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Antibacterial, Antifungal and Antiviral Activities of *Euphorbia Greenwayi var. Greenwayi* Bally & S. Carter

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Abstract

The interest in many traditional natural products is increasing. Natural products continue producing bioactive agents owing to the remarkable available chemical diversity. They were evaluated as prospective therapeutic candidates for the treatment of human and animal infectious diseases. *Euphorbia*ceae, the spurge family, holds a significant place in the domain of plant families, with scientific evidence of antiviral, antibacterial, anticancer, cytotoxic and antitumor properties. In this regard, the current study intends to investigate the antibacterial, antifungal, antiviral and cytotoxic properties of *Euphorbia greenwayi var. greenwayi* Bally & S. Carter. The dried aerial parts of *E. greenwayi var. greenwayi* Bally & S. Carter were used, then extracted with 70% ethanol, solvent was distilled off till dryness. The antimicrobial activity of the extract and both MIC and MBC were evaluated against one strain of Gram-positive bacteria: *Staphylococcus aureus* ATCC9144; four strains of Gram-negative bacteria: *Klebsiella pneumonia* ATCC10031, *Escherichia coli* ATCC10536, *Salmonella typhi* ATCC14028, *Pseudomonas aeruginosa* ATCC9027and yeast: *Candida albicans* ATCC10231. The antiviral activity of hydroalcoholic extract against Rotavirus infection was determined as well as the cytotoxic properties. The antibacterial examination revealed potential activity of the hydroalcoholic extract against all tested species with the inhibition zone ranged from 14.7 to 29.7 mm. The highest activity was against *S. aureus* and *C. albicans.* MIC and MBC results proved that the extract is potentially bacteriostatic and bactericidal agents against both Gram-positive and Gram-negative bacteria and against the tested yeast. Also, the extract has the ability to prevent Rotavirus attachment with the cell host. This research revealed that the hydroalcoholic extract of aerial parts of *E. greenwayi var. greenwayi* Bally & S. Carter has significant antimicrobial potential that can be implemented in different pharmaceutical formulations.

Keywords: *E.greenwayi* var. Greenwayi Bally & S. Carter, Antimicrobial Activity, Antiviral Activity, Plant Extract, Cytotoxicity

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INTRODUCTION

The ancient Egyptians utilized a variety of herbs in their medicines, which were clearly documented in their papyri.[1] The Egyptian flora contains over 2185 species, which provide as a valuable source of medically important compounds.[2]

Medicinal plants are still one of the most important therapeutic tools in traditional medicine in Egypt, as they are in other developing countries. The Egyptian flora provides great opportunities for the identification of novel chemicals with a variety of therapeutic properties. Egyptian medicinal plant's antibacterial activity has been reported on multiple occasions.[3-5]

The spurge family *Euphorbia*ceae is considered a large flowering family of plants with 300 genera and 5000 species; having milky poisonous juice. *Euphorbia* genus is one of the largest genera in the Egyptian flora and it is distributed in the Nile Delta and Upper Egypt[6] with nearly 2000 species worldwide, 750 species in Africa where 42 species of them are in Egypt. Some species of *Euphorbia*ceae have been used in treatment of paralysis, dermatosis, and body pain; also, they have been used as a poultice for treatment of skin ulcerations. A number of biological activities have been approved recently ranging from antispasmodic, cytotoxic, anti-inflammatory, hepatoprotective, antibacterial, anti-mutagenic, antifungal and antiviral.[7] *E.greenwayivar.greenwayi* Bally & S. Carter belongs to family *Euphorbia*ceae. It is a semi-perennial succulent plant. Its flowers are small, yellow in color and grow alongside the thorns.

The antimicrobial activity of *Euphorbia* species has been previously reported, beside the MIC values of different extracts,[8-12] herein, it is the first report on the antimicrobial activity of ethanol extract of *E.greenwayivar.greenwayi* Bally & S. Carter on Gram-positive, Gram-negative bacteria and yeast, and to correlate the chemical components of this species with the biological activity in order to develop a new antimicrobial natural drugs.

