) mg/g d.w	Total chlorophylls T-Chl (b) mg/g d.w	Total carotenoids (TCAR):
Ulva lactuca L.	286.1 ± 0.6	21.08 ± 2.69	3.77±0.36	10.57±0.85

All values show mean of three replicates, ± standard deviation

The unique chemical, physical and biological structure of polyphenols can divide them into different classed like phloroglucinols and phlorotannins. In recent times, various studies showed multiple beneficial therapeutical actions for phenolic compounds found in marine algae, such as antioxidant, antibacterial, anticancer and anti-inflammatory activities.

Marine algae organisms can synthesize different pigments such as carotenoids, phycobiliproteins and chlorophylls [19]. The green pigment for *Ulva lactuca* L. is given by the presence of *clorophylls a* and *b* [19].

Antioxidant activity

Samples were prepared and the results were expressed as nmol Trolox equivalents/sample volume, according to Table 4.

Table 4. Maximum inhibition of free radicals and total antioxidant capacity of *Ulva lactuca* L. extracts

Extract samples	Max. Inhibition of free radicals	Total antioxidant capacity (nmol equiv. Trolox/sample volume)
Ulva lactuca extract 2.5g/100mL ethanol 50%, cold maceration	0.213	0.109
Ulva lactuca extract 5g/100mL ethanol 50%, cold maceration	0.257	0.161
Ulva lactuca extract 10g/100mL ethanol 50%, cold maceration	0.178	0.023
Ulva lactuca extract 2.5g/100mL ethanol 70%, cold maceration	0.381	0.285

Ulva lactuca extract 5g/100mL ethanol 70%, cold maceration	0.245	0.138
Ulva lactuca extract 10g/100mL ethanol 70%, cold maceration	0.024	0.022
Ulva lactuca extract 2.5g/100mL ethanol 70%, Soxhlet extraction	0.140	0.035
Ulva lactuca extract 5g/100mL ethanol 70%, Soxhlet extraction	0.231	0.111
Ulva lactuca extract 10g/100mL ethanol 70%, Soxhlet extraction	0.311	0.272

In the hydroalcoholic extracts of *Ulva lactuca*, obtained by cold maceration, a significant total antioxidant capacity was recorded for *Ulva lactuca* extract 5 g/100 mL ethanol 50% with 0.161 nmol equiv. Trolox/10 µL sample and for *Ulva lactuca* extract 2.5 g/100 mL ethanol 70% with 0.285 nmol equiv. Trolox/10 µL sample.

In the hydroalcoholic extracts of *Ulva lactuca*, obtained by reflux, Soxhlet extraction, a significant total antioxidant capacity, compared to extracts obtained by cold maceration, was recorded for *Ulva lactuca* extract 10 g/100 mL ethanol 70%, with 0.272 nmol equiv. Trolox/10 µL sample.

The highest antioxidant activity was obtained through cold maceration for *Ulva lactuca* extract 2.5 g/100 mL ethanol 70% sample, with a value of 0.285 nmol equiv. Trolox/10 µL sample.

The results obtained emphasize that extracts obtained in ethanol 70% induce the highest values of total antioxidant activities for different concentrations of the samples.

Antioxidants are very important compounds that can stop the production of free radicals and the chain reactions that may affect important cells in the organism through oxidation. They are also used in food and pharmaceutical preservation, with increasing percentage in recent times because of the toxicity and danger to human health caused by the use of synthetic antioxidants [20]. Seaweeds represent an important natural source for antioxidants with very little side effects. In human organism antioxidants are significant in their activity of regulating the oxidative stress related diseases [20, 21].

Ulva lactuca L. contain a varied compounds array with antioxidant activit such as, tocopherols, carotenoids and polyphenols. An important compound with antioxidant potential in green seaweeds is represented by ulvan, a sulfated heteropoly-saccharide obtained from *Ulva lactuca* [22].

Antioxidant potential was measured by scavenging activity of hydroxyl and superoxide radicals, metal chelating potential and superoxide radicals. Sulfate contents in ulvan has been altered by using trioxide/*N,N*-dimethyl formamide. After the modification all the parameters studied gave better results, higher scavenging activity, better reducing power and stronger chelating ability [22].

From this study we draw the conclusion that even if sulfates are already in the composition of ulvan, by increasing the number of sulfate radicals we can enhance the potential of its antioxidant activity [22].

Antibacterial activity

Many species of marine algae have been studied in order to examine their antibacterial and antiviral properties. A lot of polysaccharides have been found in the composition of seaweeds, from which we highlight some that could potentially exhibit antibacterial and antiviral activity: alginic acid, agar, laminarin, fucoidan, galactans, carrageenan, and mannans [23]. In this present study we explore the potential antibacterial activity of green seaweed *Ulva lactuca* L. Review of literature has highlighted possible antibacterial activity in different compounds present in the green algae. In the view of this, an effort was made to verify some of the isolated compounds from *Ulva lactuca* L. for their antibacterial activity. The area of inhibition of microorganisms under standardized conditions was used in order to demonstrate antibacterial action for the various compounds. For present work, efficacy of eight compounds were detected against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Escherichia coli.*, see Fig. 4. The concentration used for the test compound was 1mg/mL. and we took Ampicillin as the standard drug.

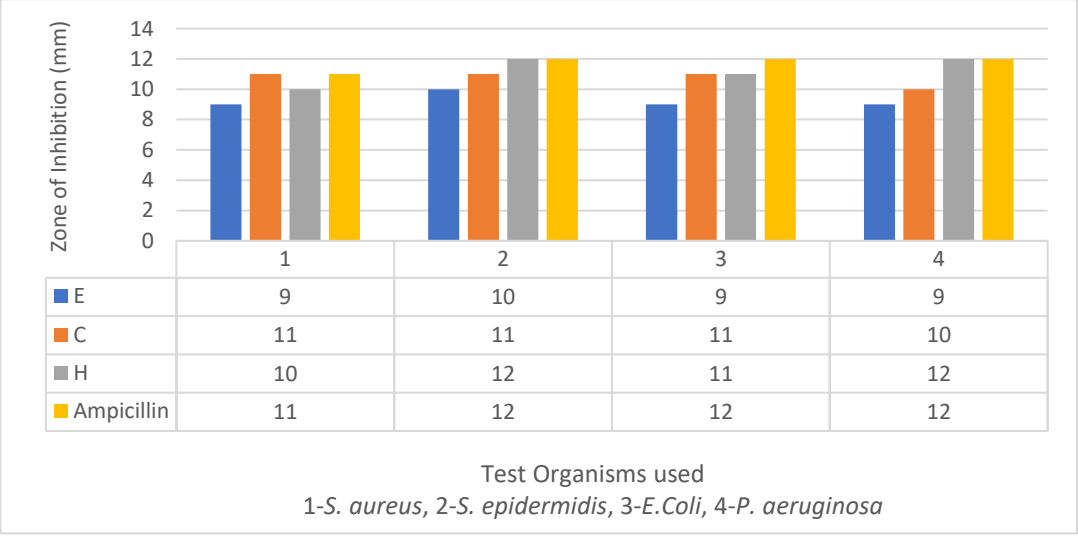


Fig. 4. Antibacterial activity of *Ulva lactuca* L. species

Against *Staphylococcus aureus* the chloroform extract showed the highest inhibition zone (11 mm) comparable to that of the standard ampicillin. In the case of *Staphylococcus epidermides* and *Pseudomonas aeruginosa,* the extract in *n*-hexane achieved the largest inhibition zone (12 mm) comparable to that of th*e* ampicillin standard. In the case of *Escherichia coli*, the extracts in *n*-hexane and in chloroform achieved the largest areas of inhibition (11 mm) but are below the values presented by the ampicillin standard. From the experiments we concluded that *n*-hexane and chloroform extracts had antibacterial activity comparable to the standard drug, ampicilin.

Conclusions

Green algae *Ulva lactuca* L. shows promising potential for both antioxidant and antimicrobial activity, that are due to the existence of known bioactive chemical compounds that support these characteristics. *Ulva lactuca* L. is also a source for many essential bioactive compounds ranging from antioxidants and essential minerals to the highly signified and complete profiles of amino acids and fatty acids. In the present study we determined the total phenolic content, total chlorophylls T-Chl (*a + b*) and total carotenoids. We managed to demonstrate that various compounds from *Ulva lactuca* L. exhibit antioxidant activity which is given by various compounds such as phenolic constituents, pigments and other active principles and we showed that the *n*-hexane and chloroform extracts display good antibacterial activity on various gram positive and negative bacteria.

References

[1] Thomas, N.V., Kim, S.K. Potential pharmacological applications of polyphenolic derivatives from marine brown algae, Environ. Toxicol. Pharmacol. 32, p. 325–335, 2011.
[2] White, W.L.; Wilson, P. World seaweed utilization: A summary. J. Aquac. Res. Dev. 2015, 6, 7–25.
[3] Sirbu, R., Tomescu, A., Jurja, S., *et al*., Study of bioactiv pharmaceutical components from seaweeds from the Black Sea, 15-th International Multidisciplinary Scientific Geoconference - SGEM, Albena, Bulgaria, Nano, Bio and Green - Technologies for a Sustainable Future, vol. I, 567-574, 2015.
[4] Sirbu, R., Negreanu-Pirjol, T., Paris, S., Negreanu-Pirjol, B.S., Jurja, S., Tomescu, A., Important bioactive compounds from marine algae – potential source of pharmaceutical industry, 14th International - SGEM Conference Proceedings, Bulgaria, vol. I, 381-388, 2014.
[5] Carl, C., de Nys, R., Lawton, R.J., Paul, N.A., Methods for the Induction of Reproduction in a Tropical Species of Filamentous *Ulva*, Plos On, Vol. 9, Iss. 5, e97396, 2014.
[6] Hayden, H.S., Blomster, J., Maggs, C.A., Silva, P.C., Stanhope, M.J., Waaland, J.R. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. Eur. J. Phycol. 2003; 38:277–294.

[7] Stanojkovic, T.P., Konic-Ristic, A., Kljajic, Z., Grozdanicstanisavljevic, N., Srdic-Rajic, T., Zdunic, G., Savikin, K.J. Nanomater. Bios. 9, 2014.
[8] Cadar, E., Mustafa, A., Tomescu, A., Cherim M., Studies regarding polluting agents in Black Sea algae, Journal of Science and Arts, vol. 1, p. 255-264, 2018.
[9] Claudio, S., Gabriela, L., Erasmo, M., Doris, O., Waldo, Q., Murray, B., Variation in Patterns of Metal Accumulation in Thallus Parts of *Lessonia trabeculata* (Laminariales; Phaeophyceae): Implications for Biomonitoring, 2012.
[10] Chevassus-au-Louis, B., Andral, B., Femenias, A., Buvier, M. Bilan des Connaissances scientifiques sur Les Causes de Prolifération de Macroalgues Vertes, Conseil Général de L'environnement et du Développement Durable; Paris, France: 2012. Rapport Pour Le Gouvernement Français, 2012.
[11] Li, J.-Y., Yang, F., Jin L., Wang, Q., Yin, J., He P., Chen, Y. Safety and quality of the green tide algal species *Ulva prolifera* for option of human consumption: A nutrition and contamination study, Chemosphere, 210, 1021-1028, 2018.
[12] Pengzhan, Y., Quanbin, Z., Ning, L., Zuhong, X., Yanmei, W., Zhi'en, L., Polysaccharides from *Ulva pertusa* (Chlorophyta) and preliminary studies on their antihyperlipidemia activity, Journal of applied phycology, 15, 21-27, 2003.
[13] Sirbu, R., Negreanu-Pirjol, T., Paris, S., Negreanu-Pirjol, B.S., Jurja, S., Tomescu, A., Important bioactive compounds from marine algae – potential source of pharmaceutical industry, 14th International - SGEM Conference Proceedings, Bulgaria, vol. I, 381-388, 2014.
[14] Sirbu, R., Cadar, E., Erimia, C. L., *et al*., Studies on the importance of vitamins from Black Sea marine algae, 3rd International Multidisciplinary Scientific Conference on Social Sciences and Arts, SGEM, Bulgaria, Conference Proceedings, II, 941-948, 2016.
[15] Singleton, V.L., Orthofer R., Lamuela Raventos, R.M., Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178, 1999.
[16] Hanaa, Abd El-Baky, Farouk K., El Baz, Gamal, El Baroty, Evaluation of Marine Alga *Ulva lactuca* L. as A Source of Natural Preservative Ingredient, Electronic Journal of Environmental, Agricultural and Food Chemistry 3(11):434-444, 2008.
[17] AOAC. Official Methods of Analysis. Association of Official Analytical Chemists, 16th Ed., K Hlrich. Arlington, Virginia, 1995.
[18] Quideau, S. P., Deffieux, D., Douat-Casassus, C. L., Pouységu, L., Plant Polyphenols: Chemical Properties, Biological Activities and Synthesis, Angewandte Chemie International Edition, 50 (3): 586–621, 2011.
[19] Pérez, M. J., Falqué, E. Domínguez, H, Antimicrobial action of compounds from marine seaweed. Mar. Drugs, 14(3), 52, 2016
[20] Negreanu-Pirjol, T., Negreanu-Pirjol, B., Sirbu, R., Paraschiv, G.M., Meghea, A., Comparative studies regarding the antioxidative activity of some therapeutic

marine algae species along the Romanian Black Sea coast, J. Environ. Prot. Ecol., vol. 13 (3A), 1744-1750, 2012.

[21] Negreanu-Pirjol, B.S., Negreanu-Pirjol, T., Mirea, M., Vasile, M., Cadar, E., Antioxidant capacity of some marine green macroalgae species fluid extracts, 18th International Multidisciplinary Scientific Conferences on Earth & GeoSciences – SGEM Vienna Green 2018, 3 – 6 December 2018, Vienna, Austria, Hofburg Congress Center, Conference Proceedings, Volume 18 - Nano, Bio, Green and Space Technologies for a Sustainable Future, Issue 6.4, Section 8 „Advances in Biotechnology", paper 51, p. 63 – 69.

[22] Indana Mo'o FR, Wilar G, Devkota HP, Wathoni N. Ulvan, a Polysaccharide from Macroalga *Ulva* sp.: A Review of Chemistry, Biological Activities and Potential for Food and Biomedical Applications, Applied Sciences, 2020.

[23] Kolanjinathan, K., Stella, D., Comparitive Studies on Antimicrobial Activity of *Ulva reticulata* and *Ulva lactuca* against Human Pathogens, International Journal of Pharmaceutical & Biological Archives, 2011, 2(6):1738-1744, 2011.

Importance of Bioactive Compounds of *Ganoderma lucidum* Extract in Medical Field

Carolina Pascale

University of Medicine and Pharmacy Carol Davila, Bucharest, Romania,

Rodica Sirbu

University of Medicine and Pharmacy Carol Davila, Bucharest, Romania,

Emin Cadar

Ovidius University of Constanta, Faculty of Pharmacy, Romania,

Abstract

Ganoderma lucidum is a well-known medicinal mushroom with a long history, used as an ancient remedy for promoting health and increasing longevity. This species of *Ganoderma* genus is important for our study because it has an interesting chemical composition and pharmacological benefits such as immune suppressor, hypocholesterolaemia, hypotensive, antitumoral, antioxidant, anti-inflammatory agents, and more others. *G. lucidum* chemical composition has many compounds such as vitamins (mainly Vitamin E), polysaccharides, triterpenes and each one of them is offering a distinctive pharmacological effect. This species of *Ganoderma* medicinal mushroom is used extensively for its internal effect on improving immune system, hepatoprotective benefit and additionally, a large variety of people consume *Ganoderma lucidum* as tea infusion and coffee because its antioxidant and reducing stress effects.

Keywords: *Ganoderma lucidum*, antitumoral, polysaccharides, anti-inflammatory, antioxidant.

Introduction

Ganoderma lucidum (*G. lucidum*) is the most known medicinal fungus due to its impressive natural ingredients. Its pharmacological application dates back nearly two thousand years ago [1] and it is known as Lingzhi from Chinese population and as Reishi in Japan [2].

G. lucidum is known as basidiomycete and according to Li, has been called as the "Mushroom of Immortality" [1]. This mushroom has been used in China for healing purpose and has been predominantly used by Asian population for an alternative of

chemotherapy treatment to inhibit cancer or to handle side-effects from chemotherapy medicine [3]. Nowadays, usage of this fungus increased because its potential of reducing obesity, cholesterol, for gout health, decreasing cardiovascular disorders, controlling the diabetes and other metabolical diseases, stimulation of probiotics [4].

William Curtis was a researcher who discovered *Ganoderma lucidum* in 1781 based on grown material in England [2]. According to Moncalvo 1995, in that time confusions were wide between *Ganoderma tsugae* and *Ganoderma lucidum* because of chlamydospore production regarding the distinction of both species. European *G. lucidum* does not produce ovoid chlamydospore like *G. tsugae*, but an interesting fact is American *G. lucium* produces ovoid chlamydospore. Many other species have been researched and beside these, Adaskaveg 1989 reported that North American *G. lucidum* and European *G. resinaceum* belong to the same species, but Moncalvo 1995 hasn't reported any evidence of these [5].

Work by Sun (2014) clarify difficulties associated with the separation and identification of polysaccharides from *Ganoderma* spp. and settle a high-performance liquid chromatography (HPLC) based methodology to characterise species from *Ganoderma* genus using polysaccharide fingerprint profiling [6].

Such as *Ganoderma* polysaccharides, many other papers were published to value triterpenes, especially Ganoderic Acids, to differentiate between *Ganoderma* species. A study released by Chen 1999 shows a method of analysis of triterpenoids to differentiate between *G. tsugae* and *G. lucidum* [7].

Even though it has a variety of components, polysaccharides and triterpenoids are the most important compounds from this fungus and some authors consider it being responsible for the most of its pharmacological activity [8].

Over the years, *G. lucidum* defines its potential due to its largely acceptance of the population and usage of it for many human conditions. Nowadays, it is known that this fungus has numerous pharmacological effects such as: immunomodulating, anti-atherosclerotic, anti-inflammatory, analgesic, chemo preventive, anti-tumor, radio-protective, sleep-promoting, antibacterial, antiviral, hepatoprotective, anti-diabetic, anti-aging, antioxidative, hypoglycaemic [9].

A study published by Gerenutti et al. 2021, shows an *in vitro* activity of *G. lucidum*, used for evaluation of antitumor, immunomodulatory, antioxidant, neuronal cell protection, antifungal, and antimicrobial activity. Based on author's research in literature, all authors reported in vitro antitumoral activity, positive effect of *G. lucidum* extract on immunomodulation, antioxidant activity of the extract, benefits as adjuvant in neurodegenerative diseases, antimutagenic activity of the extract of the body and the mycelia of *G. lucidum* [10].

It is known that the common way to administrate this supplement is oral, but dosage and posology varies from person to person. Searching in literature, *in vivo* tests aren't

so common. So, based on Gerenutti et al. 2021 work, are related some interesting in vivo activity for *Ganoderma lucidum* extract. And these are: evidence-based antitumoral activity against breast cancer, lung cancer, leukemia; testosterone inhibition activity with the result of reducing the malignancy of prostate cancer; protection against idiosyncrasies side effects induced by antitumoral agents and, protection against radiotherapy; hepatoprotective effect was also reported after drug induced hepatotoxicity [10].

Materials and Methods

G. lucidum is found all over the world, growing on multiple hosts. In Europe, it grows on living trees or stumps of oaks or chestnuts, rarely on coniferous trees [11]. The study area is Sub Carpati Region of the Romanian montain chain. We choose this surface in order to contibuite at the development of wild products in the region of Romania and their use in the medical field.

Botanical description

This species belongs to kingdom Fungi, phyla Basidiomycota, Class Agaricomycetes, Order Polyporales, Family Ganodermataceae, Genus Ganoderma and Species *Ganoderma lucidum* [10]. Fungus of this Genus have a shiny surface that is associated with the presence of thickwalled pilocystida embedded in an extracellular melanin matrix [12]. *Ganoderma* is characterised due to its shape and exceptional colors (red, black, blue, white, yellow, purple) of the fruiting body. These characteristics differ between species due to their geographical origins and cultivation environments [13].

Figure 1. ***Ganoderma lucidum***

General methods for obtaining composition data

The standard AOAC International (Association of Official Analytical Chemists) (2016) procedures were applied in order to determinate carbohydrates, proteins, lipids and ash. The Kjeldahl method of protein estimation was applied based on the calculation

of total N (Azote). Total lipid content was made by Soxhlet extracting method with petroleum ether. The ash content was determined gravimetrically after incineration at 600 ± 15˚C. The carbohydrate content was determined by calculation. The NaCl content was determined by the Mohr method with the modifications made by Taofiq et al. (2017) [14].

G. lucidum ethanolic extract

A simple method of extraction using ethanol is described by Taofiq [14]. The dried powder of *G. lucidum* (3g) was extracted in Soxhlet apparatus for 5-6h (35 ± 5 min/ cycle) using ethanol. In the end of this procedure, the final solvent was evaporated under reduced pressure to obtain the dried ethanolic extracts. In addition, to characterise phenolic and triterpenoid compounds, the obtained extracts were dissolved in ethanol (5.5 mg/mL), followed by a filtration through 0.22 µm nylon syringe filter and analysed by high performance liquid chromatography equipped with photodiode array detection-mass (HPLC-DAD-ESI/MSn). Alongside with characterising these two bioactive compounds, antioxidant, antityrosinase, anti-inflammatory, antimicrobial activity, and cytotoxicity in human tumour cell or in non-tumour cells lines can start being analysed with this ethanolic extract method, according to Taofiq [14].

Extraction of polysaccharides

A method that determinate the total polysaccharides from *G. lucidum* sample is described by Taofiq, with some modifications of Vazirian et al. 2014, method. Shortly, method is based on the dry sample (1.5 g) extracted by maceration with boiling water and then filtered under vacuum. In addition, this step was repeated twice. Next, filtered extract was lyophilized and fraction of polysaccharide is dissolved in water at a concentration of 5mg/mL. To the obtained solution (1mL), phenol (4%, 25µL) is added, followed by sulphuric acid (1mL). The final solution is stirred for 30 seconds and then measured using a UV-VIS spectrophotometer, absorbance is read at 548 nm against a blank solution (distilled water). This method uses Starch (5-50 µg/mL) as a standard and results are expressed in mg of starch equivalents per g of dw (dry weight) [14].

Extraction of triterpenoids

Nowadays, even the technology arises, for terpenoids, remain these two classical methods of extraction. Colorimetric method using vanillin-perchloric acid spectroscopic method, which can be estimated the total triterpenoids content, and the other one with determinating individual triterpenoids using high-performance liquid chromatography (HPLC) or thin layer chromatography (TLC). For both methods, many authors reported severe conditions of high temperature and strong acid and these methods are considered destructive methods. According to Chen 2012, near infrared (NIR) spectroscopy offer some advantages such as high efficiency, low cost, easy operation, less or no sample preparation and quick data analysis [15].

Results and Discussions

Ganoderma lucidum is a type of medicinal mushroom full of bioactive compounds. In addition to the mainly compounds, there are other biological components of pharmaceutical interest, such as: fat, proteins, ash, carbohydrates, and sodium chloride that are presented in Table 1.

