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& ΤΗΣ ΕΛΛΗΝΙΚΗΣ ΦΑΡΜΑΚΕΥΤΙΚΗΣ
ΕΤΑΙΡΕΙΑΣ

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NOBEL PRIZE 2018.

Discovery of Cancer Therapy by Inhibition of Negative Immune Regulation.

New class of drugs for activated immune system as an effective strategy for harnessing attacks on cancerous cells

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KEYWORDS:
Malignant neoplasm; cancer; anticancer therapy; immune system; T cells; braking mechanisms

SUMMARY
Malignant neoplasm (commonly called cancer) comprises various types of cancerous cells which are characterized by accumulation of DNA mutations and uncontrolled proliferation of abnormal cells spreading to healthy organs and tissues. In the last decades many different anticancer therapeutic approaches were developed, including surgery, radiation, and anticancer drug strategies. Some of these discoveries earned the Nobel Prize to the research scientists. Huggins earned a Nobel Prize (1966) for the discovery of hormonal treatment of prostatic cancer, discovery of chemotherapy (Nobel Prize, Elion and Hitchins, 1988), and Thomas ED, earned a Nobel Prize in 1990 for bone marrow transplantation as a successful treatment for leukemia and other blood conditions. In 2018 the Nobel Prize in Physiology or Medicine was awarded jointly to researchers-professors James P. Allison (USA) and Tasuku Honjo (Japan) "for their discovery of cancer therapy by inhibition of negative immune regulation." The discovery is transforming cancer treatments and has led to a new class of drugs that work by switching off the braking mechanism, prompting the immune cells to attack cancer cells. Professor Allison JP (immunology at University of Texas, USA) with his studies realised the potential of harnessing the immune system to destroy cancer cells. But cancer cells have sophisticated ways to hide from immune attacks by unleashing a braking mechanism (various proteins that function as brakes on the T cells, inhibiting immune activation). Professor Honjo T (immunology at Kyoto University, Japan), working in the same field discovered a different protein on immune cells that also appeared to operate as a brake, but with a different mechanism of action. These anticancer drugs have significant side-effects, but have been shown to be effective – including, in some cases, against late-stage cancers that were previ-
1. Introduction

The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to professors James P. Allison and Tasuku Honjo “for their discovery of cancer therapy by inhibition of negative immune regulation.”  

On the 1st of October 2018 the Nobel assembly at the Karolinska Institute in Stockholm for the Nobel Prize in Physiology or Medicine announced that an American and a Japanese scientist have won the 2018 Nobel Prize for their discovery of a revolutionary approach to cancer treatment. The two scientists have been awarded the prize for their discovery that the body’s immune system can be harnessed to attack cancer cells. The human immune system under normal physiological conditions seeks out and destroys mutated cells, but cancer cells find sophisticated ways to hide from immune attacks, allowing them to thrive and grow. Many types of cancer do this by ramping up a braking mechanism that keeps immune cells in check.1

The discovery came to fruition after 20 years of research. The practical applications of the methodology transformed cancer treatment and has led to a new class of drugs that work by switching off the braking mechanism, prompting the immune system to attack cancer cells. The drugs have significant side-effects, but have been shown to be effective, including, in some cases, against late-stage cancers that were previously untreatable. The human immune system is critical in fighting inflammation and cancer, because it has been designed to recognize native and non-native cells. The immune system is an efficient biological machine that protects the human body from germs and fights off viruses and infections and in turn may cause fevers, pains, inflammation and swelling.2

The teams of researchers on the subject of fighting cancer through the immune system were numerous, but the two scientists awarded the Nobel Prize (2018) were prominent in the discovery of the new therapeutic methodology. Professor Allison (professor and chair of immunology at the University of Texas’s MD Anderson Cancer Center, USA) investigated in the 1990s a known protein that functions as a brake on the immune system. He realised the potential of releasing the brake and thereby unleashing the human immune cells to attack cancerous tumours. He then developed new understanding on the biology of immune T cells that can travel in human bodies and attack cancer cells. Meanwhile,
Professor Honjo (immunology department at Kyoto University, Japan), discovered a different protein on immune cells that also appeared to operate as a brake to the immune system attacking tumours, but with a different mechanism of action.\(^3\)

2. Activation of the immune system. New therapeutic approaches to cancer treatment

Cancer (malignancy) comprises many different diseases (there are more than 200 types of cancer), all characterized by uncontrolled proliferation of abnormal cells with capacity for spread to healthy organs and tissues. In the last decades a number of therapeutic approaches are available for cancer treatment, including surgery, radiation, chemotherapy and other strategies, some of which have been awarded previous Nobel prizes. These include methods for hormone treatment for prostate cancer (Huggins, 1966), chemotherapy (Elion and Hitchins, 1988), and bone marrow transplantation for leukemia (Thomas 1990). However, advanced cancer remains immensely difficult to treat, and novel therapeutic strategies are desperately needed.\(^4\)–\(^8\)

In the 1990s researchers investigating cancer mechanisms and therapeutic means, approached the concept that activation of the immune system might be a strategy for attacking and destroying tumour cells. Attempts were made to infect patients with bacteria to activate the defense. These efforts only had modest effects, but a variant of this strategy is used today in the treatment of bladder cancer. It was realized that more knowledge was needed. Many scientists engaged in intense basic research and uncovered fundamental mechanisms regulating immunity and also showed how the immune system

---

**Figure 2.** Immune checkpoints are signaling pathways responsible for downregulating the immune response to avoid destruction of endogenous targets, and tempering the peripheral immune response. During typical interaction of T cells with antigens, complex cytokine signaling provides the immune system confirmation of correctly targeting only non-self antigens, thereby preventing a state of auto-immunity. [antigen presenting cells = APC], T-lymphocyte-associated protein-4 = CTLA-4); T-cell receptor = TCR; Programmed cell death-1 = PD-1; Programmed cell death ligand-1, PD L-1].
can recognize cancer cells. Despite remarkable scientific progress, attempts to develop generalizable new strategies against cancer proved difficult.9,10

The use of immunotherapy for cancer has become widespread as a therapeutic method in recent decades and is used to treat both solid and hematological malignancies. Immune checkpoint inhibitors, in particular, have demonstrated considerable promise in their recent approval for the treatment of melanoma, non-small cell lung cancer, and other cancers.11,12

These new anticancer drugs have been approved by the Food and Drug Administration (FDA, USA). FDA approved in 2011 the therapeutic antibody drug ipilimumab (trade name Yervoy® Bristol-Myers Squibb Co) which is a monoclonal antibody that works to activate the immune system by targeting CTLA-4 (T-lymphocyte-associated protein), a protein receptor that downregulates the immune system. It is used for the treatment of melanoma, a type of skin cancer. As also, is undergoing clinical trials for the treatment of non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer and metastatic hormone-refractory prostate cancer. The concept of using anti-CTLA-4 antibodies to treat cancer was first developed by James P. Allison.13,14

Also, in 2014 FDA approved PD-1 inhibitor pembrolizumab (trade name Keytruda®, Merck Sharp & Dohme Corp.). It is a humanized antibody used in cancer immunotherapy. It is an IgG4 isotype antibody that blocks a protective mechanism of cancer cells, and allows the immune system to destroy those cancer cells. It targets the programmed cell death 1 (PD-1) receptor of lymphocytes. The FDA initially approved it to treat metastatic melanoma. In 2017 the FDA approved it for any unresectable or metastatic solid tumor with certain genetic anomalies (mismatch repair deficiency or microsatellite instability). It was the first time the FDA had approved a cancer drug based on tumour genetics rather than tissue type or tumour site.15

Programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) axis stands out as a valuable therapeutic target because it not only plays a key role in physiological immune homoeostasis, but

Figure 3. The immune response depends on two types of lymphocytes: B lymphocytes, or B cells, and T lymphocytes, or T cells. Both types of lymphocytes are produced in the red bone marrow. Both B and T cells can recognize and respond to specific pathogens. On the right: scanning electron micrograph of a human T cell.
also appears to be a means through which cancer cells evade the immune system. The introduction of PD-1 or PD-L1 inhibitors into clinical practice has had a revolutionary effect on cancer treatment, but favourable long-term outcomes are only observed in a fraction of patients. Also, immunotherapy is associated with several immune-related adverse events and has an estimated cost of more than $300,000 per quality adjusted life year.16

Meanwhile, with more than a dozen new molecules identified to be involved with signaling at the interface of T cells, the discovery of many more therapeutic leads are anticipated. Combination therapy comprised of multiple checkpoint inhibitors.17

3. What are immune checkpoints. Accelerators and brakes in human immune system

The human immune system has the fundamental ability to discriminate "self" molecules from "non-self" molecules so that invading bacteria, viruses and other dangers can be attacked and eliminated. Self-nonself discrimination is achieved by both central thymic selection and peripheral immune regulation. T cells, a type of white blood cell, are key players in this defense. T cells were shown to have receptors that bind to structures recognized as non-self and such interactions trigger the immune system to engage in defense. But additional proteins acting as T cell accelerators are also required to trigger a full-blown immune response. Many scientists contributed to this important basic research and identified other proteins that function as brakes on the T cells, inhibiting immune activation. This intricate balance between accelerators and brakes is essential for tight control. It ensures that the immune system is sufficiently engaged in attack against foreign microorganisms while avoiding the excessive activation that can lead to autoimmune destruction of healthy cells and tissues.18

Immune checkpoints refer to a plethora of inhibitory pathways hardwired into the immune system that are crucial for maintaining self-tolerance and modulating the duration and amplitude of physiological immune responses in peripheral tissues in order to minimize collateral tissue damage. Scientists understand now why malignant tumours co-opt certain immune-checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumour antigens. Because many of the immune checkpoints are initiated by ligand–receptor interactions, they can be readily blocked by antibodies or modulated by recombinant forms of ligands or receptors. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) antibodies were the first of this class of immunotherapeutics to achieve the Food and Drug Administration (FDA, USA) approval. Preliminary clinical findings with blockers of additional immune-checkpoint proteins, such as programmed cell death protein 1 (PD-1), indicate broad and diverse opportunities to enhance antitumour immunity. Knowledge of the importance on checkpoints and accelerators on harnessing the T cell response of the immune system lead scientists to the innovation on cancer immunotherapy.19-21

