科技部補助專題研究計畫成果報告
期末報告

以甜菜根與麻芋之天然或合成之抗氧化劑作為抗凝血劑之研究

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 106-2320-B-040-015-
執 行 期 間 ： 106年08月01日至107年07月31日
執 行 單 位 ： 中山醫學大學營養學系（所）

計 畫 主 持 人 ： 王進崑

計畫參與人員： 此計畫無其他參與人員

中 華 民 國 107 年 10 月 02 日

中 文 摘 要 ： 根據世界衛生組織報告，缺血性心臟病已佔所有死亡率的13%。抗血液凝集藥物是這類心臟疾病的常用藥，而這類藥物有來自天然，也有來自人工合成。許多天然或合成的抗氧化物也同時被發現具有抗血液凝集的效果，甚至有加乘之功效。來自甜菜根與麻薏的植化素已被發現具有良好的抗氧化與抗發炎功效，本研究取得來自甜菜根的4種(betanidin, isobetanidin, vulgaxanthin-I, vulgaxanthin-II)以及來自麻薏的3種(chlorogenic acid, myricetin, cinnamic acid)具最佳抗氧化性的成份，分別測定其對癌細胞的毒性同時測定其抗凝血之功效，結果顯示，所收集的7種化合物與正對照組相比較，皆具有較強的癌細胞的毒性(Doxorubicin)，與抗凝血之功效(aspirin)。尤其麻薏中所含的酚類化合物效果最好，顯示麻薏是極巨潛力作為開發抗血液凝集藥物的天然物。未來可針對這些成份進一部作化學修飾，尋求更好之抗血液凝集效果。

中 文 關 鍵 詞 ： 甜菜根、麻薏、抗血液凝集、抗氧化

英 文 摘 要 ： According to the data of World Health Organization, the main reason of death is ischemic heart diseases, which have caused nearly 13% of all mortality. The treatment of heart diseases anticipates usage of anticoagulants and anti-aggregating agents. The amount of natural and synthetic antioxidants admitted with food and nutritional supplements is increasing. It is well known that synergism of anticoagulant agents and various biologically active compounds

The phytochemicals in beetroot and jute were found antioxidation and antiinflammation. After evaluating the antioxidant activity of the phytochemicals I both beetroot and jute, 4 compounds in beet root (betanidin, isobetanidin, vulgaxanthin-I, vulgaxanthin-II) and 3 compounds in jute (chlorogenic acid, myricetin, cinnamic acid) were isolated for advanced study for cytotoxicity on carcinoma cells and also antiplatelet aggregation. Results clearly showed that all the isolated antioxidant phytochemicals showed cytotoxicity to all carcinoma cells, and also antiplatelet aggregation. As compared with the positive control for cytotoxicity (doxorubicin) and positive control in amtiplatelet aggregation assay (aspirin), chlorogenic acid in jute showed the best cytotoxicity on carcinoma cells and also perfect antiplatelet aggregation, especially chlorogenic acid. This study showed that jute has great potential for the application for platelet aggregation effect in the future, due to rich phenolics. The chemical modification for the phenolics in jute for advanced evaluation for antiplatelet aggregation is required in the future.

英文關鍵詞：beetroot, jute, antiplatelet aggregation, antioxidation

Final Report for MOST program

中文摘要

根據世界衛生組織報告，缺血性心臟病已佔所有死亡率的 13%。抗血液凝集藥物是這類心臟疾病的常用藥，而這類藥物有來自天然，也有來自人工合成。許多天然或合成的抗氧化物也同時被發現具有抗血液凝集的效果，甚至有加乘之功效。來自甜菜根與麻薏的植化素已被發現具有良好的抗氧化與抗發炎功效，本研究取得來自甜菜根的 4 種(betanidin, isobetanidin, vulgaxanthin-I, vulgaxanthin-II)以及來自麻薏的 3 種(chlorogenic acid, myricetin, cinnamic acid)具最佳抗氧化性的成份，分別測定其對癌細胞的毒性同時測定其抗凝血之功效，結果顯示，所收集的 7 種化合物與正對照組相比較，皆具有較強的癌細胞的毒性(Doxorubicin)，與抗凝血之功效(aspirin)。尤其麻薏中所含的酚類化合物效果最好，顯示麻薏是極巨潛力作為開發抗血液凝集藥物的天然物。未來可針對這些成份進一部作化學修飾，尋求更好之抗血液凝集效果，並作進一步開發。