Table 1. Chemical composition of *Ganoderma lucidum*

Component	Mean ± SD (g/100 g)
Lipids	2.85±0.03
Proteins	7.85±0.05
Ash	2.1±0.15
Total carbohydrates	86.64±0.2
NaCl	0.56±0.04

Ganoderma lucidum ethanolic extract reveals tyrosinase activity through the compound Ganodermanondiol which suppresses the expression of tyrosinase-related activity (TRP-1, TRP-2) and microphthalmia-associated transcription factor (MITF) thereby inhibiting the synthesis of melanin. In this case, tyrosinase inhibitors contribute to skin lightning and with the help of another existent compound in the fungus, phenolic acids, reduce the severity of hyperpigmentation and decrease melanin biosynthesis. The extract has also showed anti-inflammatory activity by inhibiting NO production and at this activity phenolic acids and triterpenic acids such as, Ganoderic Acid C1 and Lanostane are contributing to its anti-inflammatory activity. Antitumor activity is present, and also high antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MSSA) and methicillin-sensitive *Staphylococcus aureus* (MRSA) [14].

Polysaccharides from *G. lucidum*

For so long, a lot of scientific attention has been focused on polysaccharides from the *Ganoderma* spp. In fact, polysaccharides represent biological macromolecules with a complex structure of elements and a wide range of physicochemical properties [14]. The content in polysaccharides was 15.4±0.2 mg starch/g, reported by Taofiq [14].

The most important benefical function of oligosaccharides in human body are: doesn't stimulate or increase the level of blood glucose, decrease the absorption of released glucose; provide small amount of energy, improve the intestinal environment and mentain intestinal tract's health as probiotic.

Polysaccharides from *Ganoderma* spp. have a good antioxidant effect due to its potential of reducing damage, caused by the free radicals formed in the oxidation

reaction. Polysaccharides act as anti-diabetic agents with the result of lowering blood glucose. Beside these functions, this bioactive compound possesses inhibitive effect on the tumour growth and enhance proliferation of macrophage cells in the immune system as a result of antitumoral agent against breast cancer [17].

Triterpenoids from *G. lucidum*

Studies have shown that over 150 triterpenoids were found in *Ganoderma* spp. and these are: ganoderic, lucidenic, ganodermic, ganoderenic, ganolucidic and applanoxidic acids, lucidones, ganoderals and ganoderols. The content of Triterpenoids reported by Taofiq was 27.2 ± 0.7 mg ursolic acid/g and in Terpenoids was 27.2 ± 0.7 mg linalool/g [14].

Naturally, triterpenoids are a subtype of Terpene composed of one or more isoprene units. Triterpenes isolated from *G. lucidum* spores have shown significantly pharmaceutical activity against many human disorders, including cancer. Studies have shown that Ganoderic acid T, D, F provide strong cytotoxic effects at low concentrations in various human cancer cell lines. Anti-inflammatory and antioxidant properties possess remarkable pharmacological activities [15].

Regarding the benefits of bioactive components, it is known that the antitumoral effect is mostly given by β-glucans from polysaccharides. But its also worth to mention the other important bioactive component, triterpenoid which provides antitumor effect. Beside it's widely discussed and studied effect, this compound also provides antioxidant and immounomodulatory effects, proved by in vitro and in vivo studies.

Table 2. Therapeutic effects and bioactive compounds of *Ganoderma lucidum* reported in literature

Pharmacological effect	Bioactive compound	References
Immunomodulating effect	Ganoderic acids	[18]
Antitumoral, Chemo preventive, Radio preventive effects	β-D-glucans, Heteropolysaccharides, Glycoproteins.	[18][19]
Anti-HIV effect	Triterpenoids	[20]
Anti-diabetic effect	Glycans	[21]
Hepatoprotective effect	Ganoderic acids	[22]
Antiinflammatory effect	Ganoderic acids	[23]
Anti-allergic effect	Ganoderic acids	[23]

These days, *Ganoderma lucidum* and not just this species, are consumed by population almost everyday. Its usage as nutraceutical and pharmaceutical formulation increased its popularity among researchers and of course, pharmaceutical companies. Based on this popularization, it become very popular between Americans and Europeans. The "Mushroom of Immortality" [1] has been utilized as herbal extract like tea infusion and as tonic, known from Traditional Chinese Medicine (TCM), and also as an alternative treatment of neurasthenia, hypertension, diabetes, liver diseases, arthritis, bronchitis, asthma, fatigue, coughing, insomnia, and many other. Is widely used for promoting human vitality and longevity based on TCM medicine.

Nowadays, *G. lucidum* has been recognized as an alternative treatment in the prevention and treatment of leukimia, carcinoma, hearth disease, immune system enhancer, detoxify effect [9][24]. Now, there are more then a hundred brands of different products based on *G. lucidum* that can be found in the market all over the world [5]. Commercially products available are: hydroalcohol extract, dry extract capsules (different dosages), dryed extract tablets (different dosages), spore powder (capsules of 100mg). However, there are still no concret reports about its safety in pregnancy, lactation, or administration in children. In addition to the commercially available forms, various extracts of *Ganoderma lucidum* are also found in the form of coffee, powder, tea, spore products, drinks, syrup, toothpaste, soaps, and lotions [10].

Conclusions

G. lucidum is considered a very potential medicinal fungus due to the large pharmacologically bioactive compounds obtained from the fruiting body, spores, and mycelium. Our study highlights a wide variety of biochemically active compounds. The high content of terpenes, polysaccharides and triterpenoids is noteworthy. The second part of the study highlights the pharmacological effects of this fungus that have been used in Traditional Chinese Medicine, but it is also use in recent modern studies.

References

[1] Li, J., Zhang, J., Chen, H., Chen, X.Q., Lan, L., Liu, C. Complet mithocondrial genome of the medicinal mushroom *Ganoderma lucidum*. PloS ONE 8:1-11, 2013;
[2] Wang, X.-C., Xi, R.-J., Li, Y., Wang, D.-M., Yao, Y.-L. The species identity of the widely cultivated Ganoderma, *G. lucidum (Ling-zhi)*, PLoS ONE 7:1-12, 2012b;
[3] Gordan, J.-D., Chay, W.-Y., Kelley, R.K., Ko, A.H., Choo, S.P., Venook, A.-P. And what other medications are you talking? *J. Clin. Oncol.* 29:288-291, 2011;
[4] Jin, X., Ruiz, J., Sze, D.M., Chan, G.C. *Ganoderma lucidum* (Reishi mushroom) for cancer treatment. *Cochrane Db. Syst. Rev.* 6, 2012;
[5] Bishop, S.K., Kao, C., H., J., Xu, Y., Glucina, P., M., Paterson, M., R., R., Ferguson L., R. From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. *Phytochemistry*. 114:56-65, 2015;

[6] Sun, X., Wang, H., Han, X., Chen, S., Zhu, S., Dai, J. Fingerprint analysis of polysaccharides from different *Ganoderma* by HPLC combined with chemometrics methods. *Carbohydr. Polym.* 114:432-439, 2014;
[7] Chen, D.-H., Shiou, W.-Y., Wang, K.-C., Huang, S.-Y., Shie, Y.-T., Tsai, C.-M., Shie, J.-F., Chen, K.-D. Chemotaxonomy of triterpenoid pattern of HPLC of *Ganoderma lucidum* and *Ganoderma tsugae*. *J. Chin. Chem. Soc.* 46:47-51, 1999;
[8] Cheng, P-G., Phan, C-W., Sabaratnam, V., Abdullah, N., Abdulla, M. A., Kuppusamy, U. R. Polysaccharides-Rich Extract of *Ganoderma lucidum* (M.A. Curtis:Fr) P. Karst Accelerates Wound Healing in Streptozotocin-Induced Diabetic Rats. *Evidence-Based Complementary and Alternative Medicine*, 2013;
[9] Sanodiya, B., S., Thakur, G., S., Baghel, R., K., Prasad, G., B., Bisen, P., S. *Ganoderma lucidum*: a potent pharmacological macrofungus. *Curr. Pharm. Biotechnol.* 10:717-742, 2009;
[10] Gerenutti, M., Viroel, F., Jozala, A., Grotto, D., Viana, S. Therapeutic applications of *Ganoderma lucidum*: Progress and Limitations*. Produtos Naturais e Suas Aplicações: da comunidade para o laboratório.* 16:249-267, 2021;
[11] Copoţ, O., Tănase, C., Maxent modelling of the potential distribution of *Ganoderma lucidum* in North-Eastern Region of Romania. *J. Plant Development* 43:133-143, 2017;
[12] Moncalvo, J.-M., Wang, H.-F., Hseu, R.-S. Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. *Mycol. Res.* 99:1489-1499, 1995;
[13] Zhao, J., D., Zhang, X., Q. Importance, distribution, and taxonomy of Ganodermataceae in China. Proceedings of Contributed Symposium, 5th International Mycological Congress, Vancouver. August 14-21, 1994;
[14] Taofiq, O., Heleno, S. A., Calhelha, R. C., Alves, M. J., Barros, L., Gonzales-Paramas, A. M., Berreiro, M. F., Ferreira, I. C. F. R. The potential of *Ganoderma Lucidum* extract as bioactive ingradients in topical formulations, beyond its nutritional benefits. *Food and Chemical Toxicology* 108:139-147, 2017;
[15] Chen, Y., Xie, M.-Y., Zhang, H., Wang, Y., Nie, S., Li, C. Quantification of total polysaccharides and triterpenoids in *Ganoderma lucidum* and *Ganoderma atrum* by near infrared spectroscopy and chemometrics. *Food Chemistry* 135:268-275, 2012;
[16] Boh, B., Berovic, M., Zhang, J., Zin-Bin, L. *Ganoderma luicdum* and its pharmaceutically active compounds. *Biotechnol Annu. Rev.* 13:265-301, 2007;
[17] Alzorqi, I., Singh, A., Manickam, S., Al-Qrimli, H. Optimization of ultrasound assisted extraction (UAE) of β-D-glucan polysaccharides from *Ganoderma lucidum* for prospective scale-up. *Resource-Efficient Technologies* 3:46-54, 2017;
[18] Zhang, L., X., Mong, H., Zhou, X., B. Effect of Japanese *Ganoderma lucidum* on production of interleukin-2 from murine splenocytes. *Chung-Kuo Chung Hsii Chien Ho Tsa Chih* 13:613-615, 1993;

[19] Li, M., C., Lei, L., S., Liang, D., S., X., Z., M., Yuan, J., H., Yang, S., Q., Sun, L., S. Effect of *Ganoderma lucidum* polysaccharides on oxygen free radicals in murine peritoneal macrophages. *Chin. J. Pharmacol. Toxicol.* 14:65-68, 2000;
[20] Gao, Y., Zhou, S., H., Huang, M., Xu, A. Antibacterial and antiviral value of the genus *Ganoderma* P. Karst species (Aphyllophoromycetideae): a review. *Int. J. Med. Mushrooms* 5:235-246, 2003;
[21] Gao, Y., Lan, J., Dai, X., Ye, J., Zhou, S., H. A phase I/II study of Lingzhi mushroom *Ganoderma lucidum* (W. Curt.:Fr) Lloyd (Aphyllophoromycetideae) extract in patients with type II diabetes mellitus. *Int. J. Med. Mushrooms* 6(1):33-39, 2004;
[22] Chen, R., Y., Yu, D., Q. Studies on the triterpenoid constituents of the spores from *Ganoderma lucidum* Karst. *J. Chin. Pharm. Sci.* 2:91-96, 1993;
[23] Li, J., M., Lin, C., C., Chiu, H., F., Yang, J., J., Lee, S., G. Evaluation of the anti-inflammatory and liver-protective effects of Anoectochilus formosanus, *Ganoderma lucidum* and Gynostermma pentaphyllum in rats. *Am. J. Chin. Med.* 21:59-69, 1993;
[24] Zhou, X., W., Su, K., Q., Zhang, Y., M. Applied modern biotechnology for cultivation of *Ganoderma* and development of their products. *Appl. Microbiol. Biotechnol* 93:941-963, 2012;
[25] Adaskveg, J., E., Gilbertson, R., L. Cultural studies, and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of *G. lucidum* complex. *Mycologia* 78:694-705, 1986;
[26] Gupta, A., Kirar, V., Keshir, G. K., Gola, S., Yadav, A., Negi, P. S., Misra, K. Wound Healing Activity of an Aqueous Extract of the Lingzhi or Reishi Medicinal Mushroom *Ganoderma Lucidum* (Higher Basidiomycetes). *International Journal of Medicinal Mushroom*, 16(4):345-354, 2014;
[27] Paterson, M., Russell, B. *Ganoderma* – A therapeutic fungal biofactory. *Phytochemistry* 67:1985-2001, 2006;
[28] Wachtel-Galor, S., Yuen, J., Buswell, J. A., Benzie, F. F. I. Ganoderma Lucidum (Lingzhi or Reishi): A Medicinal Mushroom. *Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition*, 9, 2011.

Method for Obtaining and Physico-Chemical Characterization of Collagenic Extract of Rhizostoma Pulmo from the Black Sea

Ana-Maria Pesterau

University of Medicine and and Pharmacy *Carol Davila*, Bucharest, Romania

Rodica Sirbu

University of Medicine and and Pharmacy *Carol Davila*, Bucharest, Romania

Emin Cadar

Ovidius University of Constanta, Faculty of Pharmacy, Constanta, Romania

Abstract

Rhizostoma pulmo is a jellyfish from the Black Sea basin that can be a source of natural bioactive compounds with substantial beneficial implications. It is important to use under-exploited marine resources in areas such as pharmaceuticals industry, medicine, cosmetics and dermatology. Marine collagen can be obtained from various sources. Several studies have focused on marine collagen, namely its extraction from alternative sources: fish, invertebrate marine animals such as sea sponges or jellyfish. The novelty is the extraction of marine collagen obtained from jellyfish of the species *Rhizostoma pulmo* found in the Black Sea and along the coast, the physico-chemical characterization, comparison with other types of collagenic extracts from fish and finally the formulation of a pharmaceutical preparation with medical applications.

Keywords: *Rhizostoma pulmo,* jellyfish, marine collagen, bioactive compounds, medical applications.

Introduction

Collagen is the most studied protein, with a wide range of applications, including the pharmaceutical, biomedical and cosmetic industries due to its special characteristics, which are of high biocompatibility, qualitative bioactivity and poor antigenicity [1]. Although there are various and abundant sources of collagen, the existence of various diseases among terrestrial animals is a threat to its use in our daily lives. The research aims to find an alternative that would reveal the many untapped marine sources, such as fish, jellyfish and some marine mammals. A brief description of collagen, its characteristics, sources of marine origin, extraction, collagen peptides and their biological activities, as well as potential applications in various fields, is desired.

Hot water jellyfish (*Rhizostoma pulmo* Macri, 1778). Taxonomic classification: Kingdom of *Animalia*, *Cnidaria*, Class *Scyphozoa*, Subclass *Discomedusae*, Order *Rhizostomeae*, Suborder *Daktyliophorae*, Family *Rhizostomatidae*, Genus *Rhizostoma* [2].

Large jellyfish easily recognizable by the narrow band colored in blue, purple or pink that borders the slightly lobed edge of the umbrella. It is a very common species, numerous in the Black Sea, Mediterranean Sea, Sea of Azov and North Sea. It has a tall bell with a regular diameter of 20-30 cm, with 16 marginal lobes and 8 lobes. The buccal tentacles are overgrown and form a long and voluminous handlebar, fringed at the edges, so the axial length of a specimen can reach 50-60 cm [3].

It is a mycrophage, the primitive mouth being replaced by a pore system. A common species in the Mediterranean, we find it in the Black Sea only in the warm season, brought by hot water currents. Swimmers or those who bathe in the sea water, touching this jellyfish, can get a very unpleasant burn, caused by its bladder cells.

Collagen extraction from different jellyfish species

Collagen is the most abundant protein found in the body. These proteins form fibers that help build and maintain parts of the body, it is also found in the muscles, hair, skin, bones, tendons and ligaments. Researchers have identified at least 28 types of collagen, but those classified as type I, II and III represent 80% to 90% of total collagen [4]. All collagen molecules contain a triple helical domain, are generally involved in the formation of supramolecular networks and are made up of three α chains that may or may not be identical. These α chains contain at least one collagen domain or a triple helix motif characterized by the succession of Gly-Xaa-Yaa triplets where Xaa and Yaa are often Pro and Hyp residues, respectively [5].

Collagen currently has a wide range of applications in various areas of health, namely in cosmetics, the pharmaceutical industry and in healthcare (including plastic surgery, orthopedics, ophthalmology and dentistry).

In non-medical sectors, a notable use of collagen is in the food sector (food processing, as an additive and nutraceuticals), but most often in the form of gelatin, ie in its distorted form.

Fibrillar collagens are the most abundant extracellular proteins, they form a specific family of metazoans. Their structural and physiological properties have been used successfully in the cosmetics, pharmaceutical and food industries. The increase in the number of jellyfish has led us to appreciate this marine life as a natural product for food and medicine. Different species of Mediterranean jellyfish have been tested to investigate the economic potential of their collagens. Different methods of collagen purification (tissues and experimental procedures) were analyzed. The best collagen yield was obtained using the oral arms with predilection for *Rhizostoma pulmo* and the pepsin extraction method (2-10 mg collagen/g wet tissue) see table Nr.1 [6].

Rhizostoma pulmo has been used in other experiments, these drugs are considered harmless to humans and for the abundant source of collage material [6]. The biological properties of *R. pulmo* were compared with mammalian fibrillar collagens in cell cytotoxicity and cell adhesion tests. There were no statistical differences in cytotoxicity ($p > 0.05$) between *R. pulmo* and rat type I collagen [1]. Their structural and physiological properties have been used in the food, cosmetics and pharmaceutical industries.

Materials and Methods

The characteristics of collagen are unique, both *in vivo* and in tissue engineering that attempts to attenuate the role of collagen in the extracellular matrix, based on cell cultures that verify the quality and properties of processed collagen.

Due to the fact that there are so many sources from which collagen can be extracted and so many types, it is essential to analyze the characteristics carefully and accurately [7].

There are several techniques that can be used to characterize collagen samples (solid or in solution) addressing morphological, structural and chemical characteristics [8]. With a precise characterization and a better knowledge of the sample, it is easier to obtain a desired biological response or to establish a relationship between the results and the characteristics of the structure of the product obtained.

The aim is to continuously monitor the results, trying to control the characteristics of the sample, resulting in a more predictable and accurate biological response.

The extracting marine collagen from jellyfish

A widely used acid solution for collagen solubilization is also used for the extraction phase, which is also called acid-soluble collagen (AUC), [9].

Skierka and Sadowska [9] tested the influence of various acids on the extraction of collagen from cod skin, including hydrochloric, citric, acetic and lactic acids, of which acetic and lactic acids have been shown to produce higher collagen extraction yields. Unfortunately, the process of collagen extraction normally results in a low yield.

To overcome this impediment, scientists applied an enzymatic treatment using non-collagen-specific proteolytic enzymes to aid the solubilization process, such as trypsin, pancreatin, ficin, bromelain, papain or pepsin, the latter being the most widely used. By applying pepsin, the resulting extract is called Pepsin Soluble Collagen (PSC) or atelo-collagen [10].

This treatment is very useful because it cleaves peptides specifically from the telopeptide region of collagen, which are non-helical ends, and thus, by hydrolysis of non-collagenous proteins, increases the purity of collagen. It results in a much more efficient collagen extraction, as it prepares the sample for solubilization, while reducing the antigenicity caused by telopeptides [11].

For this reason, it is common to use this proteolytic process after AUC extraction, thus obtaining the PSC mentioned above. The antigenicity of collagen is not only derived from its telopeptides, but is also linked to the presence of non-collagenous proteins, cells and cell debris, being the method of NaHO treatment of raw materials[12] important for removing this source of antigenicity. For the recovery stage, the collagen must be precipitated, usually obtained by adding NaCl to a final concentration which can vary between 2.3 M and 2.6 M in Tris-HCl (pH 7.5). The resulting precipitate is collected by centrifugation, dissolved in 0.5 M acetic acid, dialyzed and lyophilized, thereby obtaining a collagen which is soluble in dry acid and soluble in pepsin [13].

Jellyfish collagen is an alternative being an available and efficient source to use as a component of the matrix for tissue engineering, because it has a small amount of impurities. Due to the dry weight of the edible jellyfish, over 40% of it is collagen in an animal in which 95% is water [14].

From jellyfish large part of the body is called the umbrella, which is divided into a major component called the mesoglea and the outer skin: exumbrella and subumbrella collagen is obtained from the mesoglea, following a methodology based on solubilization in acetic acid.

Solution, usually time the extracts are then dialyzed against Na_2HPO_4 solution, resulting in precipitated collagens, which can be separated by centrifugation.

The collagen produced can then be purified by a re-precipitation method: solubilization in acetic acid and precipitation by the addition of solid NaCl. AUC can also be digested with pepsin to obtain atelo-collagen[15].

Purification methods

Jellyfish collagen is an available and viable source to use as a component of the matrix for tissue engineering, as it has a small amount of impurities. One of the methods used to characterize the purity and degradation of collagen is polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE).

By this method - gel electrophoresis, proteins and their fragments can be separated, depending on their size. Protein fragments can be observed by loading protein samples into the small wells of the gel, which, under an applied electric field, pass through the gel matrix according to their size: the smaller ones go further than the large ones, which remain trapped in the net of gel.

Some types of collagen obtained from different sources can be compared to existence data. The type of collagen (I, II) can be identified when the collagen bands are similar. In the case of collagen of the same type, but of different species, in which the amino acid sequence is altered, although the same chain types are present (e.g. α1, α2 and β chains in type I collagen), the position of the bands is slightly altered can be observed [16].

Stomolophus nomurai meleagris jellyfish collagen was prepared by lyophilization and crosslinking with 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide hydrochloride (EDC/NHS) for use in tissues with bioengineering applications [17].

Jeong and his collaborators [18] developed scaffolds collagenous extracts from jellyfish and poly (d, l-lactide-co-glycolide) (PLGA), by lyophilization and electrospinning, proposing their application on vascular grafts.

Addad has proposed the use of EDC-NHS crosslinked jellyfish collagen in various reports as a replacement for bovine or human collagen in biomedical applications [6]. More recently, porous scaffolds composed of refibrillated collagen, previously extracted from *Rhopilema esculentum* jellyfish, exhibited elastic behavior and were able to support hMSC culture, with overexpression of chondrogen markers under chondrogen stimulation, suggesting the use of cartilage tissue engineering structures [19].

Collagen extracted from other jellyfish species has also been proposed for later use in various applications, such as those extracted from *Cyanea nozakii* [20].

Results and Discussions

Marine collagen sources are known to have low antigenicity, *in vivo* studies should be considered before performing their use in engineering and tissue regeneration applications to verify the feasibility of using selected collagen in human implants.

In order to use alternative sources of collagen, all potential commercial alternatives to collagen must be considered. Collagen cannot be easily and advantageously replaced with other molecules, in their existing or developing compositions, it is necessary to verify whether the proposed options are economically viable and have similar biocompatibility profiles, physico-chemical and biological properties. Collagen also poses some risks due to its human or animal origins.

Table Nr.1 Extraction yields of collagen from different species of jellyfish expressed mg/g

Species	**Collagen extracions(mg/g)**	**Organ**	**Number ofanimals used**
Rhizostoma pulmo	0.83 to 3.15	umbrella	(3 animals)
Rhizostoma pulmo	2.61 to 10.3	oral arms	(5 animals)
Cotylorhiza tuberculata	0.453	umbrella	(1 animal)
Cotylorhiza tuberculata	1.94	oral arms	(1 animal)
Pelagia noctiluca	0.074	whole body	(1 animal)

Aurelia aurita	0.0079	whole body	(1 animal)

The primary biomedical applications of collagen were in biomaterials, especially as drug and gene carriers, tissue engineering, absorbable surgical suture, osteogenic and bone filling materials, hemostatic agents, immobilization of therapeutic enzymes, and burn/wound cover dressings [21].