4. A new principle for immune therapy of cancer

During the 1990s, Professor Allison JP (immunology laboratory, University of California, Berkeley, CA, USA) did some fundamental research on the function of the T cell protein CTLA-4 (T-lymphocyte-associated protein-4). He was one of several scientists who had made the observation that CTLA-4 functions as a brake on T cells in their fight to harness cancerous tumours. CTLA-4 is a well-known regulator of T cell activation. Although originally cloned from a CD8 CTL library, it was subsequently shown to negatively regulate CD4+ T cell expansion by recruitment of phosphatases to the TCR signaling complex (T cell receptor). Because of this, the requirement of CTLA-4 for adaptive tolerance has been examined in a number of experiments.22,23

Other research teams exploited the mechanism as a target in the treatment of autoimmune disease. Professor Allison, however, had an entirely different idea. He had already developed an antibody that could bind to CTLA-4 and block its function. The next phase of his research focused to investigate if CTLA-4 blockade could disengage the T cell brake and unleash the im-
mune system to attack tumour cells. Prof. Allison and co-workers performed advanced experiments in experimental animals (mice, 1994), and in their excitement the results were spectacular. Mice with cancer had been cured by treatment with the antibodies that inhibit the brake and unlock antitumour T cell activity. But despite the importance of these therapeutic results the pharmaceutical industries (some of the big pharma have headquarters in the USA) showed little interest to explore the innovative anticancer potential. Prof. Allison continued his intense efforts to develop the immunology strategy into a therapy of some difficult to treat solid and hematological cancers. Promising results soon emerged from several groups, and in 2010 an important clinical study showed striking effects in patients with advanced melanoma, a type of skin cancer (by the drug ipilimumab). In several patients signs of remaining cancer disappeared. Such remarkable results had never been seen before in cancer patient groups.24-27

A few years after the discovery of Prof. Allison for the role of immune system in fighting cancer, Prof. Tasuku Honjo and co-researchers discovered (1992) another protein PD-1 which functions in a similar way blocking the anticancer function of T cells. The immunoregulatory roles of PD-1 are responsible for limiting excessive T cell activation to prevent immune-mediated tissue damage. Programmed death-1 (PD-1) is a cell surface molecule that regulates the adaptive immune response. Engagement of PD-1 by its ligands PD-L1 or PD-L2 transduces a signal that inhibits T cell proliferation, cytokine production, and cytolytic function.28,29

Prof. Honjo and his co-workers were determined to unravel PD-1 role. So, they meticulously explored its function in a series of elegant experiments performed over many years in his laboratory at Kyoto University. The results showed that PD-1, similar to CTLA-4, functions as a T cell brake, but operates by a different mechanism. The research laboratory of Honjo performed animal experiments and established that PD-1 blockade was also shown to be a promising strategy in the fight against cancer; the same results were demonstrated by other research groups. This paved the way for utilizing PD-1 as a target in the treatment of cancer patients. Clinical development ensued, and in 2012 a key study demonstrated clear efficacy in advanced melanoma, a type of skin cancer. In several patients signs of remaining cancer disappeared. Such remarkable results had never been seen before in this patient group. The anticancer results were dramatic, leading to long term remission and possible cure in several cancer patients with metastatic cancer, which was considered essentially untreatable. Scientists concluded that “Anti–PD-1 antibody produced objective responses in approximately one in four to one in five patients with non–small-cell lung cancer, melanoma, or renal-cell cancer; the adverse-event profile does not appear to preclude its use. Preliminary data suggest a relationship between PD-L1 expression on tumor cells and objective response. (Funded by Bristol-Myers Squibb and others; ClinicalTrials.gov number, NCT00730639.).30

A large number of recent scientific papers focused on the efficiency and cancer immunotherapies targeting the PD-1 signaling pathway. All scientific reports put emphasis on immunotherapy that has moved to the center stage of cancer treatment with the recent success of trials in solid tumours.31-34

In the last decade anticancer research is focused on understanding the impact that targeted and conventional cancer therapies (chemotherapy and radiation) have on the generation of an antitumour immune response. Protective tumour immunity is thought to require innate immune stimulation, a robust cytotoxic T cell response and overcoming an immunosuppressive tumour microenvironment. The most important future challenges for fighting human cancers are to develop rational combinations, to understand the impact that all cancer therapeutics have on patients’ immune systems, optimizing the therapeutic window of treatment through appropriate dosing and temporal sequencing, and prioritizing the rapidly growing number of combination therapy trials.35

5. Conclusion

Immune checkpoint therapy for cancer today and
in the future will advance with new discoveries and it can be proved to be the most effective therapeutic methodology in combination with conventional therapies. After the initial studies showing the effects of CTLA-4 and PD-1 blockade, the clinical development has been dramatic. Anticancer specialists now know that the treatment, often referred to as “immune checkpoint therapy”, has fundamentally changed the outcome for certain groups of patients with advanced cancer. It must be clear that these methods have adverse side effects which can be serious and even life threatening. They are caused by an overactive immune response leading to autoimmune reactions, but are usually manageable. Many research groups worldwide continue to investigate and elucidate mechanisms of action, with the aim of improving therapies and reducing side effects. Of the two treatment strategies, checkpoint therapy against PD-1 has proven more effective and positive results are being observed in several types of cancer, including lung cancer, renal cancer, lymphoma and melanoma. New clinical studies indicate that combination therapy, targeting both CTLA-4 and PD-1, can be even more effective, as demonstrated in patients with melanoma. The initial discoveries of professors Allison and Honjo have inspired other research efforts to combine different strategies to release the brakes on the immune system with the aim of eliminating tumour cells even more efficiently. For more than 100 years cancer specialists attempted to engage the immune system (especially T cells) in the fight against cancer but with modest results. The seminal discoveries of recent years by many research groups made progress into clinical development of new drugs a reality. Checkpoint therapy has now revolutionized cancer treatment and has fundamentally changed the way we view how cancer can be managed.

**Athanásios Valabanidés**

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**ΠΕΡΙΛΗΨΗ**

Η κακοήθης νεοπλασία (κοινώς αποκαλούμενος καρκίνος) αποτελείται από διάφορους τύπους καρκινικών κυττάρων που χαρακτηρίζονται από συσσώρευση μεταλλάξεων στο DNA και ανεξέλεγκτο πολλαπλασιασμό «επιθετικών» κακοήθων κυττάρων που διαχέονται σε υγιείς ιστούς και οργάνα. Τις τελευταίες δεκαετίες αναπτύχθηκαν διάφορες αντικαρκινικές
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Βελτιστοποίηση του Φαρμακολογικού Προφίλ Φυσικών Προϊόντων μέσω Εγκλωβισμού τους σε Κυκλοδεξτρίνες

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1. Εισαγωγή

1.1 Λιπόφιλα Φυσικά Προϊόντα: Τα φυσικά προϊόντα διαδραματίζουν ζωτικό ρόλο στη θεραπεία πλήθους ασθενειών. Αποτελούν ανεκτίμητη πηγή καθώς πολλά από τα χρησιμοποιούμενα φάρμακα στην κλινική προέρχονται απευθείας ή με τροποποίηση της δομής φυσικών προϊόντων.1,2 Ένας τρόπος ταξινόμησης του τεράστιου αριθμού φυσικών προϊόντων είναι σε διαφορετικούς δομικούς τύπους σύμφωνα με τις βιοσυνθετικές οδούς, όπως φλαβονοειδή, καροτενοειδή, αλκαλοειδή, φυσικές φαινόλες, τερπενοειδή, λιγνάνες, κουμαρίνες, αιθέρια έλαια, αμινοξέα, πεπτίδια κλπ. Έχει επιβεβαιωθεί ότι τα φυσικά προϊόντα ανάλογα με τις δομές τους παρουσιάζουν διαφορετικές βιολογικές και φαρμακολογικές δράσεις.3,4 Η δράση πολλών από τα φυσικά προϊόντα που είναι λιπόφιλα αυξάνεται σε περιβάλλοντα αυξημένης περιεκτικότητας λίπους. Απαιτείται δηλαδή κάποιος λιπαρός φορέας για να αποδεσμευτεί το φυσικό βιοδραστικό προϊόν από τους φυτικούς ιστούς και να απορροφηθεί από τον οργανισμό. Για παράδειγμα έχει διαπιστωθεί ότι η βιοδιαθεσιμότητα του λυκοπενίου είναι μεγαλύτερη από μαγειρεμένη τομάτα ή σάλτσα (κ.λ.π.) με κάποιο λιπαρό τρόφιμο.5 Τα μόρια των φυσικών προϊόντων όπως φλαβόνες, καροαλκαλοειδή, κουμαρίνες, λιγνάνες, τερπενοειδή, αμινοξέα, πεπτίδια και άλλα συχνά διαθέτουν ένα ή περισσότερα χειρόμορφα κέντρα που εμφανίζουν δύο ή περισσότερα οπτικά ισομερή (τουλάχιστον δύο εναντιομερή).6,7 Συχνότερα, δύο εναντιομερή εμφανίζουν πολύ διαφορετικές βιολογικές και φαρμακευτικές δραστηριότητες. Τα cis-λυκο-
πέντε παρουσιάζουν χαμηλότερο σημείο τίτλους και μεγαλύτερη λιποφιλικότητα σε σχέση με τα trans λυκοπένια (αυτό οφείλεται στη δομή τους: τα cis δεν κάνουν καλή πάκτωση σε σχέση με τα trans). Έχει διαπιστωθεί ότι το λυκοπένιο που βρίσκεται στους λιπαρούς ιστούς και στο αίμα του ανθρώπου είναι κατά πολύ πλουσιότερο σε cis-μορφές και κυρίως σε 5-cis-λυκοπένιο, το οποίο παρουσιάζει και τις ισχυρότερες αντιοξειδωτικές ιδιότητες.8

Γενικά η λιποφιλία εκφράζεται ως η λογαριθμική τιμή του συντελεστή μεταβολής κατανομής / νερού (logP<sub>oct</sub>), είναι μια θεμελιώδης παράμετρος που μοντελοποιεί τη βιολογική κατανομή της δράσης των μορίων του φαρμάκου. Για παράδειγμα, η απορρόφηση του φαρμάκου και ο συντελεστής μεταβολής “logP<sub>oct</sub>” σχετίζεται άμεσα λόγω της παθητικής διάχυσης κατά μήκος της κυτταρικής μεμβράνης. Η βιοδιαθέσιμη κατανομή του φαρμάκου είναι μια από τις σημαντικότερες ρόλους στη δράση των φαρμάκων, καθώς διακρίνεται ως ισχυρός φορέας της δραστικότητας. Οι κυκλοδεξτρίνες είναι δυνητικά υποψήφιες θέσεις στόχους για ένα ορισμένο χρονικό διάστημα, επειδή η ικανότητά τους να αποδεσμεύουν στο υδρόφοβο περιβάλλον της κοιλότητας στην ενδοεσωτερική ουσία.
μεταβολή των φυσικών, χημικών και βιολογικών ιδιοτήτων των φιλοξενούμενων μορίων και να αποκτήσουν τελικά σημαντική φαρμακοδυναμική. Πρόσφατα, σχεδιάστηκε ένας αριθμός τροποποιημένων κυκλοδεξτρινών και σύμπλοκα πολυμερών-κυκλοδεξτρινών για να επιτευχθούν μεγαλύτερες δυνατότητες εγκλεισμού απ’ ότι οι πρόδρομες κυκλοδεξτρίνες [Πίνακας 1].23-24 Η β-CD είναι η πιο κοινή μητρική CD και περίεξε 21 ομάδες υδροξυλίου, 7 από τις οποίες είναι πρωτοταγείς και οι υπόλοιπες 14 δευτεροταγείς (Σχήμα 1). Όλες αυτές οι ομάδες υδροξυλίου είναι διαθέσιμες ως σημεία εκκάθαρσης για δομικές τροποποιήσεις. Επομένως διάφορες λειτουργικές ομάδες έχουν εισαχθεί στον μακροκυκλικό δακτύλιο. Έτσι, οι φυσικές και τροποποιημένες CDs έχουν χρησιμοποιηθεί εκτεταμένα για τη βελτίωση διαφόρων ιδιότητων του φαρμάκου, όπως η διαλυτότητα, η σταθερότητα, η ταχύτητα διαλύσεως και η βιοδιαθεσιμότητα.25-31

Στην παρούσα εργασία γίνεται μια προσπάθεια καταγραφής, ομαδοποίησης και συχνότητας κάποιων αντιπροσωπευτικών φυσικών προϊόντων που έχουν φιλεξενηθεί σε κυκλοδεξτρίνες ως προς τον προσδιορισμό της στοιχειομετρίας του σχηματιζομένου συμπλόκου του δραστικού συστατικού του φυσικού προϊόντος με την κυκλοδεξτρίνη, την τοπογραφία και τον προσανατολισμό του μορίου μέσα στην κυκλοδεξτρίνη καθώς και της βιολογικής αξιολόγησης του συμπλοκοποιημένου βιοδραστικού μορίου σε σχέση με το πρόδρομο μόριο.

Παραδείγματα Φυσικών Προϊόντων που έχουν χρησιμοποιηθεί σε μορφοποιήσεις με κυκλοδεξτρίνες

Metaxu ton χημικών τροποποιημένων κυκλοδεξτρινών, oi udroψιλοi26-37 ή alliai ointižomene28-40 omades ensiyghoun tin aporofrwsis tou farmakou, enw oi udroψiλoι μπορεί νa έχουν ευρεία εφαρμογή kai tha mporeoušan na χρησιμοποιήσουν ως νέοi

![Diagram](image_url)
φαινόλη) και η καρνοσίνη (diipentidio) σε κατάσταση σύμπλεξης με κυκλοδεξτρίνες

2.1 Η Κερκετίνη

Η Κερκετίνη ή Κουερσετίνη (QUE) είναι μία φλαβονοειδές (Φλαβονοειδές) που βρίσκεται σε πολλά φρούτα, λαχανικά, φύλλα και σπόρους. Μπορεί να χρησιμοποιηθεί ως συστατικό σε συμπλήρωμα διατροφής, στα ποτά ή τα τρόφιμα. Το χημικό της όνομα κατά IUPAC είναι 2-(3,4-διυδροξυφαινυλ)-βυδροξυπροπυλ-β-CD, (B) το λυκοπένιο (Lyc) με συμπλέξεις CD- Lyc (1/0,0026, 1/0,005, 1/0,05), β-CD-Lyc, 2-Υδροξυπροπυλ–β-CD-Lyc, μεθυλ-β-CD-Lyc, (Γ) η κουρκουμίνη (CUR) με συμπλέξεις CUR-β-CD, (Δ) η καρνοσίνη (CARN) με παράμοιες συμπλέξεις με β-CD.

Σχήμα 2: (Α) Η Κερκετίνη (QUE) με τον δακτύλιο B στη δεξιά πλευρά του σχήματος, με βάση την οποία εξετάζονται τα σύμπλοκα QUE-β-CD, QUE / Et-β-CD, QUE /γ-CD με στοιχειομετρία 1:7:1, QUE-σουλφαβαντολαιθέρ-β-CD, QUE-υδροξυπροπυλ-β-CD, (Β) το λυκοπένιο (Lyc) με συμπλέξεις CD- Lyc (1/0,0026, 1/0,005, 1/0,05), β-CD-Lyc, 2-Υδροξυπροπυλ–β-CD-Lyc, μεθυλ-β-CD-Lyc, (Γ) η κουρκουμίνη (CUR) με συμπλέξεις CUR-β-CD, (Δ) η καρνοσίνη (CARN) με παράμοιες συμπλέξεις με β-CD.

Επίσης το παραγόμενο σύμπλοκο εμφανίζει αρκετά υψηλή αντιοξειδωτική δράση και φωτοσταθερότητα. Οι παραπάνω ιδιότητες οφείλονται στην υψηλή επιφανειακή τάση και το υψηλό πορώδες που παρουσιάζει το συγκεκριμένο σύμπλοκο.

Στη μελέτη των K. H. Park et al.50 η διαλυτότητα της κερκετίνης ενισχύθηκε σημαντικά με το σχήματος ενός συμπλόκου εγκλεισμού με Et-β-CD. Αυτό το σύμπλοκο εγκλείσμου αναλύθηκε με φασματοσκοπία UV-vis, 1H και DOSY NMR, διαφορική θερμομετρία σάρωσης (DSC), υπέρυθρης φασματοσκοπίας (FT-IR) και Ηλεκτρονικής Μικροσκοπίας Σάρωσης (SEM) Ο τρόπος σύμπλεξης της κερκετίνης με Et-β-CD παρακολουθήθηκε με φασματοσκοπία 2D ROESY. Τα αποτελέσματα έδειξαν ότι τα πρωτόνια στο δακτύλιο B της κερκετίνης συμπεριλήφθηκαν στην κοιλότητα του Et-β-CD.

Το αποτέλεσμα της σύμπλεξης της κερκετίνης με Et-β-CD με βάση την αντιοξειδωτική του ύπαρξης κόκτητα προσδιορίστηκε χρησιμοποιώντας τη δοκιμασία ORAC-FL και το σύμπλοκο QUE / Et-β-CD έδειξε το υψηλότερο αντιοξειδωτικό αποτέλεσμα. Τα αποτελέσματα έδειξαν επίσης ότι επιτεύχθηκε μεγάλυτη σύμπλεξη κερκετίνης με Et-β-CD, σε σύγκριση με εκείνη με παράγωγα β-CD. Επιπλέον,
το σύμπλοκο QUE / Et-β-CD αύξησε την υδατοδιαλυτότητα της κερκετίνης έως 35,1 φορές, σε σύγκριση με τη φυσική υδατοδιαλυτότητα της κεκρκετίνης. Από αυτά τα αποτελέσματα, συμπεραίνεται ότι η χαρακτηριστική ελλειπτική κοιλότητα του Et-β-CD μπορεί να χρησιμοποιηθεί για άλλα επιμήκη μη πολικά φαρμακευτικά μόρια, τα οποία μπορούν να επεκτείνουν την ανάπτυξη σειράς παρόμοιων φαρμάκων.

Τα υδατοδιαλύματα σύμπλοκων κυκλοδεξτρίνης κερκετίνης μπορούν να παγιδευτούν στην υδατική φάση των σταθερών πολυστρωτικών λιποσωμάτων, επιπλέον της ενσωμάτωσης της κερκετίνης στην λιποσωμική διπλοστιβάδα με τη μέθοδο ενυδάτωση λεπτού υμένα. Οι φασματοσκοπικές μετρήσεις υποδηλώνουν ότι τα σύμπλοκα κερκετίνης-κυκλοδεξτρίνης ενσωμάτωθηκαν επιτυχώς στα λιποσώματα χωρίς να επηρεαστεί η μορφολογία τους. Το ποσοστό ενσωμάτωσης φαρμάκου διπλασιάστηκε στα διπλά ενταγμένα λιποσώματα σε σύγκριση με αυτά που δεν περιέχαν τα σύμπλοκα κυκλοδεξτρίνης. Τα λιποσώματα που ενσωματώνουν σύμπλοκα του φαρμάκου με κυκλοδεξτρίνη απελευθερώνουν περισσότερο φάρμακο από τα λιποσώματα που εγκλείουν μόνο το φάρμακο.

Στην εργασία των E.A. Parrila et al. μελετάται η σύμπλοξη και η αντιοξειδωτική δράση των σημαντικότερων πολυφαινολών που υπάρχουν στο μήλο δηλαδή χλωρογενικό οξύ, ρουτίνη και κερκετίνη με β-κυκλοδεξτρίνη. Οι τεχνικές που χρησιμοποιήθηκαν ήταν φασματοσκοπία φθορισμού και "Δοκιμασία Αναγωγής Σιδήρου / Αντιοξειδωτικού Δυναμικού (FRAP)". Η στοιχειομετρία όλων των πολυφαινολικών συμπλοκών είναι 1:1. Το σύμπλοκο της κερκετίνης παρουσιάζει τη μεγαλύτερη σταθερά σχηματισμού και την υψηλότερη αντιοξειδωτική δράση σε σχέση με τα υπόλοιπα πολυφαινολικά σύμπλοκα. Η σύμπλεξη επιβεβαιώθηκε με μετρήσεις διαφορικά.

Πίνακας 1. Χρήσιμα παράγωγα της β-CD που χρησιμοποιούνται σε φαρμακευτικά σκευάσματα

<table>
<thead>
<tr>
<th>Παράγωγο</th>
<th>Θέση υποκαταστάτη</th>
<th>Υποκαταστάτης</th>
</tr>
</thead>
<tbody>
<tr>
<td>Τα υδρόφιλα παράγωγα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Μεθυλωμένη β-CD</td>
<td>2,6 - 2,3,6 -</td>
<td>-O-CH₃</td>
</tr>
<tr>
<td>Υδροξυαλκυλωμένη β-CD</td>
<td>Τυχαίος</td>
<td>-O-CH₂ -CH (OH)-CH₃</td>
</tr>
<tr>
<td>Διακλαδισμένη β-CD</td>
<td>6 -</td>
<td>-Γλυκόξυλο-μαλτόξυλο</td>
</tr>
<tr>
<td>Υδρόφοβα παράγωγα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Αιθυλωμένη β-CD</td>
<td>2,6 - 2,3,6 -</td>
<td>-OC₂H₅</td>
</tr>
<tr>
<td>Υπερακυλωμένη β-CD</td>
<td>2,3,6 -</td>
<td>-O-CO(CH₃)ₙ -OH₃</td>
</tr>
<tr>
<td>Ιοντιζόμενα παράγωγα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Καρβοξυαλκόλιο β-CD</td>
<td>Τυχαίος</td>
<td>-O-(CH₃)ₙ -COONa</td>
</tr>
<tr>
<td>Καρβοξυμεθυλο αίθυλο</td>
<td>2,6 - 3 -</td>
<td>-O-CH₂ COONa-OC₂H₅</td>
</tr>
<tr>
<td>Θειικά</td>
<td>Τυχαίος</td>
<td>-O-SO₃Na</td>
</tr>
<tr>
<td>Αλκυλοσουλφονικά</td>
<td>Τυχαίος</td>
<td>-O-(CH₃)ₙ -SO₃Na</td>
</tr>
</tbody>
</table>
κής θερμιδομετρίας (DSC) και η αντιοξειδωτική δράση αυξήθηκε με τη σύμπλεξη.

Οι J. L. Koontz et al. δοκίμασαν τη σύμπλεξη κερκετίνης με γ-κυκλοδεξτρίνη με στοιχειομετρία 1:7:1 και απόδοση 21% (w/w). Με φασματοσκοπίαις ATR/FT-IR, 13C CP/MAS NMR, TGA και DSC αποδείχθηκε η αλληλεπίδραση με την κυκλοδεξτρίνη σε μοριακό επίπεδο.

Η μελέτη των Y. Zheng et al. κατέδειξε σαφώς ότι η υδατική διαλυτότητα και η χημική σταθερότητα (στο αλκαλικό pH) της κερκετίνης μπορεί να αυξηθεί ουσιαστικά μέσω σύμπλεξης με β-CDs, ιδιαίτερα με σουλφοβουτυλαιθέρα της β-CD. Οι σταθερές ισορροπίας και οι θερμοδυναμικές παράμετροι που προσδιορίστηκαν με τη μελέτη διαλυτότητας φάσης καθώς και οι σημαντικές μεταβολές χημικής μετατόπισης της κερκετίνης που παρατηρήθηκαν με τις διάφορες κυκλοδεξτρίνες σε αναλύσεις 1H-NMR συμφωνούν με το σχηματισμό συμπλόκων εγκλεισμού.

Οι Alexandra Primikyri et al. διερεύνησαν τη συμπλοκοπίπηση του Zn (II) με τα φυσικά φλαβονοειδή κερκετίνη και λουτεολίνη με τη χρήση χημικής μετατόπισης της κερκετίνης σε μοριακό επίπεδο. Αυτή η ιδιότητα ταξινομεί την κερκετίνη ως ένα φυσικό φλαβονοειδές με BH3 μιμητική δραστηριότητα ικανή να οδηγήσει τα καρκινικά κύτταρα σε απόπτωση. 

Συμπερασματικά, η συνδυασμένη χρήση της φασματοσκοπίας NMR, με έμφαση στους φαινολικούς συντονισμούς ΟΗ, παράλληλα με τους κβαντικούς υπολογισμούς θα μπορούσε να αποτελέσει ένα πολύτιμο εργαλείο για την ακριβή δομική και ηλεκτρονική περιγραφή των διαμαγνητικών συμπλοκών φλαβονοειδούς-μετάλλου.
τέσσερα παράγωγα της κερκετίνης σε σύζευξη με διαφορετικά αμινοξέα (αλανίνη, γλουταμικό οξύ, φαινουλαλινή και λευκίνη) στον δακτύλιο Τ. Τα υπολογιστικά δεδομένα MM-PBSA και MM-GBSA εξειδίκευαν ότι το σύμπλοκο Que-Glu3 παρουσιάζει την υψηλότερη δεσμοπλαστική τιμή (-47,16 kcal mol⁻¹). Τα δεδομένα φθορισμού ήταν επίσης σύμφωνα με τις θεωρητικές μελέτες και φασματομετρικά NMR.

Συμπερασματικά, φλαβονοειδή προφάρμακα συζευγιένα με αμινοξέα θα μπορούσαν να είναι μια υποσχετική προσέγγιση για την ενίσχυση του φαρμακευτικού φάσματός τους. Με μετρήσεις ρασματοθρωσκομετριαίας, τερμάδομετριαίας και μελέτες βιολογικών πειραμάτων προσομοιωμένων και με ενισχυμένη θεραπευτική δράση. 59 Παράγοντα για την ανάπτυξη βιοδραστικών ενώσεων θα μπορούσαν έτσι να προσφέρουν ένα σημαντικό μέρος στην ανάδοση και επαληθεύθηκε με τη μεταφορά του στους ιστούς. Τα φυσικά προϊόντα σε σχέση με την κερκετίνη, όσον αφορά την κυτταροφιλο αντιοξειδωτικό, παίζει καθοριστικό ρόλο στα άνθρωπα βιολογικά συστήματα.

Το 2.2 Το λυκοπένιο

Το λυκοπένιο (lycopene) είναι ένας πολυκόρεστος υδρογονάνθρακας ο οποίος περιέχει 40 άτομα άνθρακα με ονομασία IUPAC (6Έ, 8Ε, 10Ε, 12Ε, 14Ε, 16Ε, 18Ε, 20Ε, 22Ε, 24Ε, 26Ε) -2,6,10,14,19,23,27,31-οκταμεθυλοδρίτοικα, διπενίων από ντομάτα με ενθυλάκωση με α- και β- κυκλοδεξτρίνες. Χρησιμοποιήθηκαν δύο μέθοδοι για την απομόνωση, η συμβατική εκχύλιση και η Εκχύλιση με Υπερκρίσιμα Υγρά, SFE. Αποδεικνύεται ότι με τη μη συμβατική μέθοδο επιτυγχάνονται μεγαλύτερες αποδόσεις. Οι M. Vertzoni et al.61 χρησιμοποίησαν μια βελτιστοποιημένη μέθοδο ζύμωσης για την παρασκευή διαδικασιών κύκλωση-κυκλοδεξτρίνης που οδηγεί σε διαλυτοποίηση του λυκοπενίου σε νερό και σε διάλυμα δεξτρόζης 5% (w/v). Υπολογίσθηκε η συγκέντρωση του λυκοπενίου σε συζευξικούς μεταδοτικούς τοιχοδομούς με απόδοση 60 ήταν επίσης σύμφωνα με πρότυπη διαδικασία φασματοφωτομετρικής ανάλυσης παρουσίας α-κυκλοδεξτρίνης σε συγκέντρωσεις 0,4, 0,8 και 1,6% και μετρήθηκε η αντιοξειδωτική δράση του λυκοπενίου με τη δοκιμασία ORAC. Η ενσωμάτωση της β-κυκλοδεξτρίνης στην δοκιμασία ORAC οδηγείται αναλόγως συγκέντρωσης λυκοπενίου, διευρύνοντας έτσι το πεδίο εφαρμογής της δοκιμασίας ORAC ώστε να συμπεριλαμβάνει ένα επιπλέον λεπτομερές αντιοξειδωτικό.

2.3 Η Κουρκουμίνη

Πρόκειται για μία φυσική πολυφανήλη χρώματος κίτρινου που προέρχεται από τη ρίζα του φυτού «Curcuma longa». Είναι μία φυσική χρωματική που χρησιμοποιείται σε τροφικά και φαρμακευτικά παρασκευάσματα με ονομασία IUPAC (1E, 6E)-1,7-δις
(4-υδροξ-3-μεθοξυφαινυλ) επτα-1,6-διενο-3,5-δί-
όνη.

Οι C.S. Mangolim et al.63 με μετρήσεις FT-IR, FT-
Raman, φωτοακουστικής φασματοσκοπίας και πε-
rιθλασίμετρου ακτίνων Χ (XRD) ταυτοποίησαν το σύμπλοκο κουρκουμίνης-β-κυκλοδεξτρίνης. Χρησι-
mοποιήθηκε η μέθοδος της συγκαταβύθισης και πα-
ρατηρήθηκε ότι το παραγόμενο σύμπλοκο παρου-
σίαζε μεγαλύτερη σταθερότητα στο χρώμα υπό την επίδραση φωτός, στο pH, στην αποθήκευση και στη
θέρμανση από ότι η κουρκουμίνη. Για τους παραπά-
νω λόγους προάγεται για χρήση στα τρόφιμα.

Η κουρκουμίνη έχει αναφερθεί ως δυνητικά δρα-
sτική κατά του καρκίνου. Λόγω της χαμηλής διαλυ-
tότητας της κουρκουμίνης, οι N. Rocks et al.64 χρη-
sιμοποίησαν κυκλοδεξτρίνες (CDs) ως μέσο για την
αύξηση της υδατοδιαλυτότητας και της βιοδιαθεσι-
mότητας της κουρκουμίνης. Οι επιδράσεις της δια-
lυτοποιημένης κουρκουμίνης έχουν αξιολογηθεί σε
κυτταρικές καλλιέργειες καθώς και σε ένα «in vivo»
κύτταρα κότταρα του προστάτη στη σύγκριση με την ελεύθε-
ρη κουρκουμίνη.