關鍵詞:：甜菜根、麻薏、抗血液凝集、抗氧化

Abstract

According to the data of World Health Organization, the main reason of death is ischemic heart diseases, which have caused nearly 13% of all mortality. The treatment of heart diseases anticipates usage of anticoagulants and anti-aggregating agents. The amount of natural and synthetic antioxidants admitted with food and nutritional supplements is increasing. It is well known that synergism of anticoagulant agents and various biologically active compounds

The phytochemicals in beetroot and jute were found antioxidation and antiinflammation. After evaluating the antioxidant activity of the phytochemicals I both beetroot and jute, 4 compounds in beet root (betanidin, isobetanidin, vulgaxanthin-I, vulgaxanthin-II) and 3 compounds in jute (chlorogenic acid, myricetin, cinnamic acid) were isolated for advanced study for cytotoxicity on carcinoma cells and also antiplatelet aggregation. Results clearly showed that all the isolated antioxidant phytochemicals showed cytotoxicity to all carcinoma cells, and also antiplatelet aggregation. As compared with the positive control for cytotoxicity (doxorubicin) and positive control in amtiplatelet aggregation assay (aspirin), chlorogenic acid in jute showed the best cytotoxicity on carcinoma cells and also perfect antiplatelet aggregation, especially chlorogenic acid. This study showed that jute has great potential for the application for platelet aggregation effect in the future, due to rich phenolics. The chemical modification for the phenolics in jute for advanced evaluation for antiplatelet aggregation is required in the future.

Keywords: beetroot, jute, antiplatelet aggregation, antioxidation

Introduction

According to the data of World Health Organization, the main reason of death is ischemic heart diseases, which have caused nearly 13% of all mortality. The treatment of heart diseases anticipates usage of anticoagulants and anti-aggregating agents. The amount of natural and synthetic antioxidants admitted with food and nutritional supplements is increasing. It is well known that synergism of anticoagulant agents and various biologically active compounds, including antioxidants can cause adverse effects: simultaneous admission of Warfarin and wide range of herbal or vegetable may potentially increase the risk of bleeding or potentiate by altering the metabolism of Warfarin through acting with cytochrome P450, another risk are plants containing compounds with possible effects on platelet. It has been found out that classical antioxidants can serve as potential anticoagulant agents – high-dose supplementation of vitamin E decreased the γ-carboxylation and functionality of prothrombin (a vitamin K–dependent protein) among adults not receiving oral anticoagulant therapy. Antioxidant and anticoagulant properties of different polysacharides (e. g., polysaccharide from green algae *Enteromorpha linza*, fucans, isolated from brown algae *Sargassum vulgare*, sulfated porphyrans from *Porphyra haitanensis*, sulfated extracellular polysaccharide produced by the edible mushroom *Pleurotus sajor-caju* and low molecular weight fucoidan fractions extracted from *Laminaria japonica* are well known. Few studies are devoted to peptides and proteins (e.g., activated protein C having both antioxidant and anticoagulant properties.

Some polyphenol rich extracts (which are well known antioxidants) of plants are studied as anticoagulant agents: extracts of *Aronia melanocarpa* berries and *Vitis vinifera* seeds prolong clotting time and decrease the maximal velocity of fibrin polymerization in human plasma; phenolic glycoconjugates from medicinal plants of Asteraceae and Rosaceae families, as well as buckwheat, ginger and garlic antioxidants demonstrate anticoagulant/antithrombotic activity.

Dual effect of above mentioned extracts of different natural plants may to show up due to synergism of various compounds. The other cooperated laboratory of this principal (Dr. P. R. Venskutonis) also has extensive experience on studies of natural antioxidant sources among various plant materials. Numerous phytochemicals were identified and quantified in different plant species, some of these phytochemicals were reported as natural compounds demonstrating various health effects. E. g., beetroots (*Beta vulgaris*) were found to be essential for creating functional ingredients exerting anticarcinogenic and antiglycation activities, various mushroom extracts were characterized as sources of antioxidants and antiradical agents etc.

This laboratory has studied therapeutic effects of different plant material extracts, including anti-platelet aggregation of essential oils, the effect of cranberry vinegar on

the risk of cardiovascular diseases. Excellent anti-inflammation of phenolic crude extracts from five fractions of jute (*Corchorus olitorius* L.). In this study, we clearly found both beetroot and jute have great potential to find the dual effect of antioxidation and anticoagulation.