Analysis of the antioxidant activity (AA) of protein compounds in *R. pulmo* demonstrated based on the extraction and characterization of membrane fractionated proteins, both soluble and insoluble, the latter being digested by sequential enzymatic hydrolysis with pepsin and collagenase. All jellyfish proteins showed significant antioxidant activity, from low molecular weight (MW) proteins correlated with higher antioxidant activity [22].

Recent major outbreaks of communicable diseases, such as prion disease, bovine spongiform encephalopathy (mad cow disease) or Creutzfeld-Jacob disease, have severely affected bovine and human health care products and have been banned in some cases (eg human by-products), for operations on the spinal cord and brain).

Collagen from marine sources such as that extracted from jellyfish avoids major problems arising from cultural practices and religious beliefs, which may limit the use of bovine and porcine products by some consumers and in certain parts of the world.

Properties of collagen

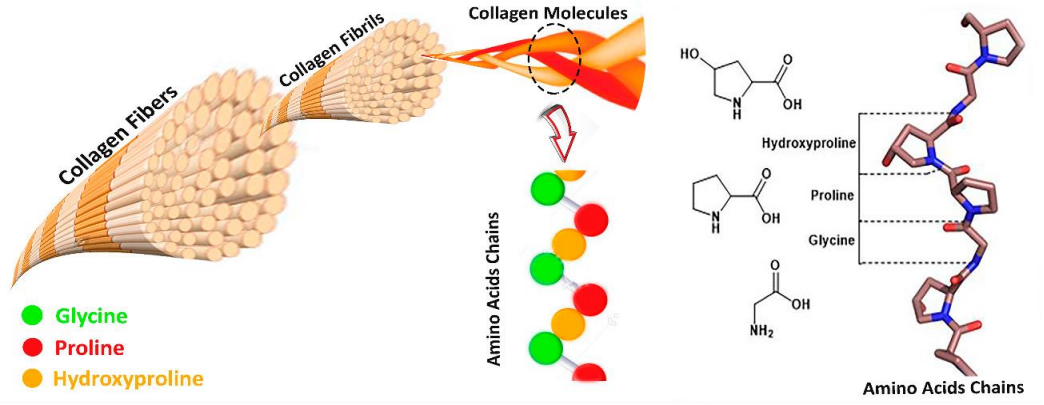


Figure 1. (**a**) Approximate content of collagen in different tissues; (**b**) Structure of collagen fibers, fibrils, triple helices of alpha chains and amino acid residues, 4-hydroxyproline (Hyp), glycine (Gly), and proline. **(c)** Amino acids chains structure of collagen. [https://www.elsevier.com/es-es/connect/medicina/colagenos-tipos-composicion-distribucion-tejidos].

Elegant and attractive alternative sources of collagen have been developed and proposed, using recombinant expression techniques. This allowed the expression of several types of human collagen in bioreactor-based eukaryotic systems and their subsequent isolation and purification [23]. Collagen-producing marine species have a distinct advantage in having a lower risk of transmitting infectious agents to humans and are considered safe for cultural and religious use.

It is anticipated that further structural stabilization of such marine collagens may be required by chemical processes, resulting in higher denaturation temperatures and increased resistance to enzyme degradation [24]. Marine collagen will provide new opportunities in health, diversification and reduction of safety risks and cultural and religious concerns, through the development of techniques for the production of collagen from alternative sources.

Medical, cosmetic, skin care and other collagen applications

Collagen is known for its biological action, with a great potential to be used in various fields such as dermatology, cosmetics, beauty. The current trend is to look for safe, high quality and reasonably priced ingredients. Marine proteins, and especially marine collagens, are nowadays found to be excellent functional ingredients for the cosmetics and pharmaceutical industries [25].

Due to water solubility, safety, biocompatibility, biodegradability, and easy extractability, as well as low immunogenicity, marine collagen has attracted scientific consideration for biomaterial applications [25]. On the other hand, due to the enormous amount of marine waste by-product such as fish skins, bones, scales, cartilage, and heads, marine-based collagen has been used in various biomaterial applications such as bone tissue engineering, skin tissue engineering and regeneration, cartilage tissue engineering, wound dressing, drug delivery, etc.[26]

The properties of collagen lead to the development of creams, gels, serums with moisturizing action, anti-aging, anti-wrinkle or protection against UV radiation.

For the cosmetics industry, marine collagen was obtained from cold-water fish skins, such as cod, haddock and salmon. In addition, it is also produced from fish scales, by decalcification and enzymatic hydrolysis [25].

Jellyfish collagen has demonstrated comparable structural properties and stability compared to mammalian collagen. Jellyfish collagen also showed comparable immunogenic responses (platelet and leukocyte activation/cell death) and cytokine release profile compared to mammalian collagen *in vitro*. Studies highlight the potential of jellyfish collagen as a safe and biocompatible biomaterial for both osteoarthritis repair and subsequent applications of regenerative medicine [26].

Another property of marine collagen used is that it heals wounds from various traumas (burns, grafts, ulcers, etc.), and collagen-based materials are used specifically to prevent moisture and heat loss from damaged tissue, while providing a microbial

infiltration barrier [27]. Collagen extracts have also been used in pharmaceutical preparations, for example, collagen barriers in ophthalmology, mini-pellets and tablets for protein delivery, gel formulations in combination with liposomes as a control material for transdermal delivery and nanoparticles for gene delivery [28].

Conclusions

Collagens are known for their biological action, with great potential for use in the biomedical, pharmaceutical and cosmetic fields. The future lies in finding viable alternative sources of safe, high-quality, low-cost raw materials.

Collagen is the most studied protein, with a wide range of applications due to its special characteristics, which have a high biocompatibility, good bioactivity and poor antigenicity. Marine proteins, obtained from various fish, jellyfish, sponges or other marine life are excellent functional products for the cosmetics industry, but also for the formulation of new pharmaceutical preparations for dermatological use.

It has been shown that the marine collagen source has applications in healing wounds resulting from various traumas (burns, ulcers, bedsores), the collagen-based materials being used mainly to prevent moisture and heat loss from the damaged tissue, while providing a barrier to microbial infiltration. Jellyfish collagen also has structural, stability properties comparable to that extracted from mammals.

The collagen extract also showed comparable immunogenic responses (platelet and leukocyte activation/cell death) and cytokine release profile compared to mammalian collagen *in vitro*. Studies highlight the potential of jellyfish collagen as a safe and biocompatible biomaterial for both osteoarthritis repair and subsequent applications of regenerative medicine.

Collagen preparations are also used in drug delivery systems, for example, collagen shields in ophthalmology, mini-pellets and tablets for protein delivery, gel formulations in combination with liposomes as a control material for transdermal delivery and nanoparticles for eyelash delivery. The marine collagen extracted from *Rhizostoma pulmo* jellyfish will offer new and real opportunities, being an alternative source, especially in the field of health due to the diversification and reduction of safety risks as well as pre-existing cultural and religious concerns over "standard" sources.

References

[1] Ramshaw, J.A.M., Peng, Y.Y., Glattauer, V., Werkmeister, J.A, *Collagens as biomaterials.* J. Mater. Sci. Mater. Med., 20 (Suppl. 1), S3–S8, 2009.
[2] https://www.gbif.org/species/5185453 (accesess 14.04.2022, 15:32).
[3] Péron F. & Lesueur C.A., *Tableau des caractères génériques et spécifiques de toutes les espèces de méduses connues jusqu'àce jour,* Annales du Muséum national d'histoire naturelle de Paris. 14, 1810, 325-366.

[4] Deyl, Z., Miksik, I., Eckhardt, A., *Preparative procedures and purity assessment of collagen proteins,* J. Chromatogr. B, 790, 245–275, 2003.
[5] Gomez-Guillen, M.C., Turnay, J., Fernandez-Diaz, M.D., Ulmo, N., Lizarbe, M.A., Montero, P., *Structural and physical properties of gelatin extracted from different marine species: A comparative study*, Food Hydrocoll., 16, 25–34, 2002.
[6] Sourour, Addad, Jean-Yves, Exposito, Clément, Faye, Sylvie, Ricard-Blum, Claire, Lethias, *Isolation, Characterization and Biological Evaluation of Jellyfish Collagen for Use in Biomedical Applications,* Marine Drugs, 9, 967-983, 2011.
[7] Skierka, E.; Sadowska, M.; Karwowska, A. *Optimization of condition for demineralization*
[8] *baltic cod (Gadusmorhua) backbone*, Food Chem.105, 215–218, 2007.
[9] Abraham, L.C.; Zuena, E.; Perez-Ramirez, B.; Kaplan, D.L., *Guide to collagen characterization for biomaterial studies,* J. Biomed. Mater. Res. Part B, 87B, 264–285, 2008.
[10] Skierka, E., Sadowska, M., *The influence of different acids and pepsin on the extractability of collagen from the skin of baltic cod (Gadusmorhua),* Food Chem., 105, 1302–1306, 2007.
[11] Lin, Y.K., Liu, D.C., *Effects of pepsin digestion at different temperatures and times on properties of telopeptide-poor collagen from bird feet*, Food Chem., 94, 621–625, 2006.
[12] Lynn, A.K., Yannas, I.V., Bonfield, W., *Antigenicity and immunogenicity of collagen*, J. Biomed. Mater. Res. B, 71B, 343–354, 2004.
[13] Sben, X.R., Kurihara, H., Takahashi, K., *Characterization of molecular species of collagen in scallop mantle*, Food Chem., 102, 1187–1191, 2007.
[14] Ehrlich, H., *Biological Materials of Marine Origin: Invertebrates*; Volume 1, Springer: New York, NY, USA, 2010.
[15] Tiago, H., Silva, Joana, Moreira-Silva, Ana, L., P., Marques, Alberta, Domingues, Yves, Bayon, Rui, L. Reis, *Marine Origin Collagens and Its Potential Applications*, Marine Drugs, *12*(12), 5881-5901, 2014.
[16] Fernandes-Silva, S., Moreira-Silva, J., Silva, S., Perez-Martin, R., Sotelo, C., Mano, J., Marques, A., Silva, T., Reis, R., *Marine collagen scaffolds crosslinked "in situ" with genipin for cartilage regeneratio,* J. Tissue Eng. Regener. Med., 6, 163–163, 2012.
[17] Song, E., Kim, S.Y., Chun, T., Byun, H.J., Lee, Y.M., *Collagen scaffolds derived from a marine source and their biocompatibility*, Biomaterials 27, 2951–2961, 2006.
[18] Jeong, S.I., Kim, S.Y., Cho, S.K., Chong, M.S., Kim, K.S., Kim, H., Lee, S.B., Lee, Y.M., *Tissue-engineered vascular grafts composed of marine collagen and PLGA fibers using pulsatile perfusion bioreactors*, Biomaterials, 28, 1115–1122, 2007.
[19] Hoyer, B., Bernhardt, A., Lode, A., Heinemann, S., Sewing, J., Klinger, M., Notbohm, H., Gelinsky, M., *Jellyfish collagen scaffolds for cartilage tissue engineering*, Acta Biomater., 10, 883–892, 2014.

[20] Stefania, De Domenico, Gianluca, De Rinaldis, Mélanie, Paulmery, Stefano, Piraino, Antonella, Leone, *Barrel Jellyfish (Rhizostoma pulmo) as Source of Antioxidant Peptides*, Mar Drugs 23;17(2): 134, doi: 10.3390/md17020134, 2019.

[21] Zhang, J.J., Duan, R., Huang, L., Song, Y.J., Regenstein, J.M., *Characterisation of acid-soluble and pepsin-solubilised collagen from jellyfish (Cyanea nozakii kishinouye),* Food Chem., 150, 20154, 22–26, 2010.

[22] Sahiner, M., Alpaslan, D., Bitlisli, B.O. *Collagen-based hydrogel films as drug-delivery devices with antimicrobial properties*, Polym. Bull, 71, 3017–3033, 2014.

[23] Zara, Ahmed, Lydia, C., Powell, Navid, Matin, Andrew, Mearns-Spragg, Catherine, A. Thornton, Ilyas, M., Khan, Lewis, W., Francis, *Jellyfish Collagen: A Biocompatible Collagen Source for 3D Scaffold Fabrication and Enhanced Chondrogenicity,* Mar Drugs. 19(8): 405, 2021.

[24] Yang, C.L., Hillas, P.J., Baez, J.A., Nokelainen, M., Balan, J., Tang, J., Spiro, R., Polarek, J.W., *The application of recombinant human collagen in tissue engineering,* Biodrugs, 18, 103–119, 2004.

[25] Pati, F., Datta, P., Adhikari, B., Dhara, S., Ghosh, K., Das Mohapatra, P.K., *Collagen scaffolds derived from fresh water fish origin and their biocompatibility*, J. Biomed. Mater. Res. A, 100A, 1068–1079, 2012.

[26] Cho, J.-K., Jin, Y.-G., Rha, S.-J., Kim, S.-J., Hwang, J.-H., *Biochemical characteristics of four marine fish skins in Korea*, *Food Chem.,* 159, 200–207, 2014.

[27] Caruso, G., *Fishery wastes and by-products: A resource to be valorised*. J. Fish. Sci, 9, 80–83, 2015.

[28] Kim, S.K., Ngo, D.H., V.o, T.S., Ryu, B., *Industry perspectives of marine-derived proteins as biomaterials*, In Marine Biomaterials: Characterization, Isolation and Applications; Kim, S.K., Ed. CRC Press: Boca Raton, FL, USA, pp. 737–746, 2013.

[29] Swatschek, D., Schatton, W., Muller, W.E.G., Kreuter, J., *Microparticles derived from marine sponge collagen (SCMPs):Preparation, characterization and suitability for dermal delivery of all-trans retino*l, Eur. J. Pharm. Biopharm., 54, 125–133, 2002.

Emergency Care Unit and Patient Satisfaction, During Covid-19 Pandemic: Durres Hospital Case

Rezarta Kalaja

Head of Department of Medical Technical Sciences, University Aleksander Moisiu, Durres.

Abstract

The outbreak of covid-19 is having a significant impact on both the physical and social environment. Over 108.2 million people still uncounted (1). Among the sectors of a hospital, the emergency service is one of the most challenging in relation to the promotion of care quality. Achieve good levels of satisfaction of patients of these services is a difficult task (2) due to weaknesses caused by overcrowding, lack of hospital beds, lack of human resources and inadequate physical infrastructure to meet all demand (3). The difficult situation created, during the pandemic outbreak, focused the entire influx of patients, in the emergency service, which encountered the initial difficulties, of facing a completely unknown and unimaginable crisis, deepening the above problems. Patient satisfaction, as a perception and an attitude that a consumer can have or view towards a total experience of health care, is a multidimensional aspect, which represents a vital key marker for the quality of health care delivery (4). The purpose of this study is to assess patient's satisfaction with the emergency service in the regional hospital of Durres, during Covid-19 pandemic period, as well as to identify the main problems that led to patient's dissatisfaction with the quality of service. In a for month period, 200 patients who received services at this hospital while affected by covid-19, were interviewed. A structured questionnaire was used to collect data from participants. The collected data were processed by SPSS statistical software. The main result of the analysis, showed that 62% of the respondents were satisfied with the service received, and that the degree of dissatisfactions on the rest of the patients was mainly related to the large number of patients hospitalized ate the same time, which created uncertainty among patients, about the service received and the fear to neglect. Preparing staff to deal with such critical situations is a must, as a good proportion of patients often perceived the insecurity of physicians and nurses in providing first aid. The overall opinions about the satisfaction level of patients for the availability and empathy of doctors in the hospital were good. As a conclusion we can say that there is a strong positive relationship between patients satisfaction and the promptness in the service as well as the necessary spaces for the treatment of patients.

Keywords: Covid-19 Emergency care unit, patient satisfaction, regional hospital Durres.

Introduction

The outbreak of covid-19 is having a significant impact on both the physical and social environment. Over 108.2 million people still uncounted (1). Among the sectors of a hospital, the emergency service is one of the most challenging in relation to the promotion of care quality. Achieve good levels of satisfaction of patients of these services is a difficult task (2) due to weaknesses caused by overcrowding, lack of hospital beds, lack of human resources and inadequate physical infrastructure to meet all demand (3). The difficult situation created, during the pandemic outbreak, focused the entire influx of patients, in the emergency service, which encountered the initial difficulties, of facing a completely unknown and unimaginable crisis, deepening the above problems. Patient satisfaction, as a perception and an attitude that a consumer can have or view towards a total experience of health care, is a multidimensional aspect, which represents a vital key marker for the quality of health care delivery (4).

In Albania covid-19 pandemic has exposed problems related to health care, and demonstrated the system problem through the patients access the health care service (5). The increases of cases with severe acute respiratory syndrome in Albania over bounded the healthcare system. We had limited human resources; general practitioners provided long time waiting form health service in the emergency room and service (6).

The impact of the COVID-19 pandemic in the health care system in Albania is noticeable. In this study our purpose is to assess patient's satisfaction with the emergency service in the regional hospital of Durres, during Covid-19 pandemic period, as well as to identify the main problems that led to patient's dissatisfaction with the quality of service.

Methods

This is a four-month study were patients who received services at this hospital while affected by covid-19, were interviewed. In total, 200 patients responses to the questionnaire from September 2020 till January 2021. The questionnaire was designed in closed questions and the patients were answered as an interview form. The survey contained a total of 15 questions including data about patient's satisfaction in emergency service in Durres hospital and about demographic data. To the question about how satisfy patients were from the health service they answered on a 5-point scale where 1 was "not satisfied" and 5 "very satisfied" with health care service. The collected data were processed by SPSS statistical software. Data were represented as mean and percentage.

Results

From 200 patients that completed the questionnaire, 64% were women and 33% were men. The mean age was 37.2 ± 13.2 years old.

Gender	Nr	%
Male	**72**	**36**
Female	**128**	**64**
Total	**200**	**100**
Mean age ± SD		**37.2 ± 13.2**
Education	**Nr**	**%**
Higher education	**96**	**48**
Secondary education	**51**	**26**
Elementary education	**37**	**18**
No education	**16**	**8**
Total	**200**	**100**
Living		
Rural	**114**	**57**
Urban	**86**	**43**
Total	**200**	**100**

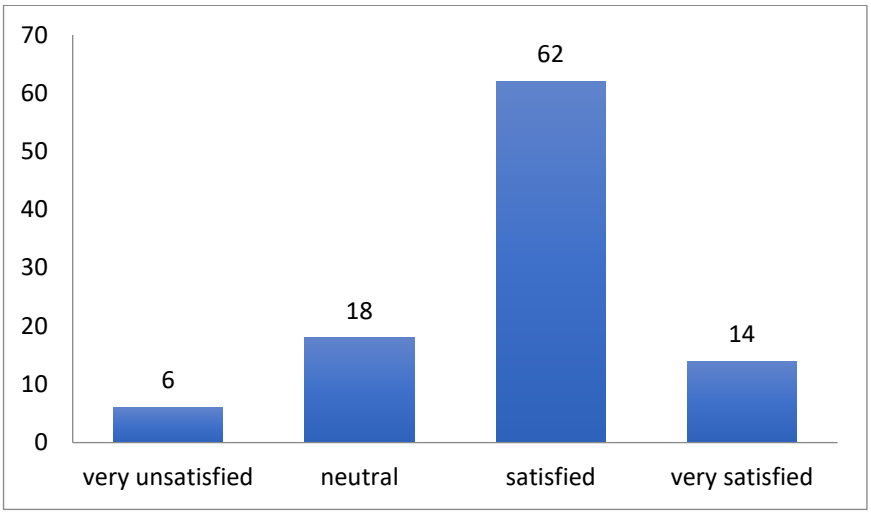
Table 1. Demographic data of patients who received emergency assistance

From table 1 we can see that 48% of patients had higher education and 8 % had no education. 57% of them responded that live in rural place and all of them received hospital emergency assistance.

Waiting time in emergency service	Nr (%)
Up to 30 min	24 (12%)
Up to 45 min	98 (49%)
Up to 60 min	41 (21%)
Up to 90 min	37 (18%)

Table 2. Waiting time in emergency service

From table 2 we have represented the waiting time for the service in emergency room in Durres hospital. As we can see 49% of patients reported that they had to wait up to 45 minutes to get health care service in emergency room.



Graph. 1. Patients satisfied from emergency service.

In graphic 1 it is represented patients satisfaction with emergency care service in Durres hospital. As we can see 62% of them answered that they were satisfied with emergency service and only 6% answered very unsatisfied.

Discussion and Conclusions:

As many study reported that their health care system was affected from covid 19 (7), (8), (9); even in our study Covid-19 has affected the health care system in Albania. As we can see from our study the time of waiting in the emergency room was up to 45 minute in 49% of the patients.

Regarding patients satisfaction during covid-19, 62% of the respondents were satisfied with the service received, and that the degree of dissatisfactions on the rest of the patients was mainly related to the large number of patients hospitalized at the same time, which created uncertainty among patients, about the service received and the fear to neglect. Other studies have reported patients satisfaction about health care system during pandemic period, where they reported a decrease of patients satisfaction (10), (11).

Preparing staff to deal with such critical situations is a must, as a good proportion of patients often perceived the insecurity of physicians and nurses in providing first aid. The overall opinions about the satisfaction level of patients for the availability and empathy of doctors in the hospital were good.

As a conclusion we can say that there is a strong positive relationship between patients satisfaction and the promptness in the service as well as the necessary spaces for the treatment of patients.

