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# *In vitro* antiviral and antimicrobial activities of *Spirulina platensis* extract

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**ABSTRACT**

*Spirulina platensis* are filamentous, undifferentiated, non-toxigenic cyanobacteria that have been used as food since ancient times. There have been numerous studies on its antioxidant and antimicrobial actions. In this study antibacterial and antiviral effect of ethanol extract of *Spirulina platensis* were tested. The reduction of infectious viral units after treatment with ethanol extract of *Spirulina platensis* was tested. Non toxic doses of *Spirulina platensis* revealed 53.3%, 66.7%, 76.7%, 56.7%, and 50% reductions *in vitro* for infectious units of adenovirus type 7, Coxsackievirus B4, astrovirus type 1, rotavirus Wa strain, and adenovirus type 40 respectively. Using disc diffusion method to show the antibacterial effect of ethanol extract of *Spirulina platensis* against different bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Enterococcus faecalis*) in addition to *Candida albicans*, inhibition zones were observed with *Enterococcus faecalis* and *Candida albicans*.

## INTRODUCTION

Early interest in *Spirulina* was focused mainly on its potential as a source of protein, vitamins, especially vitamin B12 and provitamin A (*β*-carotene), and essential fatty acids like *γ* -linolenic acid (GLA). Recently more attention has been given to study its therapeutic effects, which include reduction of cholesterol and nephrotoxicity by heavy metals, anticancer properties, protection against radiation, and enhancement of the immune system (Belay *et al.,* 1994*). Spirulina* also possesses other biological functions such as antiviral, antibacterial, antifungal, and antiparasite activities (Khan *et al.,* 2005). Cyanobacteria or blue–green algae are photoautotrophic microorganisms largely distributed in nature. Some of them have been used as human food for many years because of their high protein content (35–65%) and nutritional value. Arthrospira (Spirulina) is the best known genus and it was consumed by the Aztecs in Mexico Valley and by the Chaad lake population in Africa. At present, some countries are culturing it on a large scale (Steven and Russell, 1993). Over 100 enteric viruses species cause a wide variety of illnesses in man. These include hepatitis (e.g. Hepatitis A and E), gastroenteritis (e.g. Rotavirus, astrovirus, norovirus, and enteric adenoviruses type 40 and 41), meningitis, fever, rash, (e.g. coxsackieviruses group A and B) conjunctivitis (e.g. coxsackievirus group A) , myocarditis and may be diabetes (e.g. coxsackievirus group B) (Bosch, 1998). Enteric viruses are shed in extremely high numbers in the feces of infected individuals; patients suffering from diarrhea or hepatitis may excrete from $10^5$ to $10^{11}$ virus particles per gram of stool (Atmar *et al.,* 2008, Costafreda *et al.,*2006, El-Senousy *et al.,* 2013). Current wastewater treatments do not ensure complete virus removal and their transmission is through contaminated food or water (Blatchley *et al.,* 2007, Bosch, 2007, Ghazy *et al.,* 2008, El-Senousy *et al.,*2013). Also, Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as Streptococcus and Pseudomonas, and foodborne illnesses, which can be caused by bacteria such as Shigella, Campylobacter, and Salmonella. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy (Fisher *et al.,* 2007).

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In recent years, the pharmaceutical industry focusing on the discovery of new bioactive compounds as antiviral agents and the main focus in recent decades for pharmaceutical discovery from natural products has been on microbial sources (bacterial and fungal), dating back to the discovery of penicillin from the mould fungus Penicillium notatum in the first half of the twentieth century (Abdo *et al*., 2012). Till now, there is not any drug for enteric viruses worldwide. In Egypt, Several studies for testing the antiviral effect of synthetic compounds and natural compounds were done (Ibrahim *et al*., 2007, Esawy *et al*., 2011, Bassyouni *et al*., 2012, Hamdy and El-Senousy, 2013, Hamdy *et al*., 2013, and Salem *et al*., 2013) with some promising compounds. The aim of this study is to examine the antiviral and antibacterial activity of the ethanol extract of *Spirulina platensis*.

## MATERIALS AND METHODS

### Collection of *Spirulina platensis* samples

The algal materials were grown in the algal biotechnology unit, National Research Centre (NRC), Dokki-Cairo, Egypt. Zarrouk media was used for growing *Spirulina platensis* as was reported by Zarrouk, (1966), and Raoof *et al.*, (2006).

### Extraction of *Spirulina platensis*

After collection, *Spirulina platensis* samples were washed with fresh water several times to remove salts and debris, air dried. 500 grams of the powdered samples were macerated and mixed with 2000 ml of 95% ethanol until such time all materials were submerged and allowed to stand for 24 hours with occasional shaking in a dark condition. After 24 hours, it was filtered. The crude residue was soaked again in a fresh 95% ethanol for another 48 hours. The filtrate from the first and second soaking was then mixed together. The combined filtrates were concentrated using rotary evaporator at 50˚C. The resulting concentrated extract was further concentrated by the use of a vacuum oven to remove residual solvent (Fayzunnessa *et al.*, 2011)**.**

### Cytotoxicity test

It was done according to Simoes, *et al.,* (1999) and Walum, *et al.,* (1990). Briefly, the sample (100 mg) was dissolved in 1 ml of EtOH. Decontamination of samples was done by adding 24 µL of 100X of antibiotic–antimycotic mixture to 1 ml of the sample. Then, bi-fold dilutions were done to 100 µl of original dissolved sample and 100 µl of each dilutions were inoculated in Hep-2, BGM, MA104 cell lines (obtained from the Holding Company for Biological Products & Vaccines VACSERA, Egypt) and CaCo-2 cell line, (obtained from Prof.Dr.Albