Protein patterns and chemical constituents of *Ailanthus altissima* (Miller) Swingle and *Ailanthus excelsa* Roxb.

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Abstract

There is a growing interest in correlating biochemical constituents of plants with their taxonomic properties. Generally the genus *Ailanthus* is noted for the presence of quassinoids, alkaloids, lipids, fatty acids, terpenoids and some proteins. In this study, we aimed to investigate the similarities and/or differences in the chemical constituents and protein patterns of different *Ailanthus* species. We assume that our parameters may be used as an additional tool for chemotaxonomic studies and molecular discriminations.

Keywords*: Ailanthus*, chemical constituent, chemotaxonomy, protein pattern.
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Introduction

Ailanthus (Simaroubaceae) is a genus of tall, lofty, fast growing deciduous tree and widely distributed in Indo-Malaya, China, Japan and Australia (Kowarik 1995; Manish and Mishra 2007). The genus is noted for its antidiarrhoel and antidysenteric properties (Manish and Mishra, 2007). The two main interesting *Ailanthus* species are *Ailanthus altissima* (Miller) Swingle and *Ailanthus excelsa* Roxb. *A. altissima,* which is known as Tree-of-Heaven or Chinese Sumack is used in traditional medicine (Watt and Brever 1962; Perry 1980). *A. excelsa,* (Indian Tree-of-Heaven) is mostly distributed in India and Sri Lanka. and is also used to treat several diseases (Asolkar et al. 1992; Marish and Mishra, 2007). Previous phytochemical studies have shown the presence of quassinoids, indole alkaloids, lipids, fatty acids in *A. altissima* (Ogura et al. 1977) and quassinoids, alkaloids, terpenoids, proteins in *A. excelsa* samples (Ogura et al. 1977; Shrimali et al. 2001; Rashed et al. 2006). Nag and Mathai (1994) and Azim et al. (2002) have reported *Ailanthus* species as good sources of protein. In this study, we aimed to investigate the similarities and/or differences in the chemical constituents, protein content and protein patterns of different *Ailanthus* species to bring additional parameters for their chemotaxonomic delineation.

Material and Methods

A. altissima and *A. excelsa* leaves and stem barks were taken from the Zoo garden, Giza, Cairo-Egypt (May 2004).

Phytochemical investigations

1 kg of dried (5 hrs, 50^{o}C, Heraeus) and powdered plant material extracted with 70% MeOH (Merck) in a continuous extraction apparatus (Soxhlet Apparatus). The extract was concentrated under the reduced pressure to give a residue. The obtained residue was dissolved in 500 ml of distilled water and then it was fractionated with petroleum ether, chloroform,

ethyl acetate and butanol (60–80°C). Each fraction was dried over anhydrous sodium sulphate (Merck) and concentrated under reduced pressure to give 29 g, 14 g, 10.5 g, 32 g of petroleum ether, chloroform, ethyl acetate and n-butanol fractions, respectively.

Protein analysis

Plant protein extracts were prepared by grinding air-dried leaf samples with mortar and pestle in liquid nitrogen and ice-cold buffer (0.5 M Tris-HCl, pH: 8.6, Sigma) (Rauser 1990). The crude homogenates were cleared by centrifugation (Eppendorf Centrifuge) at 14 000 rpm for 60 min (3°C) and the obtained supernatants were kept deep-frozen (Bosch) (–20°C) until the protein analysis. Quantification of the total soluble proteins was done according to the sensitive dye-binding method of Bradford (1976). 100 µl of supernatant was mixed with the indicator solution (Coomassie Brillant Blue G-250 (Fluka), 95% ethanol (Merck), 85% H_3PO_4) and the OD of the samples was measured with spectrophotometer (Shimadzu UV-1601) at 595 nm. Different concentrations of γ-globulin phosphate and BSA were prepared as standard proteins. The obtained absorbance data were used to calculate the total soluble protein content in $mg.g^{-1}$ dw. Protein patterns of the same samples were analyzed using SDS-PAGE with monomer slab gels (16%) (Sigma) (Laemmli 1970). Gels were prepared without a stacking gel to prevent aggregation and precipitation of proteins (Walker 2003). Electrophoresis was run for 5 h (Bio-Rad Protean II xi Cell), and gels were fixed and stained with 0.1 % Coomassie Brillant Blue R-250 (Fluka). Standard curves were obtained from the Rf values of molecular weight marker proteins (69814, Fluka) which were plotted against log10 values of their known molecular weights (Weber and Osborn 1969). This standard graphic was used to detect the molecular weights (kDa) of the soluble leaf and stem bark proteins extracted from *Ailanthus* species.

Results and Discussion

Chemical constituents

There is a growing interest in correlating biochemical constituents of plants with their taxonomic properties. Generally alkaloids, flavonoids and bitter principles like quassinoids are reported in Simaroubaceae family and the genus *Ailanthus* is also noted for the presence of quassinoids, alkaloids, lipids, fatty acids terpenoids, proteins together with antidiarrhoel, antidysenteric and antileukemic properties (Ogura et al. 1977; Rashed et al. 2006; Manish and Mishra 2007). In our study, carbohydrates and/or glycosides, condensed tannins, sterols and/or triterpenes, coumarins were present in all of the *Ailanthus* extracts, but hydrolysable tannins and saponins were absent. Alkaloids and/or nitrogenous bases were present in *A. excelsa* and *A. altissima* stem bark extracts and also in the leaf extracts of *A. altissima*, but interestingly no alkaloids and/or nitrogenous bases were observed in *A. excelsa* leaf extracts. Flavonoids were present in the leaf extracts of both *Ailanthus* species, but they were present only in trace amounts in the stem bark extracts. Chemical constituents of *A. altissima* and *A. excelsa* plant extracts were shown in Table 1. The similarities and differences in the constituents may explain some taxonomical relations between species.

Protein patterns

The demonstrable differences and similarities in the biochemical compositions of the plants are very important for chemotaxonomic studies. Santoni et al. (1994) have found a promising approach to characterize proteins and have used gels to allow the characterization of the mutants and wild-type plants by a set of proteins showing differential expressions. Nag and Mathai (1994) and Azim et al. (2002) have reported *Ailanthus* species as good sources of protein. Moreover, the balance of essential

Table 1. Chemical constituents of leaf and stem bark extracts of *A. altissima* and *A. excelsa.*

Chemical Constituents	A. altissima	A. excelsa	A. altissima	A. excelsa
	Bark	Leaf	Bark	Leaf
Carbohydrates and/or glycosides	+	+	+	+
Condensed tannins	+	+	+	+
Hydrolysable tannins	-	-	-	-
Alkaloids and/or nitrogenous bases	+	+	+	-
Flavonoids	**x**	+	**x**	+
Sterols and/or triterpenes	+	+	+	+
Saponins	-	-	-	-
Coumarins	+	+	+	+

(+) presence of the constituent
(-) absence of the constituents
(x) presence in trace amounts

amino acid composition was found excellent in *A. excelsa* (Nag and Mathai 1994). Different amino acid concentrations, relative percentages and critical values of some amino acids reflecting some qualities of proteins in individual taxa were reported to be additional considerable parameters for the diagnosis of *Quercus* (Özcan 2006). According to our total soluble protein analysis, protein contents of *A. altissima* and *A. excelsa* leaves were found as 31,52 $mg.g^{-1}$ dw and 35,82 $mg.g^{-1}$ dw, respectively. We have obtained 4,895 $mg.g^{-1}$ dw total soluble protein in *A. altissima* stem barks, but the value decreased to 1,896 $mg.g^{-1}$ dw in *A. excelsa*. *A. altissima* had slightly lower protein content in leaves but higher protein content in stem barks compare to *A. excelsa*. Generally protein content of the leaves was found higher than the stem bark protein content in both species.

Among the protein pattern of the soluble proteins from *Ailanthus* leaf and stem bark extracts, the estimated molecular weights were observed similar between the plant species and also between the plant parts in some extent. But we have also observed some different molecular weights between *A. excelsa* and *A. altissima* species and also between the two different plant parts of the same species. Estimated molecular weights of the soluble proteins extracted from the leaves and stem barks of *A. altissima* and *A. excelsa* species were shown in the Figs (1, 2, 3, 4). According to the stem bark protein patterns, seven different proteins were determined between the range of 16,6-89,1 kDa for *A. excelsa* and nine proteins for *A. altissima* samples (16,6-83,2 kDa). Eight different proteins were detected (16,6 - 66,1 kDa) for *A. altissima* leaf samples, but nine proteins for *A. excelsa* (16,6-95,1 kDa).

Protein patterns may give important clues between two different species. We assume that our parameters maybe used as an additional tool for chemotaxonomic studies and molecular discriminations.

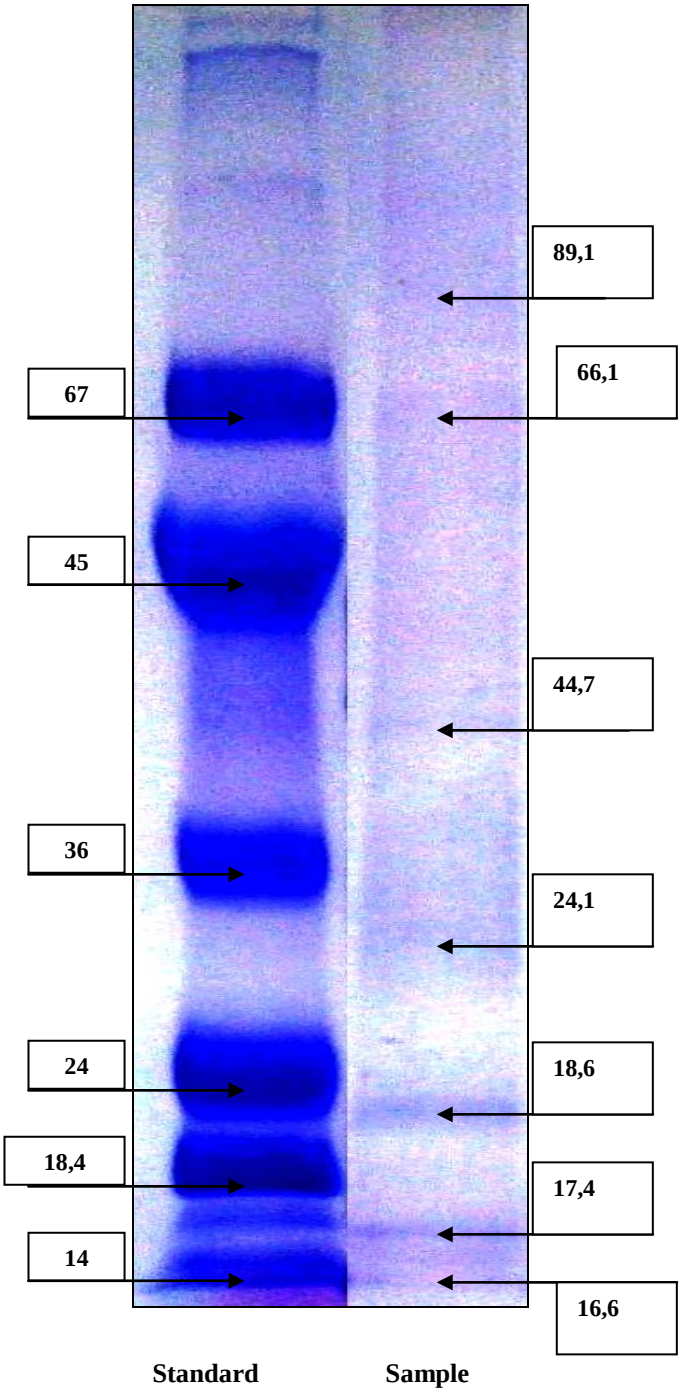

Figure 1. Protein patterns of *Ailanthus excelsa* stem bark (kDa)

