

Phytochemical Investigation of *Corchorus olitorius* L. Leaves Cultivated in Iraq and it's *In Vitro* Antiviral Activity

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Abstract

The aim of our study was to investigate the antiviral activity of the *Corchorus olitorius* family Tiliaceae cultivated in Iraq against measles virus, and to demonstrate an overview about chemical constituents and pharmacological activity of *Corchorus olitorius* L.

About 150 gm Leaves of *Corchorus olitorius* were defatted by maceration in hexane for 24 hrs. The defatted plant materials were subjected for extraction after filtration using Soxhlet apparatus, with aqueous methanol 85% as a solvent extraction for 24 hours, the extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract of about 12 gm. About 4 gm from the residue was suspended in 100ml water, about 3-4ml of 5% sodium hydroxide was added to obtain a basic solution having PH 10 and partitioned with ethyl acetate (3x100ml), the aqueous layer collected and evaporated to dryness. MTT-cell viability assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was conducted on 96-well plates (Falcon), Vero cells were seeded at 1×10^4 cells/well to obtain a multiplicity of infection (MOI 10), and at 5×10^3 cells/well to obtain a multiplicity of infection (MOI 5).

Different statistical result revealed a significant antiviral activity of the aqueous layer of *Corchorus olitorius* leaves against measles virus. The preliminary phytochemical tests showed the presence of phenols and flavonoids in the aqueous layer of *Corchorus olitorius* leaves.

The antiviral activity of *Corchorus olitorius* leaves is mainly due to the phenolics and flavonoids that detected in the aqueous layer.

Keywords: *Corchorus olitorius*, flavonoids, Phenolics, Antiviral, Measles.

التحري للمركبات الفعالة لنبات الملوخية (الجوت) المستزرع في العراق وتأثيرها المضاد ضد الفايروسات

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الخلاصة

الهدف من الدراسة هو التحقيق في النشاط المضاد للفيروسات لنبات الملوخية (الجوت) المستزرعة في العراق ضد فيروس الحصبة، ولإظهار لمحة عامة عن المكونات الكيميائية والنشاط الدوائي للنبات. تم إزالة الدهون من أوراق النبات بواسطة التنقيع في الهكسان لمدة 24 ساعة. تم اخضاع المواد النباتية منزوعة الدهون في عملية الاستخلاص باستخدام جهاز Soxhlet، المذيب الميثانول المائي 85% لمدة 24 ساعة، تمت تصفية المستخلص، وتم تبخير المذيب تحت ضغط منخفض باستخدام المبخر الدوار للحصول على مستخلص جاف حوالي 12 غرام. تم تعليق 4 غرام من المادة المتبقية في ماء 100 مليلتر، وتم إضافة حوالي 3-4 مل من 5% هيدروكسيد الصوديوم للحصول على محلول قاعدي بدلالة حامضية PH 10. تم فصله مع خلاص الأثيل (3x100ml)، تم جمع الطبقة المائية وتبخيرها حتى الجفاف. تم إجراء اختبار MTT حيوية الخلية -[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (Falcon) تحوي 96 حفرة، تم زرع خلايا Vero بمعدل 1×10^4 خلية / حفرة للحصول على تعدد العدوى (MOI 10)، و 5×10^3 خلية/ حفرة للحصول على تعدد العدوى (MOI 5).

كشفت النتائج الإحصائية المختلفة عن نشاط مضاد للفيروسات كبير في الطبقة المائية من أوراق *Corchorus olitorius* ضد فيروس الحصبة. تظهر الاختبارات الأولية الكيميائية النباتية وجود الفينولات والفلافونويدات في الطبقة المائية من أوراق الملوخية. إن النشاط المضاد للفيروسات من أوراق الملوخية يرجع أساساً إلى الفينولات والفلافونويدات التي اكتشفت في الطبقة المائية. الكلمات المفتاحية: الملوخية، الفلافونويدات، الفينولات. مكافحة الفيروسات، الحصبة.

