Qualitative, Quantitative and Cytotoxic estimation of Phytochemicals in aerial parts of Iraqi Bauhinia variegata

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Abstract:

Bauhinia variegata L., is a flowering evergreen small medicinal tree characteristic with an aesthetic appearance and the shape of their flowers which resemble the orchid plant. It is a member of Leguminosae family. Commonly called" Khuf Al-Jamal" as in Arabic and "Camel's feet" as in English. Quantitative estimation of phenolic compounds using UV spectroscopy. Also, HPLC analysis of lupeol and β-sitosterol content of aerial parts B.variegata.

Moreover, the anticancer activity of stem and leaves hexane extract against AMJ-13 cell was performed, which considered one of the first studies around the world of this plant extracts activity against AMJ-13 cell.

Key words: Bauhinia variegata , phytochemicals, anticancer, lupeol, β-sitosterol

التقدير النوعي والكمي والسمي للخلايا للمواد الكيميائية النباتية للأجزاء الهوائية لخف الجمل العراقي

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الخلاصة:

شجرة طبية صغيرة مزهرة دائمة الخضرة تتميز بمظهر جمالي وشكل أزهارها التي تشبه نبات الأوركيد. إنه عضو في عائلة Leguminosae. شاع تسميتها بـ "خف الجمل" كما في اللغة العربية و "أقدام الجمل" كما في اللغة الإنجليزية. تقدير كمي لمركبات الفينول باستخدام التحليل الطيفي للأشعة فوق البنفسجية. أيضًا ، تحليل HPLC لمحتوى lupeol و-sitosterol للأجزاء الهوائية B.variegata.

علاوة على ذلك ، تم إجراء النشاط المضاد للسرطان لمستخلص الهكسان الجذعي والأوراق ضد خلية AMJ-13 ، والتي تعتبر من أولى الدراسات حول العالم لنشاط المستخلصات النباتية ضد خلية AMJ-13

الكلمات المفتاحية: خف الجمل, كيميائية النبات, مضاد للسرطان, لوبيول, بيتاسايتوستيرول

Introduction

The growing use of medicinal plants as a source to treat various maladies is attributed to the wide diversity of phytochemical constituents of herbal drugs, which exhibit a variety of biological

activity. In addition, the physic-chemical characteristics of traditional medicine is preferred over that of synthetic one. Furthermore, the reduction in the efficacy of chemical drugs beside the rise of an adverse reaction make the need for a new effective drug with minimum side effects. This led to increase the utility of the naturally occurring drugs. [1-3]

Bauhinia variegate Linn. is evergreen small medicinal tree, compass of 300 species that are grow all over the world especially in the tropical regions. This therapeutics tree is implanted in Pakistan in plains and sub-mountainous tracks. Bauhinia has been widely cultivated worldwide due to its therapeutics value as anticancer, antioxidant, antibacterial activity. [4]

The bark of *Bauhinia variegata* Linn. is traditionally used as a tonic beside its use in the treatment of skin diseases and ulcers. The root is used as antidote to snake poison. In folklore medicine, this tree is used for the treatment of several diseases such as inflammatory conditions and bacterial infections. The present study reports the phytochemical and electrolyte contents of aerial parts of plant beside the anticancer activities of stem and leaf of Iraqi *B.variegata*.[5]

Methods and Materials

Plant material

Bauhinia variegata leaves, stem and flowers were collected from Al-Nahrain University/ college of science, Baghdad/Iraq. Authentication of plant was carried out by national herbarium in Botany directorate in Abu-Ghraibe. The aerial parts were collected during March and dried in shade at room temperature 25C, grinded as powder and weighed.

Estimation total phytochemical concentration of leaves, stem and flower of *B. variegata*:

Phytochemical concentration of different parts of *B. variegata* were estimated as follow:

Total phenolic contents

Total phenolic contents were determined by Singleton's method slightly modified by Dewanto *et al.* [6] using the Folin-Ciocalteu reagent. 0.125ml of a diluted (0.25mg ml-1) methanolic extracts of leaves, stem and flower of *B. variegata* were added to 0.5 ml of deionized water and 0.125ml of the Folin-Ciocalteu reagent. The mixture then shaken and allowed to stand for 6 min, before adding 1.25ml of 7% Na_2CO_3 solution. The solutions then adjusted to the final volume (3ml) with deionized water and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was measured at 760nm. Total phenolic contents were expressed gallic acid equivalents (GAE) per weight of dry weight (mg/g).[7]

Total flavonoid contents

Total flavonoid contents were determined by method described by Dewanto *et al.* [8]. 250µl of the aerial parts methanolic extracts, appropriately diluted to 0.25mg/ml, were mixed with 75µl $NaNO_2$ (5%). Followed by adding 150µl of 10% aluminium chloride after 6 minutes. 500µl of NaOH (1M) were added to the mixture 5 min later and finally the mixture was adjusted with distilled water to 2.5ml. The absorbance versus prepared blank was measured at 510 nm. Total flavonoid contents were expressed as rutin equivalents per amount of dry weight (mg/g). [7&9]

Extraction and detection of lupeol and β-sitosterol

Shade dried pulverized plant material (100g), include leaves, stem and flowers, were extracted separately by Soxhlet apparatus with hexane (700 ml). Each extract was filtered then evaporation of the solvent was performed under reduced pressure by using rotary evaporator.