2.4 Η καρνοσίνη

Η καρνοσίνη (β-αλανυλ-L-ιστιδίνη) είναι ένα διπε-
πτίδιο που προέρχεται από συνένωση αλανίνης και
ιστιδίνης. Βρίσκεται σε υψηλή συγκέντρωση στους
μύες και τον εγκέφαλο. Η καρνοσίνη έχει αποδειχθεί σε σειρά μελετών ότι διαθέτει αυξημένη ικα
νότητα για τη δέσμευση των ελευθέρων ριζών οξυγόνου και του ενδοκυτταρικού ρυθμιστικού διαλύματος πρωτονίων. Από την άλλη η καρνοσινάση είναι μια ειδική πεπτιδά-
sη ικανή να καταστρέφει το βιολογικά ενεργό δι-
πεπτίδιο.

Για να ξεπεραστεί αυτός ο περιορισμός, η β-κυ-
κλοδεξτρίνη (β-CD) συνδυάστηκε με καρνοσίνη για
να δώσει τις ακόλουθες νέες ενώσεις: 6Α - [(3 -{(1S)
-1-καρβοξυ-2- (1Η- ιμιδαζολ- 4- υλ) αιθυλ} αμινο} -3οξοπρο-
πυλ) αμινο] -6Α- δεοξυ- β- κυκλοδεξτρίνη (1), 6Α - [(β-
αλανυλ-L-ιστιδυλ) αμινο] -β-κυκλοδεξτρίνη (2) και (2ΑS.3ΑR) -3Α - [({1S) -1-καρ-
βοξυ-2- (1Η- ιμιδαζολ- 4- υλ) αιθυλ} αμινο] -3-οξο-
προπυλ} αμινο] -3Α- δεοξυ- β κυκλοδεξτρίνη (3). Η
έρευνα παλμικής ραδιόλυσης έδειξε ότι τα παράγω-

Η προτεινόμενη μέθοδος χρησιμοποιήθηκε για τον
προσδιορισμό της κουρκουμίνης σε κάρυ και μου-
στάρδα με ικανοποιητικά αποτελέσματα.
Lipophilic compounds such as many bioactive compounds do not present their optimum pharmacological profile because they mainly exhibit metabolic instability and toxicity, they lack selectivity and suffer from low bioavailability. Their complexes with cyclodextrins improves these disadvantages and transform them to improved molecules of.

**KEYWORDS:** Natural products; cyclodextrins, lipophilicity

**SUMMARY**

Lipophilic compounds such as many bioactive compounds do not present their optimum pharmacological profile because they mainly exhibit metabolic instability and toxicity, they lack selectivity and suffer from low bioavailability. Their complexes with cyclodextrins improves these disadvantages and transform them to improved molecules of.
pharmacological interest with good prospects for human health. In this review paper, are presented examples of representative molecules, natural products, whose properties were improved when hosted by various cyclodextrins.

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Drug-drug interactions between tyrosine kinase inhibitors and concomitant medications: drug safety in chronic myeloid leukemia treatment.

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KEYWORDS:
Drug-drug interaction; Tyrosine kinase inhibitor; Chronic myeloid leukemia

SUMMARY

Background: Clinical Pharmacist should be aware of hematologic toxicities from tyrosine kinase inhibitors (TKI) used to treat chronic myelogenous leukemia (CML). Drug-drug interactions (DDI) may be problematic.

Objective: To analyze DDI between TKI and the concomitant medication.

Setting: Retrospective observational study carried in a tertiary hospital of Spain.

Method: A bibliographic search was made on the UpToDate®, Lexicomp® and Micromedex® software platforms to search for evidence on DDI between TKI and the concomitant medication.

Main outcome measure: Number of interactions with respect to sex, to number of concomitant drugs, and to TKI used.

Results: A total of 28 patients were analyzed. 78.6% of patients had medication associated with the TKI. There was a total of 50 significant DDI, out of a total of 128 drugs, so the risk of having interaction in the study population was 39.1%. Regarding the management of the interactions by the hematologist and the acceptance of the pharmaceutical intervention: 10 patients experienced 14 high-level interactions. Of these the doctor knew 50% and had performed intervention in all cases: modify the treatment in 28.6%, consulted with service responsible for treatment in 42.8% and spaced the intake of drugs in 28.6%. It is important to periodically review concomitant medication and to have a strategy to manage interactions. The role of the clinical pharmacist is essential in communication with the patient, assessment of treatments, detecting potential interactions and disseminating information among the multidisciplinary team.
Introduction

In recent years, advances in the treatment of cancer have led to the emergence of numerous oral antineoplastic drugs. The oral route is consolidated in the first-line treatments of some carcinomas, as it has been shown that the disease-free survival and overall survival, as well as the toxicity profiles, are not different from those of the parenteral route. However, drug interactions are common and may be problematic. Concomitant use with moderate CYP3A inhibitors, strong or moderate CYP3A inducers, glycoprotein-P (P-gp) inhibitors, or narrow therapeutic index P-gp substrates should be avoided. Therefore, the pharmacodynamic characteristics of drugs do not vary over time, but plasma and tissue concentrations, as well as efficacy may be influenced as a result of concomitant treatments or specific eating habits.

In this scenario, new challenges have been posed for the clinical pharmacist, specialized in the area of oncological consultation, and in charge of the care of these patients such as the monitoring of therapeutic adherence, and management of adverse effects and interactions or safe handling of toxic waste at home. The onco-hematological patient is especially susceptible to drug interactions, as he often receives one or more antineoplastic agents, along with concomitant medications, to alleviate the pain or adverse effects of the chemotherapy itself. In addition, there are several factors derived from the disease that predispose patients to interactions, such as poor absorption, malnutrition, liver or kidney damage.

The most concerning interactions are those whose consequences are detrimental for the patient exposure to the drug, either because it is increased causing adverse effects, or because exposure is diminished causing an inadequate therapeutic response. In the case of antineoplastic drugs, this can lead to treatment failure or loss of scarce therapeutic options available, thus compromising patient’s safety.

Riechelmann et al. concluded that 67% of hospitalized cancer patients were at risk of experiencing a drug interaction. The factors that predispose them to this include the number of drugs involved in their treatment, frequent use of alternative medicines, comorbidities, organic deterioration that affects the processes of metabolism and excretion of the drugs, and finally, the fact that a large number of the recently commercialized cytostatics have not undergone extensive premarketing studies that allow proper drug interactions analysis.

This study expresses oral cytostatics involved in the treatment of chronic myeloid leukemia (CML). The introduction of tyrosine kinase inhibitors (TKIs) revolutionized the management of CML, improving the 10-year overall survival from ~20% to 80-90%. In some patients, expected survival is indistinguishable from that of the general population. These are the first drugs with a specific therapeutic target: the BCR-ABL fusion gene. The first TKI to appear was Imatinib. Despite the good results of studies with this drug, there is a group of patients in whom it is not possible to use Imatinib, either due to intolerance, adverse reactions, suboptimal effects or resistance to the drug. The options available after Imatinib resistance at maximum doses are the second-generation TKIs include Dasatinib and Nilotinib.

It is important to consider potential drug-drug interactions between TKIs and substrates, inhibitors or inducers of the CYP3A4 isoform and the P-gp. Recent studies revealed that concomitant prescription of drugs that can inhibit the effectiveness of protein

Conclusion: All patients who are prescribed oral antineoplastic drugs are provided patient education materials about TKI, which include possible interactions. Any changes in the patient’s medications prompt a review for DDI.
kinase inhibitors can vary between 23 and 57%, and drugs that can increase their toxicity between 25 and 74%\textsuperscript{10,14,15}. Patients with CML usually take several medications simultaneously with their oncological therapy therefore, taking into account the risks of polypharmacy, it is possible that there are potential pharmacological interactions between their oncological treatment and the rest of medications. To assess the impact of these interactions, we intend to carry out a study in patients treated with TKIs in a third level clinical university hospital.

The aim of this study was to analyze the presence of pharmacological interactions between TKIs and the concomitant medications used in patients with CML, and to investigate the influence of clinical pharmacist’s interventions.

Material and methods

A retrospective observational study in which 28 adult patients diagnosed with CML and treated with TKI were selected who attended the Clinical Pharmacy Service of a tertiary level Clinical University Hospital from October 1, 2016 to October 1, 2017. The data on the treatment were obtained retrospectively from the individual dispensing module of the Dominion Farmatools® program. The following variables related to the patients were collected: age, sex, type and dose of antineoplastic drug, characteristics and number of concomitant non-antineoplastic medications and number, level and type of drug-drug interactions noted. The collected information included additional medication patients could be taking for other indications such as medications prescribed by the GP/other Doctors/specialists. To detect the possible interactions between their regular medications and oncological therapy, a bibliographic search was carried out on the UpToDate® (and Lexicomp® to detect DDIs) and Micromedex® computer platforms and the information obtained with the technical data sheets of the drugs was completed. The interactions were classified according to their severity, following the classification of Lexicomp® database (LexiComp, Inc, Hudson, Ohio, 2010) and DRUG-REAX System® (Thomson Reuters, Greenwood Village, Colo, USA, 2010). Each drug-drug interaction is assigned a risk rating of A, B, C, D, or X. Monographs rated X, D or C indicate situations that will likely demand a clinician’s attention.

- Level of interaction X: significant interaction, avoid combination. The risks associated with the concomitant use of these two drugs normally outweigh the benefits. It is a contraindicated drug combination.
- Level of interaction D: significant interaction, consider therapy modification. A patient-specific evaluation should be conducted to determine if the benefits of such treatment outweigh the risks. Actions such as exhaustive monitoring, dose changes or use of alternative drugs should be carried out to obtain benefits or decrease the toxicity resulting from the concomitant use of said drugs.
- Level of interaction C: significant interaction, monitor therapy. Normally, the benefits of the concomitant use of these drugs outweigh the risks. In any case, a monitoring plan must be carried out to detect potential adverse events. Dose adjustments may be necessary in one or both drugs in a minority of patients.

