HISTORY OF PLANT METABOLITES WITH ANTICANCER ACTIVITY

*Maciej Strzemski[1], Magdalena Wójciak-Kosior[1], Jan Sawicki[1], Janusz Polkowski[1], Ewelina Rydzik-Strzemska[1], Anna Zagaja[2], Aleksandra Bożyk[3], Paweł Krawczyk[3], Ireneusz Sowa[1]**

[1]Department of Analytical Chemistry, Medical University of Lublin, Lublin, Poland
[2]Department of Ethics and Human Philosophy, Medical University of Lublin, Lublin, Poland
[3]Department of Pneumology, Oncology and Allergology, Medical University of Lublin, Lublin, Poland

*Corresponding author e-mail: i.sowa@umlub.pl

S u m m a r y. The article reviews the history of the research on anticancer drugs of plant origin. The procedures that enable the isolation of such compounds as vinblastine, vincristine or paclitaxel are presented. The procedures are based on the fractionation of plant extracts and the estimation of biological activity of the obtained fractions. In most cases, the chromatographic techniques are used to separate biologically active compounds.
K e y w o r d s: podophyllotoxin, vinblastine and vincristine, camptothecin, paclitaxel, combretastatin, jerantinines A-G.

INTRODUCTION

Neoplastic diseases are one of the biggest problems of the modern medicine. Surgery, radiotherapy, chemotherapy as well as molecularly targeted therapies and immunotherapies are currently being used in the treatment of advanced cancers. Traditional chemotherapy based on cytostatic drugs is still a key method for pharmacological treatment of cancer diseases. Despite their high toxicity, cytostatic drugs are very effective; hence, there is a constant need to search for new compounds exhibiting cytostatic activity in plant and animal organisms. The most important discoveries in this field include the isolation and identification of the structure of paclitaxel, vinblastine, vincristine, camptothecin, podophyllotoxin, combretastatin, saponin OSW-1, and jerantinines A-G.

This paper presents the history of research on anticancer drugs of plant origin. Special attention is paid to the research methods that contributed to the isolation of active compounds from plant material.

From prehistory to the second half of the 20th century

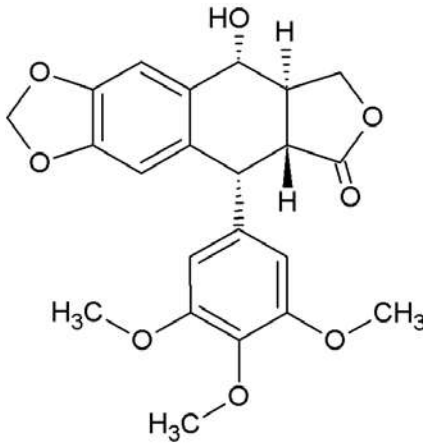
The Edwin Smith Papyrus contained notes on cancer treatment (16th-17th century BC). However, the author of the papyrus did not present a positive prognosis for people affected with neoplastic diseases. Later, Hippocrates of Kos (ca. 460-370 BC), the father of medicine, did not recommend the application of treatment to cancer patients. He even noted that untreated patients lived longer. Hippocrates believed that cancer was the result of an excess of 'black bile' in the body, and his theory has survived nearly 2,000 years. (Barrow and Mark, 1972). Pedanius Dioscorides, living at the beginning of our era (ca. 40-90 AD), cured cancer patients with red clover (*Trifolium pratense* L.) and autumn crocus (*Colchicum autumnale* L.). Ibn Sina, commonly known as Avicenna, expanded the range of anticancer drugs by including poet's narcissus (*Narcissus poeticus* L.), exploding cucumber (*Ecballium elaterium* (L.) A. Rich.), nettle (*Urtica sp*.), common figwort (*Scrophularia nodosa* L.), belladonna (*Atropa belladonna* L.), and castor bean (*Ricinus communis* L.) (Wieczorek et al., 2006; Eltoraim and Ivan 1979).