References

[1] WHO (2020), The Global Health Observatory (database), World Health Organization, Geneva, http://www.who.int/gho/database/en/ (accessed on 26 May 2020).
[2] Mitura, K. The impact of COVID-19 pandemic on critical care and surgical services availability. Crit. Care Innov. 2020, 3, 43–50. [CrossRef]
[3] AfUgglas, B.; Skyttberg, N.; Wladis, A.; Djärv, T.; Holzmann, M.J. Emergency department crowding and hospital transformation during COVID-19, a retrospective, descriptive study of a university hospital in Stockholm, Sweden. Scand. J. Trauma Resusc. Emerg. Med. 2020, 28, 107. [CrossRef] [PubMed]
[4] European Commission (2020), Albania 2020 Report, European Commission, Brussels, https://ec.europa.eu/neighbourhood-enlargement/sites/near/files/albania_report_2020.pdf.
[5] COVID-19 Health System Response Monitor (2020), Policy responses for Albania, https://www.covid19healthsystem.org/countries/albania/livinghit.aspx?Section=2.2%20Workforce&Type=Section.
[6] Sutherland, K.; Chessman, J.; Zhao, J.; Sara, G.; Shetty, A.; Smith, S.; Went, A.; Dyson, S.; Levesque, J.F. Impact of COVID-19 on healthcare activity in NSW, Australia. Public Health Res. Pract. 2020, 30, 3042030. [CrossRef] [PubMed]
[7] Butt, A.A.; Azad, A.M.; Kartha, A.B.; Masoodi, N.A.; Bertollini, R.; Abou-Samra, A.B. Volume and Acuity of Emergency Department Visits Prior To and After COVID-19. J. Emerg. Med. 2020, 59, 730–734. [CrossRef] [PubMed]
[8] Sharma, A.; Soni, D.; Dubey, P.; Sharma, R.; Bharti, A.; Singh, T.P. Satisfaction among COVID-19 positive patients: A study in a tertiary care hospital in central India. J. Prim. Care Spec. 2021, 2, 10.
[9] Dicker, B.; Swain, A.; Todd, V.F.; Tunnage, B.; McConachy, E.; Drake, H.; Brett, M.; Spearing, D.; Howie, G.J. Changes in demand for emergency ambulances during a nationwide lockdown that resulted in elimination of COVID-19: An observational study from New Zealand. BMJ Open 2020, 10, e044726. [CrossRef] [PubMed]
[10] Dicker, B.; Swain, A.; Todd, V.F.; Tunnage, B.; McConachy, E.; Drake, H.; Brett, M.; Spearing, D.; Howie, G.J. Changes in demand for emergency ambulances during a nationwide lockdown that resulted in elimination of COVID-19: An observational study from New Zealand. BMJ Open 2020, 10, e044726. [CrossRef] [PubMed]
[11] Kuisma, M.; Määttä, T.; Hakala, T.; Sivula, T.; Nousila-Wiik, M. Customer satisfaction measurement in emergency medical services. Acad. Emerg. Med. 2003, 10, 812–815. [CrossRef] [PubMed]

Valuable Bioactive Compounds Extracted from *Ceramium rubrum* on the Romanian Seaside with Medical Interest

Ana-Maria-Laura Dragan

University of Medicine and Pharmacy *Carol Davila* IOSUD Bucharest

Rodica Sirbu

University of Medicine and Pharmacy *Carol Davila* IOSUD Bucharest

Emin Cadar

Ovidius University of Constanta, Faculty of Pharmacy

Abstract

Recently in science, seaweeds are widely used because of their relevant compounds and potential origin of antimicrobial, antiviral and antioxidant activity. Seaweeds are rich in the trace elements and essential minerals that are hard to find in terrestrial plants. *Ceramium rubrum* is one of the red seaweeds that can be found in the Black Sea on the Romanian coast that has not been enough studied for their bioactive compounds. Identification and quantification of the biomolecules is carried out by specific methods. The compounds that have been found are cumarines, sterols, flavonoic aglicoles, triterpenes and polysaccharides. Is well known that this kind of biomolecules are very important in the pharmaceutical industry. Ozes and poliozes, reducing compounds and catehic tanin we're also identified from the red algae.

Keywords: *Ceramium rubrum*, red seaweed, antioxidant, flavonoids, polysaccharides, bioactive compounds.

Introduction

Nowadays, worldwide a remarkable interest of medical and pharmaceutical professionals is focused on the superior use of natural resources for therapeutic purposes. Marine ecosystems have productivity and diversity that are very important in maintaining the health of the marine and terrestrial environment and provide important sources for the cosmetics, food and pharmaceutical industries. They represent about 70% of the Earth's surface. Although the literature presents a wide range of medicines from marine resources, it is modest when it comes to the pharmaceutical use of marine resources in the Black Sea. Recently, a real field of

investigation has been developed for biological substances in marine organisms, as they have been found to be a rich source of valuable compounds. The importance of algae and phanerogams in the general bioproductivity of the marine environment, especially in shallow waters, is becoming increasingly evident both economically and ecologically. The algal macroflora of the Black Sea totals a number of 277 species of algae, of which the most common are green and red algae. Although green algae have been extensively studied for their properties, red algae have not received much attention and I believe that they should also be fully explored. The research developed in this paper treats the red seaweed existing in the territorial waters of the Romanian coast. The capitalization of seaweed is of particular importance, being a valuable resource for the medical-pharmaceutical field [1].

Ceramium rubrum belongs the red algae group: Div. *Rhodophyta*, Subcl. *Florideophycidae*, Fam. *Ceramiaceae*. This group includes pluricellular algae that can be found in an aquatic environment and it grows spontaneously and abundantly in the Black Sea [2]. The red algae (*Rhodophyta*) make a distinct photosynthetic eukaryotic lineage which consists of around 6,000 species that includes unicellular to large multicellular taxa. Red algae differ from other eukaryotes by the lack of flagella and centrioles during their entire life cycle. Because of their particular life cycles (e.g., triphasic life cycle: gametophyte, tetrasporophye and carposporophyte phases), *Florideophyceae* is one of the most important algal groups in marine environments [3]. Due to the environmental conditions, the species of *Ceramium* are easily differentiated. It is probable to provide different names, if the examinations of the samples are not done prudently [4]. The genus *Ceramium* is found in the northern sector of the Black Sea coast, this being an opportunistic species that prefers most types of substrates from the extended platform of sarmatic limestone to live or dead mussel shells. This is a constant presence on our coast and develops appreciable biomass even in waters with a high load of nutrients. All year long, generally in spring and summer, red alga richly develops along the entire Romanian coastal area, on rocks, at depths of 0,5-4,5 m [5][6][7].

Polyphenols, vitamins, polysaccharides and polyunsaturated fatty acids are the most valuable and researched bioactive compounds from marine algae. Due to the presence of non-digestible polysaccharides in the algal cell wall, algae can be considered a very good source of dietary fibers. Polyphenols are a class of compounds commonly found in plant foods, such as, vegetables, fruits, spices, herbs, tea, etc. They can neutralize harmful free radicals that would damage the human cells and increase the risk of condition like cancer, heart diseases and diabetes, they can act as antioxidants. Polyphenols are also thought to reduce the origin cause of the most chronic ilnesses, the inflammation. They can be further categorized into 4 main groups: polyphenolic acids, polyphenolic amides, flavonoids and other polyphenols. Flavonoids are an extensively distributed group of structurally associated compounds with a 2-phenylchromane skeleton that have, in the C2 or C3 position, a phenyl substituent. The subclasses that they are divided depends on the degree of oxidation of the central

pyran ring. Flavonoids play an imperative role in plants as resistance and warning compounds in pathogenesis, symbiosis and reproduction, they are of physiological and biological importance. In some recent studies, phenolic acids and specifically flavonoids have been described to exhibit various effects including antiinflamatory, antioxidant, anti-cancer, antimicrobial, anti-allergic, anti-thrombotic, anti-atherogenic and estrogenic, vasodilatory and capillary fragility and permeability lowering actions [8]. Polysaccharides are omnipresent biopolymers that can be found widely in nature. These are polymers formed with glycosidic linkages of simple sugars, which are monosaccharides. Polysaccharides can be found in different types. Distinct chemical and physical properties exist because of the structural differences. These are natural biodegradable biopolymers, nontoxic. Algae are an important source of polysaccharides, especially the red macro algae. They are largely used as stabilizers, gelling agents, emulsifiers and as thickeners in food products. Another important group are sulfated polysaccharides. These can be valuable ingredients in pharmaceutical, nutraceutical and food industry because they can act as defensive barrier against phatogen. Agar and carrageenan are the most relevant sulfated polysaccharides and both are encountered in red algae [9]. Polyunsaturated fatty acids (PUFA) play an important role in metabolism, as a regulatory molecule and as a fundamental component of all the organs membranes. These have more than one double bond in their backbone. In recent studies it has been demonstrated that PUFA have anti-cancer and antiinflammatory effects and they are regulators of lipid metabolism, notably through epigenetic mechanisms [10]. Potential sources of polyunsaturated fatty acids are the marine algae [9].

Materials and Methods

Algal Material

The marine biomass was selected manually and exposed to a pretreatment that consisted of repeated washings with potable water and finally with distilled water. Macroscopic and microscopic examinations were one of the investigations that were performed on the fresh product. Other examinations that the algal biomass was selected for are chemical, physico-chemical and microbiological analysis. For this matter, the algal material was dried at temperatures between 25-35 degrees. The dry product, by grinding, was ground to a powder and was passed through a 0.5 mm sieve to obtain a uniform powder [11], see Figure 1.

The algal flora was harvested from the Black Sea coastline in may-november period, from water at a distance of 5-25 m from the shore, from the areas of Eforie Nord, Eforie Sud, Costinesti, 2 Mai, Mangalia, Vama Veche, Năvodari and Constanţa Casino.

Figure 1. Red algae in the algal colony

Macroscopic Examination

The first phase in the study of algal products is the macroscopic examination. This is done by examinating the whole algae (cauloid, phylloid and rhizoid) both with help from a magnifying glass and with the eyes to detect its color, size, appearance, taste and smell [1][5][12]. From this macroscopic examination it was found that *Ceramium rubrum* is a red alga with a 10-15 cm height and with a philament like thalle, fixed on the substratum through the rhizoid, having a bushy appearence, with ramifications. It has an articulate aspect because of the filaments that have dichotomic and are formed of a single row of connecting cells. The entire surface of the thallus is covered by cortical cells that are formed by the continuous division of periaxial cells produced and located in the nodal area. Tow short twisted branches are situated at the end of each filament [5-7][13].

Microscopic Examination

Whole, fresh algae was used for microscopic examination and kept in seawater jars throughout the analysis. The algae were placed in a bowl of clean water after being rinsed, very well, with distilled water to remove impurities. The materials that are used in this examination are fragments of thallus from the red seaweed, a Microscope and Micros photomicroscope (10/0.25), forceps, blades, spatulate needles and Petri dishes. The microscop analysis was performed directly on fresh thallus fragments obtained by slicing it with a slice and brought into a Petri dish with distilled water, because the macrophytic algae species have single-cell or two-layer thallus.

There, in the transversal section of the thalle, in the center, is a large cell, surrounded by eight pericentral cells, which are also surrounded by another layer of cortical cells. There are large strip-shaped plastides in the central cell.

Physico-chemical Methods

Qualitative chemical analysis requires the successive and selective extraction of plant products with solvents of diverse polarities and separation using chemical methods,

followed by specific reactions with which to classify different groups of active compounds. The active principles are extracted first in order to perform the global chemical analysis. From the algae product sprayed with a non-polar solvent (chloroform, ethyl ether, petroleum ether, benzene, hexane, etc.), then with a medium polarity solvent (ethanol, methanol) and finally the following are obtained: a sol. etheric extractive, a sol. alcoholic extract and a sol. aqueous extract. In the alcoholic and aqueous extract, the hydrophilic compounds are determined, and in the etheric one, the lipophilic compounds [14-17]. The three solutions are analyzed separately, using methods corresponding to the physicochemical properties of each group of active principles [18].

Analysis of the alcoholic extractive solution

The ether-depleted algal product was used and brought to the water bath to remove traces of ether. The plant product was extracted with methanol (2 x 100 mL) by refluxing for half an hour. The combined methanolic solution was concentrated to 50 mL by distillation of the solvent and partitioned into 2 parts. One part is subjected to hydrolysis with 15 mL 10% HCl on the electric nest at 3 grade for 30 minutes and the other part is used to identify the active ingredients on the non-hydrolyzed solution (25 mL). For further extraction, the methanol depleted plant product is stored [19].

Analysis of the ether extractive solution

Weighed 20 g of freshly sprayed vegetable product from the red algae, which was initially

extracted with ethyl ether (2 x 100 mL) by refluxing for 15 minutes. The resulting solutions were filtered and collected in a fluted cube flask, after each reflux. Then the combined ether solutions have to be concentrated on a water bath to 50 ml and the first solution is obtained. It is used to perform reactions characteristic of lipophilic compounds [20].

Analysis of the aqueous extractive solution

Was used the remaining plant product from the alcohol extraction which was dried and then extracted with 100 mL of water in a water bath at 900 ° C for 30 minutes.

Results and discussions

Following the identification reactions discussed above, and the following results were obtained and they can be seen in Table 1.

Table 1. The reactions used to identify bioactive compounds [1]

Analyzed solution	Reactions	Identified active compounds
Alcoholic extract	Iron Chlorure reaction	Catehic Tanin
	Liebermann-Bouchard	Triterpenic heterozides
	Fehling	Reducing compounds
	Borntrager	Antracenozide
	Fluorescent UV(λ=365nm)	Cumarines
Etheric Extract	Fluorescent UV(λ=365nm)	Cumarines
	Liebermann-Bouchard	Sterols, Triterpenes
Aqueous Extract	Foaming	Soapozides
	Fehling	Reducing compounds
	$FeCl_3$	Catehic Tanin
	H2SO4 conc.+tymol	Ozes and poliozes

In the non-hydrolyzed alcoholic solution

The reaction with ferric chloride is blackish green, so positive for catehic tannin in this species *Ceramium rubrum*. The reaction with Styassny's reagent is negative. The analyzed species does not contain alkaloids because the residue obtained after evaporation of the non-hydrolyzed alcoholic solution was taken up with a 2% aqueous HCl solution then basified with ammonium hydroxide and extracted with ether; after evaporation of the ether solution and resumption of the residue with 2% HCl, the Mayer and Bertrand reactions were carried out and they were negative. Reducing compounds are present because by performing the Fehling reaction a reddish-red precipitate was obtained. In *Ceramium rubrum* the reaction with ninhydrine of the aqueous solution obtained from the residue of the alcoholic solution was negative.

In the hydrolyzed alcoholic solution

The Liebermann - Bourchard reaction performed on the residue of the hydrolyzed alcoholic solution did not result in a green - purple coloration, so triterpene heterosides are not present. The residue obtained after evaporation of the hydrolyzed

alcoholic solution is treated with 50% methyl alcohol; the Shibata reaction on the alcoholic solution was positive, which reported the presence of flavonoids in this species. Antracenozides are not detected in this red alga, a result of the negative Borntrager reaction. The solution shows fluorescence under the incidence of UV radiation, so cumarines are identified.

In the etheric solution

From the Lieberman-Burchard reaction performed on the ether extract, which came out positive, it appears that sterols and triterpenes are present in the analyzed algae. Carotenoids are identified in this species by the Carr-Price reaction which was positive. The residue obtained by evaporation of the ether extract was taken up with methyl alcohol; the alcohol solution was reacted with Shibata; the reaction was positive, so they contain flavonoic aglicoles. Upon evaporation of the ether extract, a residue was obtained which was subsequently taken up with aluminum hydroxide. Borntrager reagent did not stain the solution orange, which shows that the alga does not contain endemoles. The same residue taken up with ammonium hydroxide show intense fluorescence under UV radiation, so *Ceramium rubrum* contain cumarines.

In the aqueous extractive solution

The aqueous extractive solution is evaporated to the residue, then a few drops of concentrated sulfuric acid and an alcoholic solution of thymol are added - a red coloration is detected which attests the presence of oozes and polioozes. The identification reaction for starch (with Lugol's reagent) was positive for this red alga. The reducing compounds are found in *Ceramium rubrum* due to the Fehling's reaction which was positive. The foaming reaction of the soapozides was negative, that means soapozides are missing from the analyzed species. The basic alkaloids were not identified, because the reactions of Mayer and Bertrand were negative. The aqueous extractive solution reacts with dilute FeCl2 and a blackish green color appears which confirms the presence of catehic tanin.

In Table 2 are presented the results of all identifications.

Table 2. The compounds detected in *Ceramium Rubrum*

The seaweed	Catehic tanin	Oozes and polioozes	Reducing compounds	Soapozides	Flavonoic aglicoles	Cumarines
Ceramium rubrum	++	++	+	-	+	+

Polysaccharides are usually the major component of red algae. The various polysaccharides are the main composition of the cell walls of algae. The most

important polysaccharides in red algae include carrageenan and agar along with other identified polysaccharide compounds, see Table 3.

Table 3. Polysaccharides identified in red algae

Polysaccharides	Red algae
Agar	X
Carrageenan	X
Cellulose	X
Starch	X
Manan	X
Parphyran	X
Galactan sulphates	X
Xylenes	X

Carrageenans are the essential components of red algae cell walls and are linear polysaccharide chains with half sulfate esters attached to the carbohydrate unit. Thay are divided into three forms: lambda, iota and kappa, dipending on the degree of molecular sulfation [21], see Figure 2.

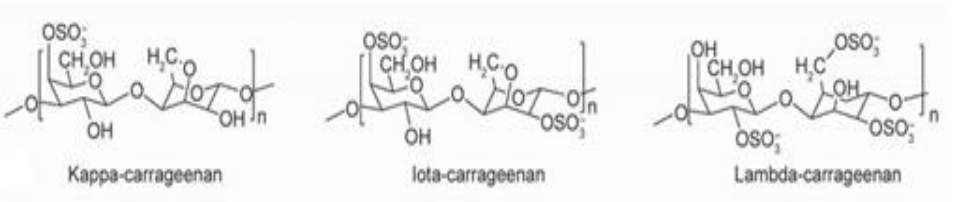


Figure 2. The structures of the three forms of carrageenan

Carrageenan is a sulfated anionic polysaccharide with a straight vertical column structure formed by bonds that alternate 3 β D-galactopyranose with residues of 4-α-galactopyranose. Many of the α-galactose residues may be in the form of 3,6-anhydrous derivatives. Sulfated ester, methyl and acetal groups of pyruvic acid and sometimes monosaccharides can substitute these derivates.

The biological activities of carrageenan

They have been tested for the treatment of hepatitis A, respiratory diseases, the H1N1 flu strain and the African swine fever virus.

The superoxidase dismutase biosensor, which is modulated by the k-carrageenan gel membrane, has been developed and has been reported to test the cleansing properties of commercial drugs. It has been used in the manufacture of the microbial temperature-time-environment indicator [22].

Agar-agar is obtained from a series of red seaweeds (aragophytes) and it is an organic product. It is formed of galactoside residues esterified at C6 with a sulfonic group. See Figure 3. Agars are a mixture of linear polysaccharide agarose, with a heterogeneous mixture of smaller molecules called agaropetin [23], see Figure 3. It has a very high gelling power.

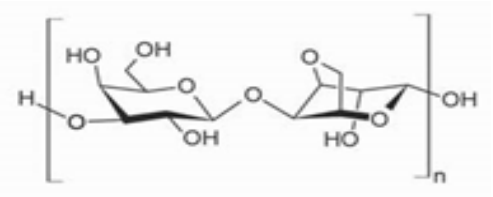


Figure 3. The sructure of the agar

The biomedical activity of the agar

Agar has been extensively used as a gelling agent in the food industry due to its hydrophilic colloidal properties, which is obtained from a mixture of agarose and pectin agar and it has the ability to form reversible gels easily by cooling the hot aqueous solution. It is frequently used as a microbiological medium to ensure the firmness of the gel. The resulting gels are transparent, relatively brittle, clear and they melt on heating. Despite its degradability and excellent gelling power, agar has not been widely used due to its poor aging properties.

Photodegradation and fluctuations in ambient temperature and humidity can modify the crystallinity of the agar, leading to the production of microfractures and the fragility of the polymers [24-26].

Conclusions

From this study, the following conclusions can be drawn: From the three extracts obtained, etheric, alcoholic and aqueous, a series of particularly important bioactive compounds, from a pharmaceutical point of view were highlighted, such as catehic tannin, reducing compounds, cumarines, flavonoic aglicoles, ozes and poliozes. Variations in the marine ecosystem along the Romanian Black Sea coast, as well as temperature variations can lead to different results for algae species compositions, so carefull monitoring is needed each year to track changes in seaweed. These are

categories of compounds of great pharmaceutical interest that can be used, with good results in the future in topical pharmaceuticals.

References

[1] Cadar, E., Cercetarea și dezvoltarea unor sisteme farmaceutice semisolide bazate pe resurse din mediu marin (teză de doctorat). *Carol Davila* University Bucharest. 2017.
[2] Sirbu, R., Negreanu-Pirjol, T., Cadar, E., Negreanu-Pirjol, B-S., Active Principles which are Important to Human Health Obtained from Ceramium Rubrum - Seaweed in the Black Sea. *Academic Journal of Interdisciplinary Studies*, 4:253. Doi:10.5901/ajis2015.v4n1s2p253. 2015.
[3] Seckbach, J., Chapman, D-J., Evolutionary History and Taxonomy of Red Algae. *Red Algae in the Genomic Age*. Springers science. 13:25-42. Doi 10.1007/978-90-481-3795-4_2. 2010.
[4] Fieldmann-Mazoyer, G., Recherches Sur Les Céramiacées de la Méditerranée Occidentale. France. Alger Imprimerie Minerva. 1940.
[5] Cadar, E., Tomescu, A., Negreanu-Pirjol, B.S., *Journal of Science and Arts*, 3:533. 2017.
[6] Negreanu-Pirjol, B.S, Negreanu-Pirjol, T., Paraschiv, G., Bratu, M., Sirbu, R., Roncea, F., Meghea, A., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry*, 12(2), 173. 2011.
[7] Sava, C., Sirbu, R., Leon, A., *Journal of Environmental Protection and Ecology*, 13(1), 289. 2012.
[8] Alberti-Dér, Á., LC-ESI-MS/MS methods in profiling of flavonoid glycosides and phenolic acids in traditional medicinal plants (Ph.D. Dissertation). Semmelweis University Doctoral School of Pharmaceutical Sciences. 2013.
[9] Udayan, A., Arumugam, M., Pandey, A., Nutraceuticals From Algae and Cyanobacteria. *Algal green chemistry*. Chapter 4. 65-89. 2017.
[10] Tollefsbol, T., Medical Epigenetics- 1st edition. AL. United States. 2016.
[11] Grasshoff, K., Ehrhardt, M., Kremling, K. (Eds.), Methods of seawater analysis. 1983.
[12] Cadar, E., Axinte, E-R., Amzoiu, M., Jurja, S., Melat, C., Preliminary study on the marine algae from the romanian Black seacoast. *Journal of Science and Arts*. 4(49):990. 2019.
[13] Sava, C., Sirbu, R., Dumitrescu, C., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry*, 7(4), 785. 2006.
[14] Sirbu, R., Bechir, A., Negreanu-Pirjol, T., Sava, C., Negreanu-Pirjol, B., Zaharia, T., Ursache, C., Stoicescu, R., *Scientific Study and Research-Chemistry and Chemical Engineering Biotechnology Food Industry*. 12(3), 221. 2011.
[15] Sirbu, R., Zaharia, T., Bechir, A., Lilios, G., Nicolaev, S., *Journal of Environmental Protection and Ecology*. 13(1), 190. 2012.
[16] Stoicescu, I., Popescu, A., Sirbu, R., Rosca, C., Doicescu, D.N., Bendic, V., Bala, C., *Revista de Chimie*. 63(9), 865. 2012.

[17] Stoicescu, I., Popescu, A., Sirbu, R., Bala, C., *Analytical Letters*, 45(17), 2519. 2012.
[18] Bintintan, A., Gligor, M., Dulama, I.D., Teodorescu, S., Stirbescu, R.M., Radulescu C., *Revista de Chimie*, 68(4), 847. 2017.
[19] Ciulei I., Istudor V., Palade M., Albulescu D., Gârd C. E., Analiza farmacognostică şi fitochimică a produselor vegetale. Tehnoplast Company, Bucureşti, vol. II, 409 – 418. 1985.
[20] Bruneton, I., Pharmacognosie, Phytochimie, Plantes Medicinales, Technique et Documentation – Ed. Lavoisier, Paris. (218, 249, 258, 498, 690). 1993.
[21] Vera, J., Castro, J., Gonzalez, A., and Moenne, A., Seaweed polysaccharidesand derived oligosaccharides stimulate defense responses and protection against pathogens in plants. Mar. Drugs 9, 2514–2525. doi: 10.3390/md9122514. 2011
[22] Choi, D.Y., Jung, S. W., Lee, D. S., Lee, S.J., Fabrication and Characteristics of Microbial Time Temperature Indicators from Bio-Paste Using Screen Printing Method. *Packaging technology and Science.* 7:33-3012. https://doi.org/10.1002/pts.2039. 2014.
[23] Kumar, M., Kumari, P., Trivedi, N., Shukla, M.K., Gupta, V., Reddy, C.R.K., Jha, B., Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India. J Appl Phycol 23:797–810. 2011.
[24] Abdul Khalil, H.P.S., Saurabh, C.K., Adnan, A.S., et al. A review on chitosan-cellulose blends and nanocellulose reinforced chitosan biocomposites: Properties and their applications. Carbohydr Polym. 150:216–226. 2016.
[25] Joye, I.J., McClements, D.J., Biopolymer-based nanoparticles and microparticles: fabrication, characterization, and application. Curr Opin Colloid Interface Sci. 9(5):417–427. 2014.
[26] Abdul Khalil, H.P.S., Tye, Y.Y., Leh, C.P., Saurabh, C.K., Ariffin, F., Mohammad Fizree, H., Mohamed, A., Suriani, A.B., Cellulose Reinforced Biodegradable Polymer Composite Film for Packaging Applications. *Bionanocomposites for Packaging Applications.* 49-69. doi: 10.1007/978-3-319-67319-6_3. 2017.

Spectral Studies of Analgesic, Antipyretic and Anti-Inflammatory Drugs Used in Medical Therapy in Romania

Sirbu Rodica

University of Medicine and Pharmacy Carol Davila, Bucharest, Romania

Ana Maria Ionescu

Ovidius University of Constanta, Faculty of Medicine, Constanta, Romania

Sanda Jurja

Ovidius University of Constanta, Faculty Medicine, Constanta, Romania

Cezar Laurentiu Tomescu

Ovidius University of Constanta, Faculty of Medicine, Constanta, Romania

Abstract

Human health decline is most commonly manifested by pain and sometimes fever in the initial phase of the disease. Analgesics, antipyretics and anti-inflammatories serve as drugs with various chemical structures which showcase in different proportions their main actions. They represent medicines that suppress pain and fight fever. The aim of the paper is studying the usual types of therapeutic indications using modern spectral analysis, based on the analgesic, antipyretic and anti-inflammatory actions. The following spectral analysis methods are used to control the chemical compositions of the studied drugs: UV-VIS analyses and IR analyses.

Keywords: UV-VIS spectra, IR spectra, analgesic, antipyretic, anti-inflammatory.

Introduction

Analgesics and antipyretics are drugs that reduce or suppress pain and fight fever. They are a group of drugs with various chemical structure and have associated in different proportions the following main actions: analgesic, antipyretic and anti-inflammatory. Pain is an important alarm system in body protection [1, 2]. Triggers defense reactions to remove the harmful agent [2, 3]. Provides useful guidelines for diagnosis [4, 5]. Acute pain causes an increase in heart rate, heart rate and blood pressure, mydriasis, sweating, hyperventilation, mental anxiety [4]. It is possible to reduce or suppress pain, using drugs that act at different levels, on the links involved in the formation and conduction of nerve influx and in the perception of pain [3, 4]. To combat the pain you can intervene:

1. Preventing the formation of nerve influx, in sensitive endings: local anesthetics, muscle relaxants, vasodilators, anti-inflammatory drugs.

2. Preventing the transmission of nerve influx through sensitive fibers - the case of local anesthetics.

3. Prevention of pain perception, at the level of integration centers: general anesthetics, antipyretic analgesics, morphinomimetic analgesics.

Analgesic - Antipyretic - Anti-inflammatory Actions

Antipyretic-analgesics act analgesically only at the talamus level, raising the threshold of pain perception, without influencing the reaction to pain. The analgesic effect, for some substances in this group, is more evident in somatic pain, localized and superficial (examples: neuralgia, arthralgia, headache), with or without an inflammatory component and is weaker in visceral pain, deep and generalized. In some cases, a peripheral, anti-inflammatory mechanism would be added to the central, thalamic mechanism [5, 6].

Antipyretic analgesics also influence the thermoregulatory center, without having any other effects on the CNS. They do not produce sedative effects, drowsiness and sleep. It does not affect other types of sensitivity and sensory functions. It has no peripheral effects on the digestive, respiratory, cardiovascular systems. Some analgesics-antipyretics have anti-inflammatory effect, [4, 7].

Antipyretic analgesics reduce the fever by acting on the thermoregulatory centers, they decrease their functional level by tending to restore it to normal values. As a result, peripheral vasodilation, sweating, and decreased metabolism occur. The substances have no effect when the thermoregulatory centers function normally, so they do not lower the normal body temperature, they are not hypothermic but only antipyretic [4, 8].

The action of analgesics-antipyretics on fever is nonspecific and occurs directly through the central mechanism. Fever can also be reduced by drugs that act specifically, but indirectly, on biological pathogens, for example by antibiotics and chemotherapeutics [8, 9].

In acute viral infections of the respiratory tract, analgesics - antipyretics are the medication of choice, in mild and moderate forms, uncomplicated, in patients who do not have organic suffering and in whom antibiotics and chemotherapeutics are not necessary. In infectious fevers with antibiotic-sensitive or chemotherapeutic germs, specific medication will be administered. Antipyretics are associated with them only in cases of high fever, with repercussions, on the CNS and cardiovascular system [1, 4, 8].

Qualification

Chemical structure is an important classification criterion. Thus we have [4]:

Salicylic acid derivatives: Acetylsalicylic acid, Aspirin Direkt, Upsarin, Alka Seltzer

(effervescent) Lysine acetylsalicylate, Salicylamide, Diflunisal, Benorilate (acetylsalicylic acid ester with paracetamol)

Pyrazolone derivatives: Phenazone, Aminophenazone, Noramino-phenazone-Metamizole- (Algocalmin ampoules, Metamizole sodium suppositories, Algozone, Novalmin, Novalgin), Propiphenazone (Propiphenazone suppositories).

Aniline (p-aminophenol) derivatives: Phenacetin, Paracetamol (Paracetamol, Paracetamol suppositories, Efferalgan, syrup, or suppositories)

Quinoline derivatives: Glafenine

Non-narcotic analgesics are fundamentally different from morphinomimetic drugs, as they do not produce euphoria, tolerance, physical and mental dependence, so they do not cause drug addiction [5].

Therapeutic Indications

Analgesics-antipyretics have several types of therapeutic indications, based on analgesic, antipyretic, anti-inflammatory, antispasmodic actions. For each of these types of indications, the substances can be used either alone, in isolated administration or in combination, in complex formulas [4, 9÷ 11]. In principle, within the associations it is possible to achieve:

An additive effect, when substances from different chemical groups are used, but act on the same substrate. The advantage of the combination is that lower doses of each substance are used than in their case in isolation.

A potentiating effect, more effective than the previous one, when substances with action on different substrates are used.

Therapeutic indications based on analgesic action (sometimes with an anti-inflammatory component) Neuralgia (dental, intercostal, sciatica). Arthralgias (arthritis, osteoarthritis, spondylosis). Myalgias. Orthopedic disorders (sprains, dislocations, fractures). Postoperative pain. Headache. Dysmenorrhea, [10, 11].

Indications based on analgesic and antispasmodic actions Colic (renal, biliary), dysmenorrhea [4, 11].

Methods and equipment

The spectral method of analysis is one of the most widely used methods for obtaining data on the structure and chemical composition of medicinal substances [12÷15].

UV-VIS and IR spectral analysis methods are often used in drug control to obtain reliable data on the structure of compounds [13,14]. IR spectrophotometry is mainly used to identify drug substances without destroying the integrity molecules. IR absorption is characteristic of a wide range of functional groups, bonds, and structural

units. Analyzed drugs by UV-VIS and IR techniques are: aspirin, paracetamol, nimesulide and sodium diclofenac.

UV-VIS spectroscopy

In the UV-VIS analysis spectra, a molecule absorption spectrum is obtained and the Lambert-Beer law is used to obtain quantitative data. Upon impact between a photon and a molecule, the photon undergoes either diffusion (an elastic shock without loss of energy) or absorption (it increases the internal energy of the molecule). Energy absorption occurs when the energy of the photon corresponds to the energy difference between two possible energy levels. The absorption spectrum of a substance is obtained by recording in a graph the variation of a quantity that characterizes the absorption of light (eg. extinction, absorbance, A, extinction coefficient ε, depending on the wavelength or number of waves, expressed in convenient units for the respective spectral domain [13÷15].

Minimum and maximums appear in the spectra thus obtained; the latter, called absorption bands, correspond to the regions of maximum absorption. The temperature can significantly influence the measured absorbance values. By observing this process with the help of a spectrophotometer is obtained an absorption spectrum of the molecule, that is, a representation of absorption as a function of frequency or wavelength. The Lambert-Beer Law is used to obtain quantitative data,

$$A = log\frac{I_0}{I} = \varepsilon cl \quad (1) \qquad \frac{I}{I_0} = T \quad (2)$$

where: A is absorbance, T is transmitance, I_0 and I are the intensities of light before and after the passage of a solution, ε is called the absorption coefficient or molar absorbability. c is the concentration of solution, l is the thickness of the analysis vessel.

The analysis equipment is the GBC Cintra 10e UV-VIS Spectrophotometer which has the following characteristics: it is a double-beam UV-VIS spectrometer, with monochromator, with direct recording of the ratio of test and reference signals and very high scanning speed. Fully automated, it is controlled by an external computer.

IR spectroscopy

The infrared spectral range, IR, ranges from 780 nm to 300 nm, but a narrower range of 2.5 μm to 1.5 μm is used for analytical determinations. There are three areas in IR, namely near IR, middle IR, and far IR. For structural analyzes, including detection, only the average IR region is of interest, which provides the most analytical

information. The near IR spectral range is less exact in specific information than the average one, being used mainly for quantitative determinations, and the far IR range is used only in research [13÷15]. IR spectrophotometry is mainly used for the identification of organic substances, including drugs, without destroying the integrity of molecules and less for dosing. IR absorptions are characteristic of some functional groups, bonds, and structural units that the IR spectrum can be thought of as a fingerprint of the molecule studied, which makes it easier to deduce structural details and recognize them. The Jasco IR 4200 Spectrometer Analysis Equipment has the following features: 7800-350 cm^{-1} wavelength range, single beam system, high intensity ceramic radiation source, DLATGS Detector (standard).

Results and discussion

Following the spectral analysis, we obtained the following results, which are systematized according to the method and technique of analysis that we used to obtain the spectra.

UV-VIS spectrum of aspirin

The range of ultraviolet in the visible spectrum is 200-350 nm. The UV-VIS spectra of aspirin in hydrochloric acid solution (0.1 N HCl) and in neutral solution are shown in Fig. 1. The maximum ultraviolet absorption of aspirin was found at 230 nm and 278 nm in acids (0, 1 N HCl) and 225 nm and 276 nm in the methanol solution. The results obtained for the spectra with the wavelength for maximum absorption of aspirin in the solvent-aspirin mixture were compared with the aspirin reference spectrum [10, 11, 15].

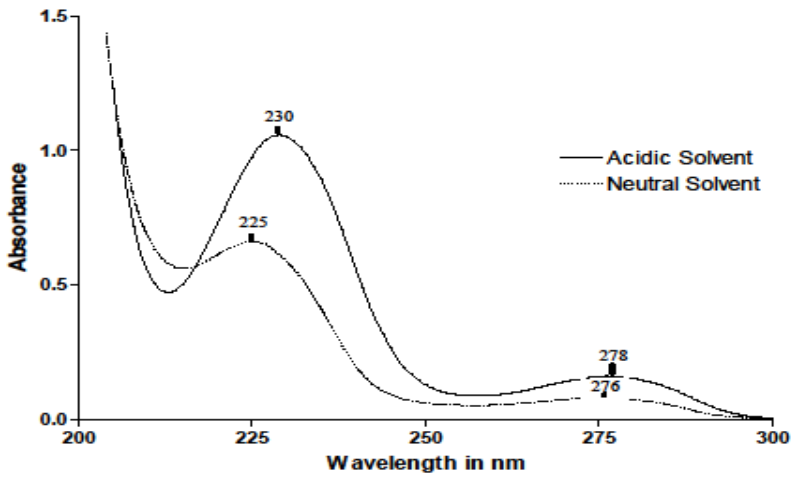


Fig. 1 UV-VIS spectrum of aspirin

The UV-VIS spectrum of paracetamol

The range of ultraviolet in the visible spectrum is 200-350 nm. The UV-VIS spectra in hydrochloric acid solution (0.1 N HCl), in alkaline solution (0.1 N NaOH) and in neutral solution are shown in Fig. 2. The molecular structure for paracetamol is shown in Fig. 2, also.

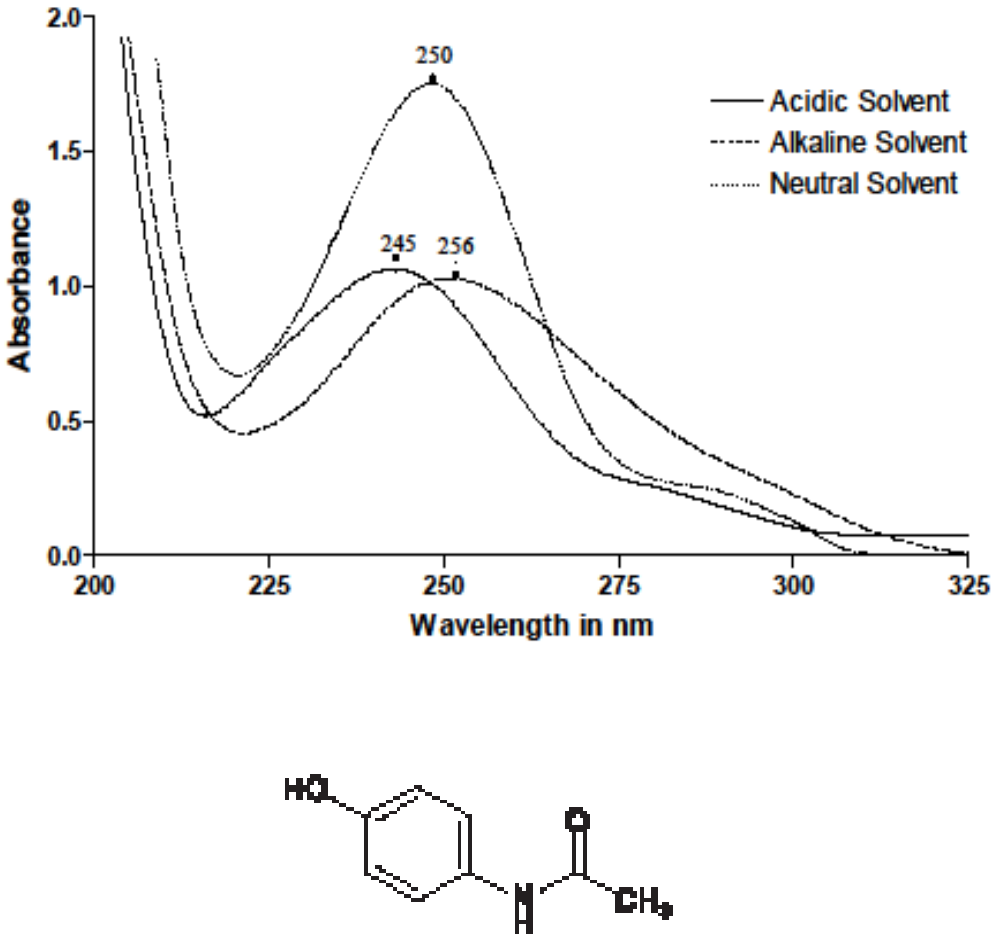


Fig. 2. UV-VIS spectrum and structure of paracetamol

The maximum ultraviolet absorption of paracetamol was found at walvelenghts 245 nm, 256 nm and 250 nm in acid solution (0.1 N HCl), in alkaline solution (0.1 N NaOH) and in neutral solution. The results obtained for the spectra with the maximum wave absorption in the acidic and alkaline solutions of the solvent-paracetamol binary systems were compared with the paracetamol reference spectrum [10,11].

UV-VIS spectrum of nimesulide

It is a non-steroidal anti-inflammatory drug (NSAID) with analgesic (pain relieving) properties. It is used to treat acute pain and painful osteoarthritis symptoms. The range of ultraviolet in the visible spectrum is 200-350 nm. Nimesulide's molecular structure and the UV-VIS spectra in acid solution (0.1 NHCl), in alkaline solution (0.1 N NaOH) and in neutral solution are shown in Fig. 3.

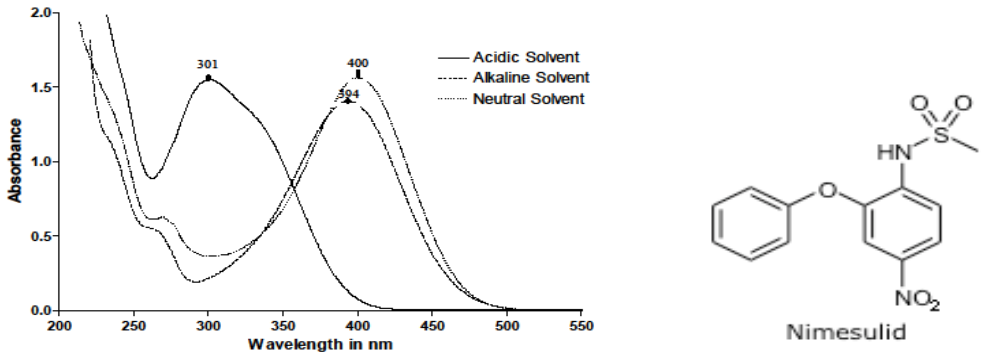


Fig. 3 UV-VIS spectrum and nimesulide structure

Ultraviolet absorption maxima of Nimesulide were found at walvelenghts 301nm, 394 nm and 400 nm in acid solution (0.1 N HCl), alkaline solution (0.1 N NaOH) and neutral solution, respectively. The results obtained for the spectra with the maximum absorption for nimesulide in the acid and alkaline solutions were compared with the nimesulide reference spectrum [10, 11].

UV-VIS spectrum of diclofenac sodium

The range of ultraviolet in the visible spectrum is 200-350 nm. The UV-VIS spectra of diclofenac sodium in acid solution (0.1 N HCl), in alkaline solution (0.1 N NaOH) and in neutral solution for solvent-diclofenac sodium binary systems are shown in Fig. 4. Maximum absorption of diclofenac ultraviolet was found at walvelenghts 273 nm, 275 nm, 279 nm in acidic (0.1 N HCl), alkaline (0.1 N NaOH) and neutral binary systems, respectively. The results obtained for the maximum absorption spectra were compared with the reference spectrum of diclofenac sodium [10, 11].

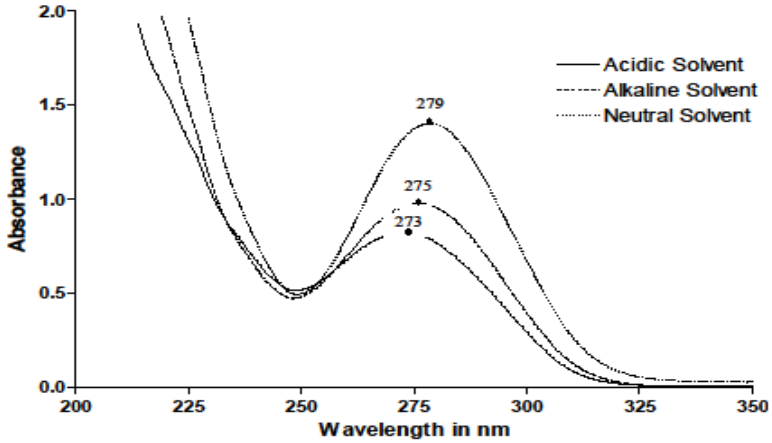


FIG. 4. UV-VIS spectrum of diclofenac sodium

IR spectrum of aspirin

The infrared spectrum of aspirin is shown in Fig. 5. The main wave numbers obtained in the infrared spectrum and their corresponding assignment (bond, type and combined functional group) were characteristic for aspirin. In the range 490 cm^{-1} - 1740 cm^{-1} are combined functional groups with transminttance under 65%T. At 2820 cm^{-1} a level of transmittance 85%T is recorded.

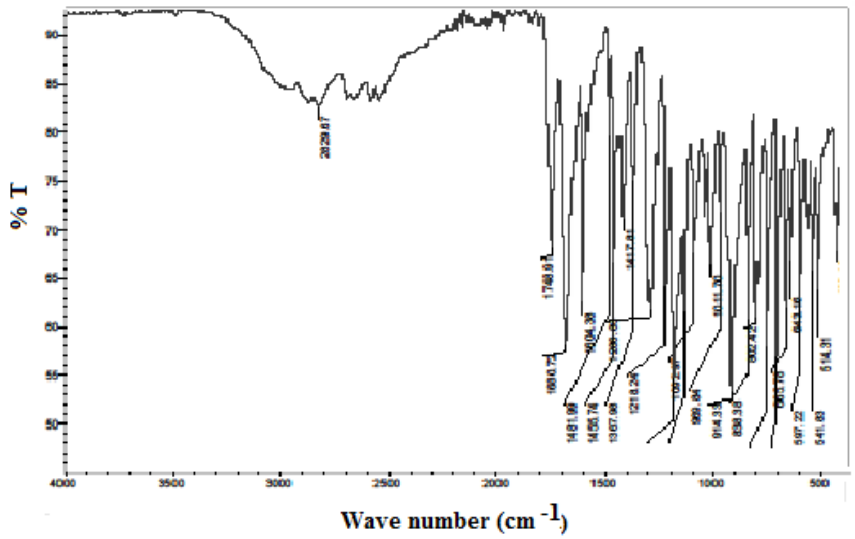


Fig. 5. IR spectrum of aspirin

IR spectrum of paracetamol

The infrared spectrum of paracetamol is shown in Fig. 6. The main wave numbers obtained in the infrared spectrum and their corresponding assignment (bond, type and combined functional group) were characteristic of paracetamol. In range 484,2 cm^{-1} - 1661cm^{-1} are combined functional groups with transminttance under 60%T. At 3109 cm^{-1} and 3319 cm^{-1} WE find two functional groups with transmittance 74 %T and 68 %T respectively.

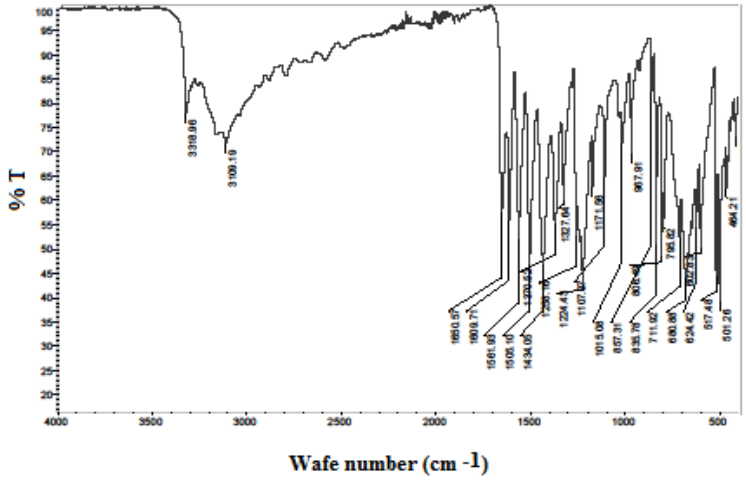


Fig.6. The IR spectrum of paracetamol

IR spectrum of nimesulide

The infrared spectrum of nimesulide is shown in Fig. 7. The main wave numbers obtained in the infrared spectrum and their corresponding assignment (bond, type and combined functional group) were characteristic of nimesulide.