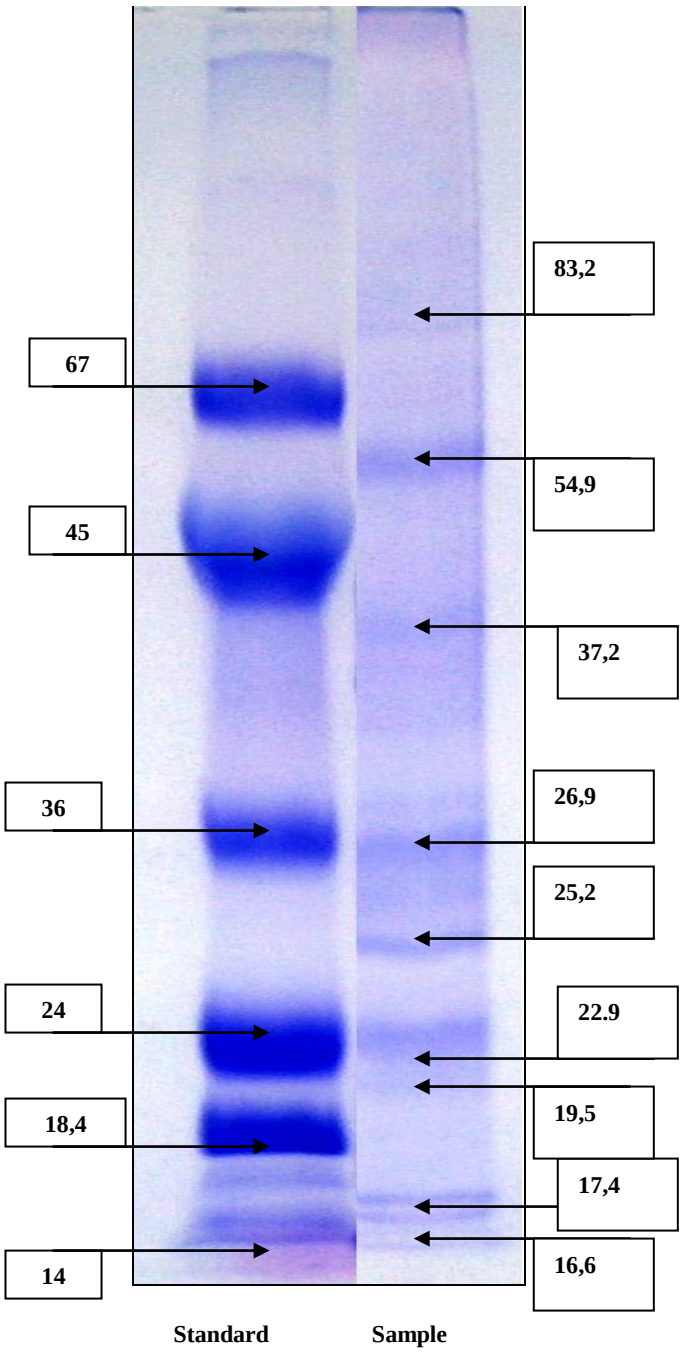

Figure 2. Protein patterns of *Ailanthus altissima* stem bark (kDa)

