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Comparison of Different Methods' for Extraction of Taxol from *Taxus Baccata*

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ABSTRACT

Development of a optimal method for purification of taxol is very important due to its demonstrated antitumor activity. In this study, two extraction methods (calsic or maceration and soxhlet methods) was compared. For this, extraction was done by maceration and soxhlet methods and two different solvents, ethanol hexane. The raw extracts were applied on a 25 cm×4.6 mm with a pre-column, Eurospher 100-5 C18 analytical column provided by Knauer (Berlin, Germany). Data acquisition and integration was performed with Millennium 32 software. The results showed that the aquired taxol from soxhlet method was more than clasic method, but this difference was not significant. Both two methods have good conditions for preparation of raw extracts for HPLC stage by ethanol solvent. The change of solvents had no effect on taxol content of extract. But this change lead to reduction of taxol in clasic method. There was significant diffrerence between obtained taxol by soxhlet and clasic methods in presence of hexane solvent. According to aquired data, for extraction of taxol by HPLC, the best solvent for preparation of raw extract is ethanol. Also, because of shorter period, soxhlet method is preferred to maceration method.

Key words: *Extraction, Taxol, Maceration, Soxhlet, Solvent*

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INTRODUCTION

Yew plant with scientific name, Taxus baccata is a conifer. Natively, this plant grow in different parts of world, especially in western, central and southern Europe, northwest Africa, and southwest Asia (Panzeri and etal, 2002). Northern Iran is the natural habitat of Taxus baccata in Iran (Alami and etal , 2014). This plant is a small to medium-sized evergreen tree with average of 28 metres tall. The one major of current chempthrapic drug, eg taxol, was purified and identified from this plant. So, natural substances derived from Taxus baccata have a special place among anti-cancer drugs. Rsearchers have shown that all parts of the this plant contain poisonous alkaloids. The potent compounds of this plant are podophyllotoxin, lignan, and desoxypodophyllotoxin (Kulaand etal , 2009;Goleniowski, 2000 ; Durakand etal , 2014). Taxol is a cancer drug that purify from this plant. This drug use for treatment of ovarian, breast, lung, pancreatic and other cancers. This agent can be used in AIDS-related Kaposi's sarcoma (Horwitz, 1994;Weaver, 2014; Weaver, 2006;Croteau and etal, 2006). Development of a optimal method for purification of taxol is very important due to its demonstrated antitumor activity. Reverse phase liquid chromatography is major important method for isolation and purification of taxol and analogues of taxol from a crude extract of Taxus species). The one of important stages of isolation is praparation of crude extract (Pyo MKand etal , 2011; Memarpoor-Yazdi and etal, 2013; Daneshmand and etal , 2013). There are two methods for this porposes: maceration and sucsulate methods (Zare-Zardini and etal, 2013). These two methods were divided to different subgroups based on used solvents. Each method has advantages and disadvantegse. In this study, we comprised maceration and sucsulate methods as well as effect of solvents on preparation and purification of taxol.

MATERIALS AND METHODS

The plants were identified by a herbalist. A voucher specimen was deposited at Herbal Research Center Laboratory in Sahid Sadoughi University of Meidcla Sciences, Yazd, Iarn.

Preparation of crude extract

In this study, Taxus baccata was supplied by two methods: clasic or soaking method and modern or Soxhlet method.

Soxhlet method

The sample is poured into cartouche and placed in the Soxhlet. The solvent Extraction, e.g. ethanol, was poured into flask. The temprature of instrument was set on 150ºC. The extraction was done for 6 hours. Rotary devices was used to separate the alcohol from the extract at 50ºC. the possible lowest

temprature was used to prevent damage of extract. Similar procidure was done by hexane instead of ethanol.

Clasic or Soaking Method

The sample was poured in to 85% alcohol (ethanol) and mixed by shaker for 24 hours. After this time, the obtained solution was filtered by filter paper and dryed by Rotary devices at 40 ºC. Similar procidure was done by hexane instead of ethanol.

HPLC analysis

A WATERS liquid chromatography apparatus consisting of a separations module: WATERS 2695 (USA) and a dual absorbance detector WATERS 2487 (USA) was used for the HPLC analysis. Injection was auto sampler injector equipped with a 100 µl loop. Separation was achieved on a 25 cm×4.6 mm with a pre-column, Eurospher 100-5 C18 analytical column provided by Knauer (Berlin, Germany). Data acquisition and integration was performed with Millennium 32 software. The chromatographic assay was performed on a 25 cm×4.6 mm with pre-column, Eurospher 100-5 C18 analytical column provided by KNAUER (Berline, Germany) reversed phase matrix (5 µm) (Waters) and elution was carried out in a gradient system with acetonitrile as the organic phase (solvent A) and distilled water (solvent B) with the flow-rate of 1 mL min-1. Peaks were monitored at 230 nm wavelength. Injection volume was 20 µL and the temperature was maintained at 25°C. All injections were repeated three times (n=3). Calibration graphs were plotted subsequently for linear regression analysis of the peak area with concentration 1, 10, 25, 50, 80, 120, 150 and 200 mg L–1.

