International Journal of Pharmacology

ISSN 1811-7775

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.835.840

Research Article
Effects of Some Adsorbents on the Pre-purification of Taxol (Anticancer Drug) from Hazelnut Nutshells

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Abstract

Background and Objective: The most important pharmaceutically active substance in plant-derived chemotherapy drugs is paclitaxel. Taxane compounds such as paclitaxel, cephalomannine, baccatin III and 10-Deacetylbaccatin III are found in hazelnut shells of Turkey's Black Sea region. This study aimed to determine the most effective adsorbent on the purity and efficiency of these taxane compounds from nutshells. **Materials and Methods:** The adsorbent effect on the purification of taxane compounds from hazelnut shells was studied using different adsorbent materials. For this purpose, extracts were obtained from samples of hazelnut shells collected, dried and ground into solvents. The resulting extract was concentrated into a stock solution. Samples taken from the stock solution were separately treated with seven different adsorbent materials and then filtered and analyzed with HPLC (High Performance Liquid Chromatography). **Results:** The HPLC analysis of taxane compounds determined on taxane, both the purification and whether or not the substance was lost, by measuring the efficiency at the same time. Graphene-oxide was found to be the adsorbent with the highest positive effect on the purification of paclitaxel in hazelnut nutshells in all cases. Graphene-oxide also had the least loss in terms of paclitaxel yield efficiency in hazelnut nutshells. **Conclusion:** The effects of adsorbent treatment increased both the purity and efficiency of taxane compounds and was, thus, a useful pre-purification method for shells obtained from Turkish hazelnuts. This shell waste material may prove to be a good alternative source of taxol as an anticancer drug.

Key words: Hazelnut shells, graphene-oxide, adsorbent, paclitaxel, 10-Deacetylbaccatin III

Received: January 08, 2018 Accepted: March 29, 2018 Published: July 15, 2018

Citation: Sibel Bayil Oguzkan, Seref Karadeniz, Bora Karagül, Ayse Uzun, Elif Sine Aksoy, Özen Özensoy Güler, Ümit Çakir and Halil Ibrahim Ugras, 2018. Effects of some adsorbents on the pre-purification of taxol (anticancer drug) from hazelnut nutshells. Int. J. Pharmacol., 14: 835-840.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of plant materials as dietary regimens and preservatives is mainly due to their antioxidant, antimicrobial and other biological potentials[1]. Paclitaxel is a complex compound that carries a total of 11 stereo-centers with eight oxo groups and one side chain (N-benzoyl phenylisoserine), with a rarely seen taxon diterpene skeleton (pentamethyltricyclo [9.3.1.0-3-8]-pentadecenes, IUPAC nomenclature). Paclitaxel belongs to a broad class of diterpenoid known as taxanes. The active ingredient in the anti-cancer drug preparation known as taxol is paclitaxel. Taxol is a very important natural anticancer drug used today for the treatment of ovarian and breast cancer, in particular[2,3]. Paclitaxel and its derivatives were isolated for the first time from *Taxus brefilova*[4]. Stierle *et al*.[5] discovered paclitaxel in *Taxomyces andreanae* culture in 1993. Recently, taxanes have also been isolated from some fungi[6]. In 1998, Hoffman *et al*.[7] obtained paclitaxel from various types of nut trees grown in America. The paclitaxel was purified for the first time and then analyzed using HPLC and mass spectroscopy. As a result of this analysis, the chemical structure of paclitaxel was discovered and the amounts of the taxane compounds found in the various solvents were determined[7]. This substance has emerged as a significant problem due to the fact that it is found in the extracts of bark, the tree is rare, the tree takes a very long time to grow and a large amount of shell is needed. In previous studies by the present authors, paclitaxel and its derivatives, baccatin III, cephalomannine and 10-Deacetylbaccatin III, were also found to be present in hazelnut shell extracts, which are common in Turkey. Various separation and purification processes are required to obtain the taxol derivatives from the appropriate organic extracts in high purity. In these methods, organic solvents with the highest amount of taxol are usually used in the most appropriate optimization. The purpose of the organic extraction and final purification method is to obtain these components in both high purity and quantity. In a study by Carver *et al*.[8], paclitaxel was isolated using a semi-permeable membrane and reverse osmosis apparatus, however, this method was problematic due to the high cost of the membranes and the difficulties in applying the reverse osmosis apparatus. Elsohly *et al*.[9] used normal-phase liquid chromatography for the paclitaxel purification of the *Taxus* genus plant from methanol extraction but the paclitaxel obtained were detected in different quantities of interfering substances, such as methylene chloride and ethyl acetate. In paclitaxel purification processes, achieving high efficiency success through the sole use of solvent extraction and chromatography has been difficult and laborious. At the same time, in such chromatographic methods, interference of paclitaxel-related compounds such as terpenoid, lipid, chlorophyll and phenols has occurred and the purity has decreased accordingly. Therefore, the amount of paclitaxel obtained through direct chromatographic methods is low without any pre-purification method and also the purity decreases due to interference. In addition, chromatographic methods may not be controlled and extra steps, such as crystallization, may be required to achieve high purity[9]. In previous studies, the application of purification directly with HPLC without applying a pre-purification process has led to some economic problems. For example, if the samples are purified directly with HPLC without being subjected to pre-purification, the organic solvent consumption increases and the life of the column material quickly decreases, causing extra costs[10]. However, using the pre-purification process before the final purification method with HPLC was found to obtain a purity higher than 50% and then cause very little loss. Positive effects on the purity and efficiency of adsorbent use in the pre-purification process have been reported in various literature sources[11]. In a study by Oh *et al*.[12], silica (SiO_2) was used as an adsorbent in the pre-purification of paclitaxel and the paclitaxel purity obtained after HPLC analysis was found to be quite high. In a study on the effect of adsorbents on paclitaxel purification from cell cultures of the *Taxus chinensis* tree, the use of sylopute, active carbon and active clay resulted in high purity paclitaxel[13]. The development of an effective method for the industrial assessment of paclitaxel requires different adsorbents to be tested. In order to obtain taxol derivatives in organic solvents both in high purity and high amounts, it is necessary to carry out a final purification process after a pre-purification process. In these steps, it can be said that the pre-purification process is extremely important.

