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The Value of Communities of Practice as a Learning Process to Increase Resilience in Healthcare Teams

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Abstract

This paper addresses the role that communities of practice (CoP) can have within the healthcare environment when facing uncertainty and highly emotionally impactful situations, such as the current COVID-19 pandemic. The starting point is the recognition that CoPs can contribute to build resilience among their members, and particularly moral resilience. Among others, this is due to the fact that they share a reflective space from which shared knowledge is generated, which can be a source of strength and trust within the healthcare team. Specifically, in extreme situations, the CoPs can contribute to coping with moral distress, which will be crucially important not only to facing crisis situations, but to prevent the long-term adverse consequences of working in conditions of great uncertainty. The purpose of this paper is to analyze how CoP can support healthcare professionals when building moral resilience. To support that goal, we will first define CoP and describe the main characteristics of communities of practice in healthcare. Subsequently, we will clarify the concept of moral resilience, and establish the relationship between CoP and moral resilience in light of the current COVID-19 pandemic. Finally, we analyze different group experiences that we can consider as CoP which emerged in the midst of the COVID-19 pandemic to navigate moral problems that arose.

Keywords: communities of practice, vulnerability, moral distress, COVID-19, moral resilience

Introduction

Vulnerability is a fundamental aspect in health care (Gjengedal et al. 2013; Delgado 2017). The recognition of our corporeality, dependence and fragility is everywhere in hospitals and health institutions. In this context, healthcare professionals do suffer or witness suffering on a regular basis: they confront death and fragility in a more noticeable way than in daily life (Delgado et al. 2020). To be witness to all of these circumstances in patients' lives has an impact on healthcare professionals' own lives, and it constitutes a form of vulnerability. Healthcare professionals may themselves be prone to *more-than-ordinary vulnerability*, since they are routinely exposed to stressors that are not ordinarily encountered by most people in their everyday life (Carel 2009). Since these situations cut deeply into the most existential aspects of human life, and place the professionals in a unique position of vulnerability, clinicians suffer when interacting with human health and illness (Ulrich and Grady 2018). In this regard, vulnerability is experienced by professionals because of their profession, as part of their work. Not recognizing this vulnerability may come at a cost not only for healthcare staff, but also for patients and their families. That is, clinician burnout and fatigue are separately associated with major medical errors and perceived medical errors (Tawfik et al, 2018; Welp 2014)

In a public health emergency, such as the COVID-19 pandemic, healthcare professionals are even more prone to moral suffering (Rushton 2018), which include vulnerability experiences and moral distress. Moral suffering is the anguish that occurs when healthcare professionals have to deal with adversities that challenge their own integrity (Rushton 2018). Facing dramatic situations, frontline workers who are directly involved in the diagnosis, treatment and care of patients with COVID-19 are at risk of developing mental health problems (Lai et al. 2020) and moral distress (Cacchione 2020). There are at least three ethical issues that are likely to affect healthcare professionals globally: a) their own safety, and the safety of patients, colleagues and families; b) the allocation of limited resources; and c) the changing nature of relationships with patients and their families (Morley et al. 2020). Due to that, there is a necessity for healthcare institutions and professionals to seek sources of support during this pandemic. However, what kind of support can healthcare professionals find, to face these ethical problems? Moral resilience may be an outcome of addressing moral suffering. We understand moral resilience as the ability to effectively navigate crisis situations in response to the moral complexity, confusion, anguish or setbacks of practice (Baratz 2015; Rushton 2016). The question is how in a public health emergency such as the pandemic COVID-19 moral resilience can be fostered in health professionals who face these challenges?

A Community of Practice (CoP) is a group of people interested in the same problem, technique or question that interests them, and who interact regularly to learn together and from each other (Casado and Uria 2019). In the healthcare settings, a CoP constitutes an intentional space to promote the exchange of experiences arising in clinical practice (Delgado et al. 2020). Because of that, our hypothesis is that CoP can be of great value in addressing the moral suffering inherent to the healthcare practice, which is manifested as vulnerability, moral distress, and sometimes burnout. As spaces of openness to share different ethical experiences arising from the practice, especially in unknown and highly emotionally impactful situations, CoPs offer an opportunity to learn together in order to increase resilience collectively.

Our goal is to explore the role that communities of practice (CoP) can have within the healthcare environment when facing unknown and highly emotionally impactful situations, such as the current COVID-19 pandemic. To this end, we first explain what is a CoP, and more particularly a healthcare CoP. Then, we address some modes in which CoPs can promote moral resilience in healthcare professionals to cope with moral distress. We then proceed to analyze these particular problems of moral distress and moral resilience in the context of the current pandemic COVID-19, which show us that moral distress is an issue that must be prevented always, but more intensively in situations which involve a huge emotional impact in healthcare professionals. Finally, we analyze different group experiences that we can consider as CoP which have emerged in the midst of the COVID-19 pandemic to navigate moral problems that arose.

Understanding the key elements of a community of practice

The notion of CoP was originally introduced to literature by Lave and Wenger in 1991. In this early work, CoP were understood as a type of informal organization oriented to learning with focus on the practical aspects. Since then, the term has been extensively used in education and business sectors, and also in the healthcare field. Lave and Wenger (1991) initially focused on how novices participate in practice, beginning at the periphery of professions, using culturally and historically rich examples. In this context, the situated learning emphasizes the social interactions that support learning within a community of those who practice similar professions or in similar fields (Delgado et al. 2020). Nowadays, CoP can facilitate the ongoing learning process for all professionals, and not only novices.

Following the early work of Wegner et al. (2002), CoP share a basic structure combining three main elements: a domain of knowledge; a community of people who are concerned or interested in the domain; and a set of shared practices that they develop to be effective within the domain. By interacting, the members of CoP obtain several benefits: they share information; help each other to solve problems; share situations, aspirations and needs; explore new ideas; create tools and documents; accumulate knowledge and associate informally. All of this reflects the value that the CoP create for individual members, as well as for the system or organization, the process of learning together. Consequently, the value of CoP is based on (Wegner et al. 2002):

Connect different or isolated professional experts, Diagnose and address recurrent problems whose root causes barriers between teams,

Analyze why work units with similar tasks offer different results and work to achieve the highest possible quality and efficiency standard,

Relate and coordinate unrelated activities within the same domain of knowledge.

A CoP has been defined as a group of people who share a practice, care about the same topics, share tacit knowledge and meet regularly to guide each other through their understanding of mutually recognized real-life problems (Pyrko et al. 2017). Pyrko et al. (2017) point out that both the intention to foster trust and the mutual engagement of all members are essential features of a CoP. They proposed developing CoP by "thinking together", in order to advance the understanding of the nature of CoPs and their fundamental learning processes. Some reasons for that are:

Thinking together entails a trans-personal process. Through this process, the members of the CoP thoroughly learn together and from each other in practice, and in this way they become more skillful professionals.

This idea of thinking together additionally emphasizes the possibility of developing learning partnerships and a sense of community through mutual identification. This way of learning is not only related to technical, practical or theoretical knowledge, but also to the understanding of relevance relationships and communities to any particular field of practice.

More particularly focusing on the healthcare field, usually, healthcare CoPs arise to share clinical information about relevant problems in daily work (de Carvalho-Filho et al. 2019), and sometimes it is an urgent clinical problem that initiates the CoP. In these cases, usually a particular patient´s case is at the core of the CoP (Young et al. 2018). Accordingly, CoPs constitute an intentional and determined space which allows the exchange of experiences that arise in clinical practice (Delgado et al. 2020). We propose that these experiences could also be a space to exchange thoughts and feelings about the ethical dimension of healthcare, present in all clinical practice, but has not yet been considered in the literature about healthcare CoP. We envision that through "thinking together" about ethical dilemmas in daily work, healthcare professionals will learn together through a process that may foster resilience.

In the next section, we will address why it is so important to deepen understanding the particular circumstances why healthcare professionals face moral suffering (Rushton 2018), and consequently, can experience moral distress.

A silent reality: healthcare professionals´ vulnerability, moral distress and burnout

Healthcare professionals´ vulnerability arises from their everyday practice, from the fact that they confront suffering, pain and death day by day. In addition, professionals bring their own vulnerabilities to their encounters with patients. Carel (2009, 218) argues that there is "a vulnerability that arises out of the experience of others' vulnerability, and this type of vulnerability may require more recognition by the profession. Working as a nurse brings with it an almost daily reminder of the fallibility of human flesh and spirit and the fragility of human life and goods. This, in turn, is a lesson in vulnerability". This learning cannot be explicitly addressed in training, supervision or practice. On the other hand, Carel also maintains that the lesson of vulnerability is not a pessimistic one: vulnerability also suggests a relationship of openness to the world. In order to flourish we must let ourselves be vulnerable: this vulnerability is also the gate to creativity and flourishing (Carel 2009). According to vulnerability theory[1], vulnerability is not simply a negative condition, but it must be accepted and not ignored (Fineman 2013; 2014) . Indeed, recognizing the positive aspects of vulnerability can improve the experiences of people in terms of isolation and exclusion, because vulnerability is also generative. "Importantly, our vulnerability presents opportunities for innovation and growth, creativity and fulfilment" (Fineman 2012, 96). Some of these positive aspects of vulnerability can improve the relationships in the field of healthcare. To recognize it, we have to consider that there is a shared vulnerability between patients and professionals. Nevertheless, as Barnard maintains, "the ability to translate shared vulnerability into therapeutic relationships requires continuing self-awareness and self-care" (Barnard 2016, 297). Some difficulties seem to appear regarding how to allow healthcare professionals in practice to talk and express their own and shared vulnerability.

Nissim et al. (2019) have developed a qualitative study to evaluate a group intervention based on mindfulness, called Compassion, Presence and Resilience Training (CPR-T), for interprofessional oncology teams. Shared vulnerability emerged in that study as a challenge identified by the participants. The authors recognized three key elements to analyze this experience of vulnerability: a) an organizational culture that does not allow the professionals to show their feelings in adversity, b) vulnerability management in sessions and c) the paradoxical benefits of sharing vulnerability within the team. Regarding the first aspect, the participants worried about being open and showing vulnerability in the group, since they considered that this could diminish their ability to function effectively within the healthcare team when they return to work. Usually in the healthcare environment, any expression of emotionality is traditionally seen as a weakness, although that reality is changing. The participants pointed out that vulnerability is something tacit, which they do not share with patients or colleagues, much less with superiors, since it is seen as a negative feature. Furthermore, people with leadership roles were uncomfortable opening up in front of direct reports and vice versa. Regarding vulnerability management in the sessions, participants described a gradual change through which they could express their vulnerability as the CPR-T was developed.

[1] The Vulnerability and the Human Condition (VHC) Initiative at Emory University has been developed over the last decade under the leadership of Woodruff Professor of Law Martha Albertson Fineman. See https://web.gs.emory.edu/vulnerability/index.html (Last visited July 8, 2020). In addition, for a broad understanding about vulnerability theory and bioethics, see Delgado 2017.

Although the participants were concerned about the possible consequences of demonstrating vulnerability in front of their team members, they noted that the sessions facilitated mutual trust, empathy and understanding, so that even communication with their colleagues had improved their work (beyond the study group). Finally, regarding the paradoxical benefits of sharing vulnerability within the team, participants commented that CPR-T helped them to recognize the commonalities that they share with their team members, which made them feel more connected to their colleagues and develop a non-critical attitude towards them. All this helped them to build cohesion as well as improve communication between different professions. The participants expressed their surprise upon learning that "they are also vulnerable" (Nissim et al. 2019, 9) and how this awareness helped normalize their own sense of vulnerability and initiate mutual dialogue and learning. Although participants expressed concern about showing vulnerability to their supervisors, participants in managerial positions noted that they became more understanding and responsive to the needs of others.

In addition to the vulnerability experienced by healthcare professionals because of their professional role, moral distress is another source of moral suffering. Moral distress occurs "when a health professional, as a moral agent, cannot or does not act in accordance with his moral judgments (or what he believes to be correct in a particular situation) due to institutional restrictions or internal "(Ulrich and Grady 2018, p1). In other words, moral distress occurs when health professionals recognize ethical conflicts and their responsibility to respond to them, but cannot make their moral choices. Moral distress can arise when the professionals cannot perform their work in accordance with their moral values. In many cases, the reasons may be directly related to the institution.

We have many examples about health care professional vulnerability, moral distress, and burnout experienced in the workplace, unfortunately, increasing during last years (Dyrbye et al. 2017; Davidson et al. 2018; Squiers et al. 2017). In this regard, there are institutional factors that generate impotence, burnout or moral distress: lack of personnel, lack of administrative support, imbalance in power, inadequate organization of work, lack of communication, work overload, etc. (Moreno 2016). These system problems can lead to feelings of impotence, fear or frustration in the individual healthcare professional. In addition, the perception of an unsafe environment for patients, and the fact that professionals cannot challenge these conditions can exacerbate moral distress (Berlinger 2016). Some of the institutional factors that can trigger moral distress are lack of staff and resources, lack of administrative support, imbalance in power, some styles of leadership, poor organization of work, poor relations between members of the interdisciplinary group, lack of communication, work overload and the precariousness of personnel, among others. In addition, there are also some institutional policies or legislation that can generate moral stress (Moreno 2016).

Moral distress can be a contributor to burnout (Fumis et al. 2017) as well as unrecognized vulnerability. Burnout is a psychological syndrome that arises in response to chronic stressors at work, a condition in which professionals lose concern and emotional feelings for the people they work with. As a consequence, they come to treat patients as dehumanized persons (Maslach et al. 2001). Burnout is a three-dimensional syndrome: (1) emotional exhaustion, (2) cynicism and depersonalization and (3) lack of accomplishment and inefficacy (Maslach et al. 2001, 2016; Fumis et al. 2017). Most burnout research has focused on its profound prevalence rather than seeking to identify the origin of the burnout epidemic, and these efforts are usually focused on increasing resilience and wellness among participants rather than combating problematic changes in how medicine is practiced by physicians nowadays (Squiers et al. 2017).

However, there is an increasing recognition that healthcare organizations need to face burnout and foster well-being, as well as help clinicians to provide the best care to patients, through collective action and targeted investment. In the United States, healthcare organizations are implementing committees and supporting groups in an attempt to reduce burnout among their clinicians, nurses and physicians. The National Academy of Medicine (NAM), has a strong commitment on addressing these problems, and they have designed the vast initiative "Action Collaborative on Clinician Well-Being and Resilience", which is one of the most important initiatives developed in this area. As part of this project, the NAM is promoting a network of organizations of the Action Collaborative on Clinician Well-Being and Resilience[1]. Another initiative to address burnout has been the Institute for Healthcare Improvement Framework for Improving Joy at Work (Perlo et al, 2017).

The COVID-19 pandemic complexity as a moral suffering trigger for healthcare providers

In exceptional situations of great physical and emotional burden, such the pandemic COVID-19, ethical questions involving huge emotional suffering increases exponentially. No other previous situation has explicitly exposed the vulnerability of healthcare professionals worldwide to the public. With the current public health crisis, several factors that increase stress, fear and moral distress in health professionals, increasing the mental load of health workers, has been added to their more than ordinarily vulnerable everyday practice. During the peak period of the pandemic, the increasing number of cases, overwhelming workload, lack of personal protective equipment (PPE), media coverage, lack of specific medications, and feelings of being inadequately supported has been identified as factors associated with experiences of psychological burden among healthcare workers exposed to COVID- 19 (Lai et al. 2020).

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[1] The information about the Action Collaborative on Clinician Well-Being and Resilience, developed by the NAM, in available in: https://nam.edu/initiatives/clinician-resilience-and-well-being/

These are some aspects that have been identified as triggering moral distress during the current pandemic:

a) The need to prioritize scarce resources such as ventilators, intensive care beds, blood, etc., generates moral distress (Berlinger et al. 2020; DePierro et al. 2020). In addition, the decision making process about the withdrawal of life support treatments, in this case, would occur despite the fact that the treatments are not objectively futile and that the patients do not reject these interventions, but mainly due to the lack of availability of resources. In Intensive Care Units, during the COVID-19 pandemic, professionals have experienced feelings of disorientation, worry, loss of control, and helplessness (Kok et al. 2020).

b) Primary Care professionals have been overwhelmed, having to face complex decision-making that generates great moral distress, since it is a new disease with great uncertainty regarding treatment, which entails establishing a relationship with patients different from usual practice (having to do triage, telematic consultations, distance or using PPE), and in a context of scarce resources for both care and protection of professionals (Melguizo et al. 2020).

c) Health professionals cannot refuse to care for patients. However, if there is a lack of personal protective equipment PPE and they are at risk of contracting the disease, should they refuse to treat their patients? When does work-based risk become unacceptable? Is there a time when health professionals have the right not to treat seriously ill patients if their PPE is inadequate or if they do not have it? It is essential to treat patients regardless of their disease. But are there limits to this duty? How much risk is too much risk? (Kok et al. 2020; Sheather and Chisholm 2020). The lack of PPE highlights the obligations of healthcare professionals to take care of themselves (Declaration of the World Medical Association in Geneva 2017; Parsa-Parsi 2017) not only because it is necessary to improve the work life of healthcare providers as part of the quadruple aim (Bodenheimer and Sinsky 2014), but also because they are extremely valuable assets for treating patients in the context of a pandemic. The Canadian Medical Association conducted a poll of Canadian physicians showing 74% were somewhat anxious or very anxious about PPE supply (CMA April 2020), and that three quarters of physicians working hospitals were uncertain of their PPE stock or supply (CMA April 2020). In any case, this kind of uncertainty about PPE availability and difficult decision-making generates moral distress, as healthcare professionals feel obligated to continue to provide care.

Moral distress has been identified as a predictor of burnout (Rushton et al. 2015; Fumis et al. 2017) and research has explored the prevalence of burnout in healthcare professionals during the COVID-19 pandemic. In a public health crisis as the current pandemic, healthcare professionals have to contribute increased efforts into their activities for extended working hours. In addition, the constant use of PPE, and the physical fatigue and mental pressures on the unknown disease make the working hours tremendously exhausting (Talee et al. 2020). Other studies have noted the psychological impact of COVID-19 to healthcare workers. A study in Italy where 49% showed post traumatic stress, 25% depression, 20% anxiety, and 22% high perceived stress (Rossi et al. 2020). A study in China showed that of healthcare workers treating patients with COVID-19, 50% reported symptoms of depression, 34% reported insomnia, 45% reported symptoms of anxiety, and 72% reported distress (Lai et al. 2020). Another study of frontline nurses in Wuhan China reported that nurses experience moderate burnout and a high level of fear, with half of nurses reporting moderate or high burnout in all burnout dimensions (Hu et al. 2020). According to the results of a study in Ecuador during the COVID-19 pandemic (Vinueza et al. 2020), more than 90% of medical and nursing professionals had moderate-severe burnout syndrome. These results were associated with profession (physicians experienced higher burnout than nurses), age (the youngest were the most affected) and gender (women were more affected than men). In a systematic review addressing the prevalence of depression, anxiety, and insomnia among healthcare professionals during the COVID-19 pandemic, the evidence suggests that a significant proportion of healthcare professionals have experienced mood and sleep disorders, highlighting the need to establish ways to mitigate mental health risks and adjust interventions to cope with it and minimize the risks factors (Pappa et al. 2020). In addition, medical and nursing students have also experienced stress and anxiety during the pandemic (Al-Rabiaah et al. 2020). A study in Iran of hospital workers showed 53% experienced high levels of burnout during the COVID-19 pandemic (Jalili et al, 2020). These studies are worrisome, as the existing rates of mental health illness and occupational burnout in healthcare prior to the pandemic were significant, with over half of physicians and one third of nurses experiencing burnout in the United States (McHugh et al. 2011, Shanafelt et al. 2012). An editorial in a Canadian newspaper highlights the potential crisis of worsening mental health issues from the pandemic, in physicians already experiencing high mental health and burnout rates (Horton 2020). The parallel pandemics of burnout and post traumatic stress disorder received further attention following the death by suicide of Dr Lorna Breen, the medical director of the emergency department of New York Presbyterian Hospital[1]. Further research is ongoing exploring the psychological impact and effect of burnout from the pandemic. For example, a psychiatrist in Montreal is recruiting healthcare workers for a study on factors associated with burnout (https://burnout.mhicc.org/), while a team in Halifax is conducting research on burnout in healthcare workers. (https://researchns.ca/2020/05/05/preventing-burnout-among-front-line-care-workers-to-fight-covid-19-screen-and-intervene/)

Considering all this complexity, to address the psychological and emotional needs by providing the healthcare professionals and students with adequate support is essential to improve the management of this situation. Some ways that have been proposed to support them are: a) considering their conditions, b) presenting

[1] The New York Times published the report about this case on April 27, 2020. Available in: https://www.nytimes.com/2020/04/27/nyregion/new-york-city-doctor-suicide-coronavirus.html

solutions, c) increasing their awareness, d) encouraging them, and e) acknowledging their importance (Talee et al. 2020). In addition, to increase the resilience of healthcare professionals seems to be a necessary goal to cope with the specific difficulties triggered by public health emergencies. To foster healthcare professionals' resilience, we show how CoP can provide the adequate space for building the healthcare professionals resilience.

How to foster moral resilience through a CoP in the context of the COVID-19 pandemic

The most common approach when talking about ethics in organizations is the use of an individualistic vision in which each person is morally responsible for their behavior; consequently, the interventions focus on the health care of the professional. However, another way to approach ethics in health organizations is to see each person as a member of a community or team, where the understanding of individual ethical behaviors must be complemented by knowledge and exploration of the organization's moral and social structure (Moreno 2016). Despite being a source of suffering for healthcare professionals, vulnerability and moral distress can also act as a spring to open the field of reflection and dialogue from which to generate change at the collective or institutional level (Carel 2009, Fineman 2013, 2014, Moreno 2016). In this regard, our thesis is that CoP can be a source of moral resilience for health care professionals to cope with vulnerability, moral distress and other forms of moral suffering.

In the healthcare environment, resilience plays an important role for workers. A way to foster workplace well-being and engagement is training for resilience, developing good mental health and subjective well-being. Resilience training has a number of wider benefits that include enhanced psychosocial functioning and improved performance (Robertson et al. 2005). All professions in healthcare experience similar effects in relation to the stressful conditions of work. This common aspect offers an opportunity to design and implement interprofessional approaches that can enhance the capacity for resilience among teams of coworkers. For this purpose, it is necessary an institutional culture that prioritizes training and cultivating specific skills and attitudes for promoting resilience to all members of the health care team, which also include students (Haramati and Weissinger 2015). Resilience has been studied mainly in regard to stress. But what about ethical conflicts and problems that workers have to deal with?

Initially the term of moral resilience was developed as moral courage. Lachman (2007, 131) defines moral courage as the "capacity to overcome fear and stand up for his or her core values; the willingness to speak out and do what is right in the face of forces that would lead a person to act in some other way; it puts principles into action". In her latest work, she also develops the concept of moral resilience, defining it as "the ability to deal with an ethically adverse situation without lasting effects of moral distress and moral residue" (Lachman 2016, 123). She adds that this requires morally courageous action, activating needed supports and doing the right thing. In addition, she argues "the virtue of moral courage is necessary to meet the ethical obligations of the profession" (Lachman 2016, 123). Rushton (2016) highlights that moral resilience is a concept under construction, and it is a way to transform the deep despair and impotence associated with morally distressing situations. Moral resilience can be understood as the ability to preserve or restore integrity in response to various types of moral adversity (Rushton 2018).