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Introduction

Therapeutic plants have been used since ancient times for the treatment of a variety of diseases⁽¹⁾. Current estimates of the number of types of flowering vegetation range between 200 000 and 250 000 in some 300 families and 10 500 genera, and despite a rapidly expanding books and researches on phytochemistry, only a tiny percentage of the entire varieties have been examined chemically⁽²⁾.

Almost all organisms need to convert and interconvert a great number of organic and natural substances to permit live, grow, and reproduce. In contrast to these major metabolic pathways (primary metabolite), which synthesize, degrade, and generally interconvert compounds commonly encountered in all organisms, there also exists an area of metabolism concerned with substances that have a very limited spread in nature. Such compounds, called "secondary metabolites", which gives almost all the pharmacologically active natural products⁽³⁾.

The genus *Corchorus* (jute) which belongs to the family of Tiliaceae, involves about 50-60 species allocated in the tropics, subtropics and warm temperate areas of the world⁽⁴⁾. *Corchorus* species is one of the main genera containing cardiac glycosides, especially in the seeds⁽²⁾. *Corchorus olitorius* are tall, annual herbs, reaching a height of two to four meters. The plant could be unbranched, or with only a few side divisions. The leaves alternately distributed, simple, lanceolate, finely serrated or lobed margin. The flowers are small (2-3 cm in diameter) and yellow, it has 5 petals.

The fruit of the plant contains many seeds inside in the form of a capsule, *Corchorus olitorius* is an important green leafy vegetable in many areas including Egypt, Southern Asia, Japan, India, China, Lebanon, Palestine, Syria, Jordan, Tunisia, and Nigeria, it is a cultivated plant here in Iraq. It has diverse common names bush okra, nalta jute, jute mallow and Jew's mallow, ewedu, melokhya and moroheiya^(4,5).

Taxonomy of *Corchorus olitorius* L. family tiliaceae

Kingdom: Plantae, Subkingdom: Viridiplantae, Infrakingdom: Streptophyta, Phylum: Magnoliophyta, Division: Tracheophyta, Subdivision: Spermatophyta, Class: Magnoliopsida, Order: Malvales, Family: Tiliaceae, Genus: *Corchorus*, Species: *Corchorus olitorius* L.⁽⁶⁾.

Traditional use

Different parts of *Corchorus olitorius* have been utilized to relieve pain, aches, chronic cystitis, dysentery, enteritis and pectoral pain⁽⁷⁾,

the leaves have been utilized in case of gonorrhea, chronic cystitis, fever, and tumors⁽⁸⁾, the seeds were utilized as demulcent, diuretic, purgative, also used in chronic cystitis, in cases of cardiac diseases like heart failure due to its content of cardenolides cardiac glycosides^(5,9). It is highly consumed in Japan as "healthy vegetable" due to its high content of carotenoids, vitamin C, B1, B2 and E, many minerals and bioactive compounds^(5,10).

Measles is one of the most common communicable diseases around the world and may cause serious complications and sometimes death, about 350 children loss his life every day around the world⁽¹¹⁾.

Corchorus olitorius is one of the plants that's used in folk medicine for the treatment of measles⁽¹²⁾.

Chemical composition of *Corchorus olitorius* L.