The range of wave numbers 400-1600 cm^{-1} is a group of functions combined with transmittance below 70% T. At 3277.2 there is a functional grouping with 77% T.

IR spectrum of diclofenac sodium

The infrared spectrum of diclofenac sodium is shown in Fig. 8. The main wave numbers obtained in the infrared spectrum and their corresponding assignment (bond, type and combined functional group) were characteristic for diclofenac sodium.

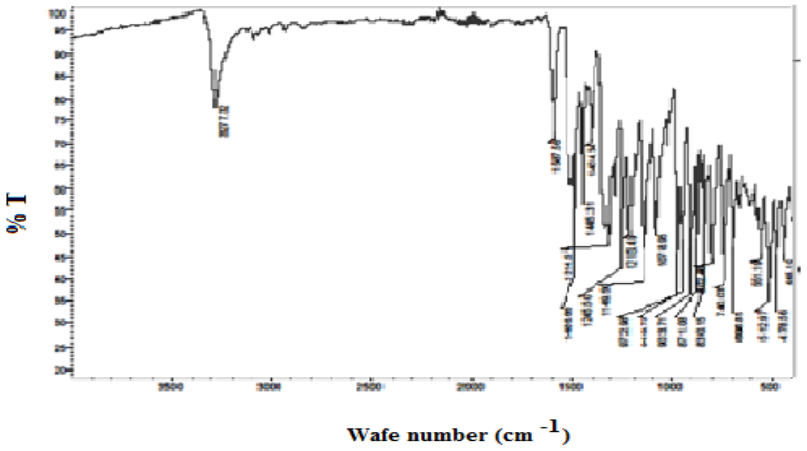


Fig.7 IR spectrum of nimesulide

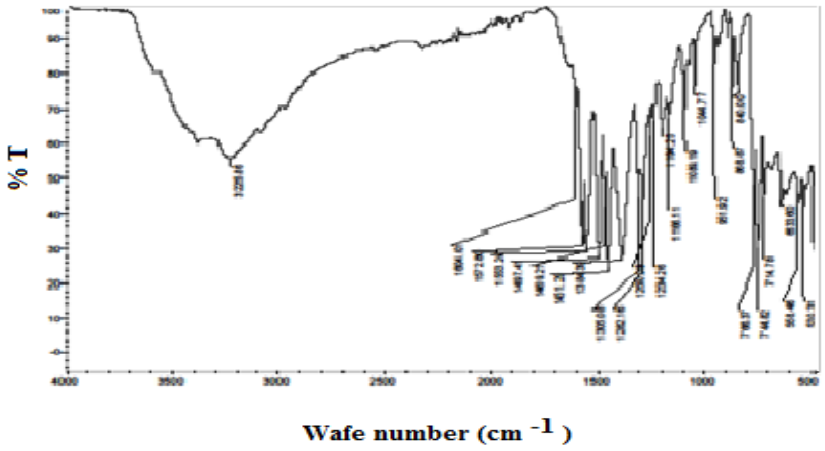


Fig. 8 IR spectrum of diclofenac sodium

The range of wave numbers 890 cm^{-1} - 1804 cm^{-1} is a group of functions combined with transmittance below 45% T. At 3205 there is a functional grouping with 55% T

Conclusions

Pain is one of the most common symptoms that cause a patient to see a doctor. Pain is an important alarm system in the protection of the body. Medications aimed at reducing or suppressing pain and fighting fever.

Antipyretic analgesics reduce fever by acting on the thermoregulatory centers that is, they decrease their functional level by tending to return it to normal values.

The spectral method of analysis is one of the most widely used methods of quality control for obtaining data on the structure and chemical composition of medicinal substances.

Our study can contribute to a spectrum atlas that can be used in drug control.

References

[1] Barbara A. Levey, OVER-THE-COUNTER MEDICATIONS, in Pharmacology and Therapeutics, Principles to Practice, Ed. Elsevier, 87, 1221-1224, 2009.
[2] Bojiță, M., Roman L., Analysis and control of drugs - Theoretical and practical bases, vol.1, Ed. Intelcredo, Deva, 2003.
[3] Brune Hanns, K., Zeilhofer, U., - Antipyretic analgesics, in Handbook of Pain Management, Chapter 22 Ed. Churchill Livingstone, 341-35, 2003.
[4] Cristea A. N., Pharmacology Treatise, Ed. I, Medical Publishing House, Bucharest, 2006.
[5] Cristea A.N., General Pharmacology, Didactic and Pedagogical Publishing House, Bucharest, 2nd Edition, 2009.
[6] European Pharmacopoeia 7th edition, Council of Europe, Strasbourg, 2010.
[7] Hall G.D., Reiss B.S, Appleton and Lange Review of Pharmacy, McGraw-Hill Medical; 8th edition, 2004.
[8] Katzung B.G. Basic & Clinical Pharmacology, 10th Ed., The McGraw-Hill Companies, 2007.
[9] Ritter, J., Flower, R., Henderson, G., Kong Loke, Y., MacEwan, D., Rang, H., Pharmacology, 9th Edition, 2018.
[10] Rodica Sirbu, Physical Chemistry - Ovidius University Press Publishing, 59-83, 2001.
[11] Romanian Pharmacopoeia 10th edition, Medical Publishing House, Bucharest, 2008.
[12] Sîrbu, R., Cadar, E., Methods of physico-chemical analysis of pharmaceutical compounds, Ed. Muntenia Constanta, 62-115, 2019.
[13] Sirbu, R., Negranu-Pirjol, T., Stoicescu, I., Negranu-Pirjol, St. B., Lupu, C., E., Methods and techniques of physic-chemical analysis of pharmaceutical compounds, Ovidius University Press Publishing, 179-213, 2010.
[14] Stroescu V., Pharmacology, 5th edition, Ed. All, 2001.
[15] The United States Pharmacopeia XXX edition, The National Formulary XXVII, Unites States Pharmacopoeial Convention, Inc., Rockville, M. D., 2007.

Early Detection of Mild Cognitive Impairment, Dementia and Alzheimer's Using qEEG

Norsiah Fauzan

Faculty of Cognitive Science and Human Development,
University of Malaysia Sarawak

Nur Hurunain Amran

Faculty of Cognitive Science and Human Development,
University of Malaysia Sarawak.

Abstract

This article reports the biomarkers of the Mild Cognitive Impairment (MCI) among the elderly group aged around 60 -75 years old by analyzing the EEG signals recorded by using the quantitative electroencephalograph (qEEG). There is growing evidence that EEG analysis in resting state condition are useful in early detection of neural signatures of Alzheimer's and dementia. EEG findings and analysis shows potential of discriminating MCI, Alzheimer's and dementia. In this research, the purpose is to 1) develop the indexes for each of the EEG bands and sub-bands such as delta (1.5 – 3.5 Hz), theta (3.3 – 7.5 Hz), alpha (8 – 12 Hz), beta 1 (15 – 18 Hz) and beta 2 (22 – 30 Hz); 2) provide reference for early diagnosis; 3) extract and analyze the brainwave pattern of MCI and cognitively healthy group. This study involved 19 channel resting state EEG from a total of 30 subjects, 18 diagnosed as having MCI and 12 cognitively healthy elderly with criteria for inclusion if the mini mental state examination (MMSE) score is more than or equal to 28, based on the age and educational level. Development of qEEG index started by decomposition of EEG by performing Fourier analysis, averaging and normalizing the value from the 19 channels to obtain the z-score. Findings showed promise of utility in early detection of Alzheimer's. Notably, 1) Increases in delta/ theta posterior temporal and prefrontal area i.e., H(6.64) vs. MCI(26.29); beta temporal region, 2) Decreases in alpha at sensory motor region i.e., H(0.27) vs. MCI(0.23).

Keywords: Mild cognitive Impairment, qEEG, Alzheimers

1. Introduction

Early detection of MCI can help in slowing down the memory loss and can maximize the efficacy of treatment in future (Milwain, E. (2000)). Burns and Zaudig (2002)

defined MCI as a transitional stage between normal ageing and dementia, and reflect the clinical situations where a person has memory complaints and objective evidence of cognitive impairment but no evidence of dementia. MCI is important in terms of recognising memory loss among the elderly as well as identifying a group of individuals at high risk of developing dementia and who may benefit from preventive strategies.

There is evidence in electroencephalography that alpha, theta and delta band oscillations reflect cognitive and memory performances. The most usual EEG findings are the displacement of background frequency into delta and theta ranges and the decrease or dropout of alpha central frequency (Klass & Brenner, 1995). Pucci et.al (1999) proposed the "alpha" rhythm as a diagnostic AD marker as there is a decrease in the alpha frequency to 6.0-8.0 Hz in mild AD patients. However, none of the research provides a reliable indexes for each of the band as a basis of comparison from the cognitively healthy normals. It is necessary to develop the indexes for each of the EEG bands to indicate the level of cognitive impairment and memory loss among the elderly group.

The main idea of the study is to develop an index as a reference between MCI and cognitively healthy elderly. This study will benefit most to the community as it is a practical contribution to the early detection of cognitive deficit and slowing down the progress of Alzheimers In addition, this is a feasible study- community approached. Therefore, the first aim of this study is to develop an index for each EEG band and sub-band, i.e. alpha, beta 1, beta 2, delta and theta. The second aim is to provide the reference for earlier diagnosis of Alzheimer's diseases and basis comparison to other EEG measures or different psychological tests. The final aim is to extract and analyse the brainwave patterns of MCI and cognitively healthy elderly group from same bended EEG.

2. Definition of Terms

2.1 Mild Cognitive Impairment (MCI)

MCI is define as a cognitive function deficit in spite of age and education level, but does not interfere the daily activity but in clinical view, MCI is a transition between early normal elderly cognition and late severe dementia and considered different because some MCI sufferers develop dementia (Al- Qazzaz et. al, 2014). Several studies suggest 5 to 20 percent of the elderly have some form of mild cognitive impairment of one form at any one time (Alzheimer's society UK, 2013).

In studies carried out at the memory clinics, 10 to 15 percent of people with MCI went on to develop dementia in each year (Alzheimer's society UK, 2013). Since the number of individual with AD is expected to increase considerable in the near future, reliable treatment and early diagnosis of MCI is critical. Early detection helps in obtaining the maximum treatment before significant mental decline occurs (Al- Qazzaz et. al, 2014).

2.2 Quantitative Electroencephalogram (QEEG)

Even though a clinical diagnosis accuracy approximately 85 percent of detection rate is commonly achieved, by a procedure of exclusion after structural or functional imaging tests – including quantitative electroencephalogram (QEEG), laboratory, and psychometric test, there are no consensus on methods to estimate and measure the diagnosis and progression of patients with MCI.

Recent research has demonstrated that QEEG is useful for investigating AD (Leucter, 1993; Babiloni et al., 2004). Topographical EEG power changes are believed to reflect early signs of the cortical atrophy and / or compensatory cortical reorganization during the early stages of the disease. More specifically, it is commonly believed that AD induces enhanced mean power of slow rhythms (0.5 – 8 Hz) and loss of fast (8 – 30 Hz) rhythms.

In the EEG of healthy subjects, recorded in resting condition with closed eyes, usually, the alpha rhythms are mostly distributed in the occipital area; in AD patients, the alpha rhythms increasingly relocate towards anterior areas as the disease progresses. Associated early stages of AD have been linked with an increase of theta activity and a decrease of alpha activity. In a more severe condition of AD, there is an increase in theta and delta activities and vice versa in alpha and beta frequency (Knott et al., 2001; Kwak et al., 2006).

2.3 Spectral analysis of EEG

According to a longitudinal study, the mean posterior dominant frequency declined by 0.08 Hz per year over 60 years (Wang & Busse, 1969). There is great evidence in the literature to consider an average alpha frequency of less than 8.5 Hz as abnormal, measured with the patient fully alert. Although posterior frequency decline is usually unspecific and cannot differentiate any particular disorder, it has been common electroencephalographic sign described in many conditions evolving to cognitive alterations, and is frequently encountered in AD individuals (Raicher et al., 2008). Current study reported increase of high alpha frequency power band on the occipital region in MCI subjects compared to normal and Alzheimer's disease patients.

2.4 Power Spectral Density (PSD)

Power spectral density function (PSD) shows the strength of the variations (energy) as a function of frequency. In other words, it shows at which frequencies variations are strong and at which frequencies variations are weak. The unit of PSD is energy per frequency (width) and you can obtain energy within a specific frequency range by integrating PSD within that frequency range. Computation of PSD is done directly by the method called FFT or computing autocorrelation function and then transforming it.

In this context, Qualitative electroencephalogram (Qeeg) measures different brainwaves within the brain where electrodes are placed based on the 10 – 20 system.

Qeeg measured the voltage of electrical impulse signal μV^2 is propositional to the power of the signal on specific sites on scalp to detect and record the electrical impulses. PSD resulted in the strength of a signal which is distributed in the frequency domain, relative to the strength of other ambient signals (Oppenheim, A. V., & Verghese, G. C., 2010). Delta and Gamma (30 - < 60 Hz) denoted an adult slow component, which is sparsely represented whereas the Alpha and Beta is a predominate and a fast component wave.

3. Methodology

3.1 Subjects

3.1.1 Sample size

For the purpose of this research, sample size estimation and statistical power analysis has been done using Gpow3 software power analysis. Statistical power (P) can be defined as P = 1- β, where 0.95 or 95% has been set for this conventional purpose and another 5% is a possibility to accept the null hypothesis (Prajapati, Dunne, & Armstrong, 2010). However, it is a must to consider a potential error. Then, with the large effect size (d), then the numbers of the sample would be 35 in each group, (Normal vs. MCI). However, this study involved 30 participants, 18 diagnosed as having MCI and 12 cognitively health normal screened using mini mental state Examination (MMSE) score is more than or eqal to 28, based on the age and educational level.[1]

3.2.2 Demographic data

All the subjects recruited in the study were pensioners' age 60 to 75 years old in Sarawak. All experiment protocols had been approved by the local ethics committee. Informed consent were obtained from all participants before recording takes place. The difference in the size of the populations is due to the technical reasons linked to the EEG analysis.

Table 1: Demographic data

Group	N	Age	MMSE
MCI	18 (3 Female)	61.89	24.56
Health	12 (1 Female)	65.17	26.25

3.2.3 EEG Recordings

All recordings were obtained in the morning with subjects resting in the chair comfortably. The EEG activity was recorded continuously from 19 sites by using electrodes set in an elastic cap positioned according to the 10- 20 International systems for 25 minutes (Electro - cap International, Inc). Data were recorded with a band- pass filter of 0.3 – 70 Hz and digitized at a sampling rate of 250 Hz (BrainAmp, BrainProducts, Germany). The electrodes skin impedance was set below 5kOhm. The

recording lasted 10 minutes, where 5 minutes with subjects eyes open (EO) and another five with eyes close (EC) task. The EEG data of eyes close task were then analysed and artifacts were discarded.

4. Result and Discussion

4.1 QEEG Patterns of MCI compared to Normal group

After recordings, data during eyes close (EC) were then extracted and analysed. Eyes close task is chosen because it is the best condition, where the minimum noise and artifacts either from muscular or non muscular tension produced. Findings revealed the presence of excessive fast wave activity (beta 1 and beta 2) at the left anterior and central, generally and specifically at point FP1, F3, F7, Fz, C3, T3, T4,and C4). The power spectral density for the five EEG bands were plotted to allow visual inspection of Qeeg patterns of MCI compared to the normal group. Refer to Figure 1,2,3,4 and 5)

Figure 1

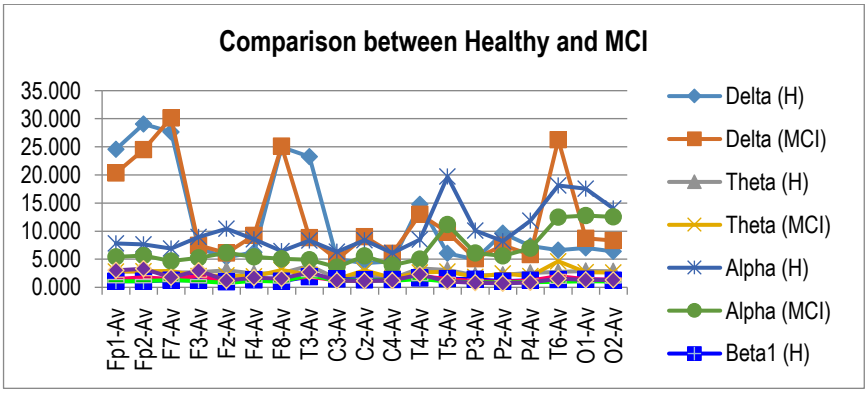
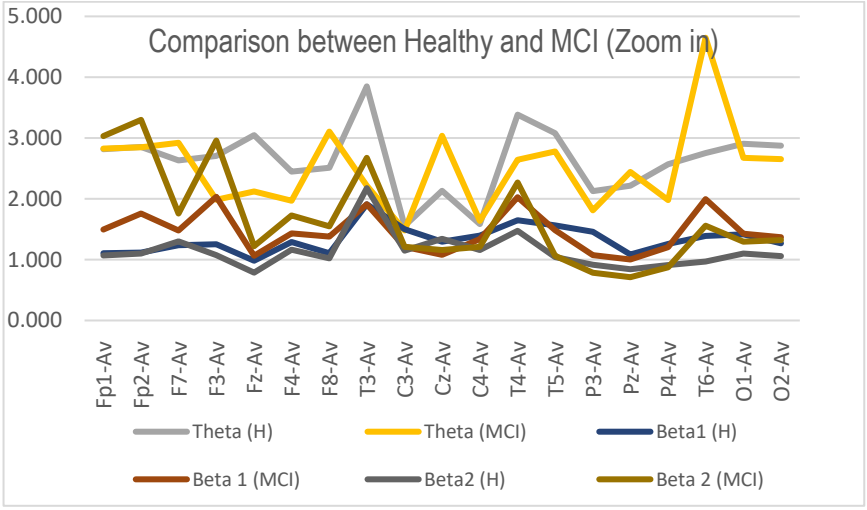


Figure 2



Findings showed promise of utility in early detection of Alzheimer's. Notably, 1) Increases in delta/ theta posterior temporal and prefrontal area i.e., H(6.64) vs. MCI(26.29); beta temporal region, 2) Decreases in alpha at sensory motor region i.e., H(0.27) vs. MCI(0.23**) . Look at Figure 4a, 4b, 4c.**

Figure 3a: Delta Anterior

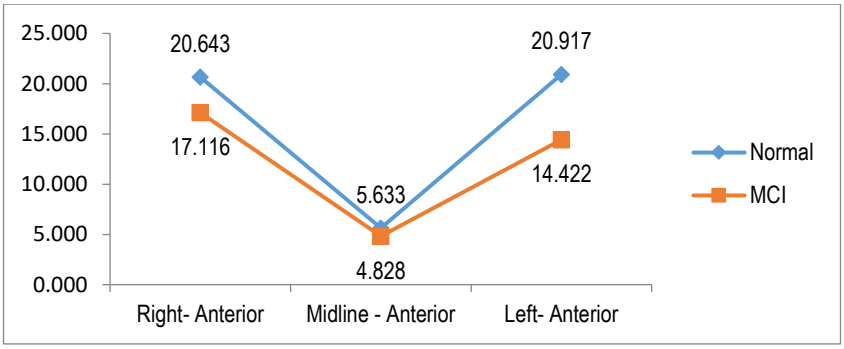


Figure 3b: Delta Central

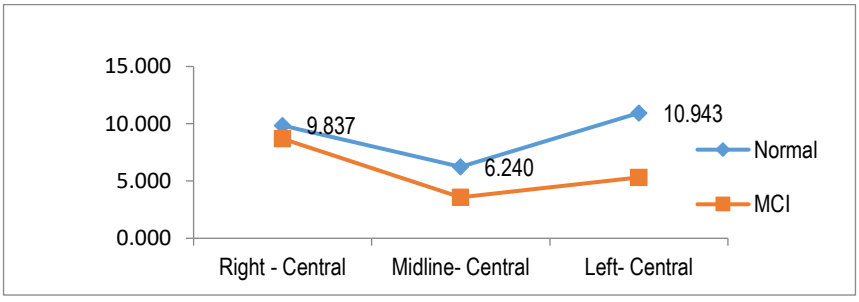


Figure 3c: Delta Posterior

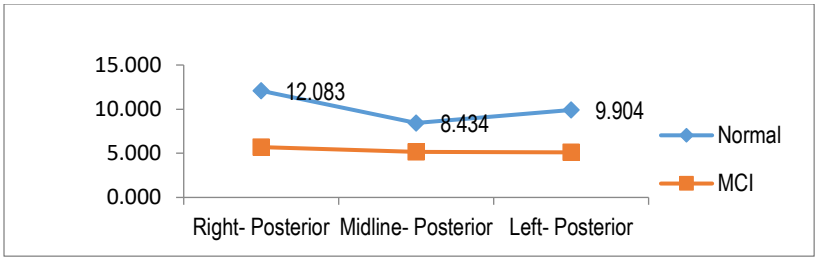


Figure 4a,4 b, 4c

Increasing Delta/theta at posterior temporal & Prefrontal Decreasing alpha wave at Central region

Frequency Bandwith

Figure 4a

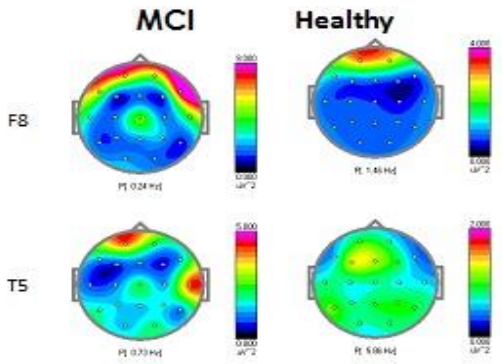


Figure 4b

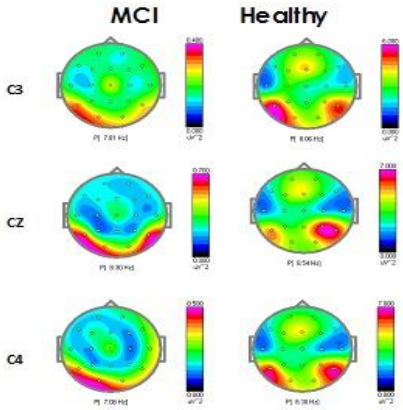
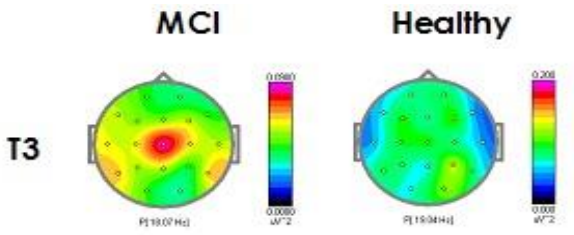


Figure 4c: Increase in beta in Temporal region



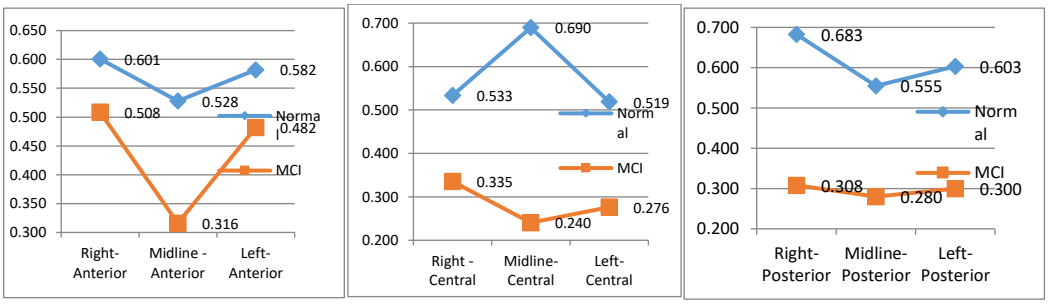
4.1.1 Delta wave

Anna Wise, 2004 in her book 'The High Performance Mind' reported that delta wave needed in order to produce hormones i.e human growth hormone. In addition, delta wave can boost anti ageing hormones. Refer to Figure 3a and 3c, Delta wave for MCI is excessively low at the left and right posterior compared to the normal.