We acknowledge that healthcare professionals are thought to be highly resilient. A Canadian study of physicians showed that despite over 30% experiencing high levels of burnout, 60% of physicians said their overall mental health was flourishing and 82% reported high levels of resilience (CMA 2018). In the COVID-19 pandemic, Hu et al. (2020) found a moderately negative correlation between frontline nurses' burnout, anxiety, and depression with the self-efficacy and resilience. Thus, as nurses have greater more self-efficacy and resilience, they may experience less mental health problems.

Gujral et al. (2020) have proposed some strategies to increase resilience in healthcare professionals during the COVID-19 pandemic:

a) Find time for self-care: give employees the opportunity to attend the practice of meditation, acupuncture, yoga therapy or massage therapy as well as a weekly mindfulness meditation session guide.

b) Breathing practice: offering 15-minute breathing sessions three times a week by teleconference for anyone working within the healthcare system as a reminder of focus on breathing practice.

c) Gratitude practices: Finding the opportunity for gratitude as a powerful practice to heal, energize, and empower.

Although these interventions are important, all of them are focused on an individual perspective, that is, to promote individual moral resilience. They must be complemented with a collective perspective. In addition, these interventions do not address the systemic factors which are thought to contribute to moral distress and burnout. A collective and systemic perspective is of great importance, given recent research that finds physicians have greater resilience than the general working population (West et al, 2020) and that even highly resilient physicians may experience burnout. Thus, individual resilience alone is not sufficient to prevent burnout or moral distress. Collective perspectives also have the potential to improve systems through advocacy. It is also necessary to consider whether resilience as currently assessed, equates with moral resilience.

In the light of this way of thinking, and according to Delgado et al. (2020), the exchange of experiences that is shared within the CoPs is an essential factor in building and maintaining moral resilience. It allows for a change in relationship from a distressing situation by shifting the mindset that the distressing experience is completely negative. From this starting point, strategies to collectively navigate ethically complex situations can be developed. Culture and systems play a crucial role in supporting physicians' moral resilience, in terms of building an environment of ethical practice. In this regard, CoP seems to be one ideal strategy for the flourishing of resilience among the healthcare team. Fostering CoPs as a process that encourages healthcare professionals to address ethical dilemmas together has the potential to build culture and system change, which reciprocally enhances personal resilience.

One concern that can arise regarding the CoP as a strategy to collectively build resilience is if this process can imply some risks. We believe that CoP can facilitate the increase of resilience among the participants through the relationships, dialogue, trust and continuity (Delgado et al. 2020). However, who should facilitate or moderate these discussions? Since the emotional management of these groups is complex and can be iatrogenic if they are not carried out by people trained, CoP pursuing the goal of increasing moral resilience should be facilitated by experts with experience facilitating groups, addressing emotional needs, understanding ethical complexities and building personal resilience. The question now is who should be these experts? The selection and composition of these experts influence effectiveness of the CoP to achieve its goal of moral resilience.

Practical approach: analyzing examples in the context of the COVID-19 pandemic

During the COVID-19 pandemic, the healthcare providers have shown great strength and resilience. Liu et al. (2020) found that healthcare nurses and doctors working in Hubei, China, used multiple support systems and self-management skills to relieve stress. However, the sense of helplessness over the suffering of the patients and the sudden loss of life were identified as painful. Some professionals believed that they could cope with their emotional stress without professional support. However, professional psychological counseling and support systems and crisis interventions should be made available to those seeking formal assistance. "With logistical support from their hospital and peer support and encouragement among colleagues, they had a sense of safety and felt they were not alone" (Liu et al. 2020, e795).

Across the world, healthcare providers were called to step up to the surge of patients requiring hospitalization. Many were redeployed from community settings, often without direct experience caring for patients with infectious or respiratory disease. The sense of responsibility to provide care in a competent manner led to the provision of multiple educational resources. Many medical organizations, educational institutions, and healthcare organizations provided educational webinars to learn more about COVID-19 with a focus on clinical aspects of managing patients with COVID-19. These webinars provided an opportunity for healthcare professionals to share experiences within their respective clinical settings. Many international physicians provided opportunities to share their experiences with others around the world via teleconference to support others' efforts to contain the pandemic[1]. The emergence of online forums to share experiences related to COVID-19 was observed, as the Doctors of British Columbia initiative (https://www.doctorsofbc.ca/news/new-online-forum-physicians-collaborate-covid-19). An international online forum for critical care physicians provided an opportunity to share experiences surrounding healthcare personnel management, isolation and quarantine procedures, respiratory therapy, antivirals, and indications for Intensive Care Unit admission and discharge (Bo et al. 2020). A report of G-MED's Global Physician Online Community showed physicians from over twenty countries contributing information about four themes: epidemiology, guidelines, preparedness, and treatment approaches (IpsosMORI 2020). The use of social media, including Facebook and Twitter to gain information from other healthcare professionals has been noted in a New York Times article[2]. Although not formally called CoPs, we witnessed healthcare professionals engage with colleagues around the world to share information and learnings together with the common goal of curbing the pandemic. In this regard, Tan and Roach, who met via Twitter, co-wrote a piece on Allyship (Tan & Roach 2020) as global anti-racism protests enhanced awareness of the greater likelihood of Black Americans, indigenous people and people of color, experiencing not only discrimination and police brutality, but also developing COVID-19 itself and its adverse outcomes, including death (Thakur et al, 2020; Elbaum 2020).

More specifically, CoPs that address healthcare professionals' well-being, with the intention to prevent burnout and increase ability to cope with moral distress have been developed in response to the COVID-19 pandemic. Experts or groups of experts as in the examples below, of the Virtual Moral Resilience Rounds and the COVID Ethics series are helpful to providing examples of learning from experience and conveying diverse ways of thinking about moral challenges. In groups, expert facilitators may support validation of and exploration of challenges or encourage others to provide perspectives. These examples of CoPs addressing moral distress and building resilience are described here:

Virtual Moral Resilience Rounds have emerged at Johns Hopkins Hospital (US) to proactively have discussions about the COVID-19 pandemic[3]. These weekly one-hour sessions invite multidisciplinary clinicians to attend to discuss ethical challenges, with the goal of acknowledging distress and finding solutions. These sessions are held on Zoom and facilitated by C. H. Rushton, a physician, and a philosopher, with broad expertise on moral distress and moral resilience.

[1] More information is available in these websites: a)https://news.cgtn.com/news/2020-03-07/China-Italy-doctors-share-experiences-of-COVID-19-control-online-OFi0gryDVS/index.html; b) http://en.people.cn/n3/2020/0324/c90000-9671699.html

[2]Information available in: https://www.nytimes.com/2020/03/18/well/live/coronavirus-doctors-facebook-twitter-social-media-covid.html

[3]Information available in: https://www.advisory.com/research/physician-executive-council/prescription-for-change/2020/05/moral-resilience-rounds.

In Calgary, Canada, a team of psychiatrists, mental health clinicians, and family physicians established a partnership to provide an opportunity for connection and support amongst healthcare professionals during the COVID-19 pandemic. Online spaces offer physicians, and separately, continuing care facility staff, the chance to meet and share experiences with peers. The online space for physicians is called "Virtual Doctors' Lounge" and acknowledges the challenges of providing care during the pandemic including ethically complex situations. It has been piloted to a group of family physicians providing care in hospital settings. As continuing care staff have been greatly affected in Canada with high numbers of COVID-19 cases and deaths, an online group provides the opportunity for staff to receive support. These online group sessions promote sharing of experiences, acknowledging emotional distress, normalizing experiences, and providing support to others. They are facilitated by psychiatrists and mental health clinicians with expertise in group therapy.

In Alberta, Canada, different experiences can be identified as emerging or strengthening frequency of CoP meetings in the midst of the Covid-19 pandemic to navigate healthcare professionals problems that arose.

1) Alberta Health Services (AHS) Zoom Room: This biweekly one hour session addressed numerous topics relevant to the pandemic and physicians, including but not limited to PPE availability, domestic violence during the pandemic, Zoom use. The series began with an expert-led presentation on one of the topics followed by participants' sharing of their own experiences and perspectives. Outcomes of the sessions were shared with AHS leaders and often led to Tip sheets that others could access.

2) Psychosocial oncology spontaneously developed a clinical discussion group - addressed all matters related to clinical practice including technology.

3) Psychodynamic psychotherapy clinicians who previously met bi-monthly, met weekly to share wisdom regarding practical and clinical implications of COVID-19 in psychotherapy practices. This included change to virtual practice, clinical presentations and therapist challenges related to COVID-19 including Zoom fatigue and methods to manage it. Texts, email information and papers are shared between meetings.

Dr. Mamta Gautum, a Canadian psychiatrist with expertise in physician health held daily online support groups for physicians across Canada during the pandemic. Many physicians entered the pandemic burnt out, and thus were at risk of medical errors. Four distinct stages were evident through the course of the first surge of the pandemic: readiness, response, reassurance and recovery. Challenges discussed varied from frontline issues including PPE limitations and, d for those not at the front-line, there was adjustment to clinical practice and delivering virtual care while working from home, balancing childcare or resilience, partner job loss. These sessions provided a space for physicians to share their experiences and receive expert advice on strategies to address personal challenges and build resilience.

A COVID Ethics Series at Seton Hall University and Hackensack Meridian Medical School arose in recognition of the value of many and diverse people discussing challenging ethical issues. The series was organized by Dr. Pilkington and includes a panel of experts from medicine, nursing and health sciences, as well as philosophers, ethicists, economists and lawyers. Topics included but were not limited to: Intention and Limitations of Aid, Vulnerability and Dependence during the time of COVID-19 and Discrimination intensified during Covid-19. The series aimed to enhance the capacity of students and healthcare practitioners to practically reason about morally challenging topics (Pilkington, 2020).

Conclusion

Healthcare professionals are exposed to complex challenges in daily work that increase vulnerability and moral distress, which are heightened in the situations of extreme stress, such as the current COVID-19 pandemic. We examined CoP as a process to build resilience, and provided examples of healthcare providers coming together to share information, experiences, wisdom and perspectives with a common goal. This process of sharing common experiences in a group setting can also be valuable to build resilience, not only for the individual professional, but also towards a culture of ethical practice. Using CoP, our intention is to recognize both the individual and the system's responsibility towards shaping the working environment in a way that promotes safe and effective care. CoP have demonstrated value during the pandemic and we theorize that CoP may be an effective strategy to increase moral resilience of healthcare professionals collectively, and exist beyond the duration of the pandemic. Finally, we emphasize the importance of promoting ethical reflection grounded on practice in order to respond to the everyday ethical challenges of healthcare professionals. "There are limits to thinking of professional ethics in terms of virtues- being caring, being compassionate, being respectful- if healthcare professionals see few ways to put these virtues into practice(...) Thinking about the complex systems as a "moral space" that must always be open to the discussion of questions of right and wrong action, of justice and injustice, may help us grapple with the continuing challenge of creating and sustaining health care systems that are safe, effective, compassionate and just" (Berlinger, 2016, p. 176).

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A Fashionable Mask: Branded Value Proposition During the Coronavirus Pandemic

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Abstract

The coronavirus pandemic has changed not only the social, economic, political spheres of nations, but also fashion industry, having to cancel fashion shows. Fashion brands started offering value proposition changed under new conditions to give the value to their customers with changing needs. A lot of fashion brands follow their pandemic sustainable business strategy to help medical staff with masks, gowns, and sanitizers for free, to donate to medical organizations, coronavirus research in Universities' laboratories that also increases their economic value added (EVA). A mask takes mostly two functions – utilitarian and emotional, i.e. new technological and fashionable part of the mask production/consumption. There are creative ideas in mask making by technological brands like a self-cleaning mask, collaborations of high-tech companies and fashion industry representatives, fashion brands creating fashionable masks by using double sustainable business strategy, popculture brands creating masks with their branded themes, fashion brands creating cloth masks made of the latest trends, artists creating unique masks using artisan techniques, a technological and fashionable mix like face-recognition masks, and also politicians setting up mask trends. The fashion brand analysis shows that the value proposition is based on value increase in EVA during the coronavirus pandemic, because they understand the necessity of sustainable business strategy to gain the longer-term customer loyalty.

Keywords: mask, fashion, brand, value proposition, coronavirus, sustainable business strategy, new technologies

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Introduction

The rise of coronavirus turned out unexpectedly to the biggest part of the world officially announced by China on December 31, 2019. However, later scientists and other research attempts have found its evidences in both China and Europe before its official announcement in autumn and even summer 2019. Medicine, economies and other spheres did not know how to react to the unknown aggressive virus and what measures are the best to be taken. The world needed to adapt to new realities by social distance, mask, lockdown, attempts to overcome economic crisis and so on.

Some countries provided financial support for businesses, e.g. the UK (Financial Support, 2020), Canada (COVID-19, 2020). Nevertheless, a lot of businesses, small in particular, had to be closed. However, some of them tried to overview their business model not to be forced into bankruptcy. They understood the necessity to provide value proposition to both meet changing needs of their customers and use the opportunity to attract new ones. Value proposition was to go online, to offer the things connected with protection against COVID-19 as well as to inspire mood of the customers. Fashion industry is the one that quite successfully offered its value proposition to customers.

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Literature Review

As COVID-19 is still a new phenomenon there is not much information about it in scientific literature except medical one, a few of political and economic topics (e.g. Balasubramanian, 2020; Gans, 2020; Maller, 2020; Qu et al., 2020; Saxena, 2020). There can hardly be found scientific works dedicated to the coronavirus and fashion. However, there is much information about it on media, mostly online magazines, newspapers, fashion brand websites and social media.

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E.g. Ivan Allegranti (Allegranti, 2020) articulates fashion show holding in main fashion capitals in February 2020 by wearing masks while e.g. the government of Italy was more conscious to adopt the Decree Law "Decreto Cura Italia" (March 17, 2020) according to which measures were taken to overcome economic crisis in the state because of the coronavirus. Allegranti researched the Italian coronavirus case, when the first Italian fashion brand Armani cancelled its Fall-Winter 2020-2021 runway show for the audience for safety reasons turning the show into closed-door and using new technologies to stream it on both its website and social networks (Maitland, 2020). Later other Italian fashion brands followed the Armani's strategy (Allegranti, 2020). They are Moncler (Moncler Genius 2020, February 23, 2020 Milan (https://www.facebook.com/events/viale-molise-70-20137-milano-mi-italia/moncler-genius-2020-cancellation-public-opening/907891382962332)), Laura Biagiotti (https://www.shutterstock.com/ru/editorial/image-editorial/laura-biagiotti-show-runway-fall-winter-2020-milan-fashion-week-italy-23-feb-2020-10565130g), etc. Other non-Italian brands have also joined the former to cancel their shows in Italy like Michael Kors' 007 capsule collection (Petrarca, 2020). Italian fashion brands like Max Mara, Prada, Versace, Gucci canceled their May cruise shows outside Italy beforehand in April 2020 (Turk, 2020).

Sarah Jones states that fashion companies during the coronavirus pandemic are to use the opportunity for sustainability balanced within their corporate social responsibility as "eco-friendly innovation could actually build more resilient business over the long term" (Jones, 2020).

Jasmin Malik Chua (Chua, 2020) analyzes the circular-economy commitment signed by a lot of companies in the fashion industry elaborated by the Ellen McArthur Foundation (https://www.ellenmacarthurfoundation.org/assets/downloads/emfjoint-statement.pdf) expressing the idea of the necessity of sustainable business strategies for businesses especially in the conditions of the coronavirus pandemic: "policymakers, CEOs, and other influential individuals are highlighting the circular economy as a solution to build back better in response to the economic impact of the coronavirus pandemic" (https://www.ellenmacarthurfoundation.org/news/more-than-50-global-leaders-pledge-to-build-back-better-with-the-circular-economy).

Methodology

The methodological basis for the paper research is the VARS model elaborated by Deepak Somaya (the "Business Strategy" course. University of Illinois at Urbana-Champaign, https://www.coursera.org/learn/strategy-business).

The four interconnected elements of the VARS business model are:

1. value proposition;
2. activities-resources-capabilities;
3. realization/revenue model;
4. scope of enterprise.

There are a lot of definitions of value proposition. E.g., "the value proposition or offering of the company depicts which value it intends to deliver to its customers" (Nielsen and Lund: 54).

The value in value proposition, which meets the current needs of the customers, leads to company ROI, hence success, measured by an analytical tool called economic value added (EVA). The tool was developed by Bennett Stewart III and Joel Stern in 1982 (Grant, 2003: 1-2) and is still widely used.

The main idea of value proposition is to offer such a value to customers, which develops a new business model so that EVA will show the profitability of the brand. There are three types of EVA in a new business model (Figure 1):

- value is increased;
- costs are decreased;
- value is increased, while costs are decreased.

The last type is the best for the profitability of the company. Modern new technological companies use this type of EVA in their value proposition. They are Netflix, Google, etc.

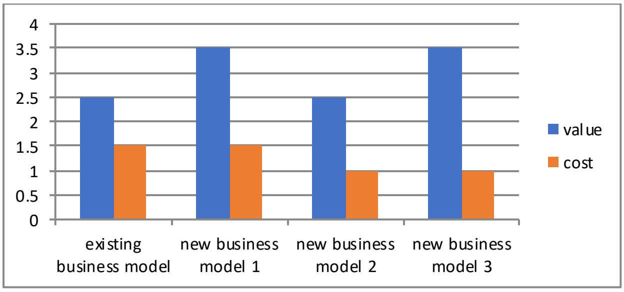

Figure 1 – Economic Value Added

In the paper there have been analyzed fashion brands to find out which type of EVA has mostly been used among them during the coronavirus pandemic.

In this paper the value proposition of a fashion brand during the coronavirus pandemic means the value the fashion brand delivers to its customers by sustainable business strategy within the COVID-19 conditions by means of supplying 1) medical staff with free medical masks, gowns and 2) customers with fashionable masks to meet their basic emotions: fear and happiness.

Fashion Brands and Sustainable Business Strategy During the Coronavirus Pandemic

Fashion brands understand the necessity to participate in social campaigns during the coronavirus pandemic to strengthen their image. They have two ways to do that: 1) to help medical staff with free masks, medical gowns, etc. and 2) to donate to buy necessary equipment, medicines, etc.

Fashion Brands and Their Free Products

Sophie Warburton stated that "fashion and philanthropy aren't two words commonly associated with one another, although many of the industry's leaders have quietly donated generously in the past. If the film The Devil Wears Prada is anything to go by, the public perception is that the fashion industry is superficial, materialistic, and self obsessed. But in the wake of Covid-19 the fashion world has shown signs of change." (Warburton, 2020). The same idea is expressed by Ellen Millard: "The luxury industry often gets a hard time for being frivolous, but its efficient and philanthropic response to the coronavirus pandemic has provided vital aid on the front line." (Millard, 2020).

Fashion brands of different countries have tried to help medical staff first of all by supplying them with hand sanitizers, masks and gowns for free. Such fashion brands are: 1) the French luxury conglomerate LVMH producing hand sanitizers on its perfume and makeup production lines commonly producing cosmetic and perfume goods for Givenchy, Christian Dior, and Guerlain to give those hydroalcoholic gels of 12 tons of its initial batch to French health authorities for free (Allaire, 2020; O'Kane, 2020) because of its shortage during the coronavirus pandemic (Allaire, 2020; George-Parkin, 2020); 2) Bulgari, the Italian jeweler brand, collaborated with ICR, "its perfume partner" to produce "75 ml recyclable bottles" of hand sanitizers of "several hundreds of thousands" to "be distributed to medical facilities through ... Italian Civil Protection Department" (Millard, 2020); 3) Ralph Lauren has produced 25.000 face masks along with 25.000 gowns (Millard, 2020); 4) Giorgio Armani switched its each factory in Italy to produce disposable medical overalls for medical staff (Millard, 2020); 5) Prada has produced 110.000 masks and 80.000 gowns for medical staff, "using a breathable, non-woven propylene fabric" as the assistance to reply to the Tuscany region's calling for help in shortage of masks and gowns for medical staff (Millard, 2020; Samaha, 2020); 6) Gap Inc. along with its partners producing gowns and masks for the front line medical staff (Yates, 2020); 7) Inditex, the Zara owner, has offered masks for both patients and medical staff, which it thinks is the priority in Spain (Yates, 2020) and also medical fabric for gowns (Bobb, 2020); 8) Gucci has made 55.000 medical gowns and over 1 million masks (Samaha, 2020); 9) H&M has produced "personal protective equipment" to be distributed among medical staff round the world (Samaha, 2020); 10) Eugenia Kim, a luxury hat and accessories designer from New York City, on perceiving the needs of the front line medical staff, after call for help by New York City governor Andrew M. Cuomo and also her Chinese hat factories having produced masks before, understood her facilities were enough to produce masks for medical staff up to 50.000 a day (George-Parkin, 2020); 11) other small US fashion brands like Collina Strada, Christian Siriano have produced masks for medical staff in New York (Bramley, 2020).

Fashion Brands and Donations

Some fashion brands sell fashionable masks to spend some part of the profit on donation against the coronavirus (Tobin, 2020). Some others donate money, e.g. 1) Giorgio Armani has donated 2 million euros to Italian hospitals and institutions (Millard, 2020); 2) "Versace made a contribution of about 144 thousand dollars to the Chinese Red Cross Foundation" (Turk, 2020); 3) Dolce and Gabbana had previously partnered with the Italian medical school, Humanitas University, and will continue working with them in the hopes of "finding a cure." (Petrarca, 2020); 4) LVMH donated 1.9 million pounds to the Chinese branch of the Red Cross Society in January, and also it gave 40 million surgical and face masks having been ordered in China to medical staff in France (Millard, 2020; Bramley, 2020); 5) Bulgari donated to the Spallanzani hospital (Italy) (Millard, 2020) to purchase a new microscope to research the coronavirus (Samaha, 2020), 6) Moncler donated 10 million euros for the hospital construction in Milan to treat those who suffer from COVID-19 (Bramley, 2020; Samaha, 2020); 7) Gucci donated in different crowdfunding campaigns against COVID-19 and organized #GucciCommunity by "asking fans to make a donation to the WHO's initiative, along with giving the agency complete access to its social media channels in the hopes of amplifying official public service information" (Samaha, 2020); 8) Ralph Lauren donated not only to different foundations that help struggle against the coronavirus, but also "gifted an undisclosed amount to the CFDA/Vogue Fashion Fund to help fashion designers hit by the economic downturn. 'We believe that no matter who you are or where you are from, we are all connected. … That is why we are taking significant action to help our teams and communities through this crisis.'" (Samaha, 2020); 9) Burberry has funded the University of Oxford coronavirus vaccine research and also donated The Felix Project and FareShare to feed those in need with meals (Samaha, 2020); 10) Bottega Veneta has provided "two-year scholarships across the cities of Veneto, Lazio, and Campania" in Italy to research the coronavirus (Samaha, 2020); 11) Dolce&Gabbana has collaborated with Sofia Vergara, the Colombian American actress, in the "Amore for Scientific Research" campaign hashtagged as #DGFATTOACASA (Made at Home) with the brand's artisans' digital workshops to unite and welcome creativity while experiencing the lockdown (Rougeau, 2020) that "celebrates life and the beauty of Italy" (Samaha, 2020) adding "The whole world and all of our lives will never be the same again. We truly hope that all of this will lead to a rebirth and want to do our part so that all of us, especially future generations, can one day benefit from these fundamental scientific discoveries" (Bonagofsky, 2020) with the Devotion bag online sales some portion of which is to be donated to the COVID-19 research conducted by Humanitas University (Samaha, 2020; Bonagofsky, 2020), and also the brand has collaborated with INTESA San Paolo Bank "to facilitate crowdfunding" and be guaranteed that no commission will be charged out of the donation to the University (Rougeau, 2020).