In general, phytochemical verification that performed on the plant revealed the presence of sterols like: β - Sitosterol⁽¹³⁾, triterpenes like Ursolic acid Corosolic acid Oxocorocin⁽¹⁴⁾, coumarins like: Cichoriine, Scopolin⁽¹⁵⁾, saponins and tannins⁽¹⁶⁾, flavonoids like: Astragaln (kaempferol 3-O- β -D glucopyranoside) Tolifolin (kaempferol 3-O- β -D- galactopyranoside) Jugalanin (kaempferol 3-O- α -L-arabinopyranoside)⁽¹⁷⁾, Isoquercetin (quercetin 3-O- β -D-glucopyranoside)⁽¹⁸⁾, carbohydrates⁽¹⁹⁾, phenolics like: 5-caffeoylquinic acid (Chlorogenic acid) and 3,5-dicaffeoylquinic acid(isochlorogenic acid)^(15,18,20), cardiac glycosides like: Cannogenol- 3-O- β -D-glucopyranosyl -(1 \rightarrow 4)-O- β -D-boivinopyranoside, periplogenin- 3-O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-digitoxopyranoside⁽²¹⁾, strophanthidin glycosides like: Erysimoside(strophanthidin 3-O- β -D-glucopyranosyl(1 \rightarrow 4)-O- β -D-digitoxopyranoside), olitoriside(strophanthidin 3-O- β -D-glucopyranosyl (1 \rightarrow 4)-O- β -D -boivinopyranoside), Corchoroside A (strophanthidin 3-O- β -D- boivinopyranoside) and helveticoside (strophanthidin 3-O- β -D—digitoxopyranoside), Digitoxigenin glycosides: glucoevatromonoside (digitoxigenin-3-O- β -D-glucopyranosyl - (1 \rightarrow 4) - O - β - D - digitoxopyranoside), coroloside (digitoxigenin-3-O- β -D- glucopyranosyl -(1 \rightarrow 4)-O- β -D-boivinopyranoside), deglucoacoroloside (digitoxigenin - 3-O- β -D- boivinopyranoside), evatromonoside (digitoxigenin-3-O- β -D-digitoxopyranoside), digitoxigenin 3-O- β -D-glucopyranosyl-(1 \rightarrow 6)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-digitoxopyranoside⁽²²⁻²⁴⁾, Corchoroside (A, B, C, D, and E)⁽²⁵⁾.

Pharmacological activity of *Corchorus olitorius*

The plant has several pharmacological activities like **Antihypertensive effect** ⁽²⁶⁾, **Dermatological activity**: improve the score and symptoms of the atopic dermatitis-like lesion ⁽²⁷⁾, and as wound healing effect ⁽²⁸⁾, **Anti-tumor activity**: the plant has a considerable effect against melanoma, leukemia, osteosarcoma ⁽¹⁸⁾, Hella cells ⁽²⁹⁾, and ARH-77 (human multiple myeloma cells) ⁽³⁰⁾, **Antioxidant activity**: due to the high content of phenolics and flavonoids ^(16,20,31), **Anti-diabetic activity**: the plant shows antihyperglycemic and hypolipidemic effects ⁽³²⁾, **Antimicrobial activity**: the plant shows a significant antibacterial effect against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus cereus*, *Corynebacterium diphtheria*, *Kosuria rhizophila*, *Shigella flexneri* and *Aeromonas hydrophila*. ⁽⁸⁾, (*Escherichia coli*, and *Yersinia enterocolitica*). Also against fungal micro-organisms (*Geotrichum candidum* and *Botrytis cinerea*) ⁽⁵⁾, **Anti-inflammatory** ⁽⁷⁾, **Gastroprotective activity** ⁽³³⁾, and **Antiviral activity** ⁽¹²⁾.

The aim of this study is to:

- 1- Demonstrating an overview of chemical constituent and pharmacological activity of leaves extracts from *Corchorus olitorius*.
- 2- Evaluating the *in vitro* antiviral activity of *Corchorus olitorius* L. leaves extract against measles virus for the first time in the world.

Materials and Methods**Collection of plant materials**

Corchorus olitorius leaves were harvested from a farm in al-Utafiyah district in Baghdad City, during July 2017. The plant was identified and authenticated by Prof. Dr. Sukaena Abass / Department of Biology / College of Sciences/ University of Baghdad. Leaves were washed thoroughly, dried under shade, and grinded in a mechanical grinder to a fine powder.