RESULTS

Solvent selection in clasic Method

In clasic Method, two different solvents (Ethanol and hexan) were used for extraction of Taxol. Figure 1 and 2 show the typical HPLC chromatograms of Taxol obtained by Ethanol and hexan, respectively. In two figures, separation conditions involved C18 column (250 9 4.6 mm, 5 µm); mobile phase, composed of water/ACN in ratio of 55/45 and 70/30 for taxol and 10-DAB III, respectively. The flow rate was 1.0 mL/ min with an injection volume of 20 µL. According to these figures, there was no are presented in Taxol extraction yields in hexan suspention. But, in Ethanol extraction, there was considreable concentration of taxol compared with the hexan suspension. So, the best solvent for purification of taxol by clasic Method is ethanol.

Solvent selection in soxhlet Method

In Soxhlet Method, two different solvents (Ethanol and hexan) were also used for extraction of Taxol. The taxol concentration obtained by the different solvents is shown in figure Figure 3 and 4. Separation conditions involved C18 column (250 9 4.6 mm, 5 µm); mobile phase, composed of water/ACN in ratio of 55/45 and 70/30 for taxol and 10-DAB III, respectively. The flow rate was 1.0 mL/ min with an injection volume of 20 µL. These figures showed that there was no diffrerence effect of solvents on contents of taxol. In two figures, the concentraton of obtained taxol was similar to some extent. So, the solvent had no effect on extraction of taxol.

Comparison of extractin based on method

The extraction was also compered based on method. accordong to figure 1 and 2, the aquired taxol from Soxhlet Method was more than Clasic Method, but this difference was not significant. Both two methods have good conditions for preparation of raw extracts for HPLC stage by ethanol solvent. In figre 3 and 4, the best condition for extraction was observed for Soxhlet Method. the change of solvents had no effect on taxol content of extract. But this change lead to reduction of taxol in Clasic Method. There was significant diffrerence between obtained taxol by Soxhlet and Clasic Method.

DISCUSSION

Development of best techniques for extraction of taxol has increasingly cinsidered as challenge between scientists. Different sciences have focused on best teqniques for purification of Taxol as suitable anticancer drug. In between, HPLC is recognized as a efficient method in this field. HPLC method is simple and sensitive extraction method with less consumed reagents (Pasrija and etal, 2010;Negi and etal, 2011; Shah and etal, 2010). This method is binifitioal for quantitative determination and purification of ingeredinets of raw extratcs, because the extracts do not need purification before injection as with HPLC method. The preparation of raw extract for HPLC column is the first stage of purification protocol (Zhang and etal, 2004). There is two important methods for achievement of extract: soxhlet and maceration Methods. In each method, the condition of solvent and time can be changed. The selection of best solvents can be led to enhancment of aquired extract (Martins and da Conceição, 2015; Nagappan , 2012; Axelsson and Gentili, 2014). In this study, these two methods for preparation of raw extract from Taxus baccata as well as effect of solvent was comprised. For gaining extract, the soxhlet and maceration methods were used by the dried and powdered plants by using various solvents (ethanol and hexane). In Soxhlet method, there was no diffrerence effect of solvents on contents of taxol. The concentraton of obtained taxol was similar to some extent in two situations. However, the final taxol doses differed notably depending on the used solvent employed in clasic method. After HPLC analysis, there is no considrable taxol in HPLC graph in presence of hexan in calsic method. The first step of purification is the solid–liquid extraction. In this step, solubility of Taxol is dpended on type and polarity of solvents. Different studies showed that alcoholic solvents such as Ethanol have better characteristics as a solvent for Taxol extraction. The increase of concentrations (e.g. Ethanol 50%, Ethanol 20% and etc) lead to enhancment of extraction of Taxol in higher yields and etal, 2011;Liu , 2008). In our study, we also showed that the use of alcoholic condition can enhance the concentration of taxol in raw extract in comparison with hexan in maceration method. The effect of solvent type in soxhlet method was not significant. The aquired taxol was almost similar in two used solvents. According to this data, the use of soxhlet method can remove the effect of solvent to some extent. However, the effect of different solvents in any methods of raw preparation can not be denied. So, in various articles, There is no uniform or satisfactory protocols for isolation of Taxol. There are many studies that they used different solvents such as methanol, ethanol, acetone, water, ethyl acetate, chloroform, dichloromethane and their combinations for the extraction of Taxol (Gabetta and etal, 1999;Chen R, Kingston DG, 1994). In our study, the extraction was also compered based on method. The aquired taxol from soxhlet method was more than clasic method, but this difference was not significant. Both two methods have good conditions for preparation of raw extracts for HPLC stage by ethanol solvent. The other parameter in extraction is time. Clasic method is time consuming in comparison with soxhlet method. In other hand, the solvent has considrable effect on aquired extract (Sadeghi-aliabadi and etal, 2015). So, because of less used time and less effect of solvent, it seems that soxhlet method is more suiltabe mothod than clasic method for raw extraction. For increase of extraction quality, the optimization of solvent conditions is