A Mask: New Technologies and/or Fashion

A lot of companies are attempting to elaborate sustainable business strategy during the coronavirus pandemic to offer value proposition as, from one hand, an innovative technologies introduction in mask creation and production to medical staff as the priority and its customers, from the other hand, a fashionable mask offer to customers as the priority.

There are a lot of creative technological ideas with face masks. E.g. 1) a self-cleaning mask being disinfected under the heat of its carbon fibre inner layer by charging via its USB port connected to a standard cellphone charger for about half an hour innovated at Technion University, Haifa, Israel (Amchay, Harash et al., 2020); 2) a transparent pool mask to protect a swimmer from the coronavirus in a pool innovated by a Japanese sports company Konami Sports for the opening of pools in Japan on June 1, 2020 (Joy, 2020); 3) a ClearMask "without hiding your face" preventing from miscommunication was initially developed for medical staff and later for common customers who have some disabilities (https://www.theclearmask.com/product).

Politicians introduce fashion of masks to the public. The first one is the president of Slovakia Zuzana Čaputová who "swore in the new government wearing a matching outfit, complete with a color-coordinated face mask" making "fashion history"

(Rabimov, 2020); Japanese Chief Cabinet Secretary Yoshihide Suga who promotes the Ainu issues had a face mask with the Ainu print during a press conference, made by "an embroidery circle in Noboribetsu, Hokkaido", which got 500 orders at once and was not in time to fulfill all of them as it can make only 20 masks per day (Kyodo News, 2020).

Fashion brands create fashionable cloth face masks that "concurrent with a wave of designers who have chosen to repurpose their archival fabrics for new creations, Silvia Tcherassi's team brings back a familiar gingham print that criss-crossed her spring/summer '20 collection", combined with charity from the mask sales to Every Mother Counts (Cohen, 2020); Polka dot masks by GetUsPPE, being of an on-trend print (Cohen, 2020); Wisteria face Mask by the Vampire's Wife being of an on-trend color and print (Cohen, 2020).

Artists create fashionable and/or unique masks. E.g. Ýrúrarí, a Reykjavik knitting designer who adds knit 3D face elements to second-hand sweaters, applied her creative ideas to knit face mask, but she does not sell them, the opposite she has offered the patterns (https://www.yrurari.com/shop/digital-sleik-zine) for those who can knit to do it by themselves and calm down during the pandemic, moreover Ýrúrarí adds that this masks seem freaky thus it may cause social distance from others (Kraus, 2020).

Popculture brands create masks with their branded themes. E.g. Disney, which donates its proceeds to Med Share and also it has donated "1 million cloth face masks for children and families in underserved and vulnerable communities across the U.S., which will be distributed by Med Share. And due to overwhelming demand, the company has already reached its goal of raising $1 million in profits." (Johns, 2020).

Fashion brands create fashionable masks by using double sustainable business strategy. E.g. Brocade Mask by VPL, a sustainable brand, that cuts masks from vintage Japanese obi belts, traditionally worn over kimonos on formal occasions like wedding and also donating proceeds to the Fashion Girls For Humanity initiative and "For every four masks purchased, one isolation medical gown will be donated to" medical staff (Cohen, 2020).

There are collaborations of high-tech companies and fashion industry representatives to create masks. E.g. an AI company Anywear and a model Halima Aden created hijab-wearing masks for Arab medical female staff (Davis, 2020; Okwodu, 2020).

There are masks that meet both needs – technological and fashionable. E.g. Danielle Baskin, a San Francisco designer, established a Maskalike company that prints a customer's face or any meme on the protective face masks (https://maskalike.com), the idea based on her meeting the need of unlocking a smartphone with a facial recognition technology feature that became a problem because of a face mask on, based on a 3D-technology calling herself a maker of a "trendy dystopian product" and adding "The combination of the coronavirus, facial recognition on our phones, fear of surveillance, and late-stage capitalism really struck a chord with the internet. … The most surprising thing to me is how this spread globally so quickly. People in South Korea, Latvia, France, and China reached out to me within a few days. … Whether it's a wildfire, a virus, or a protest, I realise humans all over the world right now can relate to the symbol of the mask." (Facial-recognition Mask, 2020); Custom Face Mask as an opportunity to have the print as the customer wants by uploading the file with the preferable print on the Customishop website and also describing the idea in the box for the company's graphic designer to contact the customer and discuss the details of the mask (https://customishop.com/product/custom-face-mask/); Donald Trump Loop-Cut Face Mask in the process of giving a speech (https://customishop.com/product/donald-trump-face-mask-loop-cut-respirator-mask-filters-pm2-5) (Kyodo News, 2020).

Conclusions

To sum up, it is seen from the analysis of various fashion brands above; the value proposition they offer to customers is based on value increase in EVA during the coronavirus pandemic. It happens because they understand the necessity of sustainable business strategy to gain the customer loyalty for a longer term. The problem is to be studied further from more perspectives to understand what other value propositions can be elaborated in a business model for fashion brands to be successful under the pandemic.

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Commented [RB5]: All the references must be given according to the APA (American Psychological Association) Referencing System.

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The Nexus of Art and Science: Creating Art Medals in the Chemistry Lab, Using Graphite Molds

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Abstract

The idea of graphing what is called the lead-tin eutectic point when alloys are produced from elemental lead and tin metals may be very common and established in chemistry and materials science laboratories. Producing art medals from the alloys that are made in such experiments is decidedly not. We will present how the lead-tin eutectic experiment can be performed in the chemistry lab, and how graphite blocks can be used as both mold and heat sink for the creation of art medals, utilizing metal alloys produced by students in the laboratory. The process brings together art, science and engineering in a single experience. We conclude that this has been found to be beneficial for students, yet is a technique that can be utilized by virtually any person who wishes to experiment and create with low-melting metal alloys.

Keywords: Alloy, Low-melting alloys, Eutectic, Art medal, Heat transfer

Introduction

The modern general chemistry laboratory usually offers a variety of phenomena to students, often those which have been studied extensively, and for which the results are well known. This tends to make it easy for students to perform an experiment, and for faculty members to grade it. Over the course of decades however, as these "canned" labs have evolved, it appears that experiments in which reduced metals are used and studied have faded from the curriculum. While there appears to be a number of low melting alloys that can be utilized as is or as purchased, or adjusted in composition, all of which are safe to work with, it appears nevertheless that laboratory experiments which use metals have been deleted from many chemistry curricula.

What is known as the lead-tin eutectic lab is a series of experiments in which a known amount of one metal, lead or tin, is melted, and some known mass of the other is added to the molten sample. The resulting alloy is quenched in cold water, dried, re-melted, and the temperature taken at the initial point of melting. A further portion of the second metal is added to the first alloy, and the quench and re-melt are repeated. In this manner, a student can gather enough data points that what is called the eutectic curve can be graphed with good accuracy. The melting points of elemental tin and elemental lead can be used as the terminal points on such a graph, with the x-axis being the weight percent of one metal from 0 – 100% (which is 100 – 0% for the other metal), and the y-axis will be temperature. This experiment is often very instructive for students, because they approach it with the preconceived idea that the graph will be a straight line between the melting point of lead (328°C) and the melting point of tin (232°C), which emphatically it is not. When metals of good quality and high purity are used, the eutectic point, which is approximately 55% tin, is 176°C. When lower purity metals are used, such as lead or tin purchased from scrap yards, the eutectic point can be as low as 86°C, as seen in the past by the main author.

One of the end results of performing this experiment in a chemistry lab is leftover metal of no specific alloy. The idea of using such products of any experiment that produces this, or any low-melting metal alloys, as the start point for the production of art medals appears to be without literature precedent. At least, after a thorough search, we have found no references to this with the exception of our own, recent work.[1-5].

The technique of casting medals

Graphite has been used for generations to aid glassblowers in removing heat from semi-molten pieces of glass while they are being blown or shaped. This is because the heat capacity of graphite is high compared to many other solids. Near where the authors live is the henry Ford Greenfield Village – a museum that showcases arts and sciences of the past. Glass is still blown in a traditional manner there; and glassblowers roll semi-molten glass on graphite plates to cool the glass. The graphite absorbs the heat very well.[6]

In a general chemistry lab, the phenomenon we are trying to examine is one of a chemical change, or perhaps one involving heat transfer. The use of a graphite mold to cool molten metal is definitely a matter of heat transfer. Engineering students find this to be of particular interest, because tin-lead objects cast in a mold made entirely of graphite can be removed from a 3" cube of graphite in less than a minute and be cool to the touch.

Any number of objects can be made using a graphite mold, for example, parts for some larger, more complex machine. But such parts can be produced using other materials for the mold, and the heat transfer managed by some coolant applied to the mold. In the case of a graphite block, it can be carved directly, using any wood-carving or metal-carving tools, and used as both heat sink for the metal being poured, and mold for some design – in our case, for an art medal. We have pursued this in the freshmen-level chemistry lab, allowing students to first create some design, often simply on a page in their lab notebook, then allowing them the time to carve into the graphite block. This may take more than one lab period, but student results are often quite good, especially considering that this is the first time virtually every student making a medal has done so.

This experiment can be considered an amazingly simple one, at least in terms of its chemistry. It is the production of a series of mixtures, as alloys are generally mixtures of two or more metal elements.[7] Since there is no further use for the metal alloys when the initial lead-tin eutectic experiment is complete, this metal becomes an ideal material to use for the creation of medals.

Graphite has a specific heat of 0.720 J/g°C. This is almost 1.6 times greater than that of iron, and almost 1.9 times greater than that of copper. These two elemental metals are used as points of comparison in terms of specific heat, because molds into which metal objects are poured are often made of such metals. Thus, molten metal must cool in such molds. Using graphite as a material for the mold, and using lead-tin alloys as the metal, means that heat can be transferred to the mold, resulting in objects – medals – that can be removed from the molds in minutes, as opposed to hours or days.

The idea of using graphite as some form of heat sink certainly has an established history to it. As mentioned, glass blowers at the Henry Ford Greenfield Village in Dearborn, Michigan, USA, still use graphite plates to cool masses of semi-molten glass when they are shaping and creating objects. This is a part of the Greenfield Village experience that is seen by numerous visitors to the museum.[6] But there exists more than simply a niche use of graphite in a historical context. Graphite continues to be used in a variety of industrial applications. In this study, graphite blocks were purchased locally from a business, Graphite Products Corporation, which supplies to customers throughout the Midwest of the United States.[8]

The term "low melting alloy" is a somewhat flexible one, but from the point of view of the artist who sculpts and works with metals in creating objects, the term may be conveniently described as a metal that melts at a low enough temperature that it can be worked without having to employ the services of a foundry.[9] When working in brass or bronze, two traditional metal alloys for making art objects, some model of the object must be sent to a foundry, made into a mold using a variety of materials such as clays or resins, then poured. This can be time consuming, and may result in a finished product with which the artist finds some fault. When working with a graphite mold, which is very easy to carve, and an alloy that can be melted with a hot plate or a Bunsen burner flame, the finished object can be examined quickly, and corrections can also be made quickly if the artist so chooses. This process of pouring a medal, examining the finished product, and making changes as needed, can be repeated numerous times if the artist so chooses.

Thus, we believe we have developed a technique that is easy and direct for artists and art students to employ, that is an excellent experiment in the chemistry of solids, and that shows directly how heat is transferred, which is of interest to engineers and engineering students.

Experimental steps

The experimental procedure for the lead-tin eutectic lab, and creating alloys of the two metals, can be listed as follows:

Needed:

Lead metal

Tin metal

Thermometer or thermocouple

Crucible

Heat source – hot plate or Bunsen burner

Beaker of cold water

It is easiest to start by melting a pre-weighed piece or pieces of lead metal. The temperature does not need to be recorded in the class, because it is well-known and can be found on-line.

After the lead is molten, add a sample of tin metal of known mass.

Allow the alloy to melt.

Pour the molten alloy into cold water to solidify it.

Dry the pieces of alloy that have formed in the cold water.

Place the cooled alloy in the crucible, along with the thermometer bulb or thermocouple tip, ensuring the thermometer end does not touch the bottom of the crucible, and that it is in good contact with the solid pieces of alloy.

Heat the alloy until it is again molten. During this step, watch carefully and take the temperature reading at the first sign of any molten metal. Unlike water or other covalently bonded matter, there is no plateau of melting for these alloys. Thus, the temperature at which the first drop of metal appears will be lower than that at which the last bit of solid disappears. It is advantageous to have students work in pairs, so that one can spot the first drop of molten metal and the other can take the temperature.

After the alloy is entirely melted, add a further known mass of tin.

Repeat steps 5 – 9 as many times as desired.

Following these steps it is not difficult to obtain five data points for different alloys. When adding the known values for the melting points of lead and tin, a graph can be made with the two known values serving as the 0% point and 100% point for each metal, as the farthest points of the graph.

Importantly, adding amounts of tin to lead in a successive manner produces graphs with a wide spread of percentages. We have found that if students pre-weigh five amounts of lead and five amounts of tin, the five data points obtained tend to be clustered towards the center of any graph that is produced. It seems to be common to use roughly the same size samples if indeed five sets of lead metal and tin metal are set aside before any alloys are made.

As well, experience has taught us that it is best to run this experiment in pairs, since it can be difficult to spot the moment at which an alloy first melts and at the same time correctly read a thermometer or thermocouple. The temperature rises quickly, especially with smaller samples (10 – 20g), and students can miss the temperature at the point of first melting. It should be remembered that this is the first such experiment for many students, and thus they do not have extensive experience taking temperature readings while a material changes phase. Failure to take these readings properly tends to result in graphs that do not show the eutectic dip.

Carving graphite blocks

This aspect of the experiment does not have to be performed in the lab at the same time as the production of the lead-tin alloys. It can be performed over the remainder of a semester, since the metals that result from the eutectic experiment can easily be kept in the lab for later use. Our experience has taught us that many students prefer to work on their design over the course of several weeks, then pour a medal towards the end of the semester. Carving tools are provided in the class, but some students request to take their block back to their home because they or a relative has an extensive tool bench, which may include better carving tools than those available in the lab.

Because students generally have never carved in graphite before, we encourage them to wear older clothing, since graphite particles tend to blacken clothes and skin when it is being worked with. It washes off easily, but does stick to clothes until they can be washed. In the past, some students have chosen to wet their graphite block while carving it, to minimize the amount of graphite dust in their work area. This does work, but doing so means that metal should not be poured into the mold until it is completely dry. Pouring while it is wet tends to cause some bubbling on the surface of the finished medal.

Conclusions and future directions

Lead-tin alloys have provided excellent results when working with low-melting alloys, and can be performed even in the first year or freshmen-level chemistry laboratory. We encourage students to work in pairs when taking readings of melting points to maximize the accuracy of their data, and thus gather data that will well illustrate the lead-tin eutectic graph.

We have found that the resultant metal is an excellent source of material from which to create art medals. They are easy to produce, easy to work with, and can be poured in graphite molds even by students who have had no prior experience.

We intend to continue our exploration of low-melting alloys using others, such as Wood's metal fusible alloy, or Onion's alloy,[10,11] which we hope will provide results that are equally intriguing and educational. We also believe that the composition of such low-melting alloys can be adjusted for results that are more in line with what the artist and art student desire, specifically, adding more tin to such alloys.[12]

We have found that this experiment draws the interest of students, serves as an excellent example of solid-state chemistry that can be performed as early as the freshman year of college or university, and has strong connections between the sciences and engineering, and the arts. We encourage others to adopt this in their own teaching laboratories.

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The Impact of Covid-19 Pandemic on Africa's Healthcare System and Psychosocial Life

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Abstract

The ravaging Corona virus caused many countries of the world to impose partial or total lockdown, African nations inclusive. Most African countries are low-income countries, with most of the population being non-office workers. With the continued spread and rise of COVID-19 in many African nations, the people had no choice but to keep working for their daily survival. Moreover, the current health system in most African countries is weak and unable to tackle the emerging COVID-19 pandemic. The extra burden of the COVID-19 pandemic exposed these gaps and weaknesses. In addition to the effect of the pandemic on Africa's healthcare system, there is an equal and parallel debilitating effect of the virus on the psychosocial lives of Africans. Regardless of the several challenges that African nations face; Is there any way forward? African leaders may be able to unite and reduce their dependency on the international community for aids during health crises. They may also collectively take proactive decisions on strengthening their health systems as they work on educating their people.

Keywords: Corona virus, COVID-19, Africa, Healthcare, Psychosocial

Introduction

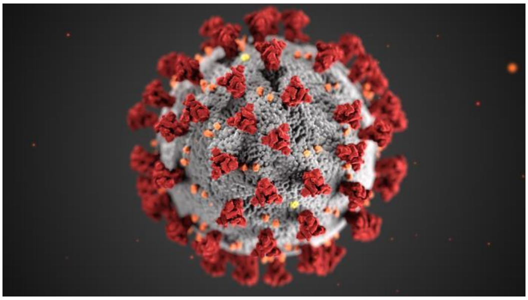
The Ebola outbreak of 2014 exposed how bad the state of health systems in Africa was. The World Health Organization (WHO) declared it a public health emergency of international concern on August 8, 2014. Barely two months later, on November 14 of the same year, more than 14,400 people had contracted the disease in eight countries, including Liberia, Sierra Leone, Guinea, and Nigeria, with a case fatality rate (CFR) of 35.92%. [1, 2]

In Nigeria, the index case of the Ebola crisis was a traveler from Liberia who collapsed at the Lagos airport; this made it easy for him and several contacts to be traced and the efficient curb. When Nigeria was announced devoid of the virus, 20 people had contracted it with up to 8 deaths, a CFR of 40%. Five of these were health workers [3]. With 2814 deaths of 6878 cases in Liberia (40.88% CFR), 1166 deaths of 1919 cases in Guinea (60.76% CFR) and 1187 deaths of 5586 cases in Sierra Leone (21.45% CFR), the feedback from the international community on the Ebola epidemic outbreak response in West Africa was that it was unsurprisingly slow, disorganized and weak [1, 4].

At the end of the Ebola crisis of 2014/2015, it was expected that Africa might concentrate more resources to the health sector and improve the overall health system. However, this was not the case. The health sectors in several African countries are still severely lacking with outdated technology and inadequate facilities, a sparse number of doctors available to treat an overwhelming amount of patients, low healthcare worker wages without insurance, and reoccurring strike action by healthcare workers in several parts of the continent. Wealthy citizens and politicians of African countries have resulted in traveling to European or American countries to get the best healthcare, neglecting to invest or contribute to the home continent's healthcare systems. Considering the current state of the health systems across Africa, the debilitating effect of the Corona virus and inability to contain it is unsurprising.

In April 2001, several leaders of the African Union had a meeting in Abuja, Nigeria. Following the meeting, African leaders promised to allocate at least 15% of each country's annual budget to the health sector [5]. Yet, according to United Nations Economic Commission for Africa (UNECA) 2019 report, Africa's global health expenditure is less than 2% while they have more than 36% of the worldwide health burden.

In a 2017 analysis by the World Bank, more developed countries spend more than 10% of the Gross Domestic Product (GDP) on health. The United States spent 17.6% on health, Germany spent 11.25%, France spent 11.31%, and Switzerland spent 12.35%. African countries, however, spent an average of 5% of their GDP on health. Nigeria in West Africa spent 3.76%, South Africa spent 8.11%, Kenya spent 4.80%, and the Central Africa Republic spent 5.82% (6). African countries are some of the most populated nations in the world. Nigeria is the most populated country in Africa, with an estimated population of 195,874,740 people in 2016, followed by Ethiopia, with 109,224,559 people and Egypt with 98,423,595 people [7].



Picture 1: structural morphology exhibited by corona viruses

Effect of the Corona Virus Pandemic on Africa's Healthcare System

South Africa is one of the African countries diverting most of their GDP to healthcare, yet they have about 0.776 doctors to 1,000 people. Mozambique has about 0.07 doctors per 1000 people. In sub-Sahara Africa, there is an average of 0.19 doctors to 1,000 people, and Africa, as a whole, has approximately one doctor to every 5,000 people [8]. Most African countries have less than one hospital bed per 1000 people. South Africa has 0.75 beds in intensive care unit (ICU) per million people, while Uganda has one bed in ICU per 1 million people. This is in contrast to 336 ICU beds per million people in the US [1; 9]. In 2013, sub-Saharan Africa had a deficit of 1.8 million doctors, and this is due to lack of funding, inadequate training, and education, international migration of health workers for several reasons and career change [10]. This was the known state of the continent before the COVID-19 virus outbreak.

In the most populated African country, Nigeria, strike action by health workers is a widespread phenomenon. It is not strange to hear about these industrial actions even during times of national health emergencies such as Ebola, Lassa Fever, or Cholera outbreaks. The most common cause of these actions in Nigeria is healthcare management and leadership issues. Demand for higher wages and infrastructural problems are also important reasons for strike actions in Nigeria and other African countries [11]. Another African country that is known for strike action is South Africa.

At a very pivotal period in the spread and maintenance of the COVID-19 virus in Nigeria, with more than 15,000 cases in June, the National Association of Resident Doctors of the country decided to embark on a strike action that lasted several days. Similarly, a significant challenge faced during the Ebola crisis of 2014 in Nigeria was the strike action during the period. Nigeria is better suited to remedial treatment rather than preventive treatments [12].

The transmission of the novel corona virus SARS-COV-2, which originated from China, started in Africa later than other countries for reasons speculated to be a warmer climate but most likely due to limited international air traffic in the continent [13]. There is, however, dense traffic, especially between China and African countries. WHO listed 13 top priority African countries including Algeria, Angola, Cote d'Ivoire, the Democratic Republic of the Congo, Ethiopia, Ghana, Kenya, Mauritius, Nigeria, South Africa, Tanzania, Uganda, Zambia. All of whom have direct traffic link with China and most of whom do not have sufficient or appropriate diagnostic capacity to handle the outbreak [14].

The first case in Africa was reported on February 14, 2020, in Cairo, Egypt. 13 days later, the first case in Nigeria was reported, an individual traveling from Italy. The most distinguishing factor of the Corona virus and pointer of its debilitating effect on Africa's health system is that Africa would have to rely on the preexisting health system to deal with the virus. The continent could not rely heavily on other continents for help as they had in previous African epidemics. This time, most countries of the world were preparing to tackle or already tackling the Corona virus with little success.

As of July 10, 2020, there are more than 550,000 cases of the Corona virus in Africa, with more than 270,000 active cases and 12,000 recoveries. South Africa has more than 250,000 cases followed by Egypt with 80,000 cases, Nigeria, with 31,000 cases, Ghana with 23,000 cases, and Algeria with 18,000 cases. On May 13, every African country had reported a case of the Corona virus [15, 16]. The risk of death from COVID-19 increases with age, with more deaths in people over 60 years old [17]. This puts Africa more at risk as the continent is experiencing the fastest growing rate of older people than any other region in the world. There are more than 43 million people over 65 years old in sub-Saharan Africa alone. The percentage of people over 65 in Africa had increased from 3.3% in 2000 to 3.6% in 2010. Also, as of 2015, there was a larger population of older men (52%) and older females (33%) that are an active part of the labor force compared to the 30% and 15% average in the world older male and female workforce respectively [18, 19]. The increasing number of cases of the virus in Africa may only serve to decimate an already overwhelmed and struggling health system in Africa.

Considering the current state of health systems in Africa, the continent can not afford to lose health workers to the pandemic. However, the most direct impact of the virus on the health system is seen on the health workers themselves. As of June 9, more than 2000 South African health workers have contracted the disease and more than 800 Nigerian healthcare workers, about 6% of the total cases. However, these are not the highest percentages of healthcare workers that have contracted

it on the continent. 19% of the total cases in Niger are healthcare workers, 12% of all cases in Sierra Leone and Liberia are healthcare workers, 10% of the cases in Namibia, and 9% of all cases in Zambia are all healthcare workers [20]. By June 29, 2020, the infected health workers in South Africa had increased to more than 3500, with 34 deaths. It is not known yet, how many more healthcare workers will be affected or die from the virus, but the number is expected to increase in the coming months as there is a shortage of protective gear among healthcare workers in the continent.

Another effect of the virus on the health system is the incapacitation of hospitals, making them unable to provide adequate care to individuals with other health issues. With most hospitals, government-owned and private, filled with Corona virus patients, hospitals are too full to admit or attend to other patients. Doctors are also careful not to clerk patients at the risk of contracting the virus. Most patients have, therefore, resorted to self-care and medication at home. This may worsen the state of health of people. Hospitals may be filled with patients suffering from severe and worsened health issues long after curbing the virus and its spread.

The Psychosocial Effect of the Corona Virus in Africa

In an attempt to curb the spread of the virus, several nations of the world quickly imposed total or partial lockdown in their cities and states. The lockdown has caused severe mental and psychosocial impacts on people in the world, such as mass hysteria, financial losses, depression, and anxiety. As the world locked out and advised people to work from home, if possible, most Africans faced anxiety as their businesses were locked up, and they descended into financial losses. How to take care of their families became a significant source of worry. Most people in Africa earn a living by practicing subsistence agriculture, selling in market stalls, or work as street vendors. People in low-income countries are six times more street vendors than people in high-income countries, and 17 times more agricultural laborers. In North America, 30% of people have jobs that allow them to work from home. This is in contrast to the meager 6% of Africans who can afford to work from home. Even so, most African cities do not have efficient social and information technology infrastructure, which allows remote working to be productive [21]. Again, there is the problem of insufficient electricity and slow internet to deal with.

Developed countries provided palliatives for their citizens to get through the lockdown period, but most Africans were thrown into starvation as the several governments could not afford palliative measures. The economic crisis arising from the COVID-19 related global lockdown could possibly increase the suicidal risk and attempts in affected individuals [22]. In addition, discrimination and social boycott of infected patients may worsen their mental health issues and suicidal risk. This is more evident in African countries where economic and palliative support was lacking.

COVID-19 lockdown affected virtually every aspect of the life of people in Africa. Schools were closed just as well as businesses, and therefore, students had to return to their homes and families. Social activities were also suspended; thus, bars and beer parlors, as well as soccer viewing centers, were closed. Family or street landlord or elders' meetings were suspended, religious activities were also suspended, and the closing of churches or mosques ensued. Wedding parties or gathering with more than 20 people were also banned in most cities. Thus, stay-at-home orders can create sudden changes to people's social life [23], especially for Nigerians who are known to have a rich sociocultural heritage of partying and hanging out (24). They are keen on maximally utilizing any social activity and networking opportunity. Thus, a negative psychological effect on the Nigerian population is expected following the lockdown, social distancing and mandatory use of face masks (24). With the COVID-19 pandemic in Nigeria, there is a significant prevalence in psychological distress: 15% for insomnia, 23% for depression and 25% for severe post-traumatic stress [24]. Olaseni, et al (2020) suggested that multiple misleading COVID-19 information from social media and the rise in confirmed cases in Nigeria could have aggravated the experience of post-traumatic stress symptoms among Nigerians [24]. Their study showed that there was no significant difference between male and female psychological distress experienced by Nigerians during the COVID-19 pandemic.

One of the basic defense mechanisms for survival adapted by animals is fear. However, when fear becomes long-standing and out of proportion it can lead to mental health issues [25]. The number of individuals affected mentally by COVID-19 is greater than those who are actually infected especially in most African countries where the infection rate is low. Another study reported a significant increase in fear and apprehension among both health workers and the entire population due to the high fatality of COVID-19, lack of preventive and protective equipment, massive misinformation/information being circulated, the negative effects caused by the lockdown and unfamiliar restrictions that infringe on personal freedom. They concluded that COVID-19 related isolation and stigma for COVID-19 are potential causes of mental health disorders like anxiety, obsessive compulsive disorder (OCD), depression, post-traumatic stress disorder (PTSD), adjustment disorders and sleep disorders [26].

Furthermore, there has been a significant rise of anxiety, stress and burn-out among healthcare professionals. A significant proportion of healthcare workers are either shielding or sick at home. Thus, the remaining workers on duty have to work under pressure with a relatively higher workload than usual. In addition, forefront healthcare workers are in constant fear of getting infected and are also psychologically distressed and traumatised by experiencing the death of infected patients [22]. There are also cases of increased malingering by front-line healthcare workers; about 25% of them hardly come to work. Most of them avoid work by giving frenzy reasons while some feign illness [26].

Nkporbu et. al, 2020 reported that common responses from people include worrying about the likelihood that their family members are infected, the fear of getting ill and dying, losing their loved ones, helplessness as they are unable to protect loved ones, stress relating to separation arising from being quarantined, boycotting health facilities due to fear of getting infected while in care and anxiety of not being able to work during isolation. In addition, uncertain prognoses, limited resources for testing and treatment facilities, and conflicting messages from authorities are also significant factors that contributed to increased COVID-19 related mental health issues (26).

Coupled with financial loss, families, and parents felt incompetent to care for their families. This is coupled with the burnout, panic, and anxiety that most people feel from fear of contracting the disease. The impact of the virus led to increased substance abuse and overall mental distress (27). The infection and measures that followed may cause new mental illnesses due to increased depression, anxiety, and substance abuse in people. It may also cause an increase in preexisting mental conditions [28].

Another impact of the virus on people is the increase of domestic and gender-based violence in Africa. Partners had to stay home during the lockdown, some of them with abusive partners. Children that moved back home from school, staying in their homes, also faced physical and mental abuse from parents or guardians. Though social isolation is effective in controlling infections, it can cause significant socioeconomic, emotional and psychological consequences which can in turn trigger family violence, especially in the background of increased use of negative coping mechanisms such as alcohol [29]. Furthermore, during isolation, there are limited opportunities to call for help by individuals living with family abuse. It should be noted that isolation helps the abused victims to keep physical and emotional signs of abuse less visible from the public. Thus, help and support from third parties are highly limited.

In the months of May and June, 2020, there was a major outrage on social media regarding the increased number of domestic abuse and rape cases in Nigeria. In March, Lagos state government of Nigeria reported 380 cases of sexual and domestic violence; this is a 60% increase in domestic violence, a 30% increase in sexual abuse, and a 10% increase in child abuse (30). South Africa, however, reported a significant drop by 69.4% from March-April 2019 and the lockdown in 2020. There have been speculations that this may be due to the ban on sales of alcohol. But this drop in sexual and domestic violence may be as a result of the total lockdown, in which victims are locked in with their abusers and too afraid to go out to report or make phone calls to relevant authorities.

With regards quarantined individuals, a study in Enugu, Nigeria proposed that in quarantined individuals, significant knowledge about COVID-19 was lower among those with psychological distress than among those without psychological distress. They suggested that it is essential to constantly educate people before, during and after quarantine [31].

On the other hand, though reduced social contact may initially result in loneliness and social isolation, recent studies suggest that a likely positive effect of COVID-19 pandemic is an increase in seeking social support or connectedness by individuals which may reduce loneliness in the long-term. Thus, long-term prospective studies are required to evaluate the course of psychological symptoms as the pandemic continues [23]. In Africa, stay-at-home orders provided an avenue for families to spend time together. Social support was mostly from nearby family members and friends, and most importantly palliatives from family members and kinsmen living abroad. However, remote social support through tele-services are lacking and even when available are inaccessible.

Picture 2: Ile-Ife town, Nigeria, during COVID-19 lockdown, May 2020.

The Way Forward

To prepare for future health emergencies, pandemics or epidemics, African governments have to review their health budgets, and GDP diverted to grow the health systems. African countries and governments should keep their 2001 promises and commitment during the Abuja declaration and meet the target 15% budget allocations to the health sector. With an increase in health budgets, appropriately allocated, and without corruption, the health facilities may be better geared. Doctors may also be encouraged to discontinue the culture of strike actions and serve their countries rather than look to migrate to countries where they can practice better and be appreciated more. There should also be more regular meetings between African leaders and health specialists on how to improve the health care systems of African countries as well as create a task force and execution team to uphold and implement the decisions made. The lessons learned from the current pandemic, as well as previous epidemics and health crises in Africa, should prepare Africa for the future and govern our decisions regarding health crisis management, even without the contributions or input of the international community.

It is also essential to set up post-COVID-19 mental health care centers to mitigate the debilitating psychological effects of the Corona virus and the strict measures taken on citizens. Several helplines such as suicide hotline, anxiety, and depression hotline should also be set up so that people get the support they need. Education of the masses should be planned so that people understand the psychological effects of COVID-19 and how best to handle the situation. Strict measures may also be put in place to discourage the spread of fake news on social media and television, as these media outlets need to be used appropriately to encourage and educate people rather than spread panic and fear. African nations should dedicate substantial portion of funds to cushion the hardship suffered by their citizens particularly the vulnerable ones. It is imperative for countries to review their restrictions as the pandemic evolves. It is also important to support healthcare workers, monitor their stress as well as cater for their welfare. Children and adolescents who have suffered the death of a loved one or friend and issues related to parental unemployment or loss of household income should be supported by state and federal governments. Again, it is equally advisable to monitor young people's mental health status over the long term, and to study how prolonged school closures, strict social distancing measures, and how COVID-19 pandemic itself affect their well-being. The provision of community-driven mental health support by trained mental health community nurses and volunteers will go a long way in educating people and mitigate the psychological burden of COVID-19.

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Total Carotenoid, Flavonoid and Phenolic Compounds Concentration in Willowleaf Cotoneaster (*Cotoneaster Salicifolius* Franch.) Fruits

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Abstract

Willofleaf cotoneaster (*Cotoneaster salicifolius* Franch.) is a low, prostrate shrub, grown in Romania as an ornamental plant. Its fruits are small, ovoid pomes. Fruit tissues were analyzed for total carotenoid content (through acetone extraction and spectrophotometry), flavonoid content (methanol extraction and spectrophotometry) and total concentration of phenolic and polyphenolic compounds (methanol extraction and spectrophotometric Folin-Ciocâlteu method). *C. salicifolius* fruits analyzed contained an average of 311.50 mg/kg DW carotenoids (heat-dried fruits – only 100.25 mg/kg). Average flavonoid concentration was 9,777.62 mg/kg in fresh frozen fruits and 4,574 mg/kg in dried ones. 25,056.09 mg/kg GAE was the average concentration of total phenolics in fresh fruits, while dry fruit powder contained 5,271 mg/kg GAE. These concentrations of bioactive compounds are comparable, or even have a higher than in pomes belonging to related species, including domestic cultivars of rowanberry (*Sorbus* sp.), wild *Sorbus* species, firethorns (*Pyracantha* sp.), or other studied cotoneasters. This shows a significant potential for these pomes, requiring further investigation. However, heat-drying was found to be an unsuitable processing method for these fruits, since it led to a 53.22-78.94% decrease in bioactive compounds concentrations.

Keywords: Cotoneaster salicifolius, fruits, carotenoids, flavonoids, phenolic compounds

Introduction

Cotoneaster salicifolius Franch. (common name: willowleaf cotoneaster or cranberry cotoneaster), belongs to the Rosaceae family, Amygdaloideae subfamily, Maleae tribe and is a common ornamental shrub.

An evergreen, or (rarely) semi-evergreen small to medium size low shrub, with extensively spreading branches, reddish to gray brown branchlets, elliptic to lanceolate glossy leaves (4-8.5 cm long), with entire margins and acute apex. White flowers are grouped in dense corymbs. Fruits are scarlet, subglobose to ovoid pomes, with 2-3 pyrenes each, ripening in September-October.

It is native to mountain areas, open slopes and mixed forests in Southern China, but grown worldwide as an ornamental plant (groundcover). It tolerates a wide variety of soils (except for those with water excess), while it is susceptible to some bacterial diseases and insect attack. With proper maintenance, it is able to thrive in urban habitats (Lu & Brach, 2003). In Romania, it is grown in parks and gardens, 'Parkteppich' and 'Repens' being the most popular cultivars (Fig. 1).

Fig.1. *Cotoneaster salicifolius* Franch.

There are no known other uses for *C. salicifolius*, however other related species are used in Asian traditional medicine, for treating bronchitis, gastritis, various infections and vascular illnesses (Liu et al., 2018).

The purpose of this research was to determine the concentration of some key phytochemical classes in fresh and heat-dried fruits of *C. salicifolius*.

Carotenoids (carotenes, lycopene, lutein, zeaxanthin among others) are common pigments in plant leaves, flowers and fruits. They represent the basis for melanin and retinol synthesis, being important for skin and eye health. Some have antitumoral properties (Eldahshan & Singab, 2013).

There are various types of phenolic and polyphenolic compounds, with protective function against pathogens and insects (Kivrak & Kivrak, 2014). Among them are flavonoids, phenolic acids, tannins, with radical scavenging, reducing and lipid oxidation inhibiting activity, thus potent antioxidants (Zymonė et al., 2018).

Among low-mass phenolics, flavonoids have antiviral, antibacterial, antifungal, antiproliferative, antitumoral and anti-inflammatory properties (Kivrak & Kivrak, 2014).

Material and Methods

Pomes were collected from several *C. salicifolius* individuals in public gardens from Constanța, Romania, in November 2018. All individuals found belonged to the 'Repens' cultivar.

Fruit pyrenes (kernels) were extracted and the fruit pulp was ground using an electrical grinder. Part of the pulp was frozen at -20°C, prior to analysis, while the rest was oven-dried at 80°C (over 72 hours).

Both fresh frozen tissue and dry one were analyzed for determining carotenoid, flavonoid and phenolic compounds concentrations (triplicate samples each).

For carotenoids, 0.1 g frozen/dry fruit pulp were ground in 10 mL acetone (80%) and filtered. Spectrophotometric absorbance was read against a blank, using a S106 WPA spectrophotometer, at 470, 647 and 663 nm (Miazek, 2011). Overall carotenoid concentration was estimated according to Lichtenthaler & Buschmann, 2001.

For flavonoids, 1 g fruit pulp was ground with 5 mL methanol and filtered. 0.5 mL of the extract was mixed with 4 mL distilled water and 8 mL methanol. Spectrophotometric absorbance was determined at 340 nm, and concentration calculated according to Szabo et al., 2012.

Total concentration of phenolic compounds was determined via a spectrophotometric Folin-Ciocâlteu method. 0.1 g pulp was ground with 10 mL methanol. 1 mL of extract was mixed with 5 mL 10% Folin-Ciocâlteu reagent and 4 mL 7.5% sodium bicarbonate. After 30 minutes, absorbance was read at 765 nm (Siddiqui et al., 2017; Stanković, 2011).

Concentrations were expressed as mg/kg DW, respectively mg/kg gallic acid equivalent DW (GAE; for phenolics, after proper calibration).

Results and Discussions

Fig. 2 shows carotenoid content in fresh frozen and dry fruits. Fig. 3 and 4 show flavonoid and total phenolic compounds concentrations.

With a 19% dry biomass percentage, the average total carotenoid concentration in fresh frozen pomes was 311.50 mg/kg DW (271.31-346.04 mg/kg in individual samples), while in dry ones only 100.25 mg/kg (92.34-114.26 mg/kg; Fig. 2).

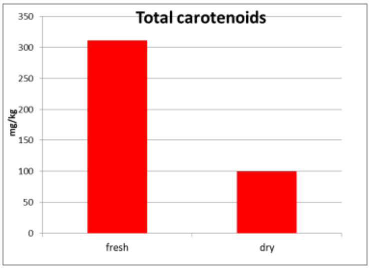

Fig.2. Total carotenoid concentration in fresh and dry *Cotoneaster salicifolius* fruits (average values; mg/kg DW).

While literature offers few data on carotenoid content in *Cotoneaster* fruits, useful comparison can be drawn with related *Pyracantha* species, with over 65 mg/kg total carotenoids in *P. angustifolia* (Zechmeister & Schroeder, 1942), and over 22 mg/kg in *P. crenulata* (a valuable medicinal species growing in the Himalayas; Pal et al., 2013).

Sorbus is another related genus (it includes rowans and service-trees), in the Maleae tribe, subject of a detailed that found a total carotenoid concentration of 39-2,659 mg/kg, in various domestic varieties (Zymonė et al., 2018).

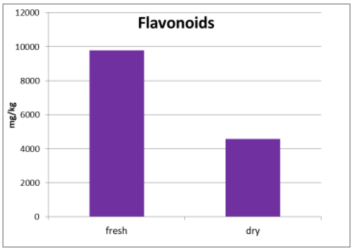

Fig.3. Total flavonoid concentration in fresh and dry *Cotoneaster salicifolius* fruits (average values; mg/kg DW).

Fresh *C. salicifolius* fruits contained, on average, 9,777.62 mg/kg DW of flavonoids (5,307.85-15,225.17 mg/kg), while in dry fruit powder, the concentration dropped to 4,574 mg/kg (4,167-4,910 mg/kg; Fig. 3). For comparison, another creeping cotoneaster species, *C. horizontalis* fruits contains around 6,800 mg/kg (Mohamed et al., 2012), while *C. multiflorus* contains 53,700 mg/kg (Liu et al., 2018).

Sorbus species pomes contain 435-37,000 mg/kg, with high variations due to species, cultivar, geographical and pedological factors (Majić et al., 2015; Zymonė et al., 2018).

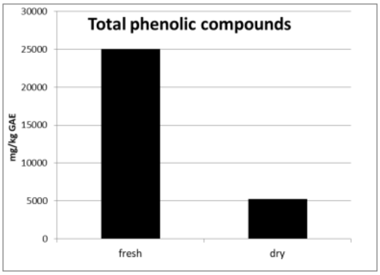

Fig.4. Concentration of total phenolic compounds in in fresh and dry *Cotoneaster salicifolius* fruits (average values; mg/kg GAE DW).

Fresh *C. salicifolius* pomes contained an average of 25,029.09 mg/kg GAE total phenolic compounds (DW; 23,056.09-27,199.39 mg/kg), while in dry fruit powder, the amount dropped to 5,271 mg/kg GAE (4,690-5,730 mg/kg; Fig. 4).

These concentrations are between those found in pomes of *C. horizontalis* (14,000 mg/kg GAE; Mohamed et al., 2012) and *C. multiflorus* (38,600 mg/kg GAE; Liu et al., 2018).

Among related genera, *Pyracantha crenulata* pomes contain 7,430 mg/kg phenolic and polyphenolic compounds (Pal et al., 2013). *S. domestica* fruits contain over 10,000 mg/kg (Majić et al., 2015) and *S. torminalis*, 19,150 mg/kg (Hasbal et al., 2015). Various domestic *Sorbus* sp. cultivars were found to contain 362-8,142 mg/kg GAE phenolics (Zymonė et al., 2018). Wild varieties of *Sorbus* contain 2,218-9,843 mg/kg GAE phenolics in their fruits (Raudonis et al., 2014).

Heat-drying of *C. salicifolius* fruits led to a 67.81% decrease in carotenoids, 53.22% decrease in flavonoids and a 78.94% loss of total phenolics. Thus, the decrease in biocative compounds concentration was a signficant one, higher than the one normal occuring in freeze-drying of various fruits (10-50% for phenolics; Shofian et al., 2011).

Conclusion

Having an average concentration of 311.50 mg/kg total carotenoids, 9,777.62 mg/kg flavonoids and 25,029.09 mg/kg total phenolic snd polyphenolic compounds in their pulp tissues, *C. salicifolius* fruits are comparable, or even have a higher content of bioactive compounds, than pomes belonging to related species, including domestic cultivars of rowanberry (*Sorbus* sp.), wild *Sorbus* species, firethorns (including the medicinal plant *Pyracantha crenulata*), or other studied cotoneasters.

This show a significant potential for these pomes, requiring further investigation.

However, heat-drying was found to be an unsuitable processing method for these fruits, since it led to a 53.22-78.94% decrease in bioactive compounds concentrations.

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Resilience of Adolescents Diagnosed with Anxiety and Their Parents in Clinical Sample

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Abstract

Anxiety disorder is the most frequent psychiatric problem among children and adolescents. Research proved that resilience can be a protective factor in coping with psychological difficulties. Our research focuses on these aspects of resilience. **Aims:** We aimed to investigate the resilience and anxiety level of families with adolescents who were diagnosed with anxiety disorders. The clinical sample included 40 adolescents who were diagnosed with anxiety disorders and who received ambulant treatment. (18 boys and 22 girls; age: M=13.37 years, SD=1.46). Members of the control group were recruited from schools and they were normally developing adolescents without any psychiatric diagnosis. (18 boys and 19 girls, age: M=13.7 years; SD=1.56) DASS-21, Ten items Connor-Davidson Resilience Scale and self-made demographic sheet were used. Regarding the resilience, a Significant difference was found between the clinical and the sample group both among the mothers and their children. However, in the case of fathers, no discrepancy was found. Our results suggest that there is a significant, moderate positive relationship between the resilience of the mother and their children. Nevertheless, similar mechanisms in the fathers' case cannot be registered. In the control group, the fathers' perception of their child's resilience proved to be the strongest predictive factor (beta=0,495). On the contrary in the clinical group, the maternal perception was more accurate. (beta=0,06). Resilience can serve as a protective factor against anxiety. Our results can be useful for practitioners and draw attention to the importance of intrafamily mechanisms in coping with anxiety and mood disorders.

Keywords: Resilience of Adolescents Diagnosed with Anxiety and Their Parents in Clinical Sample

Introduction

On the anxiety disorder in childhood

Anxiety disorder is the most frequent psychiatric problem among children and adolescents. However, these disorders are not equivalents to those in adulthood. Several researches showed that battling with anxiety disorders in childhood does not necessary carried to adulthood as many neurotic symptoms can be extremities within the normal development. While externalizing disorders cause difficulties for the social environment (Perczel, Kiss, Ajtay 2005), symptoms of childhood anxiety affect mainly the individual, so the majority can remain undiagnosed.

Prevalence and epidemics:

In the research of Costello and al. children (7-11 years) under medical treatment were examined. The prevalence of anxiety disorders was 8,9% in this population. (Costello, Egger, Angold 2004). The occurance of patological anxiety is twice more frequent among girls than boys. (Beesdo et al, 2009). Regarding the total population the prevalence of social phobia was 1%, agoraphobia 1,2%, separation anxiety 4,1% and specific phobias 9,2% (Costello, Egger, Angold, 2004).

Anxiety can be explained by family cumulations and the effect of genetic factors seems to be marginal, although they can play role in the occurance of predisposition (Hettema et al, 2001).

The role of parents in the ontology of anxiety disorders

Several studies claimed that anxiety disorders have cumulative effects within the family. The occurance of anxiety among children is higher when a parent – especially the mother – also battles with this disorder (McClure, Brennan, Hammen, Brocque, 2001). The mechanism can be explained by social learning theory (Bögels, van Dongen, Muris, 2006) that suggest that the parental stress tends to interpret situations as threats and follows the parent's avoidant and anxious behavior (Rapee, Schniering, Hudson, 2009). Nevertheless, overprotecting parental attitude also helps the development of specific fobia, panic disorder and generalized anxiety. (Kendler, Davies, Kessler, 1997 cited by Beesdo, Knappe, Pine, 2009) Parent-child relationship that is burdened by conflicts and discord also raises the prevalence of anxiety disorders (Rueter, Scaramella, Wallace, 1999 cited by Beesdo, Knappe, Pine, 2009).