Hexane extracts were analyzed for presence of lupeol and β-sitosterol by using analytical TLC with spray reagents and different solvent system

Determination of lupeol and β-sitosterol by HPLC analysis:

Chromatographic identification was performed at College of pharmacy / Mustansiriyah University. Waters symmetry shield C18 column (150 x 4.6, 5 µm) was used for the analysis. The mobile phase consisting from methanol: acetonitrile in the ratio (30:70) v/v, which was filtered through a 0.45 µm membrane filter (Millipore) and degassed by sonication. Throughout the run a flow rate of 1.0 mL min-1 was maintained. The column effluent was detected at 210 nm with L-2400 series multi-wavelength UV Detector. [10]

Cytotoxicity Assays

To determine the cytotoxic effect of leaves and stem hexane extract, the 96-well plates was used to perform the MTT cell viability assay. Cell lines were seeded and after 24 hrs. cells were treated with tested compounds at different concentration. The viability measured after 72 hrs of the treatment using MTT solution by measuring the absorbence on a microplate reader at 492 nm. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated by the following equation [11]: -

$$\% \text{ Inhibition rate} = \frac{A-B}{A} * 100$$

For visualize the shape of cells under inverted microscope, 200 µL of cell suspensions were seeded in 96-well micro-titration plates at density $1x10^4$ cells mL^{-1} and incubated for 48 hrs at 37ºC. Then the medium removed and added leaves and stem hexane extract after 24hr , the plates were stained with 50 µL with Crystal violet and incubated at 37ºC for 15 min, then the stain was removed gently by water. The cell observed under inverted microscope at 100x magnification microscope filed and photographed with digital camera.(12)

Results and discussions

Total phytochemical estimation of leaves, stem and flower of Iraqi *B. variegata*

The total concentration of phenolic acids, flavonoids and other phytochemicals that could be found in a particular plant vary according to the part of plant, cultivation soil, the weather whole the year and the time by which the flora collected. The total content of aerial parts of Iraqi *B. variegata* are shown in the table 2.

Table 1 total phytochemical concentration of leaves, stem and flowers of Iraqi *B. variegate*

Part of plant	Phenolic acid	Flavonoid
Leaves	0.721	0.240
Stem	0.830	0.391
Flowers	0.291	0

As shown in the table the higher concentration of both phenolic acid and flavonoids present in the stem. This attributed to its work, that resemble arteries and veins in human, in which it is responsible on transporting the essential nutrients, chemicals and minerals to all parts of plant therefore the essential phytochemicals are concentratrd in stem.

Detection by HPLC:

HPLC analysis used for qualitative and quantitative investigation of β- sitosterol and lupeol in hexane extract of leaves, stem and flower. However, HPLC chromatogram were determined at 6.9min and 4.6 min for β- sitosterol and lupeol respectively

The HPLC chromatogram of hexane extracts of the samples and their retention time are illustrated in figure 1. As

illustrated in the figures below the highest amount of β- sitosterol is present in stem with an area under the curve equals to 21.111. While the largest amount of lupeol was found in the flower with an area under the curve 30.379.