Equipment and chemical

The instruments used were rotary evaporator (BÜCHI Rotavapor R-205, Swiss), Microtiter reader (Gennex Lab/USA), Laminar flow hood (K & K Scientific Supplier/Korea), Cell culture plates (Santa Cruz Biotechnology/USA), Micropipette (Cypress Diagnostics/Belgium), CO₂ incubator (Cypress Diagnostics/ Belgium)

Extraction

About 150 gm of shade-dried pulverized leaves were defatted by maceration with hexane for 24 hours then filtered and allowed to dry at room temperature. The defatted plant materials were extracted using Soxhlet apparatus in which the powder packed in the thimbles and extracted

with 1.75L of aqueous methanol 85% as a solvent extraction for 24 hours. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract of about 12 gm ⁽³⁴⁾. About 4 gm from the residue was suspended in 100ml water, about 3-4ml of 5% sodium hydroxide was added to obtain a basic solution having pH 10. and partitioned with ethyl acetate (3x100ml), the aqueous layer collected and evaporated to dryness ⁽³⁵⁾.

Preliminary phytochemical investigation ⁽³⁴⁾**Test for flavonoids**

0.1 g of plant extract (aqueous layer) was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: (a) 3 ml of the filtrate was mixed with 4 ml of 1% aluminum chloride in methanol in a test tube and the color was observed. Formation of yellow color indicated the presence of flavonoids. (b) 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube and the color was observed. A dark yellow color indicated the presence of flavonoids.

Test for phenols

0.25 g of each plant extract (aqueous layer) was dissolved in 10 ml of distilled water and filtered. 1% aqueous ferric chloride (FeCl₃) solution was added to the filtrate. The appearance of intense green, blue or black color indicates the presence of phenols compounds in the test samples.

Evaluation of the antiviral activity of the aqueous layer**Experimental design**

The dried extract (about 0.9gm) obtained from the aqueous layer was used in this test and symbolled as (sample tested compound).

Cell lines and culture

The Vero (transformed monkey kidney) cell lines were obtained from the Iraq biotech Cell Bank Unit and maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged 37 times including the passage number from the reference, using Trypsin-EDTA reseeded at 50% confluence twice a week and incubated at 37 °C ⁽³⁶⁾.

Virus cells

The Schwartz Edmonston attenuated measles vaccine strain was obtained from the Iraq biotech Cell Bank Unit.

Cell viability and cytotoxicity ⁽³⁷⁾

To determine the cell cytotoxic effect of the measles virus, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) cell

viability assay was conducted as on 96-well plates (Falcon), Vero cells were seeded at 1×10^4 cells/well to obtain a multiplicity of infection (MOI 10), and at 5×10^3 cells/well to obtain multiplicity of infection (MOI 5). After 24hrs. or a confluent monolayer is achieved. Cells were treated with Cells were treated with different concentrations of the tested compounds (100 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$, 12.5 $\mu\text{g/ml}$ and 6.25 $\mu\text{g/ml}$; first: virus alone, virus with tested sample and second: the tested sample alone. The procedure was performed by adding the (virus and or tested sample) at first for 2 hrs. at room temperature to allow virus attachment and penetration. After that, cells were washed with (phosphate buffered saline) PBS and serial dilution of the tested sample was added to the non-infected and infected cells. Cell viability was measured after 72 hrs. of infection by removing the medium, adding 28 μl of 2 mg/ml solution of MTT (Sigma Aldrich-co) and incubating for 3 hrs. at 37°C. After removing the MTT solution by pouring, the crystals remaining in the wells were solubilized by the addition of 130 μl of DMSO (Dimethyl Sulphoxide) (BDH, England) followed by 37°C incubation for 15 min with shaking. The absorbency was determined by a microplate reader (Gennex Lab. USA) at 492 nm (test wavelength); the assay was performed in triplicate (36). Endpoint parameter that is calculated for each individual cell line is the percentage of cytotoxicity, it was calculated by the following equations:

$$\text{Viability percentage} = (A - B)/A \times 100$$

Where A is the mean optical density of untreated wells (control) and B is the optical density of treated (tested) wells.

$$\text{Cytotoxicity percentage} = 100 - \text{viability Percentage}$$

Control test

In order to investigate the cytotoxicity effect of the tested sample itself; the Vero cells are treated with different concentrations from the aqueous layer.

Results

The Phytochemical composition

Preliminary examination of the aqueous fraction results is shown in Table 1.

Table 1. Preliminary examination of the aqueous fraction for phenols and flavonoids.

phytochemicals	Aqueous layer
flavonoids	+
phenols	+

The Anti-Cytotoxic or antiviral effect of the plant extract

The results demonstrate the significant anticytotoxic effect of different concentrations from aqueous layer against the toxicity effect of measles virus on Vero cells using both the obtained multiplicity of infection (MOI of 5 and 10).The obtained result against the cytotoxic effect of measles virus is shown in figures (1, 2) and tables (2, 3).The results obtained from the control test are demonstrated in figure (3) and table (4).

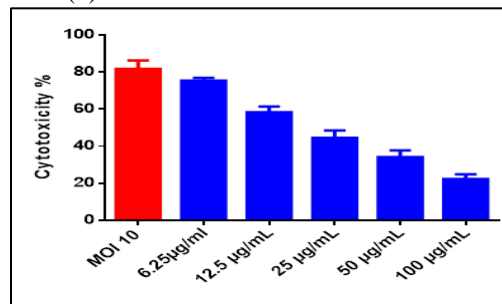


Figure1. Cytoprotecting chart; increasing the concentration of the tested sample (aqueous layer) reduce the cytotoxicity of measles virus on Vero cells using MOI of 10.

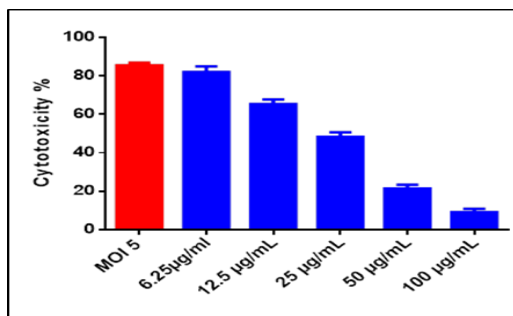


Figure2. Cytoprotecting chart; increasing the concentration of the tested sample (aqueous layer) reduce the cytotoxicity of measles virus on Vero cells using MOI of 5.

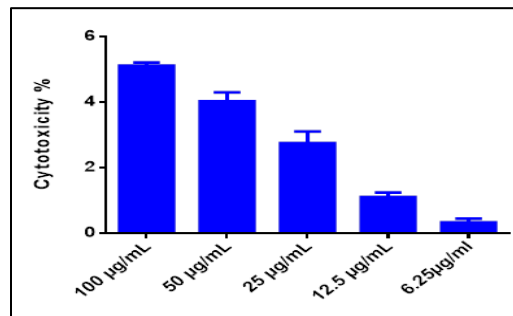


Figure 3. Control test. cytotoxicity chart using a different concentration of the tested sample (aqueous layer) against Vero cells.

Table 2. The statistical result of the tested sample (aqueous layer) against the cytotoxicity of measles virus using MOI of 10.

Con.	MOI 10	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Mean optical density of triplicate	81.80	75.33	58.37	44.49	34.08	22.20
P value	0.0010	0.0001	0.0009	0.0028	0.0038	0.0044
Significant (alpha=0.05)	Yes	Yes	Yes	Yes	Yes	Yes

Table 3. The statistical result of the tested sample (aqueous layer) against the cytotoxicity of measles virus using MOI of 5.

