Antiviral Activity and Phytochemical Analysis of Ailanthus Excelsa Roxb Bark

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Abstract—Resistance to current anti-herpetic drugs has been increasingly reported, so there is a need for discovering new antiviral agents, in particular from natural origin. The aim of the present study is to investigate chloroform and methanol (70%) extracts from Ailanthus excelsa bark for their antiviral activity against Herpes Simplex virus type 1 (HSV-1) using Plaque reduction assay. The results has shown that chloroform extract of Ailanthus excelsa has a significant anti-viral activity against herpes Simplex virus type 1 in-vitro by 82.6% at concentration of 50 µg, while methanol 70% extract was less active. Phytochemical analysis of both Chloroform and methanol extracts of Ailanthus excelsa has shown that it has interesting bioactive compounds include quassinoids (highly oxygenated triterpenes) and alkaloids and further phytochemical analysis from bio-active chloroform extract resulted the isolation of the major alkaloid compound, canthin-6-one. In conclusion, Ailanthus excelsa extracts may be an appropriate candidate for further development of anti-HSV-1 infection.

Index Terms—Ailanthus excelsa, antiviral activity, quassinoids, alkaloids, canthin-6-one.

I. INTRODUCTION

Viral infections, particularly the infections caused by herpes simplex virus (HSV), represent one of the most serious public health concerns globally because of their devastating impact. Herpes simplex virus type 1 (HSV-1) is a commonly occurring human pathogen worldwide. There is an urgent need to discover and develop new agents for the management of HSV-1 infection. Since the ancient times, natural products has served as a major source of drugs. About fifty percent of todays pharmaceutical drugs are derived from natural origin [1]. Also it is well established that natural products are an excellent source of chemical compounds with a wide variety of biological activities including antiviral and anticancer properties [2]. In our searching for new antiviral substances from plants, in particular from Simaroubaceae family, A. excelsa Roxb is a tree of rapid growth and is called tree of heaven, leaves appear in March-April, 30-90 cm long, pinnate, the flowers, small in size, yellow in colour and arranged in panicles and the fruits are formed soon after flowering. The fruits ripen in May-June, just before the onset of monsoon. A. excelsa was investigated previously to prove antibacterial [3], antifungal [4], Antifertility [5] and anticancer [6]. A. excelsa is used in treatment of skin eruption and for the cure of wounds. The bark is bitter, astringent, anthelmintic, febrifuge, appetizer, bitter tonic, taste bud stimulant, It is useful in diarrhea, amoebic dysentery, chronic giardiasis, dyspepsia, abdominal spasm anorectal disease, haemorrhoids, fistula, fissures, ulcerative colitis as mentioned in traditional medicine [7] From chemical point of view the plant is a rich source of alkaloids [8, 9] proteins [10], quassinoids [11, 12] and flavonoids were isolated from leaves [13]. In the present study, we investigated antiviral activity of chloroform and methanol extracts from A. excelsa bark against Herpes Simplex virus type 1 (HSV-1) in-vitro and also determined the major compound in the bioactive chloroform extract.

II. MATERIALS AND METHODS

Plant Material
A. excelsa bark was collected from Zoo garden, Giza, Egypt in May 2010. The plant was identified by Dr. Mohamed El-Gabaly, Professor of Taxonomy, National Research Centre. A voucher specimen no. 13241 is deposited in the herbarium of Zoo garden, Giza, Egypt.

Preparation of extracts and isolation of the major compound of chloroform extract
The Air dried powdered bark of A. excelsa 400 g was successively extracted with Chloroform and methanol70% by maceration for 24 hours and each extract was concentrated under reduced pressure to give 15 g and 23 g of crude extracts, respectively. Each extract of A. excelsa was phytochemically screened according to the following described by (Connolly et al. 1970) [14] for sterols and/or triterpenes (quassinoids); Wolf et al. 1962) [15] for carbohydrates and saponins; Harborne 1973) [16] for flavonoids and alkaloids; Farnsworth 1966) [17] for coumarins; Geissman 1962 for tannins [18] Confirmation of the presence of quassinoids and alkaloids were detected by Thin layer chromatography (TLC) by spraying with specific reagents. Chromatographic separation of chloroform extract on silical gel column chromatography (0.06-0.2 mm) eluted with chloroform: methanol (99:1)
resulted in the isolation of major compound of the chloroform extract, canthin-6-one, detection of this compound was detected by sparing with dragendorff reagent which is specific for alkaloids on TLC in which the compound gave red-orange spot.

**Preparation of the extracts for bioassay**

Extracts were dissolved as 100 mg in 1 ml of 10% DMSO in water. The final concentration was 100 µg/µl (Stock solution). The dissolved solutions were sterilized by addition of antibiotic antymycotic mixture [19] Sterility test were carried out in nutrient agar.

**Cell Culture**

African green monkey kidney-derived cells (VERO) were used. The cells were propagated in Hanks' Minimum essential medium, MEM supplemented with 10% Foetal bovine serum, 1% antibiotic-antimycotic mixture. The pH was adjusted at 7.2-7.4 by 7.5% sodium bicarbonate solution. The mixture was sterilized by filtration through 0.2 µm pore size nitrocellulose membrane.

**Viruses**

Herpes Simplex virus type 1 was obtained from Environmental Virology Lab., Department of Water Pollution Research, National Research Centre. Antiviral assay was carried by Plaque reduction assay [20].

**Plaque reduction assay**

A 6-well plate was cultivated with Vero cell culture (10^5 cell/ml) and incubated for 2 days at 37 °C. HSV-1 was diluted to give 10^5 PFU/ml final concentration and mixed with the plant extract at 100 mg in 1 ml of 10% DMSO in water and incubated overnight at 4°C. Growth medium was removed from the multwell plate virus-compound mixture was inoculated (100 µg/well). After 1 hr contact time, the inoculum was aspirated and 3 ml of Minimal Essential Medium (MEM) with 1% agarose was overlaid the cell sheets. The plates were left to solidify and incubated at 37°C until the development of virus plaques. Cell sheets were fixed in 10% formaline solution for 2 hrs, and stained with crystal violet stain. Control virus and cells were treated identically without chemical compound. Virus plaques were counted and the percentages of reduction were calculated [20].

**III. RESULTS AND DISCUSSION**

**RESULTS**

The Results of antiviral activity of Ailanthus excelsa stem bark extracts are included in table 1, it has shown that chloroform extract is more potent than methanol extract as anti-HSV-1 agent, where chloroform extract showed virus reduction by 82.6, while methanol extract showed virus reduction by 52 at the concentration of 50 µg. Phytochemical analysis of the extracts are included in table 2 which prove that each extract has interesting bio-active compounds, quassinoids, and alkaloids and the major alkaloid compound, canthin-6-one was isolated from the bioactive chloroform extract and the chemical structure of the compound was identified by ¹H-NMR, ¹³C-NMR and MS

Structure elucidation of the alkaloid compound, canthin-6-one:

Canthin-6-one: ¹H NMR (400 MHz, CDCl3): δH 6.98 (d, J = 9.9 Hz, H-5), 7.50 (t, J = 7.6 Hz, H-10), 7.68 (t, J = 7.6 Hz, H-9), 7.95 (d, J = 4.8 Hz, H-1), 8.01 (d, J = 9.9 Hz, H-4), 8.09 (d, J = 7.6 Hz, H-11), 8.64 (d, J = 8.1 Hz, H-8), 8.79 (d, J = 4.8 Hz, H-2). ¹³C NMR (100 MHz, CDCl3): δ 116.49 (C-1), 117.30 (C-8), 122.74 (C-11), 124.37 (C-11a), 125.72 (C-10), 128.95 (C-5), 130.47 (C-11b), 130.95 (C-9), 132.07 (C-11c), 136.09 (C-3a), 139.49 (C-4), 139.49 (C-7a), 145.76 (C-2) and 159.59 (C-6).