With the exception of the Caliphate, science did not develop as dynamically in the Middle Ages as during Greek and Roman Antiquity. A great breakthrough in the development of drug science was achieved by the research by Paracelsus (1493-1541), who claimed that the task of alchemy is to find novel drugs that can be extracted from plants by appropri-

ate chemical treatment. Paracelsus's postulates were implemented by Friedrich Sertürner, who isolated morphine with simple chemical methods in 1804. It was the first known pure-form plant compound with strong biological activity. Sertürner's discovery not only helped in the treatment of severe aches, including cancer pain, but also encouraged the isolation of pure compounds from plants. A real breakthrough in this field of research was provided by the investigations conducted by Mikhail Semyonovich Tsvet (1872-1919), who was the first to separate plant dyes in chalk-filled columns, thereby becoming the inventor of chromatography. This technique facilitated effective, rapid, and efficient isolation of active compounds from plant materials. It can be argued that no cytostatics would currently be available in oncology without Tsvet's discovery.

Podophyllotoxin

The first work on isolation of active compounds from *Podophyllum peltatum* L. was carried out by Podwyssotzki in 1861. By crystallisation, he obtained picropodophyllin, which he erroneously called podophyllotoxin (Fig. 1) (Imbert, 1998).

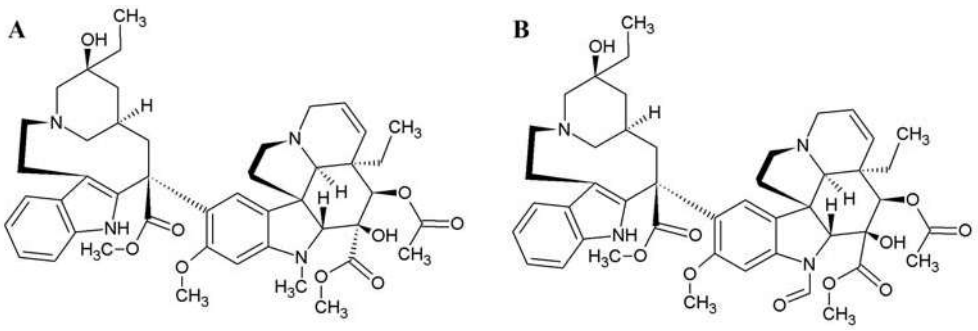


F i g u r e 1. Podophyllotoxin

Podophyllotoxin isolated 20 years later was the first pure-form compound with antimitotic activity (Wieczorek et al., 2006; Stoll et al., 1954). Isolation of the compound was challenging due to the lack of appropriate analytical techniques. There were no chromatographic techniques at that time and all isolations were performed with the classical chemical methods. The structure of podophyllotoxin was identified by Borsche and Niemann (1932). It was later modified by Schrecker and Hartwell (1956). After the verification of its structure, synthesis of the compound was carried out and etoposide (VP-16 compound) was synthesized in 1966 (Kuhn and Wartburg 1967). The activity of podophyllotoxin is based on the reversible binding to tubulin, while etoposide and teniposide inhibit topoisomerase II which mediates the cleavage of DNA. Etoposide is an effective cytostatic agent used until today in the treatment of lung cancer, especially in combination with platinum compounds in patients with small-cell lung cancer (Cragg et al., 2016).

Vinblastine and Vincristine

In 1951, Robert Noble received a letter containing *Catharanthus roseus* (L.) G. Don., leaves from his brother. The leaves were provided by a Jamaican patient, who claimed that they were used in traditional medicine as an antidiabetic drug. Investigations conducted together with Charles Thomas Beer demonstrated that extracts of the leaves were characterised by high cytotoxicity. The researchers treated the *C. roseus* leaves with a mixture of ethanol, water, and acetic acid. They evaporated the extract to dryness and suspended in a 2% aqueous HCl solution (pH 4). They centrifuged the non-dissolved fraction and determined its biological activity. It appeared to be low. Therefore, they extracted the supernatant obtained during centrifugation with benzene. After the extraction, the researchers assessed the activities of the resulting fractions. The aqueous fraction turned out to have high cytotoxic activity. It was alkalised (pH 7) and extracted again with benzene. The benzene extract contained a pure alkaloid fraction and exhibited potent cytotoxic activity. The next step was chromatographic isolation of pure vinblastine (Fig. 2A) (Noble et al., 1958).

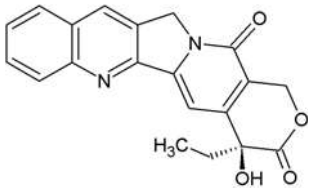


F i g u r e 2. Vinblastine (A) and vincristine (B)

Vincristine (Fig. 2B) was another alkaloid isolated from *C. roseus* that exhibited cytotoxic activity, and as it turned out later, vinblastine and vincristine destroy microtubules, which arrests the cells in metaphase stage and their subsequent death (Cragg et al., 2016). The compound was isolated by Gordon H. Svoboda from Lilly Research Laboratories (Farnsworth 1988). Vincristine and vinblastine are commonly applied in oncological therapies in combination with other cytostatics as well as in monotherapy. They have also become starting compounds for the synthesis of many derivatives, e.g. vinorelbine, vindesine, vinflunine, and anhydrovinblastine.

Camptothecin

In the 40s of the 20th century, Monroe Wall, who worked for the USDA ERRL in Philadelphia, searched for plant materials that could be important to the military industry. Doctor Wall archived samples of ethanol extracts, which were later used for the isolation of camptothecin (Fig. 3).

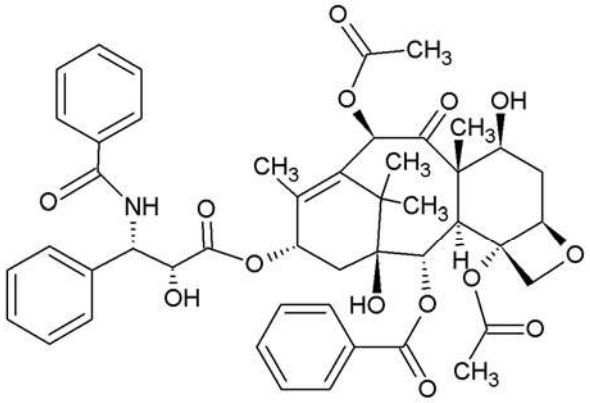


F i g u r e 3. Camptothecin

In 1957, Jonathan Hartwell from the Cancer Chemotherapy National Service Center persuaded doctor Wall to send samples of 1,000 archived plant extracts to the NCI, where their activity was assessed. One of them, i.e. the *Camptotheca acuminata* Decne extract, exhibited particularly interesting properties. However, the USDA did not support the search for anticancer drugs. Therefore, in 1960, Monroe Wall moved to the Research Triangle Institute, and in collaboration with Mansukh Wani started work on the isolation of an active compound from 20 kg of *C. acuminata* bark and wood in 1963. Both investigators adopted a research strategy employed by the discoverers of vinblastine. They repeatedly fractionated the *C. acuminata* wood and bark extract with conventional chemical techniques, each time estimating the cytotoxic activity of the fractions. In the final stage of the investigations, they employed silica gel chromatography to isolate pure camptothecin. Next, they determined the structure of this compound using X-ray analysis. Camptothecin, as a cytotoxic quinoline alkaloid which inhibits topoisomerase I, has been currently used in cancer chemotherapy (e.g. topotecan in the treatment of small-cell lung cancer and irinotecan in the treatment of colorectal cancer) (Wall et al., 1966; Oberlies and Kroll 2004).

Paclitaxel

Parallel to the work on the isolation of camptothecin, Wall and Wani started research on active compounds contained in *Taxus brevifolia* Nutt. They fractionated an alcohol extract from the bark of the tree with the liquid-liquid separation method. They purified chloroform fraction, characterised by the highest cytotoxic activity, with the chromatographic technique using Florisil, Sephadex LH-20, and silica gel adsorbents. This complex purification process yielded crystalline paclitaxel (Fig. 4) (Wani et al., 1971).

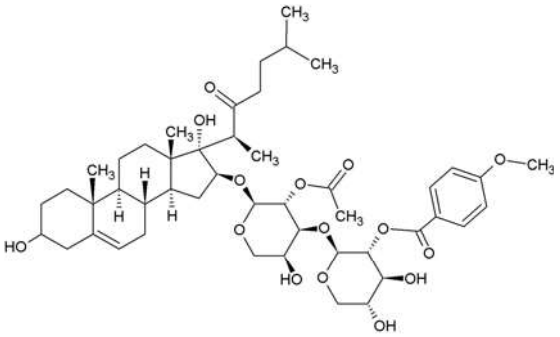


F i g u r e 4. Paclitaxel

Due to its complexity, determination of the structure of this compound proved to be extremely difficult. Dr Wall claimed that the research on paclitaxel should even be suspended, and other plant compounds should be explored. Nevertheless, dr Wani determined the structure of the alkaloid using X-ray analysis. Paclitaxel and its semi-synthetic analogue – docetaxel that work by interfering with cell division (by binding with the mitotic spindle microtubule), are commonly used in chemotherapy of breast, prostate, gastric, head and neck, and lung cancers.

Saponin osw-1

In 1992, Kubo et al. isolated saponins from *Ornithogalum saundersiae* Baker bulbs, which exhibited higher cytotoxicity than paclitaxel and cisplatin and low toxicity towards normal cells. Saponin OSW-1 (Fig. 5) appeared to be the most active compound in all the saponin family.

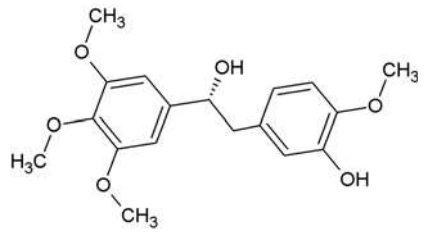


F i g u r e 5. Saponin OSW-1

To isolate it, the researchers extracted 16.2 kg of *Ornithogalum* bulbs with boiling MeOH. The extract obtained in this way was evaporated to dryness, and the residue was extracted with n-butanol. The solution was fractionated repeatedly with the silica gel chromatography method. Ultimately, milligrams of several saponins were obtained. The investigators determined the structure of the compounds with mass spectrometry and NMR techniques (Kubo et al., 1992; Kuroda et al., 2001).

Combretastatin

In the late 80s of the 20th century, George Pettit et al. isolated and determined the structure of several compounds called combretastatins (Fig. 6). They were extracting 77 kg of timber from an African tree *Combretum caffrum* (Eckl. & Zeyh.) Kuntze with 320 litres of a methylene chloride and methanol mixture (1:1) for 11 days. They fractionated the extract with the liquid-liquid separation technique using hexane and a methanol-water mixture (9:1). Each fraction was analysed for its biological activity. Finally, 827.9 g of a CH_2Cl_2 extract of the most potent activity was obtained. The extract was subjected to multistep purification with column chromatography with Sephadex LH-20 and silica gel adsorbents. The chromatographic separation yielded 0.7 g of combretastatin A-1 and 39.6 mg of combretastatin B-1. The structures of these compounds were established with mass spectrometry and NMR techniques (Pettit et al., 1987).