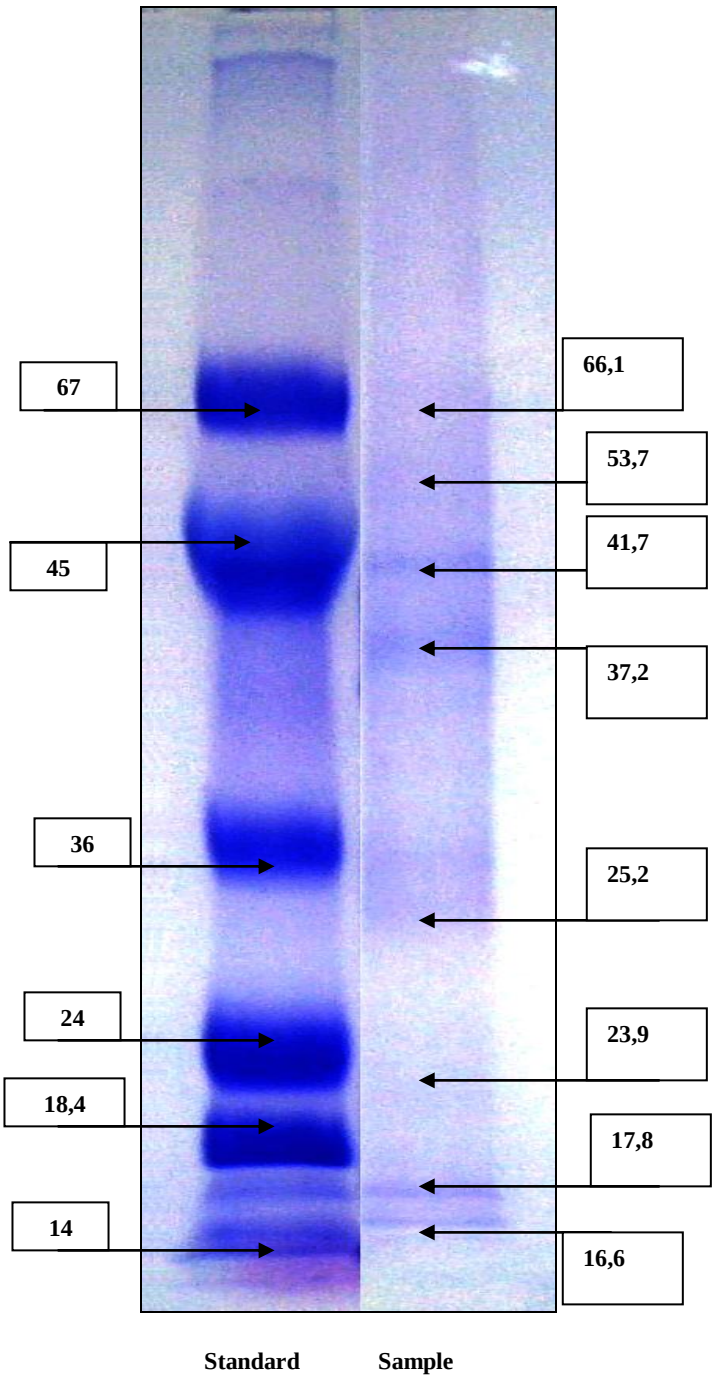

Figure 3. Protein patterns of *Ailanthus altissima* leaf (kDa)

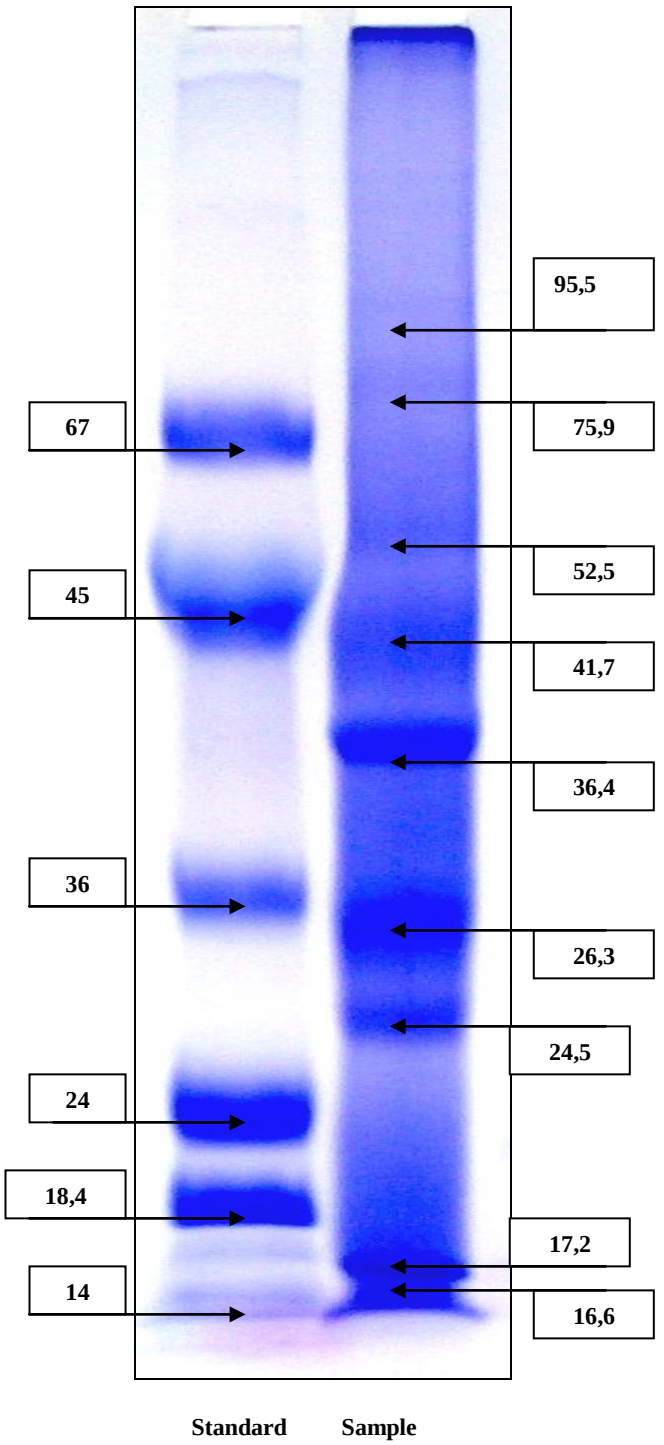

Figure 4. Protein patterns of *Ailanthus excelsa* leaf (kDa)

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