Resilience among adolescents

Nowadays increasing number of studies focus on resilience. This is an adaptive, stressresistent predisposition that helps adaptation even in hard times. The concept refers the process of overcoming negative effects, successful copong with traumas and avoiding the negative trajectories associated with risks (Fergus, Zimmerman, 2005). According to Dowrick and his collegues (2008) changing in lifestyle, the self-help attitude, the support of family members and friends helped teenagers the most in recovery from anxiety and depression. Resilience could accelerate these processes by the improvement of quality of life through promoting health protecting behaviours. Hauser (2006) found that three factors promoted the recovery from psychiatric disorders for adolescent girls: personal influence (they were able to change their situation), inner focus (they cope their inner feelings and thoughts) and establishing supportive relationships.

Norwegian research showed that higher level of resilience corralted with lower levels of anxiety, depression and obsessive-compulsive disorder (Hjemdal et al 2011). Recent study of Nagy and F. Lassú (2017) focused on inhabitants in childen's home who could cope efficiently with difficulties in life. They showed high competency in regulating emotions and impulses and were able to reframe even troublesome life situtations. They were open to spiritual experiences and expressed gratitude toward the social workers, friends or other benefactors.

So, according to the literature the resilience proves to be an important factor in coping with anxiety and other psychiatric disorders. Therefore, our study focuses on this psychological factor in the case of adolescents who were taking ambulant care after their diagnosis with anxiety disorder. We aim to elaborate the phenomenon within the dynamics of the family microsystem.

Aims

1. The examination of resilience among adolescents who were diagnosed by anxiety disorders

2. The comparison of resilience among adolescents and their parents

3. The effect of parental resilience on the resilience of the adolescent

Methods

Participants

Parents and their children between 12 and 17 participated in the research. Data collection period endured from June to December 2020. Children with anxiety disorder were outpatients of the Kertváros Pszichológiai Rendelő, Budapest. They were diagnosed but not yet treated. The control group was formed by normally developing children from primary and secondary schools in Budapest.

Criteria for the inclusion into the clinical group was anxiety disorder diagnosis based on the children's answers in DASS-21 (Lovibond 1995). Other comorbid disorders were explored from the previous clinical documentation. Inclusion into to control group was the lack of psychiatric history. Mental retardation our autistic symptoms were criteria for exclusion in both cases.

The research was permitted by the Medical Research Council (Egészségügyi Tudományos Tanács Tudományos és Kutatásetikai Bizottság; ETT, TUKEB). All the participants were informed about the research both in writing and orally.

Tools

a. Demographic sheet

Parents first provided basic demographic information, such as level of education, financial situation, the occurance of mental disorders within the family, numbers of siblings etc.

b. DASS – 21 Scale

The Depression, Anxiety and Stress Scale - 21 Items (DASS-21) is a multidimensional self-report scale designed to measure the negative emotional states of depression, anxiety and stress. The simple statements of the survey are easily comprehended by adolescents over 12 years and it makes diagnosis quick (five-ten minutes) and flexible. Dass-21 uses a dimensional concept of psychological disorders and suggests severity labels from normal to extremely severe. Hungarian adaptation of the scale was delivered by Mária Szabó on a sample of 1000 participants (Lovibond and Lovibond, 1995).

c. Ten item Connor-Davidson Resilience Scale

Connor-Davidson Resilience Scale (CD-RISC) was developed to measure resilience and positive adaptation after a stressful situation. The higher score on this scale represents higher level of resilience (Járai és mtsai, 2015; Kiss et al 2015). In our study parents filled this scale both on themselves and on their child.

Results

1.Sample

Clinical sample included 40 adolescents who were diagnosed by anxiety disorders and who received ambulant treatment. (18 boys and 22 girls; age: M=13.37 years, SD=1.46). Members of the control group were recruited from schools and they were normally developing adolescents without any psychiatric diagnosis. (18 boys and 19 girls, age: M=13.7 years;SD=1.56) Age distribution between the two groups is normal, there is no significant difference (F=0.346 and p=0.558).

Variables	Clinical group	Control group
Father’s education		
Elementary	12	5
Intermediate	21	19
Higher	7	13
Mother’s education		
Elementary	11	5
Intermediate	22	19
Higher	7	13

Table 1: Educational level of the parents

Anxeity indicators and comorbid diagnoses in the clinical group

All members of the control group provided normal level of anxiety according to their answers on DASS – 21. However, those in the clinical group showed moderate (2), severe (29) or extremely severe (9) symptoms. Table 1 contains the comborid diagnoses based on their medical history. At the time of the research they were participating in the diagnosis project and not yet received (medical) treatment.

Diagnosed psychiatric disorders	prevalence: member and %
Anxiety disorders	
obsessive disorder	6 15
social fobia	6 15
panic disorder	5 12.5
generalized anxiety disorder	3 7.5
separation anxiety	12 30
posttraumiatic stress syndrome	11 27,5
Other psychological disorders	
dysthymia	10 25
behavirural disorder	7 17,5
hypomania	6 15
major depression	12 30

Table 2: Comborbid diseases among the members of the clinical group (n=40) based on their medical history

The sample indicates that the occurance of psychiatric disorders within the family is significantly higher in the clinical group than in the control. (BM=-2,591 p=0,0119)* (Table 3)

	Members	Mean of ranks	Deviation of ranks	Kolmogorov test		
				Dmax	D*	p
Clinical group	40	43,13	10,38	0,191	1,206	0,1092
Control group	37	34.54	18,05	0,125	0,759	0,6126

Table 3: Occurance of psychiatric disorders in the families within the clinical and control group (based on the demographic sheets)

2. Resilience of parents and their children in the clinical and control group

The results on CD-RISK Scale is presented as it follows (Table 4):

Resilience	**Adolescents**						**Parents**	
	Mean	min.	max.	SD	boy	girl	mother	father
Clinical	22.68	18	30	2.576			22.27	23.20
Control	29.3	18	35	4.390			29.30	25.97
Total sample	25.86	18	35	4.860	26.8	25.07	25.65	24.53

Table 4: The deviation of means on CD-RISK, adolescents and parents

The optimal zone of resilience is between 25 and 35 points, and as the table shows the average of the control group remains within this zone. However, resilience of both the adolescents and their parents in the control group is shown low by this scale. Differences can be registered between the clinical and the control group regarding the resilience (see Table 5). Maternal results show normal distribution both in clinical and control group. However, deviation was not homogenous. Therefore Levene's test was used F(1; 59,2) = 13,428 (p = 0,0005)***). Results of the Welch's d test are: d(61,3) = 9,882 (p = 0,0000)*** So, regarding the mothers there is a signficiant difference between the two groups. The Cohen's d effect size (d=2.207) suggest that this discrepacne is notable. However, no difference can be detected between the results of the fathers'.

	N	mean	STDEV	SKEW	KURT	Kolgomorov-Smirnov test (p)	Levene test (p)
clinical	40	22.7	2.58	0.374	0.644	0.095	0.001
control	37	29.3	4.39	0.388	-0.187	0.004	
total	77	25.9	4.86	0.347	-1.079	0.003	

Table 5 Differences between adolescents in the clinical and control group regarding their results on CD-RISK

3. The relation between the parental and children's resilience

Our results suggest that there is a significant, moderate positive relation between the resilience of the mother and their children. Regarding the control group there is 57,4% chance for finding good performance on the resilience scale in both the cases of mothers and their children. Hower, good maternal performance combined with poor results by their children appears only in 23,1% of the cases. (p_poz = 57,4%,p_neg = 23,1%) Similar results can be detected in the clinical group too (p_poz = 60,4%, p_neg = 21,7%), so it might be assumed that higher maternal resilience comes with the higher resilience of their children. Nevertheless, similar mechanisms in the fathers' case cannot be registered.

4. The children's resilience as perceived by their parents

In the control group the fathers' perception of their child proved to be the strongest predictive factor (beta=0,495). In contrary in the clinical group, the maternal perception was more accurate. (beta=0,06). For the details see Table 6.

	Variables of the model	Non-standardized coefficients	Standardized coefficients	t	p
		B	Standard error	Beta	
Control group	*Constant*	4.680	7.641		0.612

	Father on the child	0.859	0.266	0.495	3.226
Clinical group	*Constant*	13.870	1.933		7.174
	Mother on the child	0.395	0.086	0.600	4.622

Table 6: Resilience of children in the control and clinical group after linear regression

Discussion

This study draws the attention to the importane of diagnosing anxiety and finding the most adequate treatment. Adolscents battling with anxiety disorder frequently produce other symptoms, that might hide the core problems. So accurate differential diagnosis is pivotal in the healing process. Nowadays, therapies focusing on anxiety and depression are highly efficient as the 70-80% of the patients recover. (Torzsa et al, 2009)

The clinical group enforces the findings of the litterature that anxiety disorder commonly coexsist with other psychological disorders (mostly dysphoria). The occurance of major depressive episodes rises the risk of alchol or drog abuse, (Kazdin, 1994) and generalized anxiety disorder is strongly associated with other comorbidities of panic disorders depressoin, dysthima, social or special fobias (Brown & Barlow, 1992; Sanderson, Beck, & Beck, 1990; Brown et al, 2001).

Resilience plays an important factor in recovery from anxiety disorders. (Kiss 2015) Our results show that the resilience of children and their parents in the clinical group is significantly lower than in the control group, and remains under the optimal zone. Resilience can be used as a resource in therapy. (Masten, 2001, Gyöngyösiné Kiss et al, 2008) Improving resilience is contributed by phsycal activity, optimist attitudes, self-confidence, spirituality and finding purposes in life. In contrary low level of resilience increases perceived stress and the use of maladaptive coping mechanisms like repression, rumination, self-reproach or even aggression (Deák 2015).

It seems trivial that those who are generally not in a state of angst provide higher levels of reseilience. But this should not lead to the conclusion that they can be left alone because they can manage all the hardships. Pivotal factors in improving resilience are strong family bounds and abundant friend relationships (Skrove, Romundstad, Indredavik, 2012). Our results also emphasize the importance of family features. The level of maternal resilience was significantly lower in the clinical than in the control group. Fathers did not show any differences.

The way how family as a microsystem reacts to the circumstances deeply affects the attitudes and coping of the family members as individuals. Resilient families tend to communicate openly and cooparte in solving problems. They also try to remain positive and emotionally warm and frequently use spiritual points of view in understanding their current difficulties. Resilient families encourining the maintance of interpersonal relationships and they are efficient in mobilizing resources even from outside the family (Walsh, 2003). Economic stability and shared time contribute resilience. Jennifer A. Theiss (2018) drew the attention to the importance of child – parent communication. Parents who accept the feeling of their children and show an example how one can express and react to feelings are cultivitating the resilience of the children who will be more efficient in managing negative life events (Gottmann, 2001 cited by Theiss 2018) Parental support can be emotional or informative. The first serves as emotional support, while the latter helps them in detecting situations and behaviours that carry risks (Fergus, Zimmermann, 2005). Other researches emphasized the importance of the father's support which can be a protective factor among adolescents against depression or suicde thoughts. (Tarver, Wong, Neighbors, Zimmerman, 2004 cited by Zimmermann et al., 2013)

Children who live in conflict-prone family environment have lower level of self-confidence. The angry or cold reactions of the parents will be part of the self-image of the children. Adolescents with highly critical mothers generally show lower level of self-esteem than those who have supportive mothers. (Neff, McGehee, 2010).

Family relationships alone can influence the inner feelings of its members (Hjemdal, Vogel, Solem, Stiles, 2011). The study of Bögels, van Dongen and Muris suggested that children learn coping by social learning and the most important role models in this process are the parents. (Bögels, Oosten, Muris, Smulders, 2001) So, it can be assumed that the parental resilience have a direct effect on the children's anxiety. Parents with low resilience generally offer negative schemes for their children that are unsufficient tools in coping hardships and therefore they rise frustration and feeling anxiety. Unfortunately, disharmonic families deprive children from other sources of coping including strong family structures or supportive relationships among the family members. (Zolkoski and Bullock, 2012). Percieved dissconance within a family directly effects the feeling of insecurity and contributes to the occurance of problems related to both externalizating and internlizating (Forman and Davies, 2003).

The attachment between father and child is shaped by those factors – acitivity, self-confidence, sense of purpose (Psychogiou et al, 2008), – that are measured by the Connor-Davidson scale. Our results show that fathers are more accurate in estimating their children's resilience than the mothers. This result enforces those researches that emphasize the importance of the paternal role in the children's development. It carries an important message to the professionals to helpi improving not only the mother-child relationship but focus on the fathers too.

The main limit of this research is the relatively low number of members within the clinical group. However, this is possibly compensated by personal testing and the involvement of families which opened the opportunity to investigate inter-family effects. Our findings provide useful evidences for promoting family therapy in healing anxiety disorders.

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Determination of Caffeine Content in Arabica and Robusta Green Coffee of Indian Origin

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Abstract

The coffee tree belongs to the Rubiaceae family, genus *Coffea*. Although more than 80 species of coffee have been identified worldwide, only two are economically important. Coffea Arabica, also known as Arabica coffee, is responsible for about 70% of the world coffee market, and Coffea Canephora or Robusta coffee represents the rest. Due to the strong physiological effects of caffeine on human physiology, the caffeine content is a very important quality parameter of processed coffee. Routine analysis of caffeine in the food industry can be facilitated using fast and reliable tests. In this article, we quantitatively determined the caffeine content using the chloroform isolation method and we also performed the qualitative determination of caffeine in green coffee of Indian origin by the UV-VIS spectrophotometric method. Following the analysis of caffeine isolate with chloroform, we obtained a caffeine content of 182 mg / 100 g for the Robusta green coffee sample and 154 mg / 100 g for the Arabica green coffee sample. Thus we can confirm the presence of a higher caffeine content in the Robusta India green coffee sample than in the Arabica India green coffee sample. In the spectrophotometric analysis we used 4 coffee samples obtained by extraction with hot distilled water and by extraction with cold distilled water. The spectral analysis confirms the presence of caffeine in both studied coffee species and agrees with the data in the literature.

Keywords: caffeine, green coffee, Arabica, Robusta, clorophorm, UV-VIS.

Introduction

Studying the impact that coffee exerts on human health is of great interest, since coffee is one of the most commonly consumed beverages in the world, accounting for 75% of the regular soft drinks consumption [Toci, A., 2016]. Drinking coffee has frequently been discouraged, due to the risks associated with the excessive consumption of this beverage (hypertension, coronary heart disease, anxiety, insomnia, depression, osteoporosis, anaemia, pregnancy-related problems [Nawrot P., 2003]. Nevertheless, the more recent information about coffee phytochemistry and biological properties has progressively led to consider coffee as a potential functional food, as its benefits on human health seem to outdo its negative effects [Messina, G., 2015]. Specifically, experimental and epidemiological studies have demonstrated that coffee (and some molecules contained in coffee beverages) can provide beneficial effects against several pathologies, among which neurological diseases (Parkinson's and Alzheimer's diseases), cancer (breast and colon cancer), psychoactive responses (alertness, mood change) and metabolic disorders (type 2 diabetes). Moreover, in recent years, the beneficial properties of green coffee for human health have been pointed out, leading to a increase in the consumption of green–coffee-based beverages [Ludwig, I. A., 2014].

Coffee species are grown in almost all countries along the equator. The people involved in the cultivation of this plant have a long history of production and an important role both in the global market and in the field of research. Coffee beans are successfully used in food, cosmetics and pharmaceuticals due to caffeine and high polyphenol content. However, the most well-known and studied phytochemical compound found in coffee is caffeine. Although other alkaloids, such as theophylline and theobromine, are also present in coffee, caffeine is found in much higher concentrations. Caffeine can also be obtained from cocoa beans, tea leaves, kola nuts, guarana fruits. Depending on the source, it has different names. Thus, the caffeine obtained from coffee and kola nuts is called caffeine; from tea it is called theine; from the red seeds fried by Paullinia cupana is called guarana [Bușuricu F., 2020].

More than 80 coffee species have been identified worldwide [Clarke, R. J. 2003], only two are economically important. Coffea arabica, also known as Arabica coffee, is responsible for approximately 70% of the global coffee market, and Coffea Canephora or Robusta coffee (commercial name of one of the main C. Canephora cultivars) accounts for the rest.

The Arabica plant is a descendant of trees native to Ethiopian coffee and is usually a large shrub that can reach a height of 4 to 6 meters. Its fruit, which has a fine, mild, aromatic taste, accounts for about 70% of world coffee production, and Arabica coffee usually has the highest prices on the market. The Arabica plant reaches maturity in 3 to 4 years and continues to bear fruit for 20-30 years. It requires a mild climate, ideal with temperatures between 15 and 23 ° C and rainfall of about 60 centimeters per year. The best Arabica coffees come from plants grown at altitudes between 2,000 and 6,000 meters. The ideal altitude in this range varies with the distance from the equator [Smith B., 2006].

The Robusta plant is similar to Arabica in size, shape and time to maturity, but produces smaller and rounder grains. Robusta plants are cheaper to grow because they are more resistant to disease and can withstand warmer climates (preferring temperatures between 23 ° C and 30 ° C). However, Arabica is much preferred over Robusta because its taste is considered superior [Farah A, 2017].

When they reach maturity, coffee trees of any kind usually bear fruit called "cherries" which turn red when ready for harvest. Arabica berries usually ripen in about 9 months, and Robusta needs 10 or 11 months. Coffee beans are the seeds of these cherries.

The main objective of this article is to offer many new opportunities and challenges for physico-chemical studies to analyze the main natural source that contains caffeine, namely green coffee belonging to the species Arabica and Robusta. In order to analyze and quantify the main component of green coffee, namely caffeine, we performed the following analyzes:

Sample preparation involves several steps, such as grinding, extraction of target compounds, pre-concentration and purification of the samples;

Isolation of caffeine with chloroform from the Arabica and Robusta green coffee sample of Indian origin;

Spectrophotometric determination of green coffee extracts in order to quantify the purine alkaloid – caffeine.

Material and Methods

Preparation of coffee extracts

The same batch of coffee was used for all extractions (100% Arabica - India; 100% Robusta - India). Each packet of coffee beans (250 g) was opened immediately before brewing to avoid oxidative damage. The beans were ground using a professional coffee grinder (DIP Grinders DK-30). The coffee was ground "coarsely" for all extraction methods: hot extraction and cold extraction.

Extraction with hot distilled water: To prepare the hot coffee extract, the distilled water was heated to 80 ± 2 ^{0}C in a heat-resistant beaker and the green coffee sample was placed in the beaker and the infusions were heated on the water bath for 5-7 minutes under continuous stirring. The sample to solvent ratio was 1:20 (g / mL). The aqueous extract was hot filtered through filter paper to remove insoluble particles. The aqueous extract obtained with hot water was named CA1 and CR1, respectively.

Extraction with cold distilled water: The cold preparation method was performed at room temperature (ranging from 21°C to 25°C during the experimental period). The coffee extract was prepared using a ratio of 1:10 (g ground green coffee / mL distilled water). The cold drink was extracted under static conditions. Ground green coffee and water were in contact for 24 hours in the refrigerator (2-8^{0}C). When the extraction was complete, the maceration was filtered through a paper filter. The aqueous extract obtained with cold water was named CA2 and CR2, respectively.

Chemicals: magnesium oxide; buffer solution: pH 6; metabisulphite of Na 0.5% solution; chloroform, caffeine standard were from Sigma – Aldrich.

Work equipment:

VWR UV-6300PC Double Beam Spectrophotometer;

Analytical balance;

Usual laboratory utensils and glassware

Caffeine isolation with chloroform

Due to the strong physiological effects of caffeine on human physiology, the caffeine content is a very important quality parameter of processed coffee. Routine analysis of caffeine in the food industry can be facilitated using fast and reliable tests. In this article, we quantitatively determined the caffeine content using the chloroform isolation method.

The caffeine isolation procedure was performed according to a method described by Buşuricu [Buşuricu F., 2008, 2020], with some modifications. The caffeine concentration was calculated according to the following equation:

mg caffeine / gram of coffee = $\frac{(A_1 - A_2) \times 10}{0.410 \times m \times V}$

where:

$A_1 = \frac{(A_{250} - A_{296})}{2}$; $A_2 = \frac{(A_{250} - A_{296})}{2}$;

10 = the volume of the solution used in the experiment;

0.410 = absorbance value for 1 mg caffeine;

m = the mass of the sample;

V = the volume of extract used in the determination;

A_1 şi A_2 = absorbance values at wavelength λ273 for solutions 1 and 2.

Working technique

Solution 1: weigh 1 g of each sample: green Arabica coffee and green Robusta coffee; bring in a heat-resistant glass, add 1.5 g of magnesium oxide in each glass corresponding to the sample I noted glass A (Arabica coffee) and glass R (Robusta coffee), and 75 mL of hot distilled water. The formed suspension is boiled for 10-12 minutes under continuous stirring. Filter the suspension obtained under low pressure by washing the filtrate several times with hot distilled water until a volume of approx. 80-90 mL of each sample. The solution obtained is brought into another heat-resistant glass, boiled for 5 minutes, cooled and brought into a 100 mL volumetric flask and made up to the mark with distilled water. From these extracts we bring in two 50 mL volumetric flasks a volume of 10 mL solution A and 10 mL solution R in which we add 5 mL of buffer solution (pH = 6) and 10 mL of sodium metabisulphite, brought to the mark with distilled water , and these will represent S1A and S1R.

Solution 2: An amount of clear extract, equal to that taken for the previous solution (10 mL), is placed in a separatory funnel and 5 extractions are made with 15 mL of chloroform. The chloroform solutions are removed and the aqueous solution is transferred to a heat-resistant beaker, the funnel is rinsed with distilled water and added to the solution in the beaker (25 mL). Bring to the boil, evaporate, cool and place in a 50 mL volumetric flask. Fill with distilled water to the mark, and these will represent S2A and S2R.

Identification of caffeine in Arabica and Robusta green coffee by UV-VIS spectrophotometric methods

Ultraviolet / Visible Spectroscopy (UV-VIS) is an important analytical technique because it can be used to identify certain functional groups in molecules, but it can also be used as a test method. The method is quick and simple and allows us to confirm the presence of caffeine in CA1, CA2, CR1 and CR2 extracts. UV-VIS spectrophotometric measurements were performed at room temperature, using a UV-6300PC VWR Spectrophotometer at a wavelength range of 200-400 nm.

Working method:

Absorption of UV-VIS measurements were performed on VWR UV-6300PC Double Beam Spectrophotometer at 200-400 nm, using halogen and deuterium sources for visible materials and ultraviolet radiation, at a controlled temperature of 25°C, using square quartz tanks with an optical trajectory of 1 cm. The determination of the molar extinction coefficients of caffeine at 272 nm was performed by applying the known Bouguer Lambert-Beer law, by linearly mounting the dependence of the absorbence on the sample concentration. The measurements were performed in triplicate and the error associated with the concentration was the standard deviation obtained.

Preparation of the standard solution: For the preparation of the standard solution, the commercial chemical standard of caffeine was used and dissolved in distilled water according to the solubility procedure of F.R. X of the monograph *Coffeinum*. Weighed 0.5 g of pure caffeine which was dissolved by heating in 45 mL of distilled water. The solution obtained is cooled to room temperature and made up with the same solvent to 50 mL.