Many *Euphorbia* species have been reported to have medicinal properties because of the presence the variety of phytochemicals such as terpenoids, flavonoids and polyphenols compounds, which exhibit a great variety of biological activities.[13] Different studies revealed the presence of number of active constituents have been isolated from *Euphorbia* species. Afzelin, myricitrin, quercitrin, quercitin, rutin, 2,4,6-tri-O-galloyl-β-D-glucose,euphorbin-A, B, C, D, kaempferol, 1,3,4,6-tetra-O-galloyl-β-D-glucose, gallic acid, protocatechuic acid, 24-methylenecycloartenol, β-amyrin,β-sitosterol, nonacosane, heptacosane, shikimic acid, tinyatoxin, camphol, choline and derivatives of quercitol containing the rhamnose acid.[14]

MATERIALS AND METHODS

Plant Extract

The plant was collected from Cactus farm in El Mansouria, Giza, Egypt. It was authenticated by Eng. Therase Labib, Senior botanist in El Orman Garden, Egypt. A voucher specimen no. (FUPD-60) was kept in Faculty of Pharmacy, Pharmacognosy Department; Fayoum University.

The aerial parts of *E.greenwayivar. greenwayi* Bally & S. Carter were used. They were cut into small pieces then air-dried and weighed. The dried powder was exhaustively extracted with 70% ethanol at room temperature. The solvent was distilled off using a rotary evaporator till dryness and kept for further studies.

Preparation of Sample for Biological Activity

A part of the dried hydroalcoholic extract was diluted in 5% Dimethyl sulfoxide (DMSO) to concentration of 50mg/ml and stored at 4°C.

Bacterial Strains

The antimicrobial activity of the hydroalcoholic extract of *Euphorbia* was evaluated against five bacterial strains: One strain of Gram-positive bacteria: *Staphylococcus aureus* ATCC9144; four strains of Gram-negative bacteria: *Klebsiella pneumonia* ATCC10031, *Escherichia coli* ATCC10536, *Salmonella typhi* ATCC14028, *Pseudomonas aeruginosa* ATCC9027and yeast: *Candida albicans* ATCC10231. All ATCC strains were provided from Microbiology and Immunology Department, Faculty of Pharmacy, Fayoum University.

Inoculums Preparation

Bacterial strains were sub-cultured at 37°C overnight in Mueller-Hilton (MH) agar slants. Next day the bacterial growth was harvested in 5 ml of sterile saline water; the viable cell count was adjusted to 10^7 CFU/ml of bacteria, 10^4 CFU/ml of yeast at 580 nm using spectrophotometer.

Antimicrobial Activity

Antimicrobial activity was determined using agar well diffusion method. A suspension of bacteria and yeast (100 µl)was inoculated into Mueller Hinton (MH) Agar (Difco) and into Sabouroud Dextrose Agar (Difco), respectively. The wells were prepared in the plates by cork-borer. The plant alcoholic extract(100 µl)was introduced into each well; thee replication for all the testing strains were used to test the activity of hydroalcoholic extract .Then the plates were incubated at 37°C, for 24 h for bacterial strains and at 25°C for 72 h for yeast. Inhibition zones against the tested species were measured to determine the antimicrobial activity.[15]

Determination of Minimum Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentration (MBC)

Microdilution method was to determine the MIC of plant extract for *S.aureus, K. pneumonia, E. coli, S.typhi, P.aeruginosa* and *C. albicans.*[16] Briefly, bacterial organisms were cultured in Muller Hinton broth, while sabaroud broth was used to culture *C. albicans* for 24 h at 37°C. The plant extract was diluted in 5% DMSO and adjusted at a concentration range from 50 to 0.39mg/ml of the original solution. One hundred µl of the extract (50 mg/ml) were added to the first wells of the microplate and then diluted two-fold serially down the wells with MH broth, one hundred µl of culture were added to the wells, extract free solvent and un-inoculated broth were used as blank, inoculated broth was used as positive control then, the plates were incubated for 24 h at 37°C. After incubation, 2 µl of tetrazolium chloride was added to evaluate microbial growth inhibition. The results were observed after 30 min. Clear wells with lowest concentration were considered as the MIC values. For MBC determination, all wells which did not show any visible signs of growth (MIC and higher concentrations), The MH agar plates were inoculated with loopful using the streak plate technique. The MBC of tested extract against tested species was determined to be the lowest concentration at which the tested organisms did not exhibit any signs of growth.[17] Each test was run three times, and the findings were presented as means. ± SD.