Work plan, methods and results

The main phases of the implementation of the project are:

- preparation of different beetroot and jute extracts: Optimize the methods of isolation and purification of bioactive phytochemicals from beetroot (betalains) and jute (cannabinoids). For this task various combinations of methods will be used, including conventional and high pressure extraction/fractionation, molecular distillation and preparative chromatography. The main results of this work will develop effective methods of isolation, fractionation and purification of bioactive principles from beetroots and jute.
- identification of the main components of extracts having highest antioxidant and anticoagulant activity: 4 compounds in beet root (betanidin, isobetanidin, vulgaxanthin-I, vulgaxanthin-II) and 3 compounds in jute (chlorogenic acid, myricetin, cinnamic acid) were found perfect antioxidation and anticoagulant activity.
- Investigating the cytotoxicity of the potential anticoagulants (natural and synthetic antioxidants - extracts and individual compounds prepared) on a series of human cancer cell lines: Hep G2 and Hep 3B (liver), MCF-7 and MDA-MB-231 (breast), and A549 (lung). Moreover, anti-platelet aggregations are also evaluated by using the platelet-rich plasma preparation anti-platelet aggregation assay carried out in this laboratory. All the results were listed as the following table. 4 compounds in beet root (betanidin, isobetanidin, vulgaxanthin-I, vulgaxanthin-II) and 3 compounds in jute (chlorogenic acid, myricetin, cinnamic acid) all showed cytotoxicity to all carcinoma cells, and also antiplatelet aggregation. Chlorogenic acid in jute showed the best cytotoxicity on carcinoma cells and also perfect antiplatelet aggregation.

Table 1 Cytotoxicity assays and antiplatelet aggregation assay of the antioxidant compounds in beet root and jute.

Compounds	IC50 (ug/mL) [a]						
	HepG2	Hep3B	A549	MCF-7	MDA-MB-231	Thrombin	Collagen
Beetroot							
Betanidin	78.75±0.21	25.36±0.41	38.24±0.43	33.29±0.83	35.65±0.57	3.98±0.12	3.48±0.33
Isobetanidin	98.31±0.45	36.78±0.36	45.21±0.22	38.67±0.75	39.26±0.25	4.61±0.29	3.94±0.45
Vulgaxanthin-I	98.61±0.34	45.27±0.69	56.72±0.74	45.66±0.87	44.36±0.87	5.44±0.67	4.52±0.55
Vulgaxanthin-II	99.17±0.68	56.36±0.97	58.74±0.76	56.34±0.54	49.68±0.33	6.98±0.38	5.14±0.64
Jute							
Chlorogenic acid	0.17±0.11	0.37±0.14	0.35±0.20	0.47±0.15	0.44±0.22	2.26±0.15	1.57±0.34
Myricetin	0.37±0.12	0.64±0.30	0.69±0.26	0.76±0.32	0.59±0.34	2.72±0.14	1.68±0.43
Cinnamic acid	0.42±0.25	0.79±0.15	0.88±0.38	0.89±0.47	0.75±0.19	2.97±0.47	2.49±0.53
Doxorubicin [b]	0.25±0.01	0.52±0.01	0.70±0.04	0.72±0.03	0.71±0.01	-	-
Aspirin [c]	-	-	-	-	-	15.75±0.21	>100

a Data expressed as mean ± SD (n=2)

b The positive control in cytotoxicity assay

c The positive control in antiplatelet aggregation assay

106年度專題研究計畫成果彙整表

計畫主持人：王進崑	**計畫編號：**106-2320-B-040-015-
計畫名稱：以甜菜根與麻芋之天然或合成之抗氧化劑作為抗凝血劑之研究	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		

	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）			即將發表		

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：□已發表　□未發表之文稿　■撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）
   論文撰寫中

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   顯示麻薏是極巨潛力作為開發抗血液凝集藥物的天然物。未來可針對這些成份進一部作化學修飾，尋求更好之抗血液凝集效果，並作進一步開發。

4. 主要發現
   本研究具有政策應用參考價值：□否　■是，建議提供機關衛生福利部,行政院農業委員會,
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：□否　■是
   說明：（以150字為限）
   根據世界衛生組織報告，缺血性心臟病已佔所有死亡率的13%。我們的研究顯示麻薏是極巨潛力作為開發抗血液凝集藥物的天然物。未來可針對這些成份進一部作化學修飾，尋求更好之抗血液凝集效果，並作進一步開發。