Results and Discussions

Caffeine isolation with chloroform

The caffeine concentration was calculated according to the equation presented, and the results were expressed in mg caffeine / gram of coffee.

Arabica green coffee = $\frac{(1.24 - 0.6065) \times 10}{0.410 \times 1 \times 10}$ = **1.54 mg caffeine / gram of coffee** - suitable for 154 mg / 100 g coffee

Robusta green coffee = $\frac{(0.98985 - 0.24745) \times 10}{0.410 \times 1 \times 10}$ = **1.82 mg caffeine / gram of coffee** - suitable for 182 mg/100 g coffee

The graphical representation of the caffeine content by chloroform extraction for the Arabica and Robusta coffee samples is shown in Fig.1.

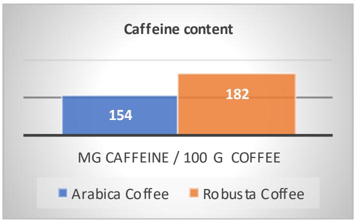

Fig. 1. Determination of caffeine content in Arabica coffee and Robusta coffee (mg caffeine / 100 g coffee)

C. Arabica and C. Robusta species differ in many ways. As the name suggests, Robusta coffee trees are more robust; that is, they are stronger, more resistant to pests and diseases, and less demanding than Arabica trees in terms of climate. Robusta coffee also contains higher amounts of antioxidant compounds and caffeine. The concentration and biological activity of caffeine depends on the following factors such as: processing conditions, agricultural practices (traditional or organic), the ideal growing climate, duration and storage conditions, and the origin of the coffee. According to the data presented in Table 1 [Smith B., 2006], the highest amount of caffeine by weight and variety is found in blueberry beans in Tanzania, with 1.42 g of caffeine per kilogram of coffee. Using the recommendation of the American Specialty Coffee Association with 10 g of ground coffee per 17 oz (6 oz) serving, Tanzania Peaberry would produce approximately 142 mg of caffeine per serving of coffee. Other high caffeine coffees include Columbia Supremo, Columbia Excelso and Indian Mysore (1.37% each), Kenya AA (1.36%) and Costa Rica Tarrazu (1.35%). At the other end of the scale with a lighter amount are Mocha Mattari (1.01%) and Zimbabwe (1.10%) coffees. In addition, the thickness of the grind also affects the caffeine content of the finished coffee.

Table 1. The amount of caffeine after the coffee mixture [Smith B., 2006]

Coffee source Varieties	Caffeine content %	Coffee source Varieties	Caffeine content %	Coffee source Dark mixes and frying	Caffeine content %
Brazil Bourbons	1.20	Kenya AA	1.36	Colombia Supremo Dark	1.37
Celebes Kalossi	1.22	Kona Extra Prime	1.32	Frying Espresso	1.32
Colombua Excelso	1.37	Mexico Pluma Altura	1.17	Frying French	1.22
Colombia Supremo	1.37	Mocha Matari (Yemen)	1.01	Frying Vienna	1.27
Ethiopian Harrar–Moka	1.13	New Guinea	1.30	Mocha-Java	1.17
Guatemala Antigua	1.32	Panama Organic	1.34	Decaffeinated: All mixtures (Swiss process)	0.2
Indian Mysore	1.37	Sumatra Mandheling–Lintong	1.30		
Jamaican Blue Mountain	1.34	Tanzania Peaberry	1.42		
Java Estate Kuyumas	1.20	Zimbabwe	1.10		

According to the results obtained by the chloroform isolation method, the caffeine content is higher in Robusta green coffee (182 mg caffeine / 100 g green coffee) than in Arabica green coffee (154 mg caffeine / 100 g green coffee). these results can be correlated with data from the literature [Crozier T. W. M, 2012].

Identification of caffeine in Arabica and Robusta green coffee by UV-VIS spectrophotometric methods

The obtained spectra are shown in Fig. 2, 3, 4, 5 and 6. In Fig. 2 it is found that pure caffeine absorbs in the wavelength range between 220 - 350 nm. Prior to spectrophotometric reading, CA1, CA2, CR1, CR2 extracts were dissolved in dichloromethane 25:25 (v / v) for better caffeine extraction from coffee. Extraction was performed 4 times with 25 mL of dichloromethane per round for each extract. Finally, the absorbance was measured with the VWR UV-6300PC Spectrophotometer in the range of 200-400 nm. According to Fig. 3 and Fig. 4. in the Arabica coffee samples CA1 and CA2 absorb caffeine in the range 220 to 350 nm, but in the case of the extract obtained by the hot process, CA1, the maximum absorbance of 3.5 (u.a) is reached, while in the cold process, CA2, the absorbance 3.5 (u.a) is exceeded, reaching values of approximately 3.8 (u.a). According to Fig. 5., Fig. 6. in the case of Robusta green coffee extracts, the wavelength range in which it absorbs caffeine is the same as 220-350 nm and the caffeine obtained by both processes reaches a maximum absorbance of 3.5 (u.a).

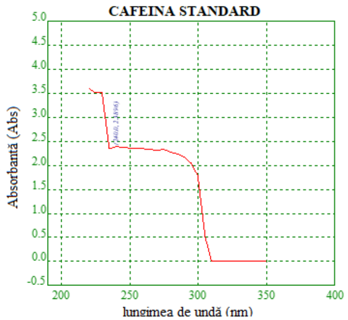

Fig.2. UV-VIS spectrum for standard caffeine

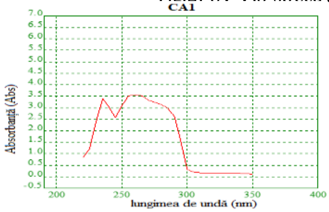

Fig. 3. UV-VIS spectrum for CA1 coffee sample

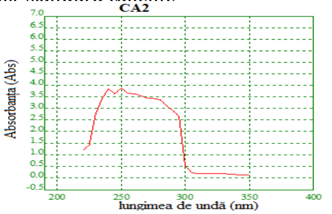

Fig. 4. UV-VIS spectrum for CA2 coffee sample

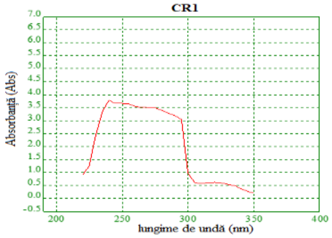

Fig. 5. UV-VIS spectrum for CR1 coffee sample

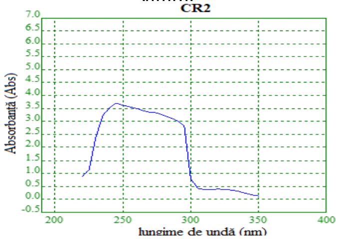

Fig. 6. UV-VIS spectrum for CR2 coffee sample

In Fig. 7., the spectrum of caffeine in Arabica green coffee extracted in water is presented and it is found that the intervals in which it absorbs caffeine are: 220-350. The maximum absorbance is (> 4 u.a.). In Figs. 8., the spectrum found in the literature is presented, namely the UV-VIS spectrum of caffeine in Arabica green coffee beans.

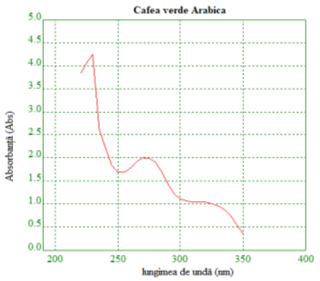

Fig. 7. UV-VIS spectrum for the Arabica coffee sample dissolved in water

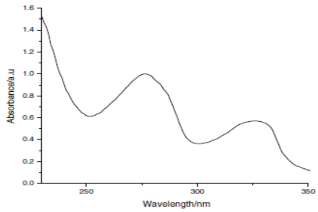

Fig. 8. UV-VIS spectrum of caffeine in Arabica green coffee dissolved in water

It is found that the extraction in water performed by us agrees with the data in the literature [Belay A,, 2008]. The differences between the spectra obtained depending on the analyzed coffee species, Arabica or Robusta are also due to the presence of other components in the coffee samples such as chlorogenic acids. Chlorogenic acids (CGAs) are classified according to the nature and number of cinnamic substituents and to the esterification position in the cyclohexane ring of the quinic acid. 5-Caffeoylquinic acid is the most widely occurring and most studied CGAs. CGAs have antioxidant properties, whichplay an important role inprotecting food, cells, andany organ from oxidative degeneration and also the coffee plant against microorganisms, insects, and UV radiation [Global Coffee Market, 2017].

The method of preparation may also affects the composition of the final coffee extract. Although coffee is generally brewed with hot water, there has recently been an increase in the consumption of cold water coffee drinks in northern European countries, the United States and Japan, due to the new preparation methods involving longer extraction times at colder temperatures, rather than rapid exposure to high temperatures [13].

The method for the qualitative determination of caffeine in coffee can simply be applied quickly after the design of a spectrophotometer which, based on a simple analysis from the reading of the spectrum, indicates the concentration of caffeine. This method incorporates an additional advantage: it is based on the use of a UV-VIS spectrophotometer, being easy, fast and available today in most laboratories. Moreover, the application makes it easier to increase knowledge to identify caffeine in various green coffee and roasted coffee products.

Conclusion

The composition of green coffee has attracted interest as a potential source of beneficial bioactive -compounds, especially through the presence of caffeine. As the content of this alkaloid is affected by the processing conditions, the main objective of this article was to determine the caffeine content of two different species: Arabica green coffee and Robusta green coffee, both of Indian production. These species are considered the most important and most used worldwide, and different in many ways, including their ideal growth climate, physical appearance, chemical composition, and infusion characteristics made from green and ground seeds. Overall, the results of this research suggest that the natural source of green coffee, belonging to the Arabica species, is an important source of bioactive compounds and is characterized by beneficial properties, with a lower average caffeine content than Robusta coffee. This finding is interesting because Arabica coffee is the most commonly used in the preparation of beverages and the most commercially valuable, due to its aromatic superiority. In the Arabica and Robusta green coffee samples we quantitatively determined the main methylxanthine (caffeine) using the chloroform isolation method and we obtained a higher concentration of caffeine in the Robusta green coffee sample (182 mg caffeine / 100 g coffee) compared to the sample of Arabica green coffee (154 mg caffeine / 100 g coffee). Also from the spectrophorometric analysis we confirmed the structure and morphology of caffeine in the Arabica and Robusta coffee samples.

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Microbiological Comparative Studies of Crude Aqueous Extracts from Arabica and Robusta Coffee

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Abstract

The aim of the present study was to determine the degree of microbial contamination of aqueous extracts of green and roasted coffee originating in India. The samples used in this analysis were obtained by two extraction methods: hot extraction and cold extraction. If a microbial load of samples was found, the same extracts were filtered using sterile 0.22 μm pore size filters from PES (Polyester) for decontamination. The working methodology was based on the Standard SR EN ISO 21149/2017 which provides general guidelines for counting and detecting mesophilic aerobic bacteria using as a medium for isolation, cultivation and counting of microorganisms (bacteria and fungi) the non-differential environment. The experimental results obtained showed microbial contamination of all samples. The level of microbial contamination is different for the extracts taken under study, depending on the type of coffee used, green or roasted, but also on other conditions, such as wet or dry processing. Roasted coffee is much less contaminated compared to green coffee, due to a thermal (incomplete) sterilization by the roasting procedure. The working conditions used, the temperature of the distilled water used to prepare the extracts, their non-sterile handling, but also the type of coffee used, are the factors that determined the microbial contamination.

Keywords: green coffee, roasted coffee, microbial contamination, aqueous extracts, Arabica, Robusta.

Introduction

Coffee is one of the most consumed beverages in the world, praised for its pleasant aroma, and its pharmacological characteristics. Recently, scientific and popular interest in its importance to health has increased due to the beneficial pharmacological properties established in clinical and epidemiological studies. The history of coffee dates back to about 900 A.D., when it was first mentioned by the Rhazes [Ukers W.H., 1948]. The coffee factory was originally from Ethiopia; from there it spread to Arabia, India, Ceylon, Java, Martinique, Suriname, Brazil, the Philippines and Mexico [Ukers W.H., 1948]. The genus Coffea belongs to the Rubiaceae family and includes up to 124 species. The most famous and used species are Coffea Arabica L. and Coffea Robusta L. Coffea Arabica (known as Arabica) and Coffea Canephora (known as Robusta), which represent 70% and 30%, respectively in global production. [Perrois C, 2015]. Coffee cherries are treated by one of two post-harvest processing methods [Silva C. F, 2008]. In Central America, Colombia and Hawaii, "wet" processing is commonly used for Arabica coffee. In the moist process, the ripe cherries are selected carefully, the pulp is mechanically separated and the fermentation follows for about 24-48 hours to remove the mucilage layer. The wet process requires more care and investment, but results in superior coffee quality [Ghosh P, 2014]. The dry method is the oldest and cheapest method of processing coffee and results in so-called natural or unspoken coffee. The dry process is preferred in countries where rainfall is scarce and extended periods of sunshine can dry the coffee properly [Silva C. F, 2008]. This method is used for about 95% of Arabica coffee made in Brazil, most of the coffee produced in Haiti, Paraguay, Ethiopia, Indonesia and some Arabica coffee made in Ecuador and India. In the dry method, the fermentation of whole fruits is essential and produces less aromatic, sweet, complex and heavy coffee in the body. The coffee fruits are placed on the ground under the sun in layers about 10 cm thick, piled up at night and spread every day to produce green coffee beans [Ghosh P, 2014]. The duration of sun drying lasts about 10-25 days. At that time, natural microbial fermentation takes place which can change the final quality of the coffee product. Fermentation of sugars generates ethanol and carboxylic acids. Endophytes involved in dry fermentation have a greater variety and complexity than those found during wet processing, but the real role of each group of microbiota during the fermentation of coffee by natural processing remains unknown [Silva C. F, 2008]. In this article we aim to determine the degree of microbial contamination of aqueous extracts from green coffee and roasted coffee of Arabica and Robusta species. This determination is an important analysis because the degree of contamination can be due to several factors such as: harvesting, method of processing, storage, handling, type of plant product, species used, processing and obtaining a plant extract that involves certain conditions and manipulation. We made this determination because it must be confirmed that, in order to ensure the quality of a plant extract, it is recommended to perform an adequate analysis of the microbiological risk. This determination will be useful in subsequent experiments and for testing antimicrobial activity but also for the use of aqueous extracts analyzed in pharmaceutical composites for topical applications.

Material and Methods

Plant material

Samples of Arabica coffee of Indian origin and Robusta coffee of Indian origin were purchased from supplier G. Bijdendijk BV, Netherlands. Plant material used was divided into 2 categories: green coffee and roasted coffee.

Green coffee beans were coarsely ground with a grinder professional coffee DIP Grinders DK-30;

The same batch of green coffee was roasted with a wood-fired roaster, which provides a increased control over the frying of the grains, ensuring that they are fried evenly, without being burned. The degree of frying was medium (at 209 ^{0}C), and the grains have were ground with the same grinder, in the form of "fine" powder.

Water-soluble coffee extracts

The aqueous extracts were obtained from Arabica and Robusta green coffee beans but also from Arabica and Robusta roasted coffee beans. From the examined material, ground "coarse" and "fine", two types of aqueous extracts were prepared. In the first extraction method we used hot water (100^{0}C), and in the second method cold distilled water (2-8 ^{0}C).

The extraction with hot water was performed for the Arabica and Robusta roasted coffee sample in the form of fine powder. Each sample was extracted with 100^{0}C hot distilled water for 5 minutes. The ratio of roasted coffee powder to solvent was about 1:20.

How to work: 6 grams of Arabica and Robusta roasted coffee powder were extracted in boiling water distilled at 100^{0}C for 5 minutes. After completion of the extraction operation, the extract obtained is separated from the roasted coffee residue by filter paper to remove insoluble particles in solution. The aqueous extract obtained with hot distilled water was named CA0 and CR0, respectively.

Maceration was the second process used for extraction. We used "coarse" ground green coffee and added the solvent in a tightly closed vessel and stored the bottle at 2-8^{0}C for 24 hours. Water quality plays an important role in the preparation of coffee beverages, so we used distilled water, freshly prepared for all samples, during the extraction procedure. The ratio of ground coffee to solvent was 1:10. The aqueous extract obtained with cold distilled water was named CA2 and CR2, respectively. After completion of the maceration operation, the coffee extract solutions are separated from the residue by filter paper to remove insoluble particles from the solution.

Determination of the degree of microbial contamination of aqueous extracts of green coffee (CA2, CR2) and roasted (CA0, CR0)

The materials used in this study to detect the degree of microbial contamination are represented by green and roasted coffee extracts: CA0, CA2, CR0, CR2. If a microbial load of samples was found, the same extracts were filtered using sterile 0.22 μm pore size filters from PES (Polyester) for decontamination for use in subsequent experiments. The working methodology was based on the Standard SR EN ISO 21149 [SR EN ISO 21149/2017] which provides general guidelines for the counting and detection of mesophilic aerobic bacteria in cosmetics, using as a medium for isolation, cultivation and counting of microorganisms (bacteria and fungi) the non-differential environment TSA (Tryptic Soy Agar) whose technical formula is in Table 1.

Preparation of TSA medium: The components (40g) are dissolved in water, the pH is adjusted if necessary so that after sterilization it is 7.3 ± 0.2 at 25 ° C. The medium is distributed in test tubes in a volume of 12-15 mL or in vials with a maximum capacity of 500 mL. Autoclave at 121 ° C for 15 minutes. It can be stored in the dark and at a temperature of 3 ± 2 ° C for 1 month.

Table 1 The technical formula for the culture medium Tryptone soya agar (TSA)

Tryptone soya agar

Composition	Amount
Tryptone	15g
Soy pepper	5g
Sodium chloride	5g
Agar	15g
Distilled water	1000 m

Procedure

Transfer, with a sterile pipette (Fig. 1.), 1 mL of test extract to a Petri dish. 15-20 mL of TSA medium, previously cooled to 48°C on the water bath, is poured into each Petri dish under aseptic conditions (in the microbiological safety niche).

Carefully homogenize (Fig. 2) the inoculum with the culture medium and allow it to solidify, placing the Petri dishes on a horizontal and cool surface. The time between the distribution of the inoculum in the plate and the pouring of the medium must not exceed 45 minutes. The samples are inoculated in triplicate.

Turn the inoculated plates with the lid down and place in the thermostat at 32.5 ± 2.5°C for 72 ± 6 hours.

After the specified incubation period, count the colonies on the plates using, if necessary, the colony counter. The plates containing at most 300 colonies are examined in diffused light. It is important that small colonies be included in the count, but it is essential to avoid confusing misleading particles of undissolved or precipitated plate material with small colonies. Extended colonies will be considered as a single colony. If less than a quarter of the plate is covered by extensions, count the colonies on the unaffected side of the plate and calculate the corresponding number for the whole plate. If more than a quarter of the plate is covered with extensive colonies, counting is not considered.

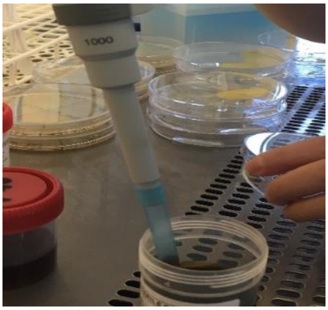


Fig. 1. Sterile transfer of test extract to Petri dishes

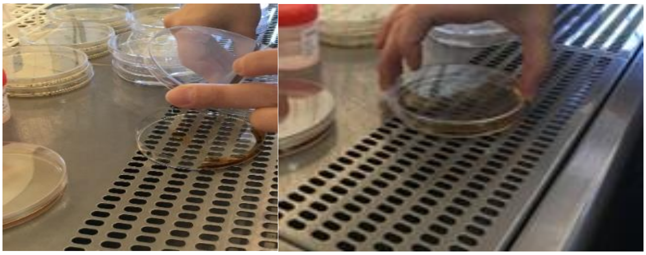


Fig. 2. Homogenization of the inoculum with the culture medium

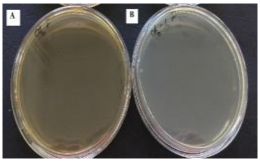
Results

After the expiration of the incubation time, the colonies in each plate were counted, before filtration (Fig.3.A. left and right), and the results were expressed in CFU / mL, as an average of 3 counts (in triplicate) + DS (Standard Deviation), the results being detailed in Table 2. The experimental results obtained showed microbial contamination of all samples and therefore required sterilization by filtration, both in the case of CR2 sample (over 3000 CFU / mL before filtration) and CA2 (110 CFU / mL), as well as in the case of aqueous coffee extract samples CR0 (2UFC / mL) and CA0 (4UFC / mL), which had a very low degree of contamination . The level of microbial contamination is different for the extracts taken in the study, depending on the type of coffee used, green or roasted, but also on other conditions, such as wet or dry processing. Roasted coffee is much less contaminated compared to green coffee, due to a thermal (incomplete) sterilization by the roasting procedure. The working conditions used, the temperature of the distilled water used to prepare the extracts, their non-sterile handling, but also the type of coffee used, are the factors that determined the microbial contamination. Since the sterilization of the extracts by filtration was efficient, after filtration no microbial colonies appeared on the culture medium (Fig. 3.B., left and right).

Table 2. Microbial load of coffee extracts

Code Extract	Microbial load (CFU / mL) before filtration, expressed as a mean of 3 counts + DS	Microbial load (CFU / mL) after filter sterilization
CA0	4+3,7	0
CR0	2+2,5	0
CA2	110+2,3	0
CR2	>3000	0

Legend: DS = standard deviation; UFC = no. Colony Forming Units



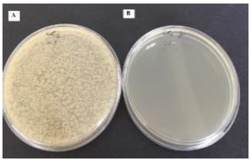


Fig.3. Extracts CA0 (left) and CR2 (right) before (A) and after (B) filtration

Green coffee beans are the final product after harvesting, washing and drying the coffee, and then roasted before grinding [Vega F. E, 2005]. Roasting seems to reduce the microbial load of CA0, CR0 coffee extracts, but does not completely eliminate it. Data from the literature indicate a 1/3 reduction in the level of contamination of roasted coffee beans compared to green coffee beans [Van der Stegen GHD, 2001].

In addition to abiotic factors, such as temperature, rainfall, cultivation practices or soil changes, microbial relationships [Berg G., 2006] can be governed by the microorganisms that make up communities during fruit ripening, fermentation and drying. At the beginning of the natural processing of coffee, simple sugars such as glucose, sucrose and fructose can be used, reducing the availability of sugars for other microorganisms. Other processes, such as cultivation practices, could have influenced the diversity of microorganisms. Environmental pollution, the use of agrochemicals and intensive soil cultivation have been shown to decrease the variety of endophytic bacteria [Mekete T, 2009].

Discussions

Endophytic bacteria are defined as those bacteria that can be found on the surface sterilized tissue of living or isolated plants inside the plant and that do not produce any apparently harmful effect on the plant [9]. They are ubiquitous in plant tissues and have been extracted from flowers, stems, fruits, seeds, leaves, roots and root nodules. Mekete et al. [Mekete T, 2009] identified a wide variety of different genera and species of endophytic bacteria from Ethiopian coffee roots. Pseudomonas has been reported as the predominant species. The next most predominant endophyte was Bacillus. Thus, it is known that a great diversity of microbes live in coffee plants, which can be essential for the influx of coffee plant microorganisms specific to the ecosystem.