Potential interactions were identified, as well as their prevalence and the risk that this could pose to the patient. They were also classified according to the type of interaction in pharmacokinetics, pharmacodynamics or others and within each of them an increase or decrease in dose was expected.

Once all the information was collected, the specialist responsible for the patients was informed about the most relevant interactions. A detailed review was done for each patient, jointly between the hematologist and the pharmacist using the clinical history, evolution of the analytical data, and medication changes, in order to design a clinical decision for each type of interaction in each patient. The clinical significance of these interactions was recorded by the hematologist and the acceptance and utility of the pharmaceutical intervention performed was evaluated.

The quantitative variables were summarized with mean and standard deviation or median and range,
and the qualitative variables with percentages. Statistical analysis was performed in the group of patients who were prescribed concomitant treatment, and the patients were grouped according to whether they had relevant interactions between the antineoplastic drugs and their concomitant drugs. Differences in sex, concomitant treatment (greater or lesser than 5 drugs) and TKI used were evaluated using the chi-square test (χ²). Statistical analysis was carried out with the statistical package SPSS 21.0 for Windows (License of the University of Zaragoza).

Ethics approval: A study with initial protocol was prepared to submit to explore confirmation by the Clinical Research Ethics Committee. Enrollment, medical, and drug files were linkable based on an encrypted patient identification number. The use and analysis of de-identified administrative claims or limited data sets; was considered exempt from review by an Institutional Review Board (IRB), as de-identified information requires personal health information (PHI) waiver of authorization.

Results

A total of 28 patients with CML treated with a TKI were analyzed. 60.7% were males with an average age of 56.5 ± 14.2 years, and only 42.8% of the patients were older than 60 years. 39.3% of patients were treated with a first-generation TKI (Imatinib), while 39.3% were being treated with Dasatinib, and 21.4% of patients with Nilotinib.

78.6% of the patients had concomitant medications that included analgesics / opioids, anxiolytics / hypnotics / sedatives and antihypertensives, followed by proton pump inhibitors (PPIs). Table 1 describes the frequencies of prescribed non-antineoplastic drugs.

The median of concomitant drugs prescribed was 4 (range 0 to 16). 21.4% of patients did not take any additional drugs, 39.3% had 1 to 5 concomitant drugs and 39.3% had ≥5 prescribed non-antineoplastic drugs.

A total of 50 TKI- no TKI interactions were recorded. These interactions occurred in 20 patients out of 28, who took a total of 128 drugs, so the risk of interactions in the study population was 39.1%. Of the total interactions detected, 72.6% were potential interactions (level C) in which a dose adjustment is not necessary but precaution and monitoring of adverse events is recommended, 15.1% were level D, in which the modification of the therapy is recommended, while the amount of contraindicated interactions (level X) was 12.2%, in which it was recommended to avoid this combination of drugs. The median of interactions was 1 (0-5). Interactions almost entirely were of Pharmacokinetic nature (90.7%), of these 87.4% involved a possible increase in concomitant drug concentrations, due to the inhibitory nature of TKI and only 12.6% a possible decrease in the concentration of TKI due to interference in absorption (antacids and PPIs). The drugs with the highest number of interactions were analgesic / opioid, antihypertensive and antipsychotic potential (Table 2).

In patients treated with Imatinib, 34.2% of the concomitant treatment could result in an interaction. Of these interactions, 84.6% were of level C, due to the moderate inhibition of cytochromes CYP3A4 (31.8%) and CYP2D6 (27.3%) and of the P-gp (4.5%). 3.8% were level D and 11.6% were level X. In this last group, interaction with Metamizole (dipyrene) stands out, as it can increase adverse reactions such as agranulocytosis and pancytopenia.

In case of Dasatinib, 48.0% of concomitant drugs had the possibility of interacting with TKIs. Of these interactions, 75.0% were of level C, due mainly to the inhibition of cytochrome CYP3A4 (44.4%) and CYP2D6 (33.3%) and the increase in the antiplatelet effect (33.3%). 8.3% were level D and 16.7% level X, highlighting the interaction with PPIs, which significantly reduce the absorption of Dasatinib, thus reducing its plasma concentration.

Finally, amongst the patients on Nilotinib, 44.4% of concomitant drugs had some type of interaction. 58.3% of the interactions were of level C, 33.3% were of level D, due to their interaction with antacids and divalent ions (66.7%) and 8.4% of level X, due to the high risk of prolongation of the QT segment on ECG and risk of developing cardiac toxicity, as with Quetiapine.
In the overall statistical analysis, there were no significant differences in the number of interactions with respect to sex \((p = 0.386)\). There were also no significant differences in the frequency of relevant interactions between patients who had less than 5 concomitant drugs and more than 5 drugs \((p = 0.603)\). As for the TKI used, no significant differences were found in patients who used first generation (Imatinib) or second generation TKI (Dasatinib / Nilotinib) \((p = 0.174)\) although there is a tendency to have more interactions with the second generation TKIs.

Regarding the management of the interactions by the hematologist and the acceptance of the pharmaceutical intervention: 10 patients experienced 14 high-level interactions (D or X). Of these the doctor managed 50% of the interactions and had made intervention in all cases: modify the treatment in 28.6%, interconsultation with service responsible for treatment in 42.8% and space the taking of drugs in 28.6%. With the other 50% of the interactions an individualized pharmaceutical intervention was carried out and recommendations included interconsultation to the service responsible for the treatment (14.3%), space the taking of the drugs (42.8%), monitor possible adverse effects due to the interaction (14.3%) and modify / reduce treatment (28.6%). All the proposals were accepted by the responsible physician.

**Discussion**

In the present study, the estimation of the risk of presenting with clinically relevant interactions between TKI and non-TKI drugs was lower than that described in the literature\(^8,9\). The percentage of interactions that increase concomitant drug concentrations was higher than that of a study presented in the USA by Bowlin et al. in 2013\(^3\), while the percentage of interactions that decrease the concentration of TKI and thus its effectiveness, was lower than that of Bowlin study. The cause of these differences may be the limited sample of patients and the fact that the study has been done only for the TKIs that treat CML. Other studies confirm that greater the number of concomitant drugs in patients diagnosed with CML on TKIs, greater the risk of interactions \([5,6]\). On the other hand, in the current study no significant differences were observed regarding the frequency of relevant interactions between patients who had less than 5 concomitant drugs and more than 5 drugs. This is because the interactions between non-antineoplastic drugs have not been included. No differences were observed in interactions when administering first generation or second generation TKI, since pharmacologically no metabolic profile more is susceptible to suffering interactions than another.

The analgesic and sedative drugs together with the antihypertensive drugs were the most frequently prescribed in the patients included in the study. Pain, insomnia and depression were one of the most frequent comorbidities in the cancer population and, therefore, analgesic and psychiatric drugs are the most frequently prescribed drugs. The concomitant drugs involved in the interactions were analgesics, antihypertensives and antipsychotics, followed very closely by anxiolytic drugs, PPIs and blood glucose lowering drugs.

Regarding the joint assessment with the responsible physician, the professionals’ concern about the interactions of this group of drugs was demonstrated and, due to the lack of time in the consultation or the limitation in access to search tools and databases, cannot be properly addressed by the hematologist. The intervention of the clinical pharmacist both at the beginning of the treatment and in successive reviews of medication could help avoid adverse events and even avoid the change to second generation TKIs, which sometimes occurs due to intolerances or adverse events of unknown origin, thus improving adherence. With this intervention, the real need of another drug, molecular response of the patient, clinical situation and, in some cases, the plasma levels of TKI, would help to predict the result of the interaction. Thus, the personalized pharmacotherapeutic follow-up of the hematological patient should lead to collaboration with the hematologist and other health professionals forming part of a multidisciplinary team.
In the present study, most of the interactions were pharmacokinetic, due to substrates, inhibitors or inducers of the CYP3A4, CYP2D6 and P-gp isoforms, confirming the information published in a review carried out in 2014⁶.

The work carried out presents a series of perfectly defined limitations. Firstly, the sample size is limited; resulting directly from the population of adult patients with treated CML in the geographic area of study. Secondly, as it is a retrospective analysis, there may be loss of information in the variables collected from the computerized medical record, since it is possible that the concomitant treatment in progress was not updated correctly in some cases. As the study was retrospective, there is also potential that the patient is no longer taking the interacting medication. Thirdly, interaction data was not always available in the databases for the different drug combinations. Fourthly, the study has been carried out for the TKIs that treat CML, there are other TKIs with other indications and most of the studies consulted analyze all the TKIs, so they cannot be compared directly. Finally, the interaction rates have been underestimated since the interactions between the antineoplastic drugs themselves or among the non-antineoplastic drugs have not been included. To sum up, this is a small study which does provide evidence of the benefit of pharmacist role in reviewing pa-
This study demonstrates that oral antineoplastics require pharmaceutical interventions aimed at preventing and/or minimizing the risk of toxicity or decreased efficacy due to interactions with other medications. Many of the drug interactions in oncology are not recognized as such since they are masked by some symptoms of the pathology itself and are even confused with the toxicity inherent to the use of antineoplastic drugs. Therefore, before introducing a new drug in onco-hematological patient therapy, it is important to question the real need for it, assessing possible safer alternatives.

The Clinical Pharmacy Services have shown that by means of the pharmaceutical intervention, the risk of an adverse event caused by a pharmacological DDI can be reduced by 25.9% \(^{17}\). This context makes the act of the pharmaceutical interview a valuable tool to detect and manage interactions involving oral antineoplastic drugs \(^{18}\). Therefore, patients with complex treatments and a high risk of potential pharmacotherapeutic problems (that may compromise the effectiveness and safety of the treatment) may benefit from this Service. In this context, clinical pharmacists have played a fundamental role in pharmacotherapeutic care and monitoring of the external onco-hematological patient.

This work could be used in the future for developments of the Clinical Pharmacy Service. Another possible future guideline with these drugs, given the wide possibility of pharmacological interaction, would be their pharmacokinetic monitoring in routine clinical practice, since target concentrations are available in terms of efficacy, and target concentrations to ensure the safety of the treatment \(^{19,20}\).