appropriate solution (Talebi and etal, 2004). Alcoholica solvents can increase the contact surface area between the plant matrix and the solvent. this status improves the extraction yield (Li H and etal, 2012; Ferreira and etal ,2000). In our study, this status was observed in comparison of hexan solvent. Because of polarity of taxol, the nanpolar solvents could not be a suitable solvent for its extraction. According to what mentioned above, it is concluded that ethanol extracts contain higher taxol as polar compound than hexane. Many articles have also confirmed that in other plant species polar solvents produce a higher yield of extract concentration compared with the non-polar ones (Trabelsi and etal, 2010; Ahmed and etal, 2015; Iloki-Assanga and etal , 2015)

In conclusion we recommend that the best way of preparation rew extract for purification of taxol by HPLC method is soxhlet method by etahnol solvent, because this method requires less time without any effect of solvent on extract content.

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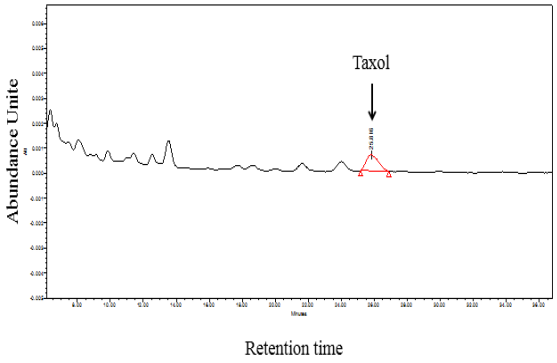


Figure 1. HPLC chromatograms of Taxol obtained by Ethanol solvent in clasic method. Separation conditions involved: C18 column (250 9 4.6 mm, 5 µm); mobile phase,composed of water/ACN in ratio of 55/45 and 70/30 for taxol and 10-DAB III, respectively. The flow rate was 1.0 mL/ min with an injection volume of 20 µL.

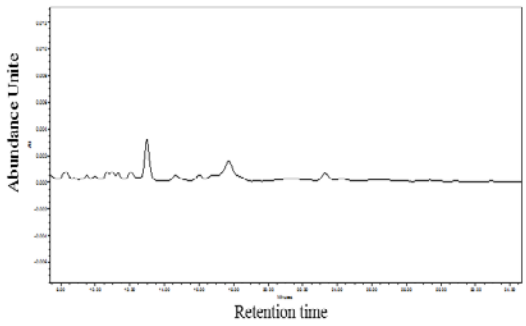


Figure 2. HPLC chromatograms of Taxol obtained by hexane solvent in clasic method. Separation conditions involved: C18 column (250 9 4.6 mm, 5 µm); mobile phase, composed of water/ACN in ratio of 55/45 and 70/30 for taxol and 10-DAB III, respectively. The flow rate was 1.0 mL/ min with an injection volume of 20 µL.

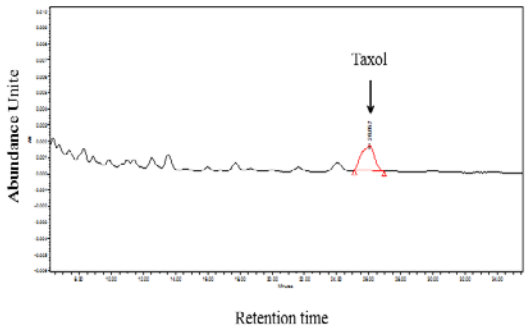
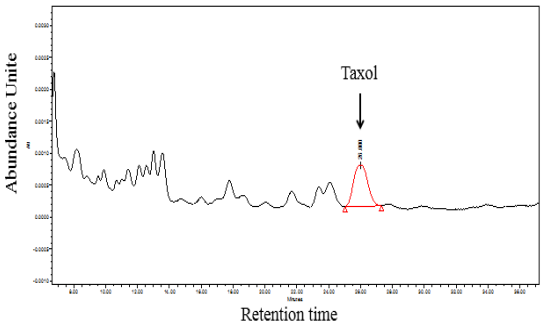


Figure 3. HPLC chromatograms of Taxol obtained by ethanol solvent in soxhlet method. Separation conditions involved: C18 column (250 9 4.6 mm, 5 µm); mobile phase, composed of water/ACN in ratio of 55/45 and 70/30 for taxol and 10-DAB III, respectively. The flow rate was 1.0 mL/ min with an injection volume of 20 µL.



1) **Figure 4.** HPLC chromatograms of Taxol obtained by hexane solvent in soxhlet method. Separation conditions involved: C18 column (250 9 4.6 mm, 5 µm); mobile phase, composed of water/ACN in ratio of 55/45 and 70/30 for taxol and 10-DAB III, respectively. The flow rate was 1.0 mL/ min with an injection volume of 20 µL.