The aim of this study was to determine how the purity and efficiency of taxane compounds in extracts from hazelnut shells were affected by different adsorbents and also to find the optimum effective adsorbent.

MATERIALS AND METHODS

Plant material: The samples were collected from different regions in Turkey at different altitudes (0-250 m, 250-500 m, 500 m+). Hazelnut shells were collected in August- September, 2015 and were dried without the sun. The collected samples were ground to powder and were eliminated so as to be smaller than 80 mesh.

All standards, adsorbents and chemicals were of analytical purity and were purchased from Sigma-Aldrich. Ultrapure water was obtained from a Milli-Q water system.

Extraction of taxane and analysis by HPLC: Taxane extractions were determined as described in a previous study by the present authors and as a result of the experiments performed[14]. Dichloromethane (DCM) was used for stock solutions for HPLC analyses and all of the adsorbent samples were analyzed. Four standard taxane compounds were used, these being paclitaxel, cephalomannine, baccatin III and 10-Deacetylbaccatin III.

Adsorbent treatment: In this study, seven varieties of adsorbent were studied that the name subsequently, reduced graphene oxide, vulcan carbon, activated carbon, graphene, graphene oxide, graphite and carbon nanotube[15]. DCM stock solution prepared for adsorbent experiments was used. The adsorbent treatment ratios were 1:0.05 (stock solution (v):adsorbent (g) (1 mL stock:adsorbent). The adsorbents from DCM stock solution were added 1 mL at the determined ratios. The mixture was stirred for 30 min at room temperature on a stirrer and then filtered. The filtrate was then used for HPLC analysis.

RESULTS

Taxane class compounds are known to be the most important anti-cancer agents containing paclitaxel, cephalomannine, baccatin III and 10-Deacetylbaccatin III. The standard chromatograms studied with HPLC analysis of the taxane components, consequently 1: 10-Deacetylbaccatin III, 2: Baccatin III, 3: Cephalomannine and 4: Paclitaxel, were shown in Fig. 1.

In this study, extraction, concentration and enrichment processes were carried out for the purification studies and this stock solution was used in the subsequent adsorbent effect and sedimentation experiments. Samples taken from the stock solution were interacted with adsorbents. After the subsequent HPLC analysis, the effects on the purification of taxane compounds were investigated. These analysis were concluded in two ways that both quantities and purity values of stock during processing and taxane derivatives after processing were measured. Thus, it was also determined whether adsorbents cause loss of material when measuring the effect on purification. In the results obtained, the highest positive effect on the purification of taxane compounds in the hazelnut shells was determined to be the graphene-oxide when used as an adsorbent in all cases as shown in Fig. 2. The graphene-oxide was the most effective on purity and yield of all the seven different adsorbents. The quantity of 10-Deacetylbaccatin III which was a taxane compound in the nut shells was found to have the highest purity efficiency with the graphene-oxide adsorbent as shown in Table 1. At the same time, the results of the yield efficiency compound of 10-Deacetylbaccatin III (5.7734 ppm) in all taxane compounds were shown in Table 2.

Table 1: Results of purity efficiency from nutshell (%) in seven different adsorbents

Adsorbents	Adsorbent (mg)	10-Deacetylbaccatin-III	Baccatin-III	Cephalomannine	Paclitaxel
Reduced graphene oxide	50	1.1749	0.7544	0.5543	0.3966
Vulcan carbon	50	0.5281	0.6522	0.5714	0.3054
Activated carbon	50	0.4235	0.2701	0.2355	0.4224
Graphene	50	1.4689	0.8082	0.4401	0.4569
Graphene oxide	50	2.0349	1.0445	1.1021	0.4880
Graphite	50	1.1324	0.7429	0.4741	0.1964
Carbon nanotube	2.5	1.4155	0.7097	0.5225	0.2883
Stock solution	---	3.0942	1.4044	0.3355	0.4559

Table 2: Results of yield efficiency from nutshell (ppm)

Adsorbents	Adsorbent (mg)	10-Deacetylbaccatin-III	Baccatin-III	Cephalomannine	Paclitaxel
Reduced graphene oxide	50	1.5578	1.9449	0.2244	0.1530
Vulcan carbon	50	0.4071	1.2317	0.2618	0.1461
Activated carbon	50	0.1754	0.3027	0.0992	0.0706
Graphene	50	0.4840	1.3151	0.1833	0.1299
Graphene oxide	50	5.7734	1.2225	1.1006	0.1765
Graphite	50	0.5437	1.8239	0.601	0.2087
Carbon nanotube	2.5	0.3993	1.1272	0.4116	0.1173
Stock solution	---	4.7391	0.6395	0.0634	0.9890

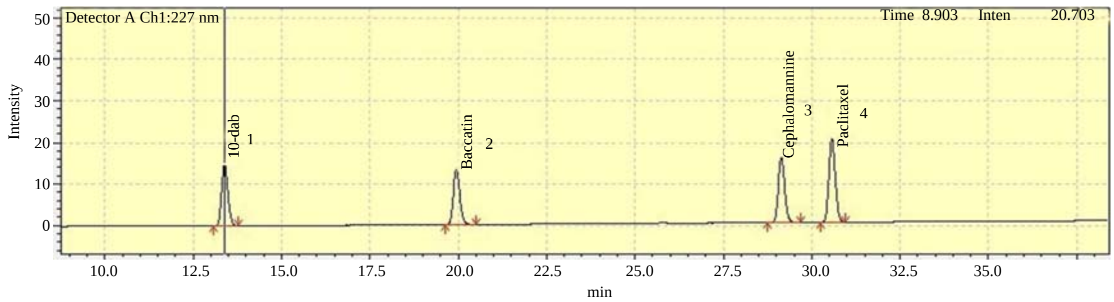

Fig. 1: Taxane compounds (1: 10-Deacetylbaccatin III, 2: Baccatin III, 3: Cephalomannine and 4: Paclitaxel) standard chromatograms

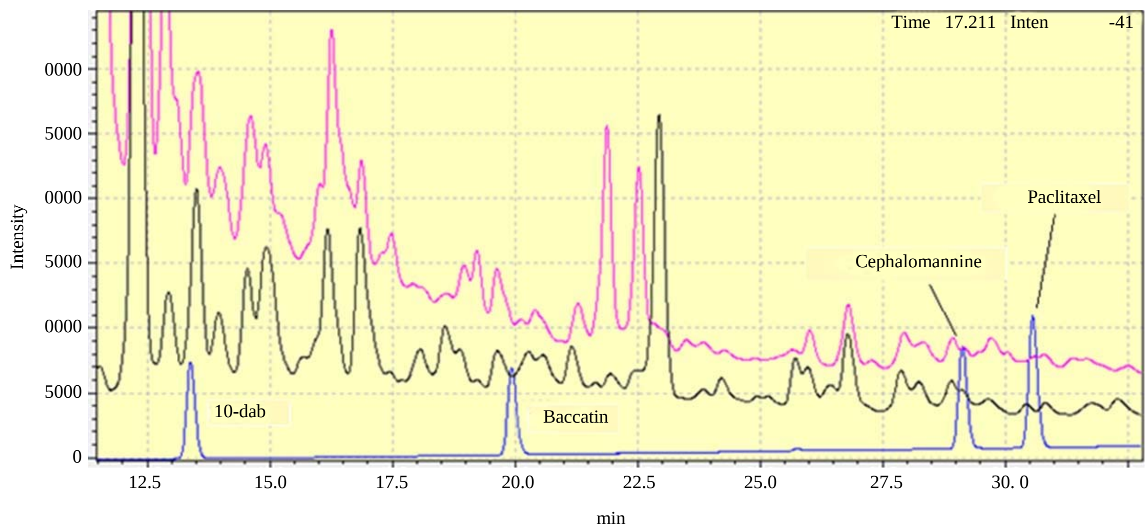

Fig. 2: Chromatogram of using graphene oxide for adsorbent (black), adsorbent-free stock solution (red) and standard compound (blue)

