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DETERMINATION OF SOME PHENOLIC COMPOUNDS IN ETHANOLIC EXTRACT OF *ELEPHANTOPUS SCABER* LINN. LEAVES

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KEYWORDS *Elephantopus scaber* L. leaves , phenolic compound, chlorogenic acid

INTRODUCTION

Phenolic compounds are a class of low molecular weight secondary plant metabolites found in most plant. Phenolic compounds can be classified into insoluble compounds such as condensed tannins, lignin, cell wall bound hydroxycinnamic acid, and soluble compounds such as phenolic acids, phenylpropanoids, flavonoids and quinones [1]. Hydroxy derivatives of cinnamic acids are a class of polyphenols having a C6-C3 skeleton such as coumaric acid, caffeic acid and chlorogenic acid. These compounds are believed to have antioxidant properties which are suggested to play an important role in preventing various diseases associated with oxidative stress such as cancer, cardiovascular, aging and neurodegenerative diseases.

Elephantopus scaber Linn. (Asteraceae) is a perennial herb distributed in the moist deciduous forests of Thailand. This plant is known as a traditional medicine and the whole plant is used as analgesic, antitussive and to promote libido. It has been reported to contain bioactive compounds such as sesquiterpene lactones (elephantopin, deoxyelephantopin, isodeoxy-elephantopin, scabertopin, isoscabertopin, scabertopinol and elescaberin) [2], triterpenoids (lupeol, epifriedelinol and stigmasterol) [3]. The leaf extracts have been evaluated for pharmacological effects such as diuretic, antiinflammatory and hepatoprotective properties. Deoxyelephantopin isolated from its leaves displayed wound-healing properties [4]. The ethanolic extracts of *E. scaber* could enhance libido [5], spermatogenesis, increase sperm density [6] and were cytotoxic against human breast cancer (MCF-7) cells by the induction of p53-dependent apoptosis [7].

MATERIALS AND METHODS

Preparation of Plant Extracts:The leaves of *E. scaber* were dried at 40 °C, ground into powder and extracted 10 times with 95 % ethanol at room temperature. The combined ethanol filtrates were evaporated under reduced pressure at room temperature to attain an ethanolic extract.

Phytochemical Screening: Phytochemical screening was performed using TLC chromatographic technique. Crude extract (10 mg) was dissolved in 1 ml of 50 % ethanol and partitioned with 1 ml of ethyl acetate. An amount of ethyl acetate fraction (15 µl) was applied to silica gel 60 F_{254} TLC plates and developed in toluene : ethyl acetate : formic acid (3:17:3), then identified by co-TLC with authentic standards (chlorogenic acid, apiginin and luteolin). Visualization of the compounds was attained by spraying the sheets with 1% methanolic diphenylboryloxyethylamine, followed by 5% ethanolic polyethylene glycol 4000. The chromatograms were examined under 366 nm UV light

Total Phenolic Content using Folin-Ciocalteu Method: The total phenolic content was determined using Folin-Ciocalteu method. The solution (50 µl) of 5 mg/ml of crude extract, 4,200 µl of Milli Q-water and 250 µl of Folin-Ciocalteus phenol reagent were mixed thoroughly. After 2 min, 500 µl of 20 % Na_2CO_3 solution were added and allowed to stand at room temperature for 1 hr. The absorbance was measure at 760 nm using UV/Vis spectrometer. The mixture without test solution was used as a blank. The total phenolic content was expressed as gallic acid in mg/100g of crude extract.

HPLC analysis: Exactly 1 mg of chlorogenic acid authentic standard (99.29%) and 20 mg ethanolic extract of *E. scaber* leaves were accurately weighed and transferred to a 5-ml volumetric flask and dissolved first in methanol, then sonicated for 20 min. The solution so obtained was filtered through filter units (0.45 µm pore size) before use. Ten microliters of varying concentrations of chlorogenic acid standard and *E. scaber* extract solution were analyzed using a Waters e 2695 separation modul equipped with Waters 2998 photodiode array detector. The chemical compounds were separated on a pHedure C-18 column (250 mm x 4.6 mm) with 5 µm (Vertisep^{TM}). The mobile phase was a gradient elution system consisting of solvent A (0.005 %TFA/H_2O) and solvent B (0.001 % TFA/ACN). The elution was as follows: 0 to 10 min, 14 % solvent B; 10 to 30 min, 18 % solvent B; 30 to 50 min, 25 % solvent B; 50 to 60 min, 35 % solvent B; 60 to 70 min, 70 % solvent B; 70 to 80 min, 100 % solvent B; 80 to 90 min, 100 % solvent B, flow rate 1.0 ml/min at 30 °C. The wavelength was monitored at 254 nm.

RESULTS AND DISCUSSION

TLC profile of crude ethanolic extract of *E. scaber* leaves showed the presence in high concentrations of phenolic compounds. TLC fingerprint is composed of chlorogenic acid (R_f = 0.18), apiginin (R_f = 0.70) and luteolin (R_f = 0.65) as shown in Figure 1.