Antiviral Activity Against Rotavirus Infection
Cells and Virus

MA104 monkey kidney cells were purchased from VACSERA, simian rotavirus (RV) SA-11 stock was acquired from the National Institute for Cholera and Enteric Diseases (NICED), Department of Virology, Kolkata, India. In a humidified incubator with 5% CO_2, Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated foetal bovine serum (FBS) was used to culture the cells, and 100 g/ml streptomycin and 100 units/ml penicillin (All purchased from Lonza, Belgium). Trypsin 10 mg/ml was used to pre-activate the Simian rotavirus SA-11 stock for 30 min at 37°C. After 72 hours of incubation, the cytopathic effect of the activated RV stock that had been diluted tenfold was assessed in MA 104 cells. The previously established Karber method (Finney, 1978) was used to determine the 50% tissue culture infectious doses/0.1 ml (TCID50/0.1ml), which were then stored in small aliquots, which can be stored at -80°C until utilized.

Cytotoxicity Evaluation

Evaluation of Cell morphology by using inverted light microscopy[18] - MA104 cell lines ($2x10^5$ cells/ml) were seeded in tissue culture plates 96-well (Corning, US). After incubation for 24 h at 37°C in a humidified 5% CO_2 atmosphere, the culture medium was discarded and replaced with 200 µl of compound dilutions (1000, 750, 500, 250, 125, 62.5 µg/ml) per well prepared in culture medium. For cell controls, adding 200 µl of culture medium without compounds. All plates completed a 72-hour incubation period at 37°C in a humidified environment containing 5% CO_2. Daily checks were made for morphological changes that could be seen under a microscope, like cell rounding and shrinkage, loss of confluence, cytoplasm granulation, or vacuolization. The antiviral assay was chosen using dilutions that

are 100% safe against the cell morphology after scoring the morphological modification.

Antiviral Activities of Compound using the Cytopathic Effect of Measurement

For $TCID_{50}$ determination, dilution from each compound that is 100% safe was selected to evaluate its activity against RV infection. 10-fold dilution of activated RV SA-11 were achieved in cell culture medium then100 µl of viral dilutions 10^{-4}–10^{-9} were incubated after addition of 100 µl of cell culture containing the tested compound for one hour incubation at 37°C in CO_2 incubator. Virus dilutions either with tested compound or without it were inoculated into four parallel wells. All plates completed incubation period of 72h at 37°C in CO_2 incubator, then, the inverted microscope was used to observe the cytopathic effect. Virus titration was calculated and then expressed as $TCID_{50}$ (50% tissue culture infection dose) using Spearman-Karber method.[19] Differences between the treated virus value and untreated virus were used in calculation of reduction in virus titer.

RESULTS

Antimicrobial Activity

Euphorbia extract was investigated to evaluate its activities against chosen strains of Gram-negative bacteria (*K. pneumonia, E. coli, S. typhi* and *P. aeruginosa*), Gram-positive bacteria (*S. aureus*) and yeast (*C. albicans*) using agar well diffusion method. Results of antibacterial activity revealed that hydroalcoholic extract of *E.greenwayi var. greenwayi* Bally & S. Carter was potentially active in suppressing microbial growth of all tested species and the inhibition zone ranged from 14.7 to 29.7 mm (Table 1). The most antimicrobial activity was against Gram-positive bacteria *S. aureus* (29.3 mm)as well as yeast *C. albicans* (29.7 mm), while effect against *E. coli.* (14.7mm) was the least one. Based on these results, the minimal inhibitory concentration (MIC) as well as the minimal bactericidal concentration (MBC) against chosen species were tested.

Table 1. Results of antimicrobial activity

Species	Inhibition Zone (mm)
S.aureus	29.3 ± 0.57
K.pneumonia	17.7 ± 0.57
E.coli	14.7 ± 0.57
S. typhi	27.7 ± 0.57
P. aeruginosa	19.7 ± 0.57
C. albicans	29.7± 0.57

*Results are means of three replicates (n = 3) ± standard deviation. * *p*-value was significant < 0.05.

MIC and MBC of Plant Extract

MIC value and MBC value of the plant hydroalcoholic extract were determined using broth Microdilution method to evaluate its bacteriostatic and bactericidal properties. Table 2 represent data (Table 2).

Table 2. MIC and MBC Results

Species	MIC (mg/mL)	MBC (mg/mL)
S. aureus	1.56	3.125
K. pneumonia	6.25	12.5
E. coli	6.25	12.5
S. typhi	1.56	3.125
P. aeruginosa	3.125	6.25
C. albicans	3.125	6.25

MIC and MBC results showed that the hydro alcoholic extract is potentially active as bacteriostatic and bactericidal against both Gram-positive bacteria and Gram-negative bacteria beside its activity against the tested yeast.