According to Chase, B., an amino acid known as GABA is produces during the sleep and 30% extra GABA is produced for a regular sleeping pattern compared to insomniacs. Many researchers agreed that during the sleep without dream, the dominant wave is delta. Volgushev et al.(2006) reported that the slow wave spread preferentially from anterior to the posterior direction.

4.1.2 Theta wave

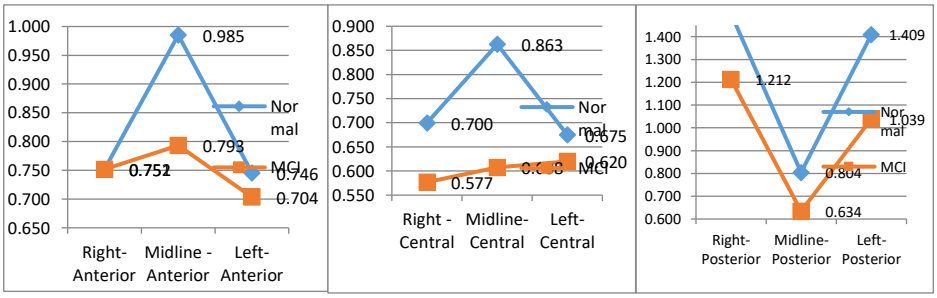
Figure 5a:Theta Anterior Figure 5b: Theta Central Figure 5c:Theta posterior



Refer to Figure 5a, 5b,5c, there is a significant difference between normal and MCI elderly theta at most region. This is supported by the research done by Prishep, L.S. et al. (2005) reported that alpha and theta mean values is significantly different from left temporo-occipital region compared to MCI. Theta waves is equivalent to the learning process as we can predicted human memory strength by the theta oscillation and medial temporal lobe is vital for learning (Rutishauser, U., Ross, I. B., Mamelak, A. N., & Schuman, E. M., 2010). Theta wave is generated by anterior cingulated cortex which the deficits at the frontal midline and central denoted them as MCI (Al- Qazzaz et al., 2014)

4.1.3 Alpha Wave

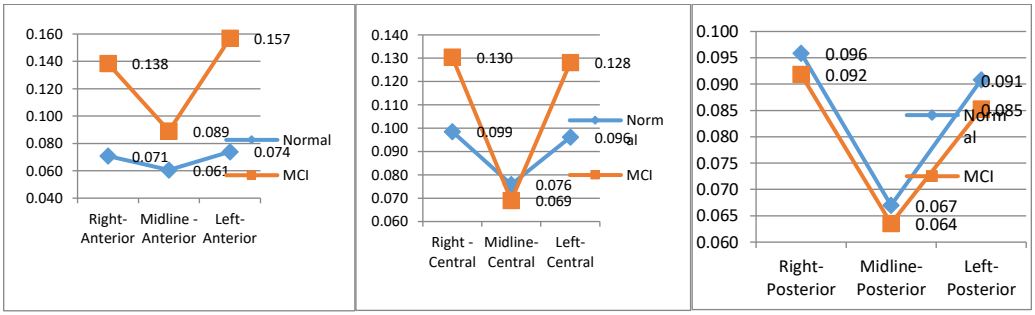
Figure 6a Alpha Anterior Figure 6b:Alpha Central Figure 6c: Alpha Posterior



Obviously, in resting state for normal elderly, alpha will dominate the brain as the eyes were close. But, in this situation, alpha for MCI is reported to be excessively low for MCI compared to the normal at the central region and notable low at the midline anterior (Refer to Figure 6a, 6b,6c). Alpha inadequacy in MCI power density can be supported by Weber, E. which reported that alpha wave worked best for incoming sensory and motor information which is synonym with the sensorimotor rhythm (SMR). Moreover, it is also claimed to disperse from the central region and mostly observed in the posterior region (Al- Qazzaz et al., 2014).

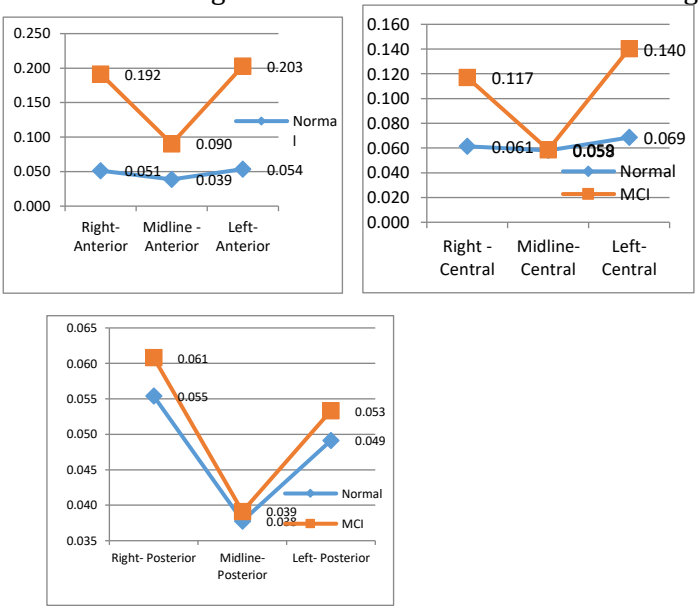
4.1.4 Beta wave

Figure 7a: Beta 1 Anterior Figure 7b: Beta 1 Central Figure 7c: Beta 1 Posterior



Occipital lobe is located at the posterior region, thus the Beta 1 posterior is not active as the beta wave will slowly disappeared as the eyes close (Refer Figure 7a, 7b dan 7c). Beta 1 anterior and central for MCI is higher than the normal group and it denoted the impairment compared to the normal. Even though beta wave is synonym with attention and concentration, but according Al-Qazzaz et al. (2014) during the resting condition, if the beta wave replaced the alpha, it remarks the cognitive impairment.

Figure 8a: Beta 2 Anterior Figure 8b: Beta 2 Central Figure 8C: Beta 2 Posterior



Most researchers reported that beta waves are observed in the parietal and frontal region and this explain the differences showed in Figure 5. The result is more likely for Beta 2, as the high beta is dominant in higher thinking order activity (Refer Figure 8a, 8b, 8c). Delta and theta activity will diminish as people reached their adulthood,

whereas those alpha and beta waves increasing linearly as an outcome of cognitive impairment

Conclusion

The observation on Mild cognitive impairment group revealed the predominance posterior slowing rhythmic frequency bands reducing the alpha and beta activities whereas the occipital activity of the alpha and beta band in the normal aging is increasing. The findings showed a predominance distribution of theta and delta power or slow cerebral rhythms for both normal and the MCI. A reduction of delta at the subfrontal regions for the normal and central regions might be related to the cognitive decline at the hippocampal area. Analysis revealed statistically significant slowing in EEG activity of the MCI group. Specifically, there is an increase in delta and theta at the posterior and left temporal region (T6, T5) and prefrontal lobe (F7,F8) among the MCI over the healthy group and increase in Beta 1 and Beta 2 over the temporal regions among MCI group. Moreover, there is a significant decrease in rhythmic alpha frequency at the central or sensory motor region (c3,cz,c4) and posterior region.

References

[1] Al Qazzaz, N. K., Ali, S. H., Ahmad, S. A., Chellappan, K., Islam, M. S., & Escuedero, J. (2014). Role of EEG as biomarker in the early detection and classification of dementia. *The scientific World Journal.* Vol. 2014. Retrieved from http://dx.doi.org/10.1155/2014/906038

[2] Babiloni, C., Binetti, G., Cassetta, E., Cerboneschi, D., Dal Forno, G., Del Percio, C. Rossini, P. M. (2004). Mapping distributed sources of cortical rhythms in Mild Alzheimer's disease. A multicentric EEG study. Mapping distributed sources of cortical rhythms in mild Alzheimer's disease. A multicentric EEG study, *22*(1), 57 - 67.

[3] Besthorn, C., Zerfass, R., Geiger – Kabisch, C., Sattel, H., Daniel, S., Schreiter – Gasser, U., Förstl, H. (1997). Discrimination of Alzheimer's disease and normal aging by EEG data. *Electroencephalography and Clinicalneurophysiology, 103*(2), 241 – 248.

[4] Brinkmeyer, J., Grass- Kapanke, B., & Ihl, R. (2004). EEG and the test for the early detection of dementia with discrimination from depression (TE4D): avalidation study. *International Journal of Geriatric Psychiatry, 19*(8), 749 – 753.

[5] Dierks, T., Ihl, R., Frolich, L., & Maurer, K. (1993). Dementia of the Alzheimer type: effects on the spontaneous EEG described by dipole sources. *Psychiatry Research - Neuroimaging*, *50*(3), 151–162.

[6] Flicker, C., Ferris, S. H., REisberg, B. (1991). Mild cognitive impairment in the elderly: Predictors of dementia. *Neurology, 41(7), 1006 – 1009.*

[7] Jelic, V., Shigeta, M., Julin, P., Almkvist, O., Winblad, B., & Wahlund, L. O. (1996). Quantitative Electroecephalograpy Power and Coherence in Alzheimer's disease and Mild Cognitive Impairment. *Dementia and Geriatic Cognitive Disorders., 7* (6), 314 – 323.

[8] Klass, D. W., Brenner, R. P. (1995). Electroencephalography of the elderly. *Journal of Clinical Neurophysiology, 12*(2), 116 – 131.

[9] Knott, V., Mohr, E., Mahoney, C., & Ilivitsky, V. (2001). Quantitative electroencephalography in Alzheimer's disease: comparison with a control group, population norms and mental status. *Journal of Psychiatry and Neuroscience*, *26*(2), 106 – 116.

[10] Kwak, Y. T. (2006). Quantitative EEG findings in different stages of Alzheimer's disease. *Journal of Clinical Neurophysiology*, 23(5), 457 – 462.

[11] Leuchter, A. F., Cook, I.A., Newton, T.F., Dunkin, J., Walter, D.O., Rosenberg-Thompson, S., Weiner, H. (1993). Regional differences in brain electrical activity in dementia: use of spectral power and spectral ratio measures.*Electroencephalography and Clinical Neurophysiology, 87*(6), 385 – 393.

[12] Moretti, D. V., Zanetti, O., Binetti, G., & Frisoni, G. B. (2008).) Quantitative EEG Markers in Mild Cognitive Impairment: Degenerative versus Vascular Brain Impairment. *Dementia & Neuropsychologia, 2*(1), 9 – 12.

[13] Petersen, R. C., Doody, R., Kurz, A., Mohs, R. C., Morris, J. C., Thal, L.inblad, B. (2001). Current Concepts in Mild Cognitive Impairment. *Journal of American Medical Association, 58*(12). Retrieved from http://archneur.jamanetwork.com/article.aspx?articleid=781015

[14] Pucci, E., Belardnelli, N., CacchiÒ, G., Angeleri, F. (1999). EEG power spectrum differences in early and late onset forms of Alzheimer's disease. *Clinical Neurophysiology, 110*(4), 621 – 631.

[15] Raicher, I., Takahashi, D. Y., Kanda, P. A. M., Nitrini, R., Anghinah, R. (2008). qEEG spectral peak in Alzheimer's disease: A possible tool for treatment follow- up. *Dementia & Neurophychologia, 2*(1), 9 – 12.

[16] Rodriguez, G., Nobili, F., Rocca, G., De Carli, F., Gianelli, V., Rosadini, G. (1998).

[17] Quantitative electroencephalography and regional cerebral blood flow: discriminant analysis between Alzheimer's patients and healthy controls. *Dementia and Geriatric Cognitive Disorders, 9*(5), 274 – 283.

[18] Rutishauser, U., Ross, I. B., Mamelak, A. N., & Schuman, E. M. (2010). Human memory strength is predicted by theta- frequency phase- locking of single neurons. *Nature* 464, 903 – 907.

[19] Vialatte, F., Dauwels, J., Maurice, M., Musha, T., & Cichocki, A. (2011). Improving
the Specificity of EEG for Diagnosing Alzheimer's Disease. *International journal of Alzheimer's Disease, 2011*(2011). Retrieved from http://www.hindawi.com/journals/ijad/2011/259069/abs/

[20] Wise, A. (1997). The High Performance Mind. Tarcher; New Ed edition (January 27,
1997).ISBN-10: 0874778506 ISBN-13: 978-0874778502

Local Anaesthetics – Substances with Multiple Application in Medicine

Rodica SÎRBU

Ovidius University of Constanta, Faculty of Pharmacy, Constanta, Romania

Emin CADAR

UMF Carol Davila Bucharest, Faculty of Pharmacy, Bucharest, Romania

Cezar Laurenţiu TOMESCU

Ovidius University of Constanta, Faculty of Medicine, Constanta, Romania

Cristina-Luiza ERIMIA

Ovidius University of Constanta, Faculty of Pharmacy, Constanta, Romania

Stelian PARIS

Ovidius University of Constanta, Faculty of Pharmacy, Constanta, Romania

Aneta TOMESCU

Ovidius University of Constanta, Faculty of Medicine, Constanta, Romania

Abstract

Local anaesthetics are substances which, by local action groups on the runners, cause loss of reversible a painful sensation, delimited corresponding to the application. They allow small surgery, short in duration and the endoscopic maneuvers. May be useful in soothe teething pain of short duration and in the locking of the nervous disorders in medical care. Local anesthesia is a process useful for the carrying out of surgery and of endoscopic maneuvers, to soothe teething pain in certain conditions, for depriving the temporary structures peripheral nervous control. Reversible locking of the transmission nociceptive, the set of the vegetative and with a local anesthetic at the level of the innervations peripheral nerve, roots and runners, a trunk nervous, around the components of a ganglion or coolant is cefalorahidian practice anesthesia loco-regional. Local anaesthetics summary and semi-summary have multiple applications in dentistry, consulting, surgery and obstetrics, constituting "weapons" very useful in the fight against the pain.

Keywords: Local anaesthetics, dentistry, consulting, surgery and obstetrics

Introduction

Local anesthesia is a useful method for performing surgical procedures and endoscopic maneuvers, in order to relieve pain in certain conditions, for the temporary depravation of peripheral structures from nervous control. Reversible locking of the nociceptive, motor and vegetative transmission with a local anesthetic at the level of peripheral nerve endings, nerve roots, of a nerve trunk, around the components of a ganglion or in the cerebrospinal fluid constitutes the practice of loco-regional anesthesia. Local anaesthetics are substances which, through local action on nerve formations, lead to the reversible loss of the pain sensation, defined corresponding with the application area [1]. The first known local anesthetic was cocaine. It is found in the leaves of a shrub scientifically called Erythroxylon coca, and more commonly coca shrub. The shrub grows wildly in the Andes Mountains of South America, but also in Ceylon and Jamaica. The natives use it by chewing the leaves, to remove the sensation of hunger, thirst and fatigue. An American author noted that natives measure the distance between villages in coca leaves [2]. Later, in 1948, xiline or lidocaine was introduced with a faster and more intense action than procaine and, also with a notable efficacy in the treatment of cardiac arrhythmias [1, 2]. Xiline was followed, in 1952, by chloroprocaine, which has, however a higher level of neurotoxicity, in 1957 by mepivacaine, acting somewhat longer than xiline and bupivacaine, introduced in 1963 [1,2]. The latter local anesthetic determines a longer action period and it is indicated to be used to remove the pain of childbirth or postoperative pains. Administered through continuous infusion, it may cause effective analgesia for several days. Unfortunately, its administration must be carefully monitored because it has side effects on the heart. For the anesthesia of the skin and of the mucous membranes were later synthesized other local anaesthetics. As it can be seen, cocaine, the first anesthetic discovered lost, ground to the newer anaesthetics, which is also because this preparation snorted through the nose causes a feeling of drunkenness and an increase of physical and mental energy, phenomena sought after avidly by addicts. The chronic use of cocaine with this purpose determines however phenomena of somatic damage, the chronic consumer having an earthy yellow color, with a total lack of appetite, physically and mentally exhausted, phenomena that can lead to death. Clinically, cocaine is now used less often, only for the surface anesthesia of the mucous membrane of the eyes and of the nasopharynx. However, the other local anaesthetics have multiple uses in dentistry, ENT surgery and obstetrics, very useful "weapons" in the fight against pain. However, most local anaesthetics are injected near the action area and they must be able to penetrate the membrane of the nerve. In general, the membranes of the nerves are composed of lipids. The increase of solubility of lipids of a series of compounds has as a result the facilitation of membrane penetration. In *in vitro* experiments involving very simple systems with isolated nerves, the potency of the compounds is directly proportional to the distribution coefficient. The *in vivo* system is more complicated and often, in a homologous series, the increase of partition coefficients leads to the increase of

potency to a maximum, afterwards the activity decreases, at the same time the toxicity increases [1, 3].

Local anaesthetics of synthesis and semi-synthesis have multiple uses in dentistry, ENT, surgery and obstetrics, constituting very useful "weapons" against pain.

In this paper the local anaesthetics are systematized depending on their structure. Assessments are made regarding the effects based on the correlations generated through the analysis of the structures from spectroscopy data [3, 4].

Research Methods

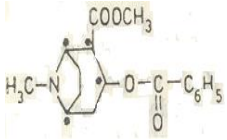
In this study the information regarding the natural and synthetic anaesthetics is systematized, with the presentation of the chemical structures, short characterizations and highlights of the beneficial and toxic effects.

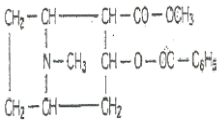
Through the spectrophotometric method in infrared were obtained spectra in infrared for the following local anaesthetics: Cocaine, Prilocaine, Lidocaine, Bupivacaine, Benzocaine, Procaine. The FT IR 4200 Jasco spectrometer with Fourier transform, manufactured in Japan, Tokyo, was used. The characteristics of the device are: the wavelength 7800 to 350cm-1; with the precision of ±0.01 cm^{-1} and the maximum resolution of 0,5 cm^{-1}[4]. Spectra in infrared were carried out for the following substances: Cocaine, Benzocaine, Procaine, Lidocaine, Bupivacaine, Prilocaine.

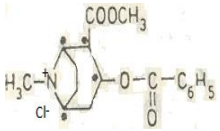
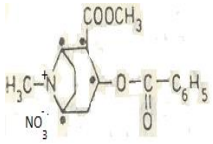
Results and Discussions

A systematization of local anaesthetics can be made after the chemical structure and the obtaining method/ In Table 1 and 2 are presented examples of local anaesthetics [3]

Table 1. Natural local anaesthetics [1,3, 5,6]

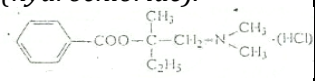
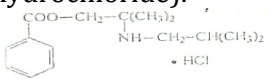
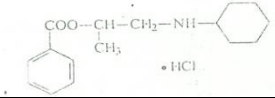
Local anaesthetics and structure	Effect on the human body
Cocaine 	Cocaine is an alkaloid isolated from Erxtroxylon coca leaves, Family Erytroxylaceae, in 1855, by Gaedcke, who assumed that it is an alkaloid related to caffeine. In 1860 Niemann obtained it by extraction from the leaves, along with other compounds with a similar chemical structure: benzoylecgonine, cynnamil cocaine. The fruits have been used by locals since ancient times, for their effects (elation and removing the sensation of fatigue, hunger and thirst). Cocaine is obtained also synthetically. The cocaine can cause very serious acute poisoning (some with a fatal outcome), especially as therapeutic accidents (overdose,

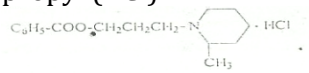
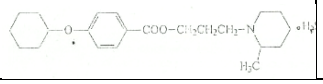
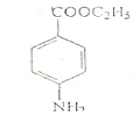
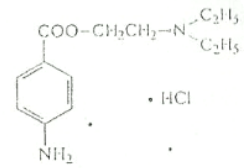
	confusion, or consecutive to the hypersensitivity of the subject), rarely because of suicide and chronic poisoning - cocainomania. Base cocaine is rarely used, as oil solutions 2%, used in ophthalmology. Its salts are frequently used: cocaine hydrochloride and nitrate. Cocaine does not penetrate intact skin, but it is quickly absorbed by the mucous membranes, as highlighted by the response given to addicts when cocaine is sniffed. The use of cocaine is limited, exclusively externally, as a surface analgesic, especially in the field of ENT. The maximum dose of local application must not exceed 30 mg for one use and 60 mg for 24 hours. **Pharmacokinetics.** It is easily absorbed at the level of mucous membranes. It is biotransformed intensely by hydrolysis in the blood, liver and gastrointestinal tract (when administered this way) forming benzoylecgonine (and methanol) and subsequently benzoic acid and ecgonine. It is eliminated through the kidneys. **Toxicodynamics.** Cocaine is toxic to the CNS, producing stimulating effects followed by depression, by depressing the vital centers of the bulb. Produces hyperthermia. At the level of the vegetative nervous system, it has a sympathomimetic action, the main effects are vasoconstriction and mydriasis. By repeated administration the tolerance increases, familiarity easily being set up, with a strong psychological dependence, the abstinence syndrome is insignificant in these events. The lethal dose (for unusual subjects) is smaller by mucous membrane or parenteral administration than orally. It is admitted that lethal doses for adults are 0.2 to 0.3 g s.c. and 1 g orally. **Treatment.** In acute poisoning by ingestion gastric lavage is done with a solution of KMnO4 1‰, followed by the administration of a purgative. Symptomatic treatment refers to combating convulsions and respiratory and cardio-circulatory assistance.

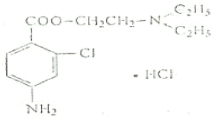
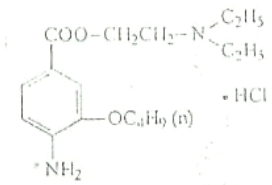
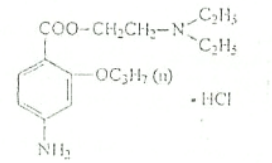
Cocaine hydrochloride 	It is presented in the form of crystals or white crystalline powder, soluble in water (1: 0.5), alcohol (1: 3.5), chloroform (1:15), and glycerin. It has a sympathomimetic action by blocking presynaptic reuptake of noradrenaline by receptors. Local anesthetic action is strong but limited by its toxicity; it is also vasoconstrictive locally, small doses causing psychomotor stimulation. It acts on the central nervous system, as a stimulant in the first phase, and then causes depression. Produces cocainomania. Inside the organism, cocaine breaks down quickly, it is eliminated partially through urine as ecgonine and benzoic acid. Its toxicity prevented its use with other purposes apart from local anesthesia and even this use is limited because of fear of producing systemic effects and addiction.
Cocaine nitrate 	It is rarely used. It has the same uses as cocaine hydrochloride. Due to the fact that the use of cocaine therapeutically is limited, presenting a number of disadvantages including euphoria and pronounced toxicity (cacoinomania); synthesis was used to obtain compounds with similar structure and activity. Research has shown that most compounds in this class can fit into a general formula, which represents only part of the cocaine molecule responsible for local anesthesia.