Coffee also contains a great variety of compounds which could be responsible for its antimicrobial activity. Several natural constituents of coffee have shown antimicrobial activity, among them caffeine, phenolic acids and trigonelline; however, products formed during coffee processing also present antimicrobial activity like thermal degradation products (nicotinic acid) and also products from Maillard reaction (melanoidins, alpha-dicarbonyl compounds), among others [Almeida A. A. P, 2004]. Antimicrobial agents have been widely used in the treatment of infectious diseases and in the food and cosmetics industry as antibiotics and preservatives, to effectively control microbial contamination and its consequences on the health of living organisms and the quality of a product, although the use of these chemicals antibiotics has led to many ecological and medical difficulties, due to their continuous toxicity, hormonal imbalance caused in host organisms, carcinogenic and teratogenic effects, etc.

The pathogens of infectious diseases have become resistant to antibiotics, which is why it has become a priority and strategic research of plant extracts with antimicrobial activity, in order to discover new biologically active substances with antimicrobial action. The natural antimicrobial capacity of plant extracts has been the basis of many applications, including raw materials and the preservation of processed foods, pharmaceuticals, cosmetics, in alternative medicine and natural therapies [Andrews J. M., 2005].

Following the analysis performed by us, we can appreciate that for all samples represented by aqueous extracts of green coffee (CA2 and CR2) and roasted (CA0 and CR0) the need for sterilization was imposed before they can be analyzed for testing antimicrobial activity and before introduction into semi-solid formulations for topical applications.

Conclusion

Determining the degree of microbial contamination of aqueous extracts from green coffee and roasted coffee is an important analysis because this degree of contamination can come from several factors such as: harvesting, method of processing, storage, handling, type of plant product, species used , processing and obtaining a plant extract that involves certain conditions of production and handling. Thus, our attempts to highlight the degree of contamination of aqueous extracts from green and roasted coffee, led us to the need to filter the samples for decontamination, especially of highly contaminated CA2 and CR2 extracts. This determination will be useful for further analyzes such as: before testing antimicrobial activity, as well as before introduction into semi-solid formulations for topical applications.

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Spectrophotometric Studies of Indolic Compounds from *Vinca Minor* L.

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Abstract

The most medically representative plant in the Apocynaceae family is *Vinca minor*. *Vinca minor* is a perennial, herbaceous plant, commonly known as Saschiu. In the present research we aimed to isolate the indole compounds from *Vinca minor* L. and to carry out some physico-chemical studies on these compounds: UV-VIS spectroscopy and the determination of the polyphenols content using Folin-Ciocâlteu method. The spectrophotometric study of the alcoholic plant extracts obtained from the leaf and stem of *Vinca minor* was performed using the UV-VIS spectrophotometric method and a VWR UV-630PC double beam spectrophotometer. Both samples of plant alcoholic extracts obtained from the leaf and from the stem of *Vinca minor* L. had the specific absorption maxima detected in the range 225-350 nm and the absorbance maximum in both cases was 3.5 (u.a-absorbency units). The total content of polyphenols was determined by the Folin-Ciocâlteu method from alcoholic extracts of different concentrations: 40%, 70%, 96% (T40, T70, T96, F40, F70, F96) obtained from the leaf and stem of *Vinca minor* plant using the spectrophotometer model JASCO- 550 UV VIS. Regarding the alcoholic extracts obtained from the leaf of *Vinca minor* L., the concentrations of polyphenols were between 812.50 mg / 100g pv and 1737.50 mg / 100g pv and and in the case of alcoholic extracts obtained from the strain, the results were between 1525.00-3962.50 mg GAE / 100 g pv, results that were in accordance with the literature.

Keywords: Vinca minor, bioactive substances, UV-VIS spectrophotometric method, Folin-Ciocâlteu method

Introduction

In recent decades, new alternative therapies using drugs containing active principles of natural origin, have been a topical field of pharmaceutical research. One of the objectives of these lines of research was to obtain bioactive compounds from natural resources. Plants are the raw material from which various active principles can be extracted, which can be used in the treatment of various diseases. Indole chemistry is a fascinating and complex field. The foul-smelling indole molecule is the cornerstone of a wide variety of natural and chemical products. Moreover, some indole derivatives are vital pharmaceuticals or important intermediates. Indole ring compounds belong to the class of aromatic heterocyclic compounds with a heteroatom and the indole ring is the most widespread heterocycle. Other indole ring alkaloids of particular importance in medicine are Vinca alkaloids [Zimerman H.J., 1999]. Vinca alkaloids inhibit cell division by blocking mitosis, also inhibiting purine and RNA synthesis and ultimately causing the death of rapidly dividing cells. Vincristine and Vinblastine, two important indole alkaloids were isolated from *Vinca Rosea* L., and their extracts have antitumor activity. Vinorelbine is a semi-synthetic indole alkaloid derived from plant extracts from *Vinca minor* L. Vincristina was approved for use in cancer chemotherapy in 1963, Vinblastine in 1965, and Vinorelbine in 1994 [Bohannon R.A., 1963]. They have become major components of many combined anticancer regimens, used especially in the treatment of acute leukemia, Hodgkin's disease and other lymphomas, various sarcomas, Wilms tumor, neuroblastoma and breast and lung cancers. Vinca alkaloids are given intravenously, usually at intervals of one or two weeks in cycles with other agents. Vinca alkaloids are available in generic forms and under the trade names Oncovin (vincristine), Velban (vinblastine), Navelbine (vinorelbine) and Oxybral (vincamine) [DeLeve L.D., 2013].

Vincamine, another extremely important indole alkaloid in medicine, was isolated from *Vinca minor* L., found under the popular name of Saschiu. *Vinca minor* L. is a perennial, indigenous plant that grows spontaneously, found in our country in the alpine and subalpine area. To date, more than 50 indole alkaloids have been isolated from the aerial parts of this plant, some of which have quaternary structures such as 4-methylraucubaininium chloride, 4-methylstrictamine chloride and 4-methylacamycinium chloride. This perennial plant contains indole alkaloids of the monomeric eburnamine type, including vincamine [Vas A., 2005] which has modulatory effects on brain circulation. In folk medicine, vincamine is used internally to treat circulatory disorders, cerebral circulatory deficiencies and support for brain metabolism [La Gow B., 2004]. A large amount of clinical evidence indicates a favorable effect of vincamine in a number of conditions in elderly patients, such as memory disorders, vertigo, transient ischemic deficiencies and headaches. Vincamine increases cerebral blood flow, oxygen consumption, and glucose utilization [Blumenthal M. 1998]. At the skin level, vincamine contributes to the healing of bleeding ulcers, open wounds, bruises, contusions, by speeding up the healing process, [Fischhof P.K.,1996].

In the present paper the research hypothesis started from obtaining the indole compounds from *Vinca minor* L. found in abundance in our country. Starting from the extraction of these compounds of pharmaceutical interest, we analyzed the physico-chemical properties of indole compounds from the leaf and stem of plant *Vinca minor* plant. The general objective of this research is to isolate the indole compounds from the *Vinca minor* L. and to perform physico-chemical studies on these compounds: UV-VIS spectroscopy and determination of the total polyphenol content using the Folin-Ciocâlteu method. This paper proposes an original approach aimed at achieving the following scientific objectives:

Obtaining and carrying out a biotechnical process of extraction of indole compounds from the leaf and stem of the *Vinca minor* L. plant.

Characterization of the extraction products of *Vinca minor* L. by comparative physico-chemical studies, using different methods of spectrophotometric analysis (absorption in UV-VIS and determining the polyphenol content using the Folin-Ciocâlteu method.

Material and Methods

Plant materials

The plant material under study consists of the leaf and stem of *Vinca minor* plant, harvested locally, Dobrogea area, depending on their growing periods. The leaves and stems of the plant were selected manually and subjected to a treatment that consisted of repeated washings with drinking and distilled water. The plant material was dried in air, at ambient temperature, in the dark. After drying, the plant material was crushed into powder and stored in paper bags as needed.

Preparation of plant extracts

The dried and crushed plant material was extracted with ethyl alcohol: 40%, 70%, 96% alcoholic extractive solutions obtained from the leaf and stem of *Vinca minor* plant were prepared according to the 10th edition of the Roman Pharmacopoeia (F.R. X.). This process involved crushing 10 g of plant product from the leaf and stem of Vinca minor L. plant, to which was added ethyl alcohol of 40%, 70% and respectively 96% to 100 mL (Ratio 1:10). The extracts were left for 10 days in optimal conditions, away from light and moisture, in a cool place. During the 10 days, the extracts were carefully monitored and shaken 2-3 times a day. At the end, they were filtered using cotton cloth filters so that they could be separated from the plant material. The liquid collected on filtration was stored in sterile, dark containers.

Chemicals

All used reagents were of analytical reagent grade. Gallic acid, Folin – Ciocalteu reagent, metanol p.a. and 20% anhydrous sodium carbonate were purchased from Sigma-Aldrich, Germany. The solution of gallic acid (standard phenolic compound) 1×10^{-2} mol$\times$L^{-1} was prepared by dissolving 0.1881g of gallic acid in 100 mL ethanol. Folin – Ciocalteu reagent was diluted with distilled water 1:2 (V:V).

Work equipment

Usual laboratory utensils and glassware;

Analytical balance;

VWR UV-630PC spectrophotometer;

JASCO-550 UV VIS model spectrophotometer.

Spectrophotometric study of plant extracts obtained from the leaf and stem of the plant *Vinca minor* L.

The analysis were carried out in the laboratories of the Pharmacy Faculty of Ovidius University of Constanta. The spectrophotometric study of alcoholic plant extracts obtained from the leaf and stem of *Vinca minor* plant was performed using the UV-VIS spectrophotometric method. A VWR UV-630PC double beam spectrophotometer was used for this analysis. The measurements were performed at room temperature, and the spectral range studied was in the range of 200-400 nm, with specific absorption maxima detected in the range of 225-350 nm. For this analysis, a double-beam VWR UV-630PC spectrophotometer was used using halogen and deuterium sources for visible materials and ultraviolet radiation, at a controlled temperature, 25 ° C, using square quartz cuvettes with an optical trajectory of 1 cm. The determination was performed by applying the Bouguer-Lambert-Beer law, known by the linear dependence of the absorbance on the sample concentration. The measurements were performed in triplicate and the error associated with the concentration was the standard deviation obtained.

Study by IR spectroscopy of 70% alcoholic extracts obtained from the leaf of *Vinca minor* L. plant

To identify the chemical structure of indole compounds extracted from the leaf of *Vinca minor* L., FT-IR spectrophotometric analysis was used. By analogy with the spectra obtained by the UV-VIS method, the IR spectrum is the graphical representation of the percentage of energy absorbed (absorbance or transmittance) depending on the wavelength, in µm, or the frequency expressed in cm^{-1} (wavelength). The spectra were recorded using a FT-IR spectrophotometer model JASCO FT-IR 4200. Standard vincamine (0.5 g) was dissolved in 20 mL of methanol and diluted to 50 mL with NaOH 1M solution. The final solution was refluxed for 8 hours at 80 ° C, after which it was found that pure vincamine (VINC) was partially degraded into degradation products (RV).

Determination of total polyphenol content

In this study was identified and determined the total phenol content by Folin-Ciocâlteu method of alcoholic extracts of different concentrations: 40%, 70%, 96% (T40, T70, T96, F40, F70, F96) obtained from the leaf and stem of *Vinca minor*.

Folin-Ciocâlteu method

For the detection of a wide range of compounds with antioxidant action (polyphenols) from a wide variety of plant species, the simplest and most used method is the Folin-Ciocâlteu method. The basic mechanism of this method is represented by an oxidation / reduction reaction, in which the metal ion is reduced and the phenolic group is oxidized. By using this method the following classes of organic compounds can be identified: flavones, isoflavones, flavanones, flavonols, pro-anthocyanidins, anthocyanins and phenolic acids.

The total phenolic content was determined using the Folin-Ciocâlteu method [14-15]. In a 50 mL volumetric flask was introduced Vi into mL of the extracts prepared for analysis, 1 mL 1: 2 Folin-Ciocâlteu reagent, 1 mL 20% sodium carbonate solution. After homogenization, the sample was allowed to stand for 10 minutes, after which the contents of the sample were labeled with distilled water and left for 30 minutes at room temperature to stabilize the color. The absorbance was read using the Jasco 550 UV VIS spectrophotometer at 681 nm. To calculate the concentration of total phenols, expressed in mg of gallic acid (GAE) per 100 g of dried plant material, equation (1) was applied:

$$\boldsymbol{TPC}\ (\boldsymbol{mg\ GAE}/\mathbf{100}\boldsymbol{g}) = \frac{\boldsymbol{V_f} \times \boldsymbol{c} \times \mathbf{5}}{\boldsymbol{V_i} \times \boldsymbol{mprob}\text{ă}} \qquad (1)$$

Where, c represents the concentration (mg / L) read on the calibration curve, Vf is the volume of filtered alcoholic extract of each sample, Vi is the volume of sample added to the 50 mL rated flask for determination and the sample is the mass of dry plant material (leaf and stem) subjected to maceration.

The samples were prepared in triplicate and the average absorbance value was obtained. The total phenol content was reported as gallic acid equivalents by reference to the linear equation of the standard curve. Then, the total phenolic content was expressed as milligrams of gallic acid equivalent per 100 g of plant product (mg GAE / 100 g of plant product).

Results and Discussions

UV-VIS spectrophotometric study

The following figures (Fig. 2., Fig. 3., Fig. 4., Fig. 5.) show the chemical structures of indole alkaloids with major importance in the medical and pharmaceutical field. Through the UV-VIS spectrophotometric analysis performed by us, the structure of the indole compounds is confirmed [Neuss N., 1980].

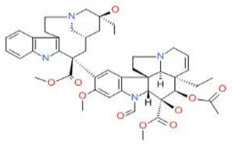


Fig. 2. The chemical structure of Vincristine

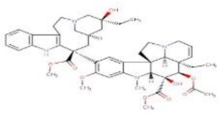


Fig. 3. The chemical structure of Vinblastine

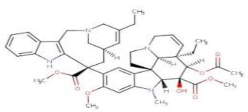


Fig. 4. The chemical structure of Vinorelbine

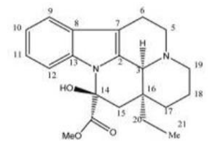


Fig. 5. The chemical structure of Vincamine

In Fig. 6. and Fig. 7. are representated the superimposed UV-VIS absorption spectra obtained for the samples of alcoholic extracts made from the leaf and stem of the *Vinca minor* plant.

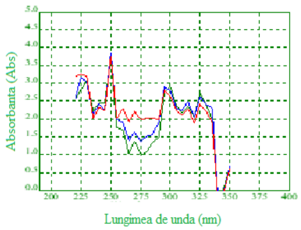


Fig. 6. Overlapping absorption spectra of alcoholic extracts of 40%, 70% and 96% concentration obtained from the leaf of the *Vinca minor* plant

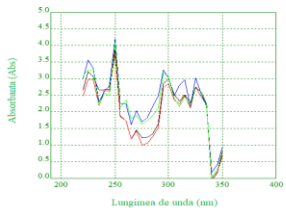


Fig. 7. Overlapping absorption spectra of alcoholic extracts of 40%, 70% and 96% concentration obtained from the stem of the *Vinca minor* plant

At the same time, we considered it important to readjust the absorption spectra on different concentration levels for the extracts from the leaf of *Vinca minor* plant. In

Figs. 8., 9. and 10. are represented the unique spectra for the samples of alcoholic extracts of concentration 40% (Fig. 8.), 70% (Fig. 9.) and 96% (Fig. 10.) obtained from the leaf of *the Vinca minor* plant. The spectra obtained in UV-VIS are consistent with the data from the literature [Wootton A., 2011].

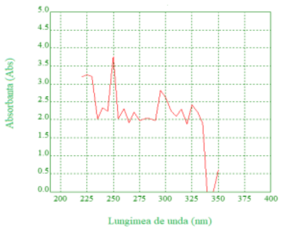


Fig. 8. Absorption spectrum of alcoholic extract with a concentration of 40% of the leaf of the Vinca minor plant

Fig. 9. Absorption spectrum of alcoholic extract with a concentration of 70% of the leaf of the Vinca minor plant

Fig. 10. Absorption spectrum of alcoholic extract with a concentration of 96% of the leaf of the Vinca minor plant

In the spectrophotometric study (UV-VIS) performed, the extracts obtained from the leaf and stem of the *Vinca minor* L. plant were analyzed. Absorbance was measured using VWR UV-6300PC spectrophotometer in the range of 200-400 nm. Both in the case of samples of plant alcoholic extracts obtained from the leaf and in the case of those obtained from the stem of *Vinca minor* L., the specific absorption maxima detected were in the range 225-350 nm and the absorbance maximum was in both cases 3.5 u.a. (units of absorbance). These results confirm the structure of indole compounds extracted from *Vinca minor* plant, [Amato A., 1983].

IR spectrophotometric study

The degradation process of pure vincamine (VINC) follows the scheme in Fig.1. These degradation products can be identified by spectrophotometric analysis in IR [S. M. Grujić,. 2015]. In Fig. 10. and 11. are represented the IR spectra for standard vincamine (VINC) and the degradation products of vincamine (RV). According to the structure of vincamine in Figs. 10., it is found that the transmittance registers peaks at wave numbers located in the range 3500 cm^{-1}–500 cm^{-1}, thus confirming the types of indole connections existing in *Vinca minor* plant.

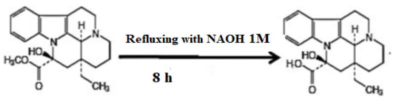


Fig. 1. Degradation scheme of Vincamine in degradation products

The peaks recorded at 3339.24 cm^{-1} - 319.42 cm^{-1} indicate the presence of primary amines. The IR spectrum of standard vincamine (VINC) shows a characteristic peak at 1680.38 cm^{-1}, which indicated the presence of the carbonyl group, existing in the structure of vincamine. In the IR spectrum of the degradation products of Fig. 11. appears slightly wider at 3411.88 cm^{-1}, indicating the presence of the hydroxyl group of a carboxylic acid (as a result of hydrolysis produced after degradation). The spectrum obtained is consistent with data from the literature [Ahmed A.B., 2016].

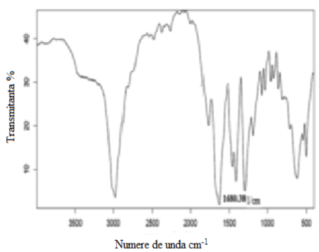


Fig. 10. IR spectrum for standard vincamine (VINC)

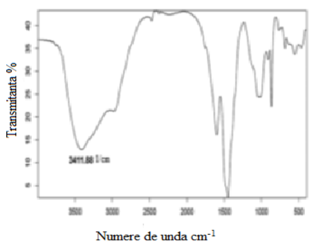


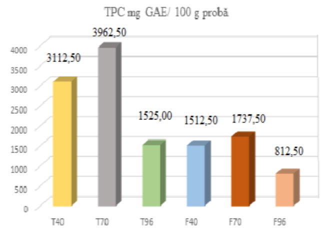
Fig. 11. IR spectrum for vincamine degradation (RV) products

Total phenolic content (TPC)

Spectral analysis was also used to determine the total polyphenol content in plant extracts of *Vinca Minor* L , by using the Folin-Ciocâlteu reagent method [9]. Plant samples were named as follows: T40, T70 and T96 – alcoholic extracts from *Vinca minor* L. plant stem. obtained in alcohol concentration 40%, 70%, respectively 96% and F40, F70, F96-alcoholic extracts from the leaf of *Vinca minor* obtained in alcohol concentration 40%, 70%, respectively 96%. Following the analysis, the following values were obtained for the total polyphenol content, for each extract analyzed, by applying the formula from equation (1) These values are represented in Table 1.

The total polyphenol content of alcoholic extracts obtained from the stem and leaf of *Vinca Minor* L. plant, between 812.50 mg / 100g pv and 3962.50 mg / 100g pv is in accordance with the literature [Nishibe S., 1996]. In the case of alcoholic extracts obtained from the stem of *Vinca minor* L., the highest concentration of total polyphenols was found in the alcoholic extract 70%., followed by the alcoholic extract 40% and the alcoholic extract 96%. Regarding the alcoholic extracts obtained from the leaf of *Vinca minor* L., the concentrations of polyphenols were between 812.50 mg / 100g pv and 1737.50 mg / 100g pv.

At the same time, it is observed that the extraction with 70% alcohol was the most efficient, both in the case of the leaf and in the case of the stem, where it results that ethyl alcohol in concentration of 70% represents the solvent with the highest extraction power of bioactive compounds.



Sample	TPC mg GAE/100 g pv
T40	3112,50
T70	3962,50
T96	1525,00
F40	1512,50
F70	1737,50
F96	812,50

Table 1. Concentration of total phenols (TPC) in ethanolic extracts obtained from the leaf and stem of *Vinca minor* L.

The results of the present study confirm the existence of a higher content of phenolic compounds in the alcoholic extracts obtained from the stem of *Vinca minor* L., which means that they showed a higher antioxidant activity.

Conclusion

Indole compounds are a valuable natural source containing numerous physical and chemical properties, associated with the source of the raw material of the analyzed samples but also with the methods of obtaining these compounds. These compounds have various fields of applicability: pharmacy, medicine, agriculture. The correct characterization of the physico-chemical properties represents a major role in establishing the field in which indole compounds can be applied. The UV-VIS and IR spectrophotometric studies performed in this research confirms the structure of indole compounds extracted from *Vinca minor* plant. Similary, the total polyphenol content of the vegetable alcoholic extracts obtained from the leaf and stem of *Vinca minor* plant was determined using the spectral method Folin-Ciocâlteu. The study carried out on the alcoholic plant extracts of *Vinca minor* plant, showed a high content of polyphenols both in the case of the stem and in the case of the leaf, a result that confirms their antioxidant action. The total concentrations of polyphenols separated by the Folin-Ciocâlteu method were higher in the case of alcoholic extracts of 70% concentration obtained both from the stem and from the leaf. We can say that ethyl alcohol in a concentration of 70% is the solvent with the highest extraction power of bioactive compounds. Following the analysis of the total concentration of polyphenols, it was found that there is a higher content of phenolic compounds in the alcoholic extracts obtained from the stem of *Vinca minor* plant than in the case of those obtained from the leaf.

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Studies on Chitosan, Chitin and Chitooligosaccharides and Their Biomedical Properties

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Abstract

Research in healthcare involves the use of natural resources in the manufacture of pharmaceutical products. Chito-oligosaccharides (CHOS) are described as homo- or heterooligomers of N-acetylglucosamine and D-glucosamine. As a starting material for CHOS production chitin or chitosan can be used. When it comes to the medical field, there are indications that CHOS may be developed and used as drugs against asthma, antibacterial agents, ingredients in wound-dressings and vectors in gene-therapy. The methods of production can rely on enzymatic conversions, chemical methods or combinations of these methods. There is ample literature concerning the biological effects of chitosans, but relatively little is known about the effects of CHOS, and the mechanisms behind observed bioactivities are generally poorly understood. This review is focused on the characterization of chitin and chitosan by presenting the biological properties, and on the enzymatic production of CHOS, and on further separation and purification methods for producing well-defined mixtures and also provides an overview of some of the most promising applications of CHOS.

Keywords: chitooligosaccharide (CHOS); chitosan; biological properties; biomedical uses; chitin

Introduction

A major interest in modern medicine is represented by the biometerials with marine origins. Among these, chitin and chitosan received special attention in the medical fields due to their unique properties.

Chitin and its deacetylated derivative, chitosan are natural polymers composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Both chitin and chitosan cannot be defined as a unique chemical structure, but as a family of polymers, due to their natural origin, and also present a high variability in their chemical and physical properties. This variability is related not only to the origin of the samples but also to their method of preparation. So, a complete characterization of the samples is necessary (Sirbu R., 2019).

Since the oligosaccharides have several interesting bioactivities, production of well-defined chito-oligosacharides (CHOS)-mixtures, or even pure CHOS, is of great interest. Understanding the mechanisms underlying these bioactivities is of major importance. It could be noted that some of the biological effects reported for chitosan in fact may be due to CHOS, which emerge when chitosan is degraded by naturally occurring hydrolytic enzymes (Berit B. Aam, 2010).

Many studies and research have been made concerning the biological effects of chitosans, but relatively little is known about the effects of chito-oligosaccharides (CHOS) (Kim S.-K., 2005). Initially it was thought that CHOS need to interact with proteins that either act on chitin (chitinases), or that are supposed to bind to chitin forms and detect the biopolymer (You Y., 2004).

In the pharmaceutical and medical fields, CHOS may be used and developed as ingredients in wound-dressings (Ribeiro M.P., 2009), drugs against asthma, antibacterial agents (Elias J.A., 2005). According to other research CHOS may also be used to reduce metastasis and tumor-growth in cancer, increase bone-strength in osteoporosis, could prevent malaria by inhibiting chitinases in *Plasmodium* parasites, CHOS help lower the levels of glucose in diabetics, had have immune modulatory effects and anti-fungal activities.

It is thought that the majority of the biological effects of chitosan are in fact due to CHOS. These effects emerge when chitosan is degraded by naturally occurring hydrolytic enzymes.

Characterization of Chitin, Chitosan and Chito-Oligosacharides (Chos)

Chitin is a natural polymer, a linear polysaccharide consisting of $\beta(1\rightarrow4)$ linked *N*-acetyl-*D*-glucosamine (Fig.1). After cellulose, chitin is the second most abundant polysaccharide in nature. It is present in the cell walls of fungi and yeasts and in the exoskeletons of insects and arthropods such crabs, lobsters and shrimps. In reference to the physico-chemical properties it can be noted that chitin is insoluble in water and exists mainly in two crystalline polymorphic forms, α and β. α-chitin is found in the exoskeleton of arthropods, in insects and in fungal and yeast cell walls. β-chitin occurs less frequently in nature than α-chitin, but can be extracted from squid pens (Mustafa A., 2015).

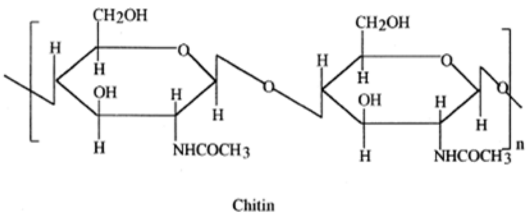

Fig.1. Structure of chitin

Chitosan is the natural derivative of chitin and it can be prepared by partial deacetylation, and is a heteropolymer of *β*(1→4) linked *N*-acetyl-*D*-glucosamine and D-glucosamine (Fig.2). In comparation with chitin, chitosan is soluble in dilute aqueous acid solutions. Chitosan can also be described as a continuum of soluble polymeric chitin derivatives. These polymeric chitin derivatives can be characterized according to the fraction of *N*-acetylated residues (F_A) or degree of *N*-acetylation (DA), the degree of polymerization (DP) or the molecular weight (M_W), the molecular weight distribution (PD, for poly-dispersity), and the pattern of *N*-acetylation (PA) or sequence (Bhatnagar, A., 2009). Due to its remarkable physic-chemical and biological properties such as non-toxicity, biocompatibility and biodegradability, chitosan is used in numerous applications and fields (pharmacy, medicine, agriculture, cosmetics, water treatment) (Kim S.-K., 2005; Vårum K.M., 2005).

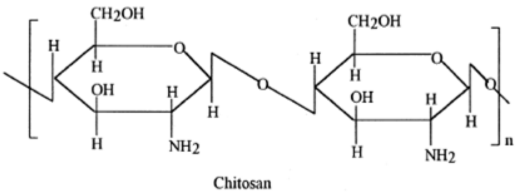

Fig.2. Structure of chitosan

Due to their natural origin, both chitin and chitosan are defined as a family of polymers which present a high variability in their chemical and biological properties such as biocompatibility, biodegradability, mucoadhesion, anticholesterolemic, antitumoral, hemostatic and antimicrobial effect (Sirbu R., 2019).

The main parameters that affect the polymer properties are DD (degree of deacetylation), Mw (molecular weight), polydispersity and crystalinity.

The purity (ash content), the moisture, the content of heavy metals, endotoxin and proteins must be determined for applications related to human consumption such as food and medical applications.

It has been demonstrated that the DD is one of the most important chemical characteristics, which could influence the performance of chitosan in many of its applications. The influence of average Mw on the viscosity development of aqueos solutions plays a significant role in the biochemical and biopharmacological significance of chitosan (Tharanathan R.N., 2003). Due to its low solubility chitin Mw is not easily determined. Table 1. shows the various methods for the determination of chitin and chitosan characteristics (Inmaculada Aranaz, 2009).

Table 1. Physicochemical Characteristics of Chitin and Chitosan and the Determination Methods

Physicochemical Characteristics	Determination Method
DD	Infrared Spectroscopy UV- spectrophotometry Nuclear magnetic resonance spectroscopy Potentiometric titration
Average Mw and/or Mw distribution	Viscosimetry Gel Permeation cromatography
Moisture content	Gravimetric analysis
Ash content	Gravimetric analysis
Protein	Bradford method

As is shown in Table.1 different result are obtained when using methods based on different principles. In present, even the best characterized chitosans available on the market are usually described olny with regard to their average degree of acetylation and their average degree of polymerization (DP), their ash content and the absence of contaminating bacteria (Moerschbacher B., 2007).

Apart from the specific characteristics, which are specific fosr each application, there is a degree of consensus regarding general characteristics that must be present in chitosan samples to be used in the field of biomedical applications: moisture content %, ash content %, protein content %, insolubility%, turbidity NTU units, DD %. For pharmaceutical applications, the chitosan requirements are: colour: white or slight yellow, particle size <0.3 mm, density 1.35-1.40 g/cm^3, pH 6.5-7.5 (Sirbu R., 2019).

Chitin and chitosan are currently receiving a great deal of interest in the medical and pharmaceutical applications due to their interesting properties that make them suitable for use in the biomedical field, such as biocompatibility, biodegradability and non toxicity. Other properties such as analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobian and antioxidant properties have also been reported. A better understanding of the mechanism of these properties makes it necessary for chitosan to be well characterized and purified from accompanying compounds (Muzzarelli R.A.A, 2005). In addition chitin and chitosans derivatized in a variety of fashions can be used to prove molecular hypothesis for the biological activity. The parameter with a higher effect is the DD, because the majority of the biological properties are related to the cationic behaviour of the chitosan. In some cases, the Mw has a predominant role. Beside the DD and Mw, other properties such as chain conformation, solubility or degree of substitution have been studied. Chitosans produced by heterogenous deacetylation, with a block arrangement of acetylated units, have a tendency to form aggregates in aqueous solutions. Table.2 shows the relationship between some chitin and chitosan biological properties and their physicochemical characteristics (Inmaculada Aranaz, 2009).

Table 2. The relationship between some chitin and chitosan biological properties and their physicochemical charateristics

Property	Characteristic
Biodegradability	DD, distribution of acetyl groups Mw
Biocompatibility	DD
Mucoadhesion	DD, Mw (only chitosan)
Hemostatic effect	DD, Mw
Analgesic effect	DD
Adsorbtion enhancer	DD (only chitosan)
Antimicrobian effect	Mw
Anticolesterolemic effect	DD, Mw, viscozity
Antioxidant effect	DD,Mw

Biological Properties of Chitosan
Biodegradability

Chitin and chitosan are absent from mammals but they can be degraded *in vivo* by several proteases (lysozyme, papain, pepsin). Their biodegradation leads to the release of non-toxic oligosaccharides of variable length which can be subsenquently incorporated to glycosaminoglycans and glycoproteins, to metabolic pathways or be excreted. A degradation role on chitin and chitosan seems to play a non-specific protease present in all mammalian tissues- lysozyme. The lengths of the chains (Mw) affects the degradation rate (Huang M., 2004). The understanding and control of the degradation rate of chitin and chitosan-based devices is of great interest since degradation is essential in many small and large molecule releaseapplications and in functional tissue regeneration applications. Ideally, the rate of scaffold degradation should mirror the rate of new tissue formation or be adequate for the controlled release of bioactive molecules (Mustafa A., 2016). Thus, it is important to understand and control both the mechanism and the rate by which each material is degraded. The degradation rate also affects the biocompatibility since very fast rates of degradation will produce an accumulation of the amino sugars and produce an inflammatory response.

Chitosan samples with low DD induce an acute inflammatory response while chitosan samples with high DD induce a minimal response due to the low degradation rate. Degradation has been shown to increase as DD decreases. Kofuji *et al.* (2005) investigated the enzymatic behaviours of various chitosans by observing changes in the viscosity of chitosan solution in the presence of lysozyme (Kofuji K., 2005). They found that chitosan with a low DD tended to be degraded more rapidly. It can be concluded that it is impossible to estimate biodegradation rate from the DD alone.

Biocompatibility

Both chitin and chitosan show very good compatibility but this property depends on the characteristics of the sample (natural source, method of preparation, Mw and DD). Due to its higher versatility and biological properties the majority of the assays have been carried out on chitosan samples. Although the gastrointestinal enzymes can partially degrade both chitin and chitosan, when both polymers are orally administered they are not absorbed. For this reason, they are considered as not bioavailable. Toxicity of chitosan is reported to depend on DD. It was reported that chitosans with DD higher than 35% showed low toxicity, while a DD under 35% caused dose dependant toxicity. On the other hand, Mw of chitosan did not influence toxicity. Residual proteins in chitin and chitosan could cause allergic reactions such as hypersensitivity. The protein content in a sample depends on the source of the sample and, especially, on the method of preparation (Mustafa A., 2015).

Hemostatic effect

Chitosan presents anticoagulant activity tested *in vitro* (Park P.J, 2004). The anticoagulant activity of chitosan seems to be related to its positive charge since red blood cells' membranes are negatively charged and chitin is less effective than chitosan. The hemostatic effect of chitosan is not related to the clasic coaulation pathways, but it can promote platelet agregation. The blood platelets play a very important role in the coagulation process and can lead to hemostatis and thrombosis. Besides platelets and erythrocytes, chitosan also accelerates thrombin generation.

Analgezic effect

It was reported that both chitin and chitosan show analgesic effect. The analgesic effect of these biopolymers on inflammatory pain has been studied due to intraperitoneal administration of acetic acid. Chitosan showed a greater effect than chitin. This difference is due to the different action mechanism of the two polymers. It was demonstrated that the main analgesic effect of chitosan is the absorption of proton ions released in the inflammatory area (Inmaculada Aranaz, 2009).

Antitumor activity

An antitumor activity of chitosan has been claimed by inhibition of the growth of tumor cells mainly due to an immunostimulation effect. chitosan oligomers possess antitumor activities tested both *in vitro* and *in vivo* (Jeon Y.J, 2002).

Studies carried out using mice that had ingested low-Mw chitosan revealed significant antimetastatic effects of chitosan against Lewis lung carcinoma. Partially deacetylated chitin as well as chitin with a carboxymethyl group have also been effective to demote tumor progression. The suggested mechanism involves immunostimulating effects of chitin and its carboxymethyl derivatives via stimulation of cytolytic T-lymphocytes. This activity increases with smaller molecular sizes and it is suggested that they have immunostimulating effects that activate peritoneal macrophages and stimulate non-specific host resistance. However, higher Mw oligomers have also exhibited antitumor activity. The effect of chitosan on tumor growth and metastasis was studied. The activation of macrophages by chitosan is suggested to mediate its antitumor effects *in vivo*, while its angiogenic inducing properties may be the harmful effects of chitosan, such as promotion of tumor growth and invasion (Ueno H., 2001).

Anticholesterolemic effect

There are several proposed mechanisms for cholesterol reduction by chitosan. The entrapment caused by a viscous polysaccharide solution is thought to reduce the absorption of fat and cholesterol in the diet. On the other hand,the presence of the amino group in its structure determines the electrostatic force between chitosan and anion substances, such as fatty acids and bile acids. The interaction between chitosan and anionic surfaceactive materials (phospholipids, bile acids) depends on its three types of reactive functional groups: the amino group at the C2 position and primary and secondary hydroxyl groups at the C-3 and C-6 positions, respectively. Although great effort has been made to find a correlation between the physicochemical characteristics of chitosan and its fat-binding capacity, only some significant relationships have been demonstrated (Inmaculada Aranaz, 2009).

Antimicrobial activity

The antimicrobial activity of chitin, chitosan, and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years (Sirbu R., 2019). Two main mechanisms have been suggested as the cause of the inhibition of microbial cells bychitosan. The first mechanism refers to the interaction with anionic groups on the cell surface. Due to its polycationic nature, it causes the formation of an impermeable layer around the cell, which prevents the transport of essential solutes. Electron microscopy demostrated that the site of action is the outer membrane of gram negative bacteria. The permeabilizing effect has been observed at slightly acidic conditions in which chitosan is protonated, but this permeabilizing effect of chitosan is reversible (Helander I., 2001). The second mechanism involves the inhibition of the RNA and protein synthesis by permeation into the cell nucleus. In this case the Mw is the decisive property (Liu X., 2001). Other mechanisms have also been proposed. Chitosan may inhibit microbial growth by acting as a chelating agent rendering metals, trace elements or essential nutrients unavailable for the organism to grow at the normal rate. Chitosan is also able to interact with flocculate proteins, but this action is highly pH-dependent.

Antioxidative activity

Chitosan has shown a significant scavenging capacity against different radical species, the results being comparable to those obtained with commercial antioxidants. Samples prepared from crab shell chitin with DD of 90, 75 and 50% where evaluated on the basis of their abilities to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical, superoxide radical and alkyl radical. The results revealed that chitosan with higher DD exhibited the highest scavenging activity (Park P.J., 2004). Chitosans of different size as well as their sulphate derivatives were assayed against superoxide and hydroxyl radicals. A negative correlation was found between chitosan Mw and activity. The chitosan sulphated derivatives presented a stronger scavenging effect on peroxide radicals but the chitosan of lowest Mw showed more considerable ferrous ion-chelating potency than others (Xing R., 2005). The chelation of metal ions is one of the reasons why chitosan may be considered as a potential natural antioxidant for stabilizing lipid containing foods to prolong shelf life. Chitosans may retard lipid oxidation by chelating ferrous ions present in the system, thus eliminating their prooxidant activity or their conversion to ferric ion.

Chito-oligosaccharides (CHOS) are oligomers prepared from chitosan by chemical or enzymatical methods. CHOS can be obtained starting from chitosan by acid hydrolysis or by enzymatic hydrolysis using chitinases or chitosanases. In result, the F_A, M_W, PD and P_A of the resulting CHOS-mixture depend on the chitosan and the specificity of the enzyme used. It can be necessary, for certain compounds to enrich the product mixtures, and for this process the chitosan-enzyme combination is optimized (Berit B. Aam, 2010).

A recent statistic estimated the annual production of chitin in nature to be approximately 1010–1011 tons per year (Berit B. Aam, 2010). It was calculated that in 2007, the amounts of chitin, chitosan and their derivatives that are used and produced in industrial processes (Sannan T. Kurita, 1976) have been estimated to be about 30 000 metric tons for chitin and about 10,000 metric tons for chitosan. It was suggested that it is a possibility to isolate chitosan directly from the cell walls of certain fungi, but chitosans that are commercially available are usually prepared from chitin by a heterogeneous deacetylation process (Sandford P.A., 2002).

There are two fundamentally different methods to prepare chitosans from chitin: homogeneous and heterogeneous deacetylation (Sannan T. Kurita, 1976). The homogeneous deacetylation process requires the chitin to be dissolved in an alkali solution during the deacetylation process (this needs low temperature and excessive stirring). The heterogeneous deacetylation process is a actually a two-phase process, in which the chitin is kept insoluble in a hot alkali solution. In order to facilitate the depolymerization to take place, some specific enzymes that act on chitin and chitosan are required: *Chitinases and chitosanases*, *Human chitinases* and *Lysozyme.*

Chito-Oligosacharides (Chos) Production

The production of CHOS is made by two methods: enzymatic and chemical.

Enzymatic methods

The research of this methods is poor in concrete results and cannot yet be considered an alternative to the chemical methods that are used in the present. In theory, chitin deacetylases could be used to produce chitosan (Tokuyasu K., 2000; Tsigos I., 2000) by hydrolyzing the N-acetyl linkage and convert chitin to chitosan, but the insolubility and crystallinity of the chitin substrate makes it very difficult. Recent studies with chitinases have related that kinetics of the degradation reactions change considerably during the hydrolysis process. The enzymes that are used for this processes have very different binding affinities for different sequences on the substrate, leading to reactions that present multiphasic kinetics, resulting very different product mixtures. The other important issue to take into hand is processivity, because the degradation process may change during a reaction. It can stat from processive hydrolysis of polymeric chains and lead to non-processive hydrolysis of intermediate products due to the exhaustion of the polymeric material. We can conclude that the outcome of the enzymatic conversion can be influenced by choosing the right starting chitosan, the enzyme and the processing time (Sikorski P., 2005).

Chemical Methods

2.1. Acid Hydrolysis of Chitosan

One of the best known chemical methods for hydrolysis of chitosan is acid hydrolysis (Einbu A., 2007). The first studies on acid hydrolysis of chitosans had demonstrated that it is possible to convert fully deacetylated chitosan to CHOS in concentrated hydrochloric acid (Berit B. Aam, 2010). In later studies (Einbu A., 2007), using a variety of chitosans, the acid-catalyzed degradation rates of chitosans were shown to depend on F_A (fraction of *N*-acetylated residues), and the initial degradation rate constant was found to increase in direct proportion to F_A.

2.2. Chemical Synthesis of Chito-Oligosacharides (Chos)

The chemical synthesis of CHOS does not represent a frequent procedure in the present days, because it requires multiple protection and deprotection steps. Although chemical synthesis of CHOS results in pure compounds this method is time consuming and requires extensive use of organic solvents. In the speciality literature there are only a few examples of chemically synthesized CHOS. One example is that Kuyama *et al*. performed synthesis of fully deacetylated chitosan dodecamers starting with glucosamine monomers using phthalimido as the amino protective group.

Another one, Aly *et al*. reported a method for synthesis of fully *N*-acetylated CHOS from GlcN monomers using dimethylmaleoyl as an amino protective group for synthesis of chitotetraose and chitohexaose. The removal of the amino protective group and *N*-acetylation was performed in a one-pot reaction in order to result in the desired products (Berit B. Aam, 2010). In theory it can be a possibility to combine the two described protection methods in order to synthesize partly deacetylated CHOS, but this has so far not been reported in the scientific literature.

Chito-oligosacharides (CHOS) purification

It is known that enzymatically or chemically produced CHOS is usually composed of a mixture of oligomers differing in DP (degree of polymerization), F_A (fraction of *N*-acetylated residues) and P_A (pattern of *N*-acetylation). Over the years, a multitude of techniques for separation and purification of CHOS have been reported, such as: gel-filtration, ultra-filtration, and ion exchange and metal affinity (Berit B. Aam, 2010) chromatography. In almost all cases is preferred to use a combination of these techniques in order to obtain homogenous CHOS fractions. All in all, as shown above, the production of pure CHOS fractions is generally a time consuming, expensive and challenging task.

Pharmaceutical and biomedical properties of CHOS

As shown above, due to its important physico-chemical properties and based on the fact that

it is a natural biopolymer, CHOS have a remarkable wide range of pharmaceutical and biomedical properties and activities.

Applications of chitosan/CHOS in wound-dressings

There are a multitude of studies that had very promissing and positive effects of chitosan used in wound-dressing (Mustafa A., 2015). Other studies developed in parallel on the same actions of CHOS had also very positive effects, in fact it was shown that the use of CHOS accelerates the wound healing process (You Y., 2004). In contact with the wound chitosan is likely to convert into CHOS by the action the the naturally occurring enzymes. This wound-healing effect that was presented initially for chitosan can be in fact the effect of CHOS converted by enzymes. The resulting conclusion is that the use of CHOS directly is more advantageous in wound dressing than chitosan, because of the more immediate effect.

Recent studies showed that hemostatic effects may also contribute to the beneficial effects of chitosan/CHOS in wound dressings. It was demonstrated that chitosan enhances platelet adhesion and aggregation and increases the release of the platelet derived growth factor-AB (PDGF-AB) and the transforming growth factor-β1 (TGF-β1) from platelets in canine blood (Berit B. Aam, 2010). These two factors retract inflammatory cells which are important in wound healing.

Tumor growth inhibition

In the 1970s it has been demonstrated that CHOS have anti-tumor effects, and reduce metastasis from tumors (Mustafa A., 2016). Initially it was believed that the anti-tumor activity was due to the cationic properties of CHOS, and later, the M_W was discovered to play a major role. Two researchers, Maeda and Kimura (Berit B. Aam, 2010) found that CHOS enhanced the natural killer activity in intestinal intraepithelial lymphocytes at the same time as they reduced tumor growth in mice, and suggested that this CHOS-activation of intestinal immune functions could be useful in treating tumors.

In recent years, the hypothesis that the anti-tumor effects of CHOS are related to their inhibitory effects on angiogenesis has received some attention. Angiogenesis is the formation of new capillary blood vessels from already existing blood vessels. This process is important for tumor formation, since tumor growth and metastasis require angiogenesis when the tumor reaches a certain size.

Asthma

AMCase is induced during T_H2 inflammation through an interleukin (IL)-13 dependent mechanism. This was shown to be over-expressed in human asthmatic tissue (Elias J.A., 2005). Inhibition of the AMCase with the chitinase inhibitor reduced the inflammation (Berit B. Aam, 2010; Mustafa A., 2015). The fact that chitinases are a factor in host antiparasite responses and in asthmatic T_H2 inflammation led to the hypothesis that asthma may be a parasite-independent antiparasite response (Elias J.A., 2005). In some studies it has been suggested that partially deacetylated CHOS can function as inhibitors of family 18 chitinases. So, we can conclude that CHOS have a great potential in use as an anti-inflammatory drug in patients with asthma.

Increased bone strength

The bone marrow is formed from the mesenchymal stem cells that are able to differentiate into chondrocytes, adipocytes and osteoblasts. Bone-tissue is mainly formed from bone matrix and osteoblasts (Mustafa A., 2015; Mustafa A., 2016). CHOS and chitosan are able to increase the differentiation of mesenchymal stem cells to osteoblasts and to facilitate the formation of bone-tissue. CHOS can increase calcium depositation in bone, because it is known that the mineralization process and bone strength are dependent on Ca^{2+} (Berit B. Aam, 2010).

Conclusion

Chitin, chitosan and CHOS have a wide spectrum of possible bioactivities, due to their remarkable properties. Although they are very versatile polymers and have promising bioactivities, the research must be continued and completed with well-defined CHOS preparations. Recent techniques for further purification of CHOS and for sequence determination are available, but are still quite challenging to exploit. Another challenge in the process of purification of CHOS is to find an economically acceptable solution. From this point of view, in the present it is cheaper to produce CHOS mixtures that require enriching for a specific bioactivity, rather than producing a pure compound form the beginning.

The vision for the future in medical health of these studies and researches is to successfully convert the abundant bio-resource that is chitin into CHOS-based medicines and pharmaceutical forms.

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