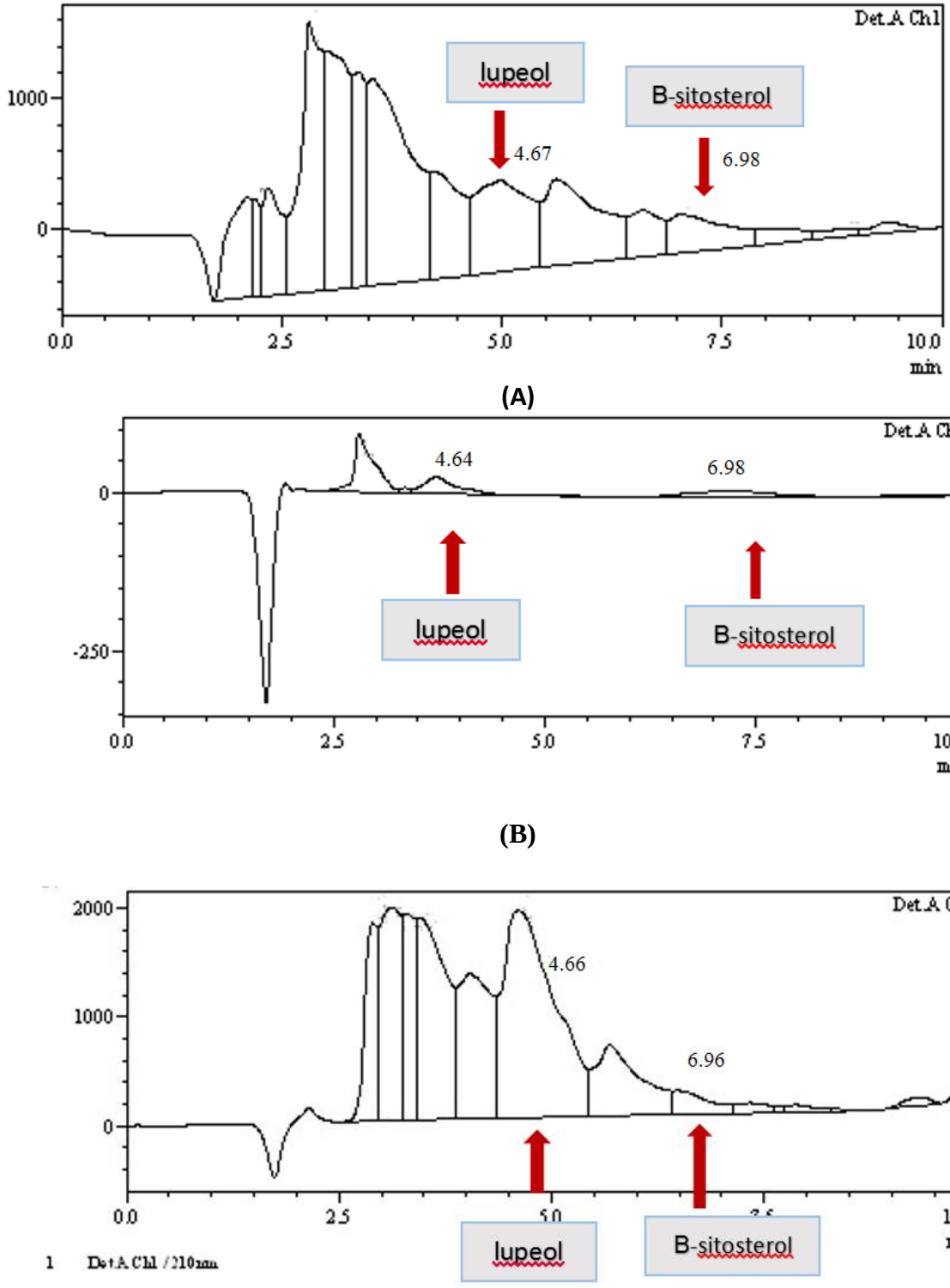

Figure (1): HPLC chromatogram of hexane extract of (A) leaves (B) stem (C) flowers

The cytotoxicity of hexane extract of Iraqi *B. variegata* against AMJ-13 cells:
Stem and leaves hexane extracts were examined on AMJ-13 cell isolated from Iraqi women having breast cancer. The results show the obvious activity of stem hexane extract over the leaves one. The estimations were performed after 72hr exposure to the extracts in 96 well plates, and as shown in the following figure 2.

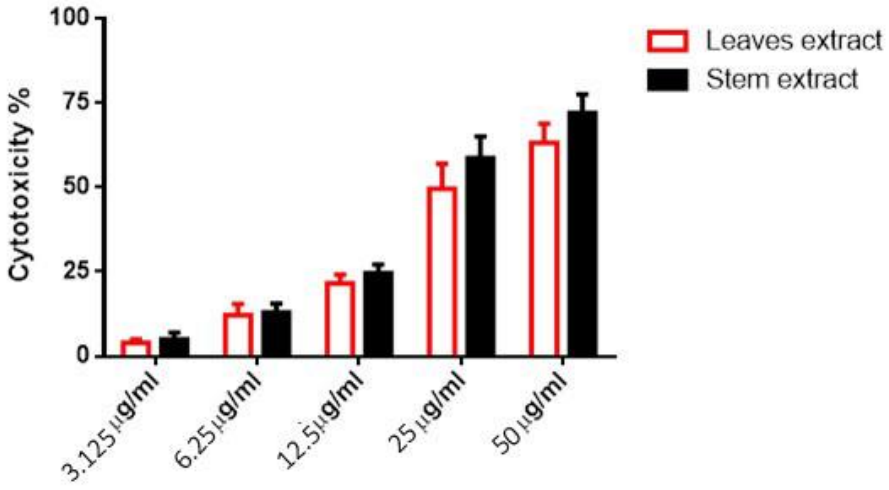

Figure (2): stem and leaves cytotoxicity action against AMJ-13 cells over a serial of diluting concentrations.

As seen in the figure 2, the cytotoxic activity of both hexane extracts was dose dependent inhibition in an ascending manner. The stem extract shows a superior action over the leaves one that reached to more than 75% inhibition of cell growth at 50 µg/ml concentration, while the leaves extract cause barely 75% inhibition. This result attributed to the fact that the stem hexane extract contains more steroids and terpens than the leaves, that they are responsible on the antioxidant and anticancer action of the plants.
The IC50 % of leaves and stem hexane extracts were calculated by unpaired t-test with Graph Pad Prism were 25.34 µg/ml and 19.88 µg/ml respectively.

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