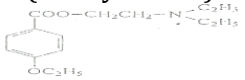
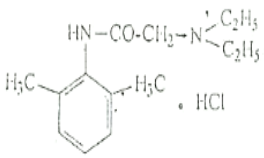
Each structural component (aryl and aminoalkyl) contributes to the stability of the molecule in lipids and it can be modified to form derivatives with an increased coefficient of distribution. The substitution of the aryl radical with alkyl, alkoxy and alkylamino leads to homologous series in which the partition coefficients increase with the number of methylene groups (-CH2-). A review of these series showed that the local anesthetic activity reached the peak for the homologues C4, C5 or C6 according to the specific nature of the series under consideration. Similarly, changes in the amino alkyl group of the molecule lead to an increase of the activity and toxicity, with the increase of the number of carbon atoms. The N-alkyl branching is often accompanied by an increase of the action. Tertiary amino group may be diethylamino, piperidino or pyrrolidino, resulting in compounds that have the same degree of effectiveness. Most local anaesthetics have PKa values between 8 to 9.5. The result is that some of the compounds with higher pKa values are ionized at a rate of 100 / pH physiologically, and thus have difficulty in achieving biophase. Substances that have a lower pHa are not sufficiently ionized and are less effective, even if they reach biophase.

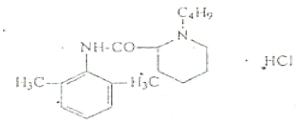
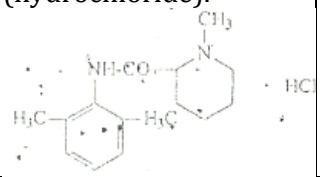
Table 2. Local anaesthetics of synthesis and semi-synthesis [1,2,3,5,6]

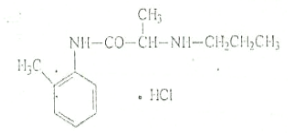
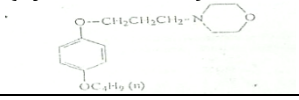
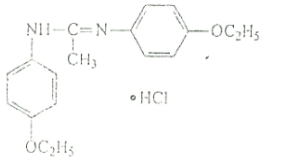
Local anaesthetics and structure	Effect on the human body
AMINO ALCOHOLS ESTERS BENZOIC ACID DERIVATIVES (BENZOATE)	
Amylocaine *(DCI), Amileina, Stovaina, Benzoate 1-Ethyl-1-methyl-2-(dimethyamino)-ethyl; 1-Dimetilamoni-2-methyl-2- benzoyl oxibutan (hydrochloride).* 	It is a local anesthetic as active as cocaine, but less toxic. Is also has cardiac analeptics properties; also, it does not produce arterial hypertension. It is administered internally in doses of 0.01-0.1 g per day in potions and externally, in the form of solutions 5-20%, 4% eye drops, 1-2% ointments and 0.02-0.04 g suppositories. It can be administered parenterally (s.c.).
Isobutene (DCI), Benzoate 2 (isobutylamine)-2-methyl-1-propanol (hydrochloride). 	White crystalline powder, easily soluble in water, alcohol and chloroform. From the structural point of view, Isobutene differs from Meprylcaine in that it has an N-isobutyl group in the place of the N-propyl group. As Meprylcaine, the action period is short. It is used in dentistry in the form of a solution of 2%.
Hexylcaine (DCI) Cyclaina, benzoate 1-(cyclohexyl-amino) -2-propanol (hydrochloride). 	It is used as a local anesthetic (1-5%), with the same intensity as Cocaine and Butacaine, and for the nerve blocking anesthesia a solution of 1-2% is used, its toxicity is comparable with that produced by Procaine and Tetracaine. It is also used in spinal anesthesia with a solution of 1-2.5%.
Piperocaine (DCI), Metycaine, metilpiperidilpropil benzoate; 3 pipecolinopropyl benzoate; benzoate 3-(2'-methylpiperidino)	It is a crystalline substance soluble in water (1: 1.5) and alcohol (1: 4.5). Aqueous solutions are slightly acidic and stable to sterilization through autoclaving. It is a very good local anesthetic recommended as solutions of 2-4% in ophthalmology, in ENT with solutions of 2-10%, for infiltrations are recommended solutions of 0.5-1%, and for spinal anesthesia solutions of 1,5% are used without exceeding 1.65 mg / kg.

propyl (HCl). 	
Cyclometicaine (DCI) Surfacaine, benzoate 3-(2-methylpiperidino) propyl-p-(cichlohrxiloxi) (sulphate). 	Cyclometicaine sulphate is in the form of a white crystalline powder, slightly soluble in water, alcohol and chloroform. It is an effective local anesthetic on altered or diseased skin and on rectal mucosa. It is used topically on burns (sun) light skin injuries, on the rectal mucosa in the form of ointments, gels, creams or suppositories. Not recommended in ophthalmology.
DERIVATIVES OF P-AMINOBENZOIC ACID (P-AMINOBENZOATE)	
Benzocaine (DCI), Anesthesine, Etophorm, p-Aminobenzoate ethyl. 	It is a local non-toxic anesthetic, readily absorbed through the altered skin and through mucous membranes. It acts only as long as it is in contact with the diseased skin or the mucous membranes. It is used externally in the form of ointments and creams at concentrations of up to 20%, 2% oil solutions and suppositories. Internally it is administered as a potions in doses of 0.3-0.6 g for ulcer pain, gastralgia.
Procaine (DCI), Novocain, Allocaine, Sy,caine, 4-amino benzoic 2-dimethylamino acetate (hydrochloride). 	Procaine is the salt of a strong acid with a weak base. The aromatic amino group causes the aqueous solution to be neutral, therefore it is well tolerated by tissues. The ester group may undergo hydrolytic cleavage in p-aminobenzoic acid and diethylaminoethanol. Procaine hydrochloride is not effective on intact skin or mucous membranes, but it acts promptly through infiltration. Its action may be extended by concomitant administration with a vasoconstrictor (adrenaline) that slows its release into the blood where it is rapidly inactivated by hydrolysis Procaine. It is used as a local anesthetic for a relatively short duration, used more in infiltration anesthesia, truncal, epidural and spinal anesthetic being weak. Administered by injection (i.v.), Procaine has an analgesic effect, causing vasodilatation and smooth muscle relaxation, depresses the heart and prevents certain ectopic arrhythmias, reduces the

	reflectivity of vegetation. Low doses over time, prevent aging, being a trophic sealant.
Chloroprocaine (DCI), Nesacaine, 2-Clhoro-4-aminobenzoate of diethylaminoethyl (hydrochloride). 	Chloroprocaine differs structurally from procaine by the fact that it has an atom of chlorine in the position 2 of the aromatic nucleus which, attracting electrons, de-stabilizes the ester group. Consequently, chloroprocaine is hydrolyzed in plasma four times quicker than procaine; the local anesthetic action is more rapidly installed and is more intense than that of procaine, but the toxicity is higher.
Oxybuprocaine (DCI), Novesine, Butoxyprocaine, Benoxinal, 3-Butoxy-4-aminobenzoate of 2-(dietilamino)-ethyl (hydrochloride) 	White, crystalline powder with a salty taste, very soluble in water and chloroform, soluble in alcohol, stable in air, light and heat. It appears that the 3-butoxy radical stabilizes the molecule, so it is hydrolyzed more slowly than Procaine, the degree of hydrolysis depending on the pH of the solution (pH≈4). Oxybuprocaine is a rapidly acting surface anesthetic. By blocking reversible transmission of stimuli via sensory nerve, it is more effective than cocaine, does not cause mydriasis and accommodation disorders, being better tolerated than cocaine or tetracaine. It is indicated for surface anesthesia of the cornea, in the extraction of deep foreign bodies, in diagnostic examinations and eye surgery. It is administered as a 0.4% solution (5 drops in 5 minutes). The application of oxybuprocaine drops must be strictly limited to preparing ophthalmic surgery or diagnostic examinations. Used in small quantities, oxybuprocaine is well tolerated; sometimes causing a burning sensation, transient hyperemia, even corneal epithelial lesions. It does not cause local irritation, vasoconstriction, specific pupil dilation or sensitivity to light.
Propoxycaine *(*DCI), Blockain, 2-proxy 4-aminobenzoate of 2-(dimethylamino)-ethyl (chlorhydrate) 	White crystalline substance, readily soluble in water, soluble in alcohol. 2% aqueous solutions have pH 5.4. The popoxy- group apparently destabilizes the ester group in the same way as the chlorine in the 2-position of the chloroprocaine's structure. This is in contrast to the apparent stabilizing effect of the butoxy group located in the 3-position on the aromatic ring. (Oxybuprocaine). The inductive and steric effects of a 2-alkoxy substituent which favors hydrolysis, apparently exerts greater influence than the positive mezomer

	stabilizing effect (resonance). These effects are minimal when the -alkoxy substituent is in position 3. Propoxycaine causes a more rapid onset of the local anesthetic effect and a longer duration of action, being more active than Procaine. The lipophilic nature justifies these advantages. It is administered injectable for nerve block and infiltration anesthetic, in the form of a 0.5% solutions, without the addition of a vasoconstrictor.
P-ALCOXYBENZOIC ACID DERIVATIVES (P-ALCOXYBENZOATES)	
Paretoxycaine (DCI), Intracaine, Maxycaine, p-Ethoxybenzoate of 2-(diethylamino)-ethyl 	The presence of the –alkoxy group instead of the –amino group makes Paretoxycaine produce less significant side effects than the esters of the p-aminobenzoic acid. It is used as a local anesthetic in ENT.
AMIDES	
Lidocaine (DCI), Xiline, Xylocaine,a-(Diethylamino)-2, 6-dimethylacetanilide, a-(Diethylamino)-N-(2,6 dimethylphenil)-acetamide (hydrochloride). 	Löfgren (1940) has researched and selected Lidocaine as the main representative of the series. Based on the iodine derivative (a- diethylaminomethylindol) with low anesthetic properties, he obtained a local anesthetic with the chemical structure similar to that of lidocaine. It has the form of a white or slightly yellow crystalline powder, insoluble in water, very soluble in alcohol and oils. Lidocaine hydrochloride is a white crystalline powder, odorless, very soluble in water and alcohol. Lidocaine (xiline) is resistant to hydrolysis because, in addition to the relative stability of the amide bond, the two methyl groups at position 2 and 6 cause the steric hindrance to the attack the carbonyl group; this explains the prolonged action. Lidocaine acts on all the nerve fibers, as it contains in its molecule a hydrophilic end represented by an amino group and a hydrophobic end represented by an aromatic radical, joined by a short chain, typically ester or amide. In tissues due to the slightly alkaline pH and buffer system, the liposoluble base is released, which is able to penetrate through the membrane of nerve fibers, where the amide undergoes a quartemizing effect. Lidocaine is used in all types of local anesthesia, it is three times

	more active than procaine and has longer action. As antiarrhythmic, it is used in ventricular arrhythmia in acute myocardial infarction, cardiac surgery or therapy with digitalis, being administered i.v., typically under EKG control, from the initial dose of 100 mg, to the reach of the effective plasma concentration, continuing with 1.5-3 mg / minute.
Bupivacaine (DCI), Sensorcaine, Marcaine, Carbostesia, (±)-N-(2,6-Dimethylphenyl)-1-butyl-2-piperidincarboxamide (hydrochloride). 	Bupivacaine is a local analgesic characterized by a long duration of action, and a relatively high installation latency. The pharmacodynamic properties are similar to lidocaine. The effect occurs slowly, in 15 minutes, and lasts 4-8 hours, due to its marked liposolubility and to its ability to bind to membrane proteins. Bupivacaine is used in local or regional anesthesia for surgery (truncal, plexicală, caudal, epidural anesthesia) and is administered parenterally; doses should not exceed 2.5 mg / kg body weight.
Mepivacaine (DCI), Carbocaine, Mepivastein, Opticain, Scandonest, (±) N-(2,6-Dimethylphenyl)-1-methyl-2-piperidine-carboxamide (hydrochloride); 1-Methyl-2,6'-pipelocoxylidide (hydrochloride). 	As with the other anilides and lidocaine, mepivacaine is highly resistant to hydrolysis, and its solutions are subjected to sterilization in autoclave, without decomposition. Mepivacaine is used as racemic because it was established that the two optical isomers have the same activity and toxicity; they are comparable to those of lidocaine. Its duration of action is considerably longer than that of lidocaine, even in the absence of a vasoconstrictor. It is recommended especially when epinephrine and its counterparts are contraindicated. Mepivacaine is a local anesthetic of synthesis whose action is due to the decrease in membrane permeability of sodium ions. It is administered parenterally 1 to 2 ml of a 3% solution.
Prilocaine (DCI), Citanest, N-(2-Methylphenyl)-2-(propilamina)-propanamide (chlorhydrate); 2-(Propilamino)-o-propionotoluidide	White crystalline powder, odorless, bitter, slightly soluble in water and alcohol. Prilocaine has stability, effectiveness, toxicity and duration of action similar to that of other anilides. Its duration of action is between that of lidocaine and that of mepivacaine. Side effects are similar to those produced by other anilides, noting that prilocaine produces methemoglobinemia. This is due to the fact that as a result of its metabolism is obtained a-toluidine- (2-methylaniline), which is

(hydrochloride) 	broken down into toxic products (phenylhydroxylamine nitrobenzene) that cause methemoglobinemia. Prilocaine hydrochloride solutions are used without addition of vasoconstrictors.
ETHERS	
Pramoxin (DCI), Pramocaine,Tronothane,4-[3-(p-Butoxyphenoxy)-propyl]-morpholine (hydrochloride) 	White crystalline powder with aromatic smell, slightly soluble in water and alcohol, the pH of THE 1% solution is 4.5. It is a topical local anesthetic, with a low index of sensitivity, with few side effects. It is used for relieving pain and itching caused by insect stings lesions, superficial injuries and hemorrhoids.
OTHER STRUCTURES	
Fencaine (DCI), Holocaine, N, N'-bis-(4-Ethoxyphenyl)-acetamidine monohydrate (hydrochloride) 	White crystalline powder, odorless, bitter-tasting, stable in air, easily soluble in water (1:50), easily soluble in alcohol. The aqueous solutions may be sterilized by boiling, but are unstable to the action of alkaline hydroxides. The local anesthetic properties of fencaine were highlighted in 1897, preceding the discovery of izogamine. Fencaine hydrochloride is a slightly irritating, causing a slight discomfort before the onset of anesthesia. It is more toxic than cocaine, and therefore cannot be used for parenteral administration; it is used as a 1% ophthalmic solution 1 to 2% ointment.

The spectrums obtained for the analyzed substances (Cocaine, Benzocaine, Procaine, Lidocaine, Bupivacaine, Prilocaine) are presented in the following figures. *IR spectrums of the analyzed compounds are presented by describing the transmittance depending on the wavelength.*

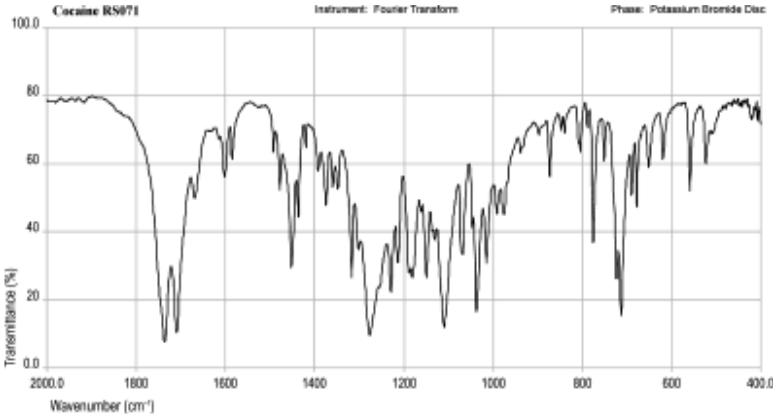


Fig. 1 IR spectrum of cocaine

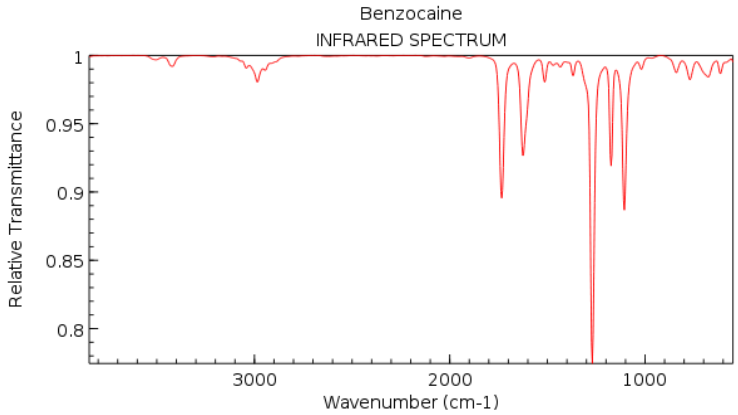


Fig. 2 IR spectrum of Benzocaine

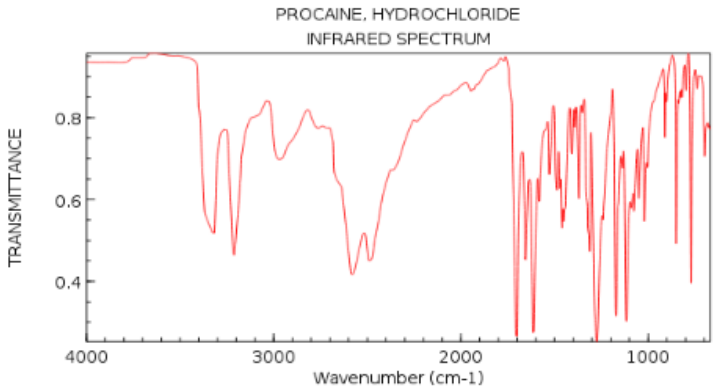


Fig. 3 IR spectrum of procaine

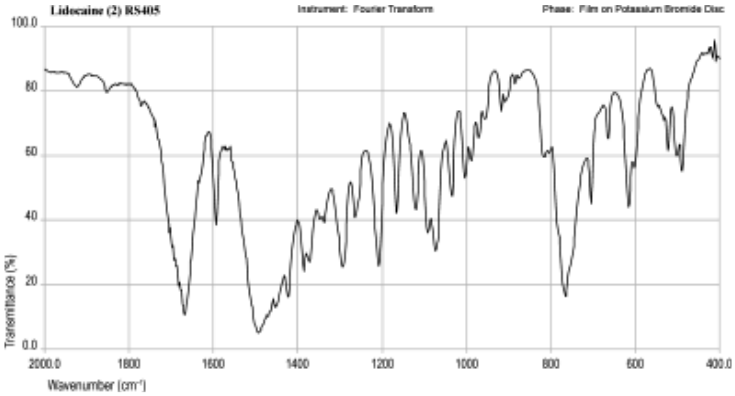


Fig. 4 IR spectrum of lidocainei

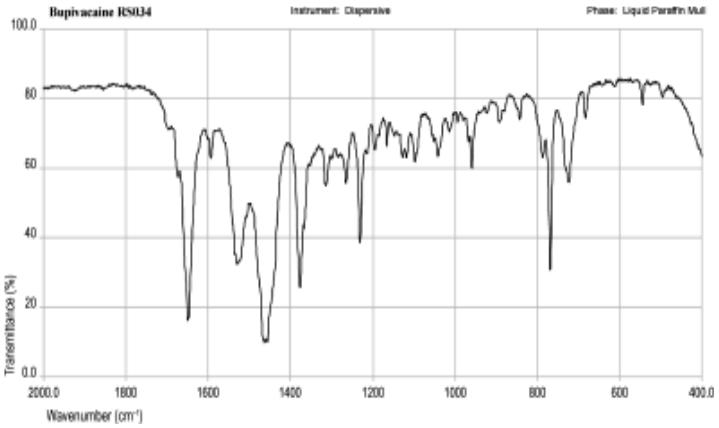


Fig. 5 IR spectrum of bupivacaine

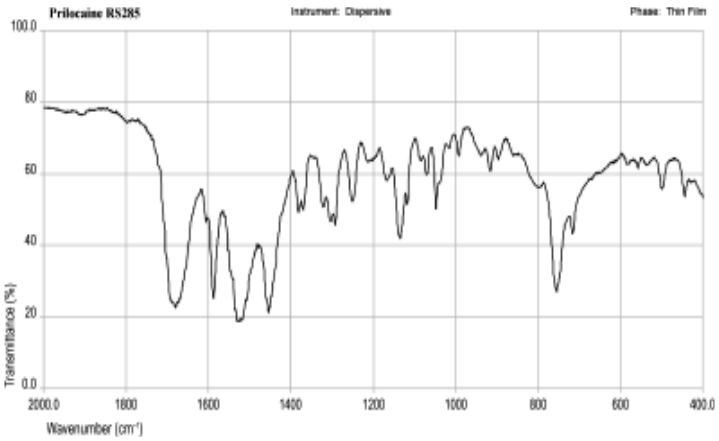


Fig. 6. IR spectrum of prilocaine

Conclusion

- Local anaesthetics may have 3 types of systemic effects, namely: central-nervous, vegetative and cardiovascular. Being absorbed at the site of applications, they cause central excitement phenomena, followed by depression. These effects may lead to serious toxic symptoms. The slowing of absorption by associating vasoconstrictors allows to prolong local action and attenuates resorption effects:
  a) at the level of the central nervous system, excitation and depression events may take place, sometimes a state of confusions (similar to intoxication), after which may follow coma and finally the paralysis of respiratory centers.
  b) at the level of the vegetative nervous system they produce a vasodilator, hypotensive effect, also having a certain antispasmodic action at the level of the smooth muscles.
  c) at the level of the cardiovascular system they cause myocardial depression by inhibiting excitability. In overdose, myocardial depression may be severe. But this effect is therapeutically exploited in cardiac arrhythmia.
- Local synthesis and semi-synthesis local anaesthetics have multiple uses in dentistry, ENT, surgery and obstetrics, representing very useful "weapons" in the fight against pain.
- The obtained specters may bring a contribution to the setup of an atlas of specters to be used in the control of medicines.

Acknowledgement

This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/187/1.5/S/156040/

References

[1] Aurelia Nicoleta Cristea – "Tratat de Farmacologie Ediţia I" – Medical Publishing House Bucharest 2005
[2] Cristea L., Noul ghid de Anestezie Terapie Intensivă. Medical Publishing House Bucharest, 1992
[3] Elena Haţieganu; Camelia Stecoza – "Chimie Terapeutică Vol. 2" – Editura Medicală Bucharest 2010
[4] Sîrbu R., Negreanu-Pîrjol T.: *Metode fizico-chimice de analiză a substanţelor farmaceutice*, Editura Matrix
Rom, Bucharest, 2004.
[5] H. Gall, R. Kaufmann, C.M. Kalveram, Adverse reactions to anaesthetics: analysis of 197 cases, J. Alergy Clin. Immunol. 4 (1996)
[6] Becker, DE; Reed, KL: Essentials of Local Anesthetic Pharmacology. Anesth Prog 53:98-109 2006