Con.	MOI 5	6.25µg/ml	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Mean optical density of triplicate	85.17	82.05	65.25	48.30	21.37	9.140
P value	< 0.0001	0.0159	0.0171	0.0224	0.0406	0.0790
Significant (alpha=0.05)	Yes	Yes	Yes	Yes	Yes	No

Table 4. The statistical result of the cytotoxicity of different concentrations from the tested sample (aqueous layer) against Vero cells.

Con.	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25µg/ml
Mean optical density of triplicate	5.112	4.026	2.750	1.100	0.3225
P value	0.0001	< 0.0001	0.0577	0.0577	0.0130
Significant (alpha=0.05)	Yes	Yes	No	No	Yes

Discussion

In the present study, maceration of plant material in hexane for overnight was used as defatting method. Next day the solvent removed by filtration and the defatted plant was dried then extracted with alcohol (85% ethanol) to get the crude extract. fractionation of the crude extract is recommended for the separation other, it depends on several factors like differences in the polarity and solubility. polyphenols like (flavonoids and phenolic acids) have acidic properties⁽³⁸⁾, adding base like 5%NaOH will produce water-soluble salts, fractionating the aqueous phase with ethyl acetate will make the lipophilic and non-polar compounds to stay in the Ethyl acetate phase leaving the water-soluble salts (flavonoids and phenolic compounds) in the aqueous phase⁽³⁵⁾. MTT is a water-soluble yellow dye that is absorbed by living cells and

reduced by the action of mitochondrial dehydrogenases. The reduction product is a water-insoluble blue formazan, that must then be dissolved for a colorimetric way of measuring by using different types of solvents like DMSO. Metabolically inactive cells do not produce significant amounts of formazan. Conversely which can be detected by the microplate reader, the quantity of formazan produced per cell in a given time depends on the metabolic activity of the cells, so Formazan production is directly proportionate to the living cell number. The MTT (Tetrazolium (MTT) Assay for Cellular Viability and Activity) procedure assesses the activity and number of living cells at the end of an experiment⁽³⁷⁾.

The results demonstrate the significant anticytotoxic effect of different concentrations from aqueous layer against the toxicity effect of measles virus on Vero cells using both the obtained multiplicity of infection (MOI of 5 and 10).

The obtained result clarifies a linear relationship between the concentration of the tested sample and the degree of cytoprotecting effect (antiviral effect) against the cytotoxic effect of measles virus. The result obtained from the control test shows no cytotoxic effect against Vero cells by the tested sample, since even in high concentration which is 100 µg/mL only five percent cells were killed, while only 1 percent cell killed at 12.5 µg/mL. This result augments the effect of the tested sample as antiviral (anticytotoxic) against measles virus. Natural flavonoid compounds with antiviral activity were identified in 1940, and many reviews were available about their antiviral activity especially against human immunodeficiency virus (HIV). Several types of research demonstrate the mechanism of action through inhibition of various enzymes involved in the life cycle of viruses such as HIV-1 reverse transcriptase, DNA polymerases, and HIV-1 enzyme proteinase⁽³⁹⁾.

In general, the presence of two vicinal hydroxyl groups on an aromatic ring is the most important structural requirement for inhibition of integrase protein (HIV-1-IN) leading to inactivate viral integration and replication which is the situation with most flavonoids. The dicaffeoylquinic and L-chicoric acid derivatives have been proved to show antiviral activity although it doesn't have such adjacent hydroxyl group⁽⁴⁰⁾. It may exert its action by inhibiting the virus-cell fusions in the early stage of the replication cycle and by inhibiting the cell-cell fusions at the end stage of the replication cycle⁽⁴¹⁾.

Conclusions

1. Preliminary Phytochemical screening is done for the aqueous layer obtained from of Iraqi plant *Corchorus olitorius* demonstrate the presence of, flavonoids and phenolics.
2. For the first time in the world, the preliminary study has gathered experimental evidence that aqueous methanolic extract of Iraqi plant *Corchorus olitorius* exhibited significant antiviral activity against measles virus.

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