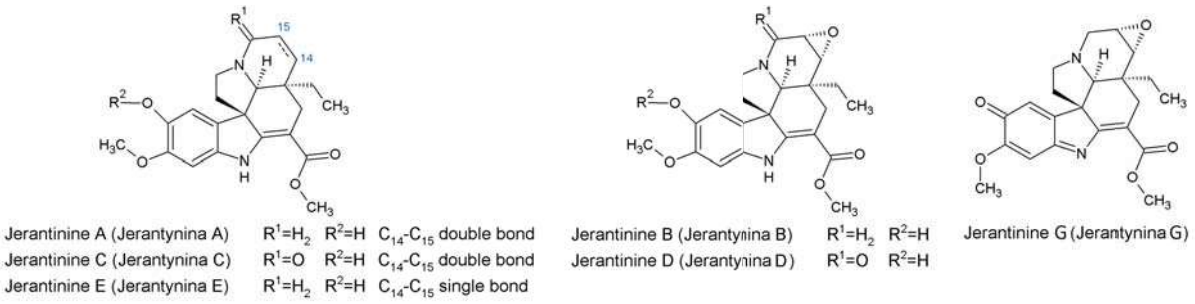
F i g u r e 6. Combretastatin

Combretastatins are promising compounds binding to the colchicine domain in the microtubules. Their semisynthetic derivatives such as combretastatin phosphate A-4, are currently undergoing phase II clinical trials in the treatment of thyroid cancer or Phase III clinical trials in the treatment of cervical, colorectal cancer, non-small cell lung cancer, ovarian and prostate cancer (Rogalska et al., 2015).

Jerantinines A-G

In 2008, a research team from Malaysia and Japan isolated and determined the structure of seven new indole alkaloids from a Malaysian plant *Tabernaemontana corymbosa* Roxb. ex Wall. The investigators fractionated ethanol extract from the leaves of the plant with column chromatography followed by multiple re-chromatography with centrifugal thin layer chromatography. As a result, a kilogram of leaves yielded milligrams and micrograms of different alkaloids. The structure of these compounds was identified with mass spectrometry and NMR techniques (Lim et al., 2008).

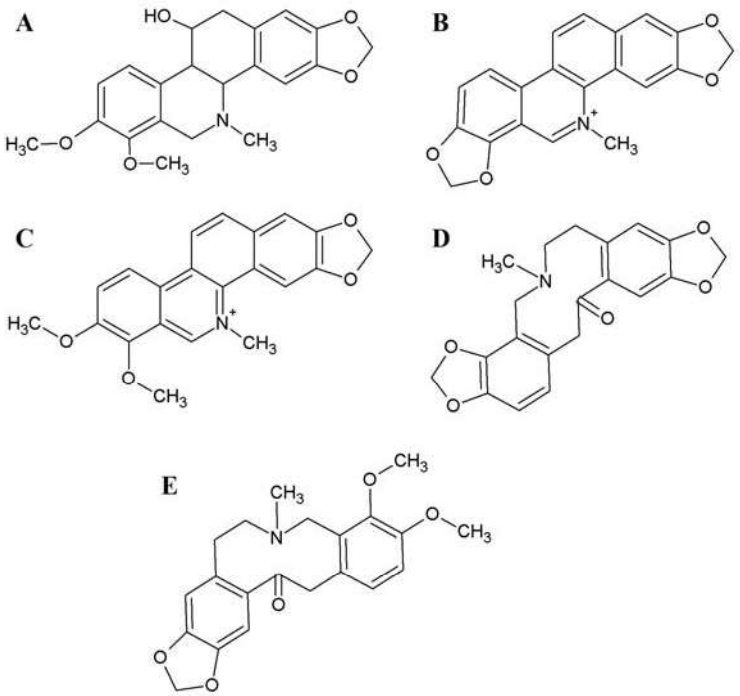
The cytotoxicity of jerantinines (Fig. 7) is currently being investigated in many *in vitro* studies. Similar to vinca alkaloids, jerantinine, which is a potent inhibitor of microtubule polymerisation, proved to be cytotoxic towards vincristine-resistant tumour cells (Rogalska et al., 2015). Jerantinine B has been shown to exhibit high cytotoxicity towards breast lung, pancreas, or intestine cancer cell lines (Qazzaz et al., 2016).



F i g u r e 7. Jarantinines

Alkaloids from Chelidonium majus

Ukrain is known as a plant derived semi-synthetic product which consists of trimers of chelidonine (Fig. 8A) alkylated with thiophosphoric acid and other alkaloids from *Chelidonium majus* L. Ukrain was developed in 1978 by Dr. Wassil J. Nowicky, director of the Ukrainian Anti-Cancer Institute of Vienna, Austria. In 2004 and 2006, Nowicky was nominated for the Nobel Prize in Chemistry. The researchers found that the most important components of *Chelidonium* include chelidonine, sanguinarine (Fig. 8B), chelerythrine (Fig. 8C), protopine (Fig. 8D) and allocryptopine (Fig. 8E) (Habermehl et al., 2006). The antitumor activity of Ukrain was based on the differences between oxygen consumption in healthy and tumor cells (experiment with normal liver cells and Ehrlich ascetic tumor cells). Studies have shown increased oxygen consumption in tumor cells at the beginning, which then droped to zero, while oxygen consumption in healthy cells returned to normal and stayed undamaged (Nowicky 1985).



F i g u r e 8. Alkaloids *Chelidonium majus*: chelidonine (A), sanguinarine (B), chelerithrine (C), protopine (D) and allocryptopine (E).

Cytotoxic properties of Ukrain are used in the treatment of colorectal, bladder, pancreatic and breast cancers (Capistrano et al., 2015). Ukraine is not approved for medicinal purposes in the EU and USA (derivatives of thiophosphoric acid can cause severe liver damage), but it is used as a standard anticancer agent in Mexico and United Arab Emirates. Dr Wassil Nowicky has submitted complaints to the European Court of Human Rights on account of 'unlawful rejection of an approval application'.

CONCLUSIONS

The intensive search for plant compounds that exhibit anti-tumour activity was initiated in the 50s and 60s of the 20th century. To date, cancer chemotherapy has been primarily based on the application of plant-originated compounds and their analogues. However, due to the serious side effects and limited effectiveness of such therapies, molecularly targeted therapy and immunotherapy are gaining popularity. However, it should be emphasised that the discovery of such compounds as paclitaxel or vinca alkaloids has saved many patients' lives. This would not have been possible without the use of appropriate procedures and analytical techniques, mostly based on fractionation of cytotoxic plant extracts and purification of the most active fractions. The fractionation procedure has usually been performed with the conventional chemical methods, e.g. liquid-liquid extraction, and pure plant metabolites have been isolated with chromatographic techniques. This approach is substantially different from the procedures adopted by phytochemists, who first isolate pure compounds from plants, determine their structure, and then evaluate their biological activity. However, screening of many plant extracts and search for active compounds after identification of the activity of the whole extract seems to be considerably more efficient.

REFERENCES

1. Barrow M.V. (1972). Portraits of Hippocrates. *Med. History.* 16 (1). p.85-88.
2. Borsche W., Niemann J. (1932). Über podophyllin. *Liebigs Ann. Chem.* 494, p.126-142.
3. Capistrano I. R., Wouters A., Lardon F., Gravekamp C., Apers S., Pieters L. (2015). In vitro and in vivo investigations on the antitumour activity of *Chelidonium majus*. *Phytomedicine.* 22. p.1279-1287.
4. Cragg G. M., Pezzuto J. M. (2016). Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents

medicinal principles and practise. *Med. Prin. Pract.* 25. p.41-59.
5. Eltorai I. (1979). Avicenna's view on cancer from his canon. *Am. J. Chinese Med.* 7. p.276-284.
6. Farnsworth N.R. (1988). Screening plants for new medicines. *Biodiversity.* 9. p.83-97.
7. Habermehl D., Kammerer B., Handrick R., Eldh T., Gruber Ch., Cordes N., Daniel P.T., Plasswilm L., Bamberg M., Belka C., Jendrossek V. (2006). Proapoptotic activity of Ukrain is based on *Chelidonium majus* L. alkaloids and mediated via a mitochondrial death pathway. *BMC Cancer.* 6 (14). p.1-22.
8. Imbert T. F. (1998). Discovery of podophyllotoxins. *Biochemie.* 80. p.207-222.
9. Kubo S., Mimaki Y., Terao M., Sashida Y., Nikaido T., Ohmoto T. (1992). Acylated cholestane glycosides the bulbus of *Ornithogalum saudersiae*. *Phytochemistry.* 11. p.3969-3973.
10. Kuhn M., Wartburg A. (1967). Podophyllumlignane. structur und absolutkon□guration von podorhizol-β-d-glucosid (= Lignan F)1). *Helv. Chim. Acta.* 161. p.1547-1565.
11. Kuroda M., Mimaki Y., Yokosuka A., Sashida Y., Beutler J. A. (2001). Cytotoxic cholestane glycosides from the bulbus of *Ornithogalum saundersiae*. *J. Nat. Prod.* 64. p.88-91.
12. Lim K-H., Hiraku O., Komiyama K., Kam T-S. (2008). Jerantinines A-G, cytotoxic aspidosperma alkaloids from *Tabernaemontana corymbosa*. *J. Nat. Prod.* 71. p.1591-1594.
13. Noble R. L., Beer C. T., Cutts J. H. (1958). Role of Chance observations in chemotherapy. *Vinca rosea*. *Ann. NY Acad. Sci.* 76. p.882-894.
14. Nowicky J. W. (1985). Cancer treatment using anticancer preparation alkaloid derivative. *Ukrain. Chemioterapia.* 4. p.1169-1170.
15. Oberlies N. H., Kroll D .J. (2004). Camptothecin and taxol. historic achievements in natural products research. *J. Nat. Prod.* 67. p.129-135.
16. Pettit G. R., Singh S. B., Niven M. L., Hamel E., Schmidt J. M. (1987). Isolation, structure, and synthesis of combretastatins A-1 and B-1, potent new inhibitors of microtubule assembly, derived from *Combretum caffrum*. *J. Nat. Prod.* 1. p.119-131.
17. Qazzaz M. E., Raja V. J., Lim K-H., Kam T-S., Lee J. B. Gershkovich P., Bradshaw T. D. (2016). In vitro anticancer properties and biological evaluation of novel natural alkaloid jerantinine B. *Cancer Lett.* 2. p.185-197.
18. Rogalska A., Miśkiewicz K., Marczak A. (2015). Inhibitors of microtubule polymerization – new natural compounds as potential anti-cancer drugs. *Postępy Hig. Med. Dosw.* 69. p.571-585.
19. Schrecker A. W. Hartwell J. L. (1956). Components of podophyllin. The absolute con□guration of podophyllotoxin and related lignans. *J. Org. Chem.* 21. p.381 -382.
20. Stoll A., Renz J., Wartrburg A. V. (1954). The isolation of podophyllotoxin glucoside. *J. Am. Chem. Soc.* 76. p.3103-3104.
21. Wall M. E., Wani M. C., Cook C. E., Palmer K. H. (1966). Plant antitumor agents I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.* 88. p.3888-3890.
22. Wani M. C., Taylor H. L., Wall M. E. (1971). Plant antitumor agents VI. The isolation and structure of taxol, a novel antileucemic and antitumor agent grom *Taxus brevifolia*. *J. Am. Chem. Soc.* 93. p.2325-2327.
23. Wieczorek M., Sobiak S., Meissner R. K. (2006). Development of research on utilization of plant-derived substances in tumor treatment. *Nowiny Lekarskie.* 75. p.407-413.