With this study we conclude that administration of concomitant drugs causes a potential risk of experiencing DDIs. In addition, it is important to periodically review the concomitant medication and have a strategy to manage those interactions and avoid them. And that the role of the pharmacist is fundamental in the communication with the patient, assessment of their treatment, and detection

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**Table 2. No tyrosine kinase inhibitors (TKI) implicated in interactions**

<table>
<thead>
<tr>
<th>NON-ANTINEOPLASTIC DRUGS</th>
<th>INTERACTIONS (N=50) % (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANALGESICS / OPIOIDS</td>
<td>20 % (10)</td>
</tr>
<tr>
<td>ANTIHYPERTENSIVES</td>
<td>16 % (8)</td>
</tr>
<tr>
<td>ANTIPSYCHOTICS / NEUROLEPTICS</td>
<td>16 % (8)</td>
</tr>
<tr>
<td>ANXIOLYtics / HYPNOTICS / SEDATIVES</td>
<td>10 % (5)</td>
</tr>
<tr>
<td>PROTON PUMP INHIBITORS</td>
<td>8 % (4)</td>
</tr>
<tr>
<td>BLOOD GLUCOSE LOWERING DRUGS, EXCL. INSULINS</td>
<td>8 % (4)</td>
</tr>
<tr>
<td>ANTICOAGULANTS</td>
<td>6 % (3)</td>
</tr>
<tr>
<td>ANTIACIDS</td>
<td>6 % (3)</td>
</tr>
<tr>
<td>STEROIDS</td>
<td>4 % (2)</td>
</tr>
<tr>
<td>ANTIBACTERIALS</td>
<td>2 % (1)</td>
</tr>
<tr>
<td>LIPID MODIFYING AGENTS</td>
<td>2 % (1)</td>
</tr>
<tr>
<td>DRUGS USED IN BENIGN PROSTATIC HYPERTROPHY</td>
<td>2 % (1)</td>
</tr>
</tbody>
</table>
of potential interactions and the dissemination of medication information among the multidisciplinary team.

Conclusions

All patients who are prescribed oral antineoplastic drugs should be provided written or electronic patient education materials about their treatment before or at the time of prescription. Patient education includes: the preparation, administration, and disposal of their antineoplastic drug; concurrent cancer treatment and supportive care medications/measures (when applicable); possible drug/drug and drug/food interactions; and the plan for missed doses. At each clinical encounter, staff must review the patient’s current medications including over the counter medications and complementary and alternative therapies. Any changes in the patient’s medications prompt, should review for drug-drug interactions, as well as communication the prescribing physicians.

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Conflicts of interest

All the authors declare that they have no conflict of interest.

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Standardization of *Citrullus colocynthis* (L.) Shrad. fruits dry extract for further study of its antidiabetic activity

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**KEYWORDS:** *Citrullus colocynthis*; dry extract; HPLC; standardization; validation; ellagic acid

**SUMMARY**

*Citrullus colocynthis* (L.) Shrad. (*C. colocynthis*) is a perennial herb of Cucurbitaceae Family native in Arabia, West Asia, Tropical Africa and in the Mediterranean region. *C. colocynthis* is a perspective medicinal plant due to its pharmacological activities in particular for the treatment of diabetes mellitus. In this study a dry extract of *C. colocynthis* fruits was obtained. Technological scheme of extract obtaining was represened. Obtained dry extract has been standardized for these parameters: solubility, pH, weight loss on drying, the content of heavy metal, total ash content, identification and determination of quantitative content of biologically active substances (BAS). *C. colocynthis* fruits dry extract was standardized with the content of ellagic acid (EA) (0.62%). High performance liquid chromatography (HPLC) method was developed and validated for quantification of EA. The validated procedure is linear, specific, and therefore can be used to determine EA in *C. colocynthis* dry extract.

**1. Introduction**

In recent years diabetes mellitus (DM) gains menacing proportions of the global pandemic. According to the International Diabetic Federation (IDF) by the year 2015 there were 415 million patients with diabetes, and according to experts in 2040 their number is expected to increase to 642 million people\(^1\). Today more attention is paid to phytotherapy of DM\(^2\)-\(^3\). Herbal preparations, unlike synthetic ones, do not have a single mechanism of action. Their influence on the organism is caused by a balanced complex of biologically active substances (BAS), which simultaneously act on both the underlying disease and the functional disorders that accompany it. It should be noted that the total effect of the action of extracted substances may differ from the effect of the basic BAS, therefore, taking into account the expected pharmacological activity, the isolation of a specific physiologically active substance is not always necessary\(^4\)-\(^5\).

Colocynthis – representative of the Cucurbitaceae family, known for its antioxidant, antihyperglycemic, antihyperlipidemic, hepatoprotective properties, used for treatment of inflammatory processes, joint pain, fever, bacterial and fungal infections\(^6\)-\(^10\).

The largest number of preclinical and clinical studies is devoted to the study of *C. colocynthis* antidiabetic activity. In the traditional medicine of the Mediterranean countries *C. colocynthis* is known as...
an antidiabetic agent\textsuperscript{11,12}. Despite this literary sources contain rather contradictory information about the optimal form of plant administration, some of which supplement the results obtained earlier, others exclude them, which leads to the continuation and conduction of new trials\textsuperscript{13-16}.

In order to study antidiabetic activity of \textit{C. colocynthis} we obtained a dry extract from its fruits, which was subsequently standardized, so reduced to a certain content of a substances with known therapeutic effect, to ensure that the object of standardization is in line with its functional purpose.

2. Materials and methods

The studied material was a dry extract obtained from \textit{C. colocynthis} fruits in the laboratory of the Department of Pharmacognosy and Botany of Bogomolets National Medical University (Kyiv, Ukraine).

2.1. Obtaining of \textit{C. colocynthis} fruits dry extract

Dry fruits of \textit{C. colocynthis}, imported from Egypt (Cairo), were grounded to a particle size of about 0.5 mm through a sieve\textsuperscript{17}. The crushed pieces of the fruits were extracted in a Soxhlet apparatus (extractant – chloroform)\textsuperscript{18}. The extaction cake was dried, and then extracted with purified water for 30 minutes (in the ratio of 1:10) in a water bath\textsuperscript{19}; the obtained extract was filtered; the filtrate was evaporated and dried to a residual moisture content of 5%. Thus, a dry extract of \textit{C. colocynthis} fruits was obtained.

2.2. Standardization of \textit{C. colocynthis} fruits dry extract

According to the Monograph «Extracts» of the State Pharmacopoeia of Ukraine (SPhU) for standardization the numerical parameters were determined in 5 batches of the extract obtained in laboratory conditions. The following indicators were determined: solubility, pH, weight loss on drying, the content of heavy metal, total ash content, identification and determination of quantitative content of BAS. The determination of solubility of dry extracts in various solvents, pH and the content of heavy metal were carried out according to the standard methods of the SPhU.

2.2.1 Weight loss on drying.

This indicator is introduced to control the content of volatile substances and / or moisture in the substance. According to the method (2.2.32) of the SPhU if there are no other indications in a separate monograph and the substance is not crystalline solvates, the weight loss on drying or the water content should not exceed 5%. Testing of the samples was determined after drying in a dryer «ШС-161». Approximately 0.2 g (precise weight) of the substance, with an accuracy of 0.002 g, was dried in a dryer at a temperature of (105 ± 1)° C to constant mass. The calculation was carried out according to the formula 1:

\[ \text{Weight loss on drying, } \% = \frac{(W1+W2) - W3}{W2} \times 100 \%, \]  
(1)

where

- \( W1 \) – the weight of the empty weighing bottle, mg;
- \( W2 \) – weight of the tested sample, mg;
- \( W3 \) – constant weight of the weighing bottle and sample after drying, mg.

2.2.2 Total ash.

The common ash was determined according to the method (2.4.16) of the SPhU after combustion of substances in a muffle furnace «МП-2У». Approximately 0.2 g (precise weight) of the substance, to an accuracy of 0.002 g, was placed in a crucible, dried to a constant mass. It was burned on a tile and placed in a high-temperature oven, burned at 600-650 ° C to constant weight (two weighings, a difference ≤ 0.0005 g). The calculation was carried out according to the formula 2:

\[ \text{Total ash, } \% = \frac{(W3-W2)}{W1} \times 100 \% , \]  
(2)

where

- \( W1 \) – weight of the tested sample, mg;
- \( W2 \) – the weight of the empty crucible, mg;
- \( W3 \) – constant weight of the crucible and ash, mg.
Figure 1. Technological scheme of production of C. colocynthis dry extract
2.2.3 Identification and qualitative determination.

For the identification of dry extracts methods of infrared spectroscopy or high-performance liquid chromatography combined with characteristic chemical reactions are commonly used. HPLC studies were performed on a Shimadzu LC20 Prominence liquid chromatograph in a modular system equipped with a four-channel pump LC20AD, column thermostat CTO20A, automatic sampler SIL20A, diode-matrix detector SPDM20A in comparison with the external standard model of elagic acid in such conditions:
- column Phenomenex Luna C18(2), size 250 mm x 4.6 mm, particle size 5 μm;
- column temperature – 35° C;
- detecting wavelength – 330 nm;
- flow rate of the mobile phase – 1 ml/min;
- volume of the entered sample – 5 μl;

Mobile phase: Eluent A: 0.1% solution of trifluoroacetic acid in water; Eluent B: 0.1% solution of trifluoroacetic acid in acetonitrile.

Identification of the components was carried out in accordance with the time of retention and compliance of the UV spectra with the substances-standards (at 254 nm and 330 nm). The HPLC method has been validated in accordance with accepted scientific practice and existing recommendations for analytical validation, conducted in accordance with the requirements of the ICH management regarding the validation of the Higher Technical Methods (HAC-CP): Text and Methodology Q2(R1) \textsuperscript{20}.

3. Results

The obtained extract is homogeneous composition, with a characteristic smell and a specific bitter taste, brownish-orange color. Technological scheme of extract obtaining is given in Figure 1.

In order to investigate the pharmacological activity of C. colocynthis dry extract, obtained for the first time, it was necessary to establish parameters for its standardization. Parameters of standardization of C. colocynthis dry extract are represented in Table 1.

