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EVALUATION OF ANTITYROSINASE AND ANTIOXIDANT ACTIVITIES OF
RAPHANUS SATIVUS ROOT EXTRACT FOR
COSMETIC APPLICATIONS

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A Thesis submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmaceutics

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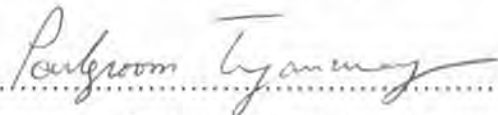
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การนำพืชจากธรรมชาติมาใช้ในผลิตภัณฑ์เครื่องสำอางเพื่อให้ผิวขาวขึ้นและลดเลือนริ้วรอยเป็นที่นิยมกันมาก เป็นเวลานานมาแล้วที่ผู้หญิงไทยนิยมนำรากหัวผักกาดขาวสดมาใช้ในการรักษาฝ้าแต่ยังไม่มีการศึกษาอย่างเป็นวิชาการ การศึกษานี้ทำการสกัดสารสกัด 2 ชนิดจากรากหัวผักกาดขาวด้วยน้ำและทำให้แห้งด้วยการ freeze dried และเมทานอลโดยเปรียบเทียบปริมาณฟีนอลิกโดยรวม ฟลาโวนอยด์โดยรวมและวิตามินซีในสารสกัดทั้ง 2 ชนิด พบว่าสารสกัดด้วยน้ำมีปริมาณฟีนอลิกโดยรวม ฟลาโวนอยด์โดยรวมและวิตามินซีต่อ 1 มิลลิกรัมน้ำหนักแห้งของสารสกัดมากกว่าสารสกัดด้วยเมทานอล โดยมีปริมาณเท่ากับ 10.09 ± 0.07 , 0.51 ± 0.007 และ 24.11 ± 0.01 ไมโครกรัมสำหรับสารสกัดด้วยน้ำ และ 6.59 ± 0.05 , 0.33 ± 0.004 และ 8.28 ± 0.20 ไมโครกรัมสำหรับสารสกัดด้วยเมทานอล ตามลำดับ สำหรับฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนสของสารสกัดทั้ง 2 ชนิดเปรียบเทียบกับสารสกัดชะเอมและวิตามินซี พบว่าสารสกัดด้วยน้ำมีฤทธิ์ในการยับยั้งเอนไซม์ไทโรซิเนสดีกว่าสารสกัดด้วยเมทานอล (ความเข้มข้นในการยับยั้งเอนไซม์ 50% เท่ากับ 3.09 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยน้ำและ 9.62 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยเมทานอล) แต่น้อยกว่าสารยับยั้งเอนไซม์ไทโรซิเนสที่ใช้เปรียบเทียบ สำหรับฤทธิ์ด้านออกซิเดชันและอนุมูลอิสระของสารสกัดทั้ง 2 ชนิดนั้นได้ทำการศึกษาฤทธิ์ยับยั้งอนุมูลอิสระ 3 ชนิดคือ อนุมูลอิสระดีพีพีเอช ซูเปอร์ออกไซด์ และซิงเกลต ออกซิเจน เปรียบเทียบกับโทรลอกซ์และวิตามินซี พบว่าสารสกัดด้วยน้ำจะให้ฤทธิ์ในการยับยั้งอนุมูลอิสระที่สูงกว่าสารสกัดด้วยเมทานอล ไม่ว่าจะเป็นอนุมูลดีพีพีเอช (ความเข้มข้นในการยับยั้งอนุมูลอิสระ 50% เท่ากับ 0.643 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยน้ำ และ 1.248 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยเมทานอล) ซูเปอร์ออกไซด์ (ความเข้มข้นในการยับยั้งอนุมูลอิสระ 50% เท่ากับ 4.20 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยน้ำ และ 6.28 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยเมทานอล) หรือ ซิงเกลต ออกซิเจน (ความเข้มข้นในการต้านออกซิเดชัน 50% เท่ากับ 1.42 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยน้ำและ 2.40 มิลลิกรัมต่อมิลลิลิตรสำหรับสารสกัดด้วยเมทานอล) แต่พบว่ายังน้อยกว่าสารต้านออกซิเดชันที่ใช้เปรียบเทียบ นอกจากนี้การศึกษาความเป็นพิษของสารสกัดทั้ง 2 ชนิดต่อเซลล์ด้วยวิธีแอลดีเอช พบว่าสารสกัดทั้ง 2 ชนิด ให้ความเป็นพิษที่ต่ำมากแม้ในความเข้มข้นที่สูง จากข้อมูลการศึกษานี้ จึงเป็นไปได้ที่จะนำสารสกัดด้วยน้ำซึ่งเตรียมได้ง่ายและราคาไม่แพง มาใช้เป็นสารยับยั้งเอนไซม์ไทโรซิเนสและสารต้านออกซิเดชันในผลิตภัณฑ์เครื่องสำอางต่อไป