EI-MS: m/z 220.

**DISCUSSION**

Present study for the first time, proved antiviral activity of A. excelsa bark extracts. The results has shown that chloroform has a good antiviral activity than methanol extract (Table 1) in a dose dependent manner. Chloroform extract of A. excelsa stem bark at concentration 20 µg has shown an inhibition for the virus by 66.5%, and at concentration of 50 µg, chloroform fraction of A. excelsa stem bark has a significant anti-viral activity by inhibition for the virus by 82.6%. Methanol extract at concentration 20 µg has shown an inhibition for the virus by 44.9%, and at concentration of 50 µg, it has shown inhibition of the virus by 52%. The anti-HSV-1 properties of A. excelsa extracts in this research could be due to multiple different components in these extracts. The phytochemical characterization of extracts and the identification of the bioactive compounds are now needed. Phytochemical analysis of the extracts has shown that both extracts have interesting bioactive compounds include quassinoids and alkaloids (Table 2). Chloroform extract contained canthin-6-one as the major component and the higher activity of chloroform extract is probably due to the higher concentration of these bioactive compounds in the chloroform extract, while methanol is less active due to its low concentrations of these bioactive compounds. Analysis of both extracts by TLC has proved many spots for chloroform extract and less number of spots for methanol extract and these spots were detected under ultraviolet (UV) light. Spraying these spots with vanillin-sulphuric acid reagent followed by heating for 5 min until the appearance of purplish-blue colours specific for...
quassinoids and Spraying with draggendorf’s reagent resulted in the formation of an orange colour specific for alkaloids. The HSV-1 activity of chloroform extract may be due to the presence of alkaloids (canthin-6-one) and quassinoids which present in considerable amounts in the extract [8], [9], [11], [4] and this is in agreement with the activity of alkaloids isolated from *Tripterygium hypoglaucum* against herpes simplex virus type 1 [21] Also the antiviral activity of alkaloids was confirmed from *Fumaria* and *Corydalis* species [22] as well Quassinoids have shown anti-HSV-1 [23] Chloroform extract of *A. excelsa* bark has considerable amounts of alkaoids and quassionoids and the major component is the alkaloid is canthin-6-one while methanol has the same bioactive chemical compounds but in less amount, so Chloroform extract has higher antiviral activity than methanol.

### Table 1

**Anti-HSV-1 bioassay in Vero cell line by plaque reduction assay of *A. excelsa* extracts**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration</th>
<th>Initial virus count (PFU/ml)</th>
<th>Virus count (PFU/ml)</th>
<th>% of Virus reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>20 µg</td>
<td>1.96 x 10⁷</td>
<td>1.22 x 10⁷</td>
<td>66.5</td>
</tr>
<tr>
<td></td>
<td>50 µg</td>
<td>1.96 x 10⁷</td>
<td>1.2 x 10⁷</td>
<td>82.6</td>
</tr>
<tr>
<td>Methanol</td>
<td>20 µg</td>
<td>1.96 x 10⁷</td>
<td>1.08 x 10⁷</td>
<td>44.9</td>
</tr>
<tr>
<td></td>
<td>50 µg</td>
<td>1.96 x 10⁷</td>
<td>0.94 x 10⁷</td>
<td>52</td>
</tr>
</tbody>
</table>

### Table 2

**Results of Phytochemical analysis of *A. excelsa* extracts**

<table>
<thead>
<tr>
<th>Chemical Constituents</th>
<th>Chloroform extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates and/or glycosides</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Condensed tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>b. Hydrolysable tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids and/or nitrogenous bases</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterols and/or triterpenes (Quassinoids)</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ denotes the presence of the constituents  
- denotes the absence of the constituents
VI. CONCLUSION

*Ailanthus excelsa* bark Chloroform extract can be as a promising source as antiviral agent and this is due to the presence of the interesting bioactive phytoconstituents as quassinoids and alkaloids (canthin-6-one) and it may be appropriate for further therapeutic studies against herpes viruses.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

REFERENCES