According to results of standardization C. colocynthis dry extract is well soluble in water and practically insoluble in alcohol. All 5 samples corresponded the parameters of standardization. The average value of pH of 10% aqueous solution of C. colocynthis dry extract was 5.0, weight loss on drying was 2.71%. The content of heavy metals was no more than 0.01%. The content of total ash in dry extract was set at 6.34%.

According to results of the HPLC the chlorogenic acid content (retention time = 20.5 min) was 0.05% and EA (retention time = 31.2 min) was 0.62% (Fig.
Other phenolic components were unknown. Taking into account research data about positive impact of EA on the course of diabetes through different ways of action in particular suppressing the activity of phosphorylase and α-glucosidase, slowing of glucose transport through the intestine, insulin-tropic action, protection of SOD from glycosylation and fragmentation and others\textsuperscript{21-28} the extract was standardized with the content of EA.

Validation parameters of the methodology for determining the EA by the HPLC method are given in Table 2.

### 4. Discussions

At the stage of preclinical experimental research medicinal plant raw materials are typically used in the form of various extracts. For the convenience to study pharmacological activity of \textit{C. colocynthis} fruits we obtained a dry extract from a pre-obtained aqueous fruit extract. The dry extract has a number of advantages over liquid extract and soft extract. Due to low moisture content (no more than 5%), the dry extract is easily transported. Dry extract can be crushed to a powder state, exactly dosage, which contributes to increasing its therapeutic effect.

In search of the most effective form of application that determines the presence of a certain type of pharmacological activity based on the composition of BAS in the received substance, the researchers proposed to use different types of extracts from medicinal plant material of \textit{C. colocynthis}\textsuperscript{13-16}. Scientists have contradictory data on the benefits of different types of extracts, their safety and efficacy\textsuperscript{29-31}. Marwat S. K. et al. in their review highlighted the positive effect of various seed extracts

---

**Table 1. Parameters of standardization of \textit{C. colocynthis} fruits dry extract according to requirements of SPhU**

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A homogeneous brownish-orange powder with a characteristic odor and a specific bitter taste</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Solubility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well soluble in water, practically insoluble in alcohol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Identification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>When interacting with cupri-tartaric solution, a red brick-red precipitate develops (reducing sugars)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Weight loss on drying</td>
<td>&lt; 4.0 %</td>
<td>3.02 %</td>
<td>2.83 %</td>
<td>2.10 %</td>
<td>2.46 %</td>
</tr>
<tr>
<td>pH</td>
<td>4.5-6.5</td>
<td>4.72</td>
<td>5.12</td>
<td>5.25</td>
<td>4.81</td>
</tr>
<tr>
<td>Total ash</td>
<td>&lt; 9.0 %</td>
<td>5.32 %</td>
<td>6.15 %</td>
<td>7.04 %</td>
<td>5.20 %</td>
</tr>
<tr>
<td>The content of heavy metals</td>
<td>&lt;0.01%</td>
<td>&lt;0.01 %</td>
<td>&lt;0.01 %</td>
<td>&lt;0.01 %</td>
<td>&lt;0.01 %</td>
</tr>
<tr>
<td>The content of ellagic acid</td>
<td>≥ 0.5 %</td>
<td>0.63 %</td>
<td>0.60 %</td>
<td>0.64 %</td>
<td>0.61 %</td>
</tr>
</tbody>
</table>

* + – Sample corresponds the parameter of standardization*
of *C. colocynthis* (aqueous extract, fat-free aqueous extract, aqueous methanolic extract, ethyl acetate and n-butanol extracts) on the following indicators: glucose tolerance, body weight, mass of the pancreas, diaphragmatic muscle tissue, serum cholesterol, triglycerides, urea, creatinine, transaminase and alkaline phosphatase in animals with diabetes. The authors concluded that the most pronounced effect in diabetic rats had aqueous and n-butanol extracts, the lowest – fat-free aqueous extract.

Investigation of phenolic compounds content was a priority for us, since it is known that they possess antioxidant, antiinflammatory and also antidiabetic properties. Rashedi H. et al. shows a significant difference in the content of flavonoids and phenolic compounds for different parts of *C. colocynthis*. The maximum content of flavonoids and phenolic compounds were observed in fruits and stems. Moreover, the content of these compounds is different depending not only on a certain anatomical part of the plant but also on the type of extract. Such facts have been proven in studies by E. Chekroun et al., A. I. Hussain et al., N. Benariba et al.

The object of our research was *C. colocynthis* fruits dry extract. We detected the presence of EA and HA in the fruits of *C. colocynthis* by HPLC method. Husseain A. I. et al. by the method of reverse phase high performance liquid chromatography (RP-HPLC) found ferulic acid, vanillic acid, p-coumeric acid, gallic acid, p-hydroxy benzoic acid and chlorogenic acid, and flavonoids quercetin, myricetin and catechin in ethanol and hexane extracts of roots, leaves and fruits. The data of the author according to the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum of absorption</td>
<td>330 nm</td>
</tr>
<tr>
<td>System suitability</td>
<td>Tailing factor = 1.19</td>
</tr>
<tr>
<td></td>
<td>RSD = 0.23 %</td>
</tr>
<tr>
<td></td>
<td>Theoretical plates = 21047</td>
</tr>
<tr>
<td>Linearity</td>
<td>Range: 2-25 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient $r^2=0.9998$;</td>
</tr>
<tr>
<td></td>
<td>Linear equation: $y_i = 0.9923x + 0.0057$;</td>
</tr>
<tr>
<td></td>
<td>Slope = 0.9923; Intercept = 0.0057</td>
</tr>
<tr>
<td>Specificity</td>
<td>Standard solution of EA: $R_t$ EA = 31.2 min</td>
</tr>
<tr>
<td></td>
<td>Placebo solution of EA: $R_t$ EA = absent</td>
</tr>
<tr>
<td></td>
<td><em>C. colocynthis</em> dry extract solution of EA: $R_t$ EA = 31.2 min</td>
</tr>
<tr>
<td></td>
<td>The method is specific</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td>2.23 % (NMT 3.0 %)</td>
</tr>
<tr>
<td>Intra-laboratory precision</td>
<td>2.89 % (NMT 3.2 %)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.14 % (NMT 0.74 %)</td>
</tr>
<tr>
<td>Stability</td>
<td>0.01 (NMT 1.024 %)</td>
</tr>
<tr>
<td>Limit of quantitation (LOQ)</td>
<td>0.143% to the concentration of the standard solution</td>
</tr>
<tr>
<td>Limit of detection (LOD)</td>
<td>0.047% to the concentration of the standard solution</td>
</tr>
</tbody>
</table>
results of the chromatographic analysis coincide with ours regarding the presence of chlorogenic acid in fruits of *C. colocynthis*. However, in the list of compounds mentioned by the author there is no elagic acid, which was obtained in our trials. Both the author and we carried out the determination of phytochemical compounds by HPLC method which was developed and validated. In trials of other scientists’ identification of phenolic compounds was carried out using the method in which Folin-ciocaltue was used. Obtained results regarding the component composition of *C. colocynthis* fruits dry extract were expected. Our previous research on the culture of pancreatic cells of Rin-m5F showed the presence of pronounced antioxidant properties of extract, which contributed to the increase in the number of living cells in the culture in the environment of prooxidant factors action. We believe that this effect was caused by EA, which is a strong antioxidant of natural origin. The ability of *C. colocynthis* fruits extract to reduce glucose levels in vitro and in vivo experiments is also likely to be in most cases related to the effects of EA.

A number of studies confirm the antidiabetic activity of EA through various mechanisms of its action. In the literature review, Gurudeeban S. et al. the antidiabetic effect of the *C. colocynthis* is also associated with the content of phenolic compounds, namely, flavonoids (isorientine and isovitexin). Some scientists argue that flavonoids have the ability to stimulate the synthesis of insulin in vitro, including apigenin and quercetin.

In any case, it is necessary to provide further researches to investigate the pharmacological activity of *C. colocynthis* dry extract to understand the mechanism of its antidiabetic activity. Another important question is the condition of raw materials of medicinal plants. It is necessary to carry out further research of the condition of raw material resources of wild growing *C. colocynthis* in Ukraine for its probable use in medicine and pharmaceutical industry.

5. Conclusions:

1. For the first time a technology for obtaining a dry extract of *C. colocynthis* fruits has been developed. *C. colocynthis* fruits dry extract was standardized with the content of ellagic acid (0.62%).
2. Methodology for determining the ellagic acid in *C. colocynthis* fruits dry extract by the HPLC method was validated. The validated procedure is linear, specific, and therefore can be used to determine elagic acid in a dry extract of *C. colocynthis*.

REFERENCES


25. Fatima N., Hafizur R.M., Hameed A., Ahmed S.,


## ΕΚΔΗΛΩΣΕΙΣ - MEETINGS

<table>
<thead>
<tr>
<th>Event Date</th>
<th>Location</th>
<th>Event Title</th>
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<tbody>
<tr>
<td>18-19 NOVEMBER 2019, PARIS, FRANCE</td>
<td>31st International Conference and Expo on Nanosciences and Nanotechnology</td>
<td><a href="https://nanotechnology.conferenceseries.com/">https://nanotechnology.conferenceseries.com/</a></td>
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<tr>
<td>20 NOVEMBER 2019, NOTTINGHAM, UNITED KINGDOM</td>
<td>Twenty Years of the Rule of Five</td>
<td><a href="http://www.rsc.org/events/detail/39112/twenty-years-of-the-rule-of-five">http://www.rsc.org/events/detail/39112/twenty-years-of-the-rule-of-five</a></td>
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<tr>
<td>16 - 17 ΔΕΚΕΜΒΡΙΟΥ 2019, ΑΙΓ ΛΗ ΖΑΠΠΕΙΟΥ</td>
<td>19ο Πανελλήνιο Φαρμακευτικό Συνέδριο</td>
<td><a href="http://www.pepharm.gr/">http://www.pepharm.gr/</a></td>
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<td>6-10 SEPTEMBER 2020, BASEL, SWITZERLAND</td>
<td>XXVI EFMC International Symposium on Medicinal Chemistry (EFMC-ISMC 2020)</td>
<td><a href="https://www.efmc-ismc.org">https://www.efmc-ismc.org</a></td>
<td></td>
</tr>
</tbody>
</table>
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