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RATTANAMANEE JAKMATAKUL: EVALUATION OF ANTI-TYROSINASE AND ANTIOXIDANT ACTIVITIES OF *RAPHANUS SATIVUS* ROOT EXTRACT FOR COSMETIC APPLICATIONS. THESIS ADVISOR: ASSOC. PROF. PARKPOOM TENGAMNUAY, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. RUTT SUTTISRI, Ph.D. 184 pp. ISBN 974-14-3405-7.

There is considerable demand for skin whitening and anti-wrinkle/anti-aging from natural plants. Thai women have long used fresh white radish root as a treatment of melasma although no systematic study has been made. In this study, two extracts (freeze-dried water extract and methanol extract) from the root of Thai radish (*Raphanus sativus* L.) were compared and evaluated to determine their role in protection of skin against melasma and aging. The two extracts were assayed for total phenolics, total flavonoids and vitamin C content. The total phenolics, total flavonoids and vitamin C content of the two extracts expressed as per 1 mg on the extract weight basis were 10.09 ± 0.07 , 0.51 ± 0.007 and 24.11 ± 0.01 μg for freeze-dried water extract and 6.59 ± 0.05 , 0.33 ± 0.004 and 8.28 ± 0.20 μg for methanol extract, respectively. Data showed that freeze-dried water extract had higher total phenolics, total flavonoids and vitamin C content than methanol extract. Anti-tyrosinase activity of the two extracts was also measured and compared with that of licorice extract and L-ascorbic acid. Freeze-dried water extract apparently showed higher extent of tyrosinase inhibition than methanol extract ($\text{IC}_{50} = 3.09$ and 9.62 mg/ml, respectively) but generally lower anti-tyrosinase activity than other reference anti-tyrosinase agent. The antioxidant properties of the two extracts were also tested for their scavenging effect on DPPH radical, superoxide anion and singlet oxygen and compared with those of Trolox[®] and L-ascorbic acid. Freeze-dried water extract exhibited higher potency than methanol extract ($\text{IC}_{50} = 0.643$ and 1.248 mg/ml for antioxidant activity against DPPH, 4.20 and 6.28 mg/ml for superoxide anion scavenging activity and 1.42 and 2.40 mg/ml for singlet oxygen scavenging activity) but generally lower than other reference antioxidants. The *in-vitro* cytotoxicity of the two extracts were determined by LDH assay. Both the freeze-dried water extract and methanol extract showed mild cytotoxicity even at high concentrations. Considering its various properties, the inexpensive and easily available freeze-dried water extract has potential for use in both the pharmaceutical and cosmetic applications.

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LIST OF ABBREVIATIONS

abs	=	absorbance
AlCl ₃	=	aluminium chloride
°C	=	degree of Celsius
cm	=	centimeter
conc.	=	concentration
CV	=	coefficient of variation
DMEM	=	Dulbecco's modified eagle medium
e.g.	=	exempli gratia, for example
<i>et al.</i>	=	et alii, and other
g	=	gram
µg	=	microgram
µl	=	microliter
H ₂ O	=	water
H ₂ O ₂	=	hydrogen peroxide
HO	=	hydroxyl radical
IC ₅₀	=	median inhibitory concentration
LDH	=	lactate dehydrogenase
m	=	meter
MeOH	=	methanol
mg	=	milligram
ml	=	milliliter
mw	=	milliwatt
MW	=	molecular weight
nm	=	nanometer
no.	=	number
NaOCl	=	sodium hypochlorite
PBS	=	phosphate buffer solution
pH	=	The negative logarithm of the hydrogenion concentration
R ²	=	coefficient of determination
¹ O ₂	=	singlet oxygen

SD = standard deviation
UV = ultraviolet