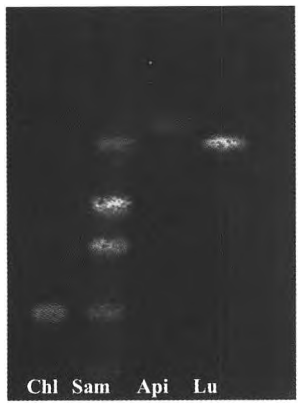

Fig 1: TLC of ethanolic extract of *E. scaber* leaves was developed using solvent system of toluene : ethyl acetate : formic acid (3:17:3), co-TLC with authentic standards (chloroginic acid and luteolin), sprayed with 1% methanolic diphenylboryl-oxyethylamine, followed by 5% ethanolic polyethylene glycol 4000 and observed under 366 nm UV light

The total phenolic content equivalent to gallic acid of the ethanolic extract of *E. scaber* leaves are expressed as 130 mg/1 g of dry crude extract. The chemical components were analyzed using HPLC technique. The chromatogram is composed of chlorogenic acid (RT: 16.28) and luteolin (RT: 61.45) in comparison with authentic standard as shown in Figure 2. The highest peak at RT 43.37 represents an unknown compound, which has UV-vis absorbance spectrum similar to chlorogenic acid as shown in Figure 3. Moreover, the color of some spots in TLC chromatogram was the same color as chlorogenic acid when observed under UV light at 366 nm. From these results, it can be comfirmed that the unknown compound (RT: 43.37) is the hydroxyl cinnamic acid derivative, chlorogenic acid.

The linear regression line of calibration curve of chlorogenic acid showed good linear relationship Y = 12,268X + 15,513 with r^2 = 0.999 in the concentration range of 25 - 100 µg/ml. The amount of chlorogenic acid presented in ethanolic extract of *E. scaber*. leaves was 11.59 mg/1g of dried crude extract.

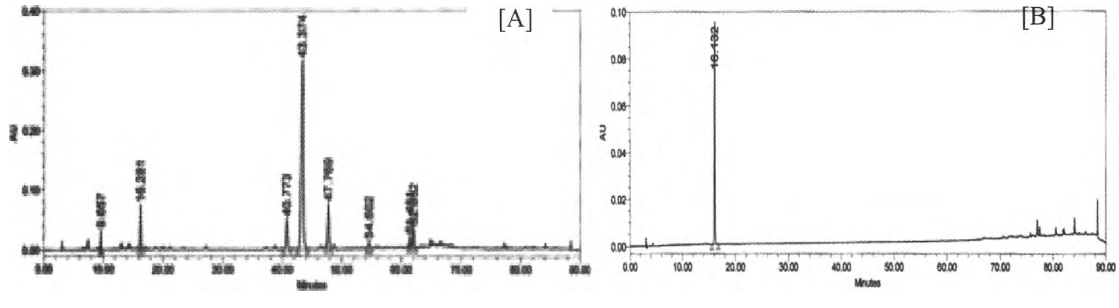

Fig 2. HPLC chromatogram [A] : *E. scaber* extract at 254 nm; RT: 16.28, chlorogenic acid; 61.45, luteolin
[B] : standard chlorogenic acid

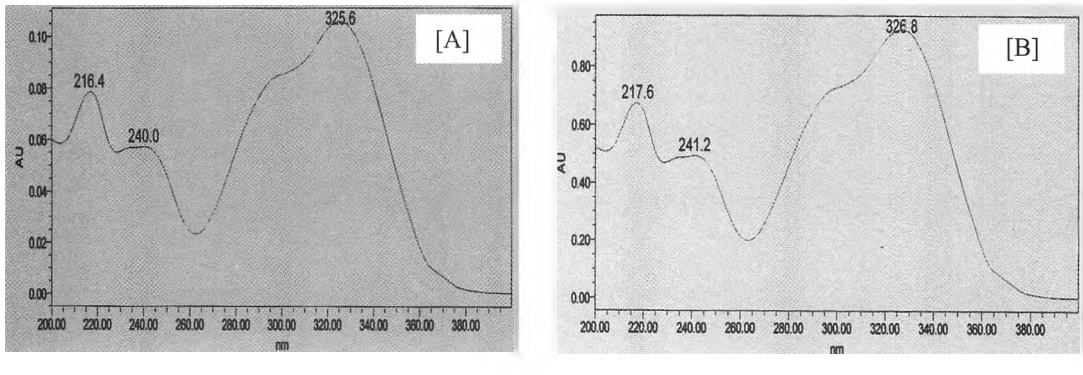

Fig 3. UV-vis absorption spectra of [A] chlorogenic acid [B] unknown compound RT: 43.37

CONCLUSION

The ethanolic extract of *E. scaber* leaves contained high amount of hydroxyl cinnamic acid derivatives such as chlorogenic acid and an unknown compound (RT: 43.37). The condition of the HPLC mobile phase solvent system used in this study was suitable for the analysis of phenolic compounds in the ethanolic extract of *E. scaber* leaves.

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