DISCUSSION

The use of plant-based cancer treatments is increasing day by day, resulting in the isolation of many natural compounds from plants. The most important pharmaceutically active substance in plant-derived chemotherapy drugs is taxol. The main source of taxol is from the *Taxus* species, which is a kind of tree, meaning that this source is very limited and non-renewable. Hazelnut nutshells are the alternative to these *Taxus* trees and therefore, the hundreds of thousands of tons of hazelnut shells generated every year in Turkey should be regarded very seriously as an alternative source of taxol. In this study, the extraction of these taxane compounds has been developed from a technique using adsorbent treatment for pre-purification methods. This pre-purification process in particular has a significant impact both on purification and efficiently yield from these compounds of the nutshells. In this study, higher purity taxane compounds were obtained by using adsorbent and then HPLC analysis. In order to increase the effectiveness of the commercial scale, more efficient methods should be developed and the most effective ones should be selected by using various adsorbents.

In the literature, it has been reported that as the use of active clay, sylopute and activated carbon as adsorbents limits color removal for the decolorization of natural compounds[16]. In another study, it was reported that the sylopute selected as a synthetic adsorbent is more effective in paclitaxel purification from plant cultures[17]. In a study performed to determine the effect of adsorbents, it was determined that synthetic adsorbents used in the pre-purification steps play an imperative role in the purity of the dried raw extracts obtained from the methanol extractions of fatty substances. It was also determined that in the processes in which active clay (Mizukalife Chemical Co., Japan) and activated charcoal were used as adsorbents, active clay was more effective on purity than activated charcoal[18]. In a study by Nair[18], using activated charcoal (3-5%) in purification, the raw paclitaxel color was removed at 25°C[18,19]. There are some reports on the uses of different kinds of absorbents which state that when an ideal absorbent is chosen, it has the ability to absorb the compounds of interest selectively and then easily desorb them[20]. This study investigated the effect of different adsorbents on the purification of various taxanes, especially paclitaxel from hazelnut residue. Simple and convincing methods have been reported in some previous studies regarding pre-purification method for pure paclitaxel obtainment (>60%) using raw paclitaxel adsorbent[21]. In the processes reported in the literature, it is very expensive to use pre-purification chromatographic methods or to purify raw extract directly with HPLC without pre-purification and this increases the cost of industrial use[22]. However, the pre-purification process in particular has a significant impact on the cost of the entire purification process. In another study, Shirshekan *et al.*[23] reported that the absorbent (Diaion® HP- 20) and a silica-based solid phase extraction were used as a new, efficient and cost-effective method for large-scale production of taxanes from natural products[23]. From the parts investigated in this study, it was found that the effect on paclitaxel purity in the shell of the hazelnut is not much changed but the use of graphene-oxide increases the purity, even if just a little. It is believed that unexpected changes in purity at this point are due to possible penetrations.

This is because the loss of paclitaxel, especially in recovery, is noticeable in all adsorbents. Particularly in cephalomannine, the effect of graphene-oxide on purification was found to be quite high. In studies on hazelnut shells, very positive results were obtained for taxane compounds, both in terms of increase in purity and recovery.

The results of this study on hazelnut shell residues, of which around 700,000 t are generated in Turkey each year are very important for investments into methods of obtaining taxane compounds.

CONCLUSION

This study presented an efficient pre-purification method for the use of adsorbents in from obtained hazelnut shells from Turkish hazelnut. Although the amounts of taxanes obtained appear to be minimal but these hazelnut shells generated very huge amount every year in Turkey that should be regarded very seriously as an alternative source of the paclitaxel. When considered in terms of its industrial aspect, a shortage of this raw material has never been experienced. Moreover, this pre-purification method as using adsorbent treatment could also be applied to other plant species.

SIGNIFICANCE STATEMENT

The use of plants increase in cancer treatment and therefore, the isolation of many natural compounds is performed by using plants. This paper clearly outlines the pre-purification method for using adsorbent to extract taxane compounds which present a more readily available source of taxol (anticancer drug) from the nut's shells. The method proposed in current study would help the researchers in purification of taxol compounds from the nut shells.

ACKNOWLEDGMENT

The authors express great thanks to the Scientific and Technological Research Council of Turkey for its financial support (TUBİTAK-114Z233 coded project).

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