Cytotoxicity and Antiviral Activity Evaluation

In this study, we used the microscopic examination in investigating the safe concentration of the tested extract. Results demonstrated that the 100% safe concentration of the tested extract was 750 µg/ml which showed 2.25 log10 $TCID_{50}$/0.1 ml reduction effect in the virus titer as represented in Table 3.

Table 3. Antiviral impact of extract on RV SA11 using TCID50 / 0.1 ml measurement

Titers of viruses without compounds	Titers of viruses with compounds	Virus titer reduction value
$10^{6.25}$	10^{4}	$10^{2.25}$

DISCUSSION

Family *Euphorbiaceae* is used in Egyptian traditional medicine for treatment against infectious disease. This study focuses on testing the activity of the aerial parts of *E.greenwayi var. greenwayi* Bally & S. Carter as antimicrobial agent against Gram-positive, negative bacteria, yeast and viruses as the first report. This study revealed that the plant hydroalcoholic extract of this species showed antimicrobial impact against both Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*K. pneumonia, E. coli, S. typhi* and *P. aeruginosa*) as well as yeast (*C. albicans*). Other *Euphorbia* species are reported for their great importance as antibacterial activity.[20-24] The market prefers natural and healthy products with no synthetic raw materials because f their side effects, thus there is indeed a growing interest in natural antimicrobial agents. The one among the most and influential studies on this areas summarized by Salehi, Iriti, Vitalini, Antolak, Pawlikowska, Kregiel, Sharifi-Rad, Oyeleye, Ademiluyi, Czopek, Staniak, Custodio, Coy-Barrera, Segura-Carretero, Cadiz-Gurrea, Capasso, Cho, Seca[13] illustrating wide range of antimicrobial activities for several *Euphorbia* species such as *E. royleana* Boiss, *E. hirta* L., *E. tirucalli* L., *E. neriifolia* L., *E. paralias* L, *E. granulate* Forssk, *E. helioscopia* L. and *E. characias* L.

The extraction technique used in this study was the same as reported by[25,26]; where, the ethanol extract of aerial part from *E. hirta* L. proved to prevent the growth of most types of bacteria and fungi in different studies.

Several *Euphorbia* spp. cultivated in Egypt are well known for their antimicrobial activity. A reported study demonstrated the antimicrobial activity against *Bacillus subtilis* of *E.helioscopia* which was attributed to the presence of the essential oil from the inflorescence.[27] The methanol extract of wild Egyptian Sahara plant *E. paralias* was reported to be active against Mycobacterium spp.[28] The biological activities of ethyl acetate and dichloromethane extracts of *E.paralias* and *E. geniculate* showed strong antibacterial effect.[29] The ethanol extract of *E. hirta* L. exhibits high antibacterial activity against *Streptococcus mutans* obtained from samples of dental plaque isolated from patients suffering from dental caries.[30]

Since an effective medicine should not exhibit either chronic toxicity or chronic adverse effects on the host, cytotoxicity testing is an important stage in the evaluation of a possible antiviral drug. The studied extract should have no or minimal effects on cellular metabolism and be completely selective for particular viral activities.[18] Since an effective medicine should not exhibit either chronic toxicity or chronic adverse effects on the host, cytotoxicity testing is an important stage in the evaluation of a possible antiviral drug. The studied extract should have no or minimal effects on cellular metabolism and be completely selective for viral activities.[31]

There are several stages in the viral life cycle, including 1) attachment, 2) penetration, 3) replication of viral proteins and genetic material, and 4) assembly and viral egress from infected cells. Anti-rotavirus medications can be used to combat these actions. SA-11 agents.[32] Our findings showed that the extract under test may influence RV infections by interacting with viral capsid and inhibiting the virus from attaching to the cell host. It is necessary to conduct additional research on the antiviral activity in cell culture to illustrate the mechanism of this extract which affects the virus replication by adapting other replication steps.

CONCLUSION

E.greenwayivar. greenwayi Bally & S. Carter aerial parts hydroalcoholic extract can be used in different pharmaceutical preparations as a natural antimicrobial agent.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed to the study conceptualization and design. Material preparation, sample collection, laboratory experiments, first draft writing were performed by RB. Editing, reviewing and supervision were done by MHH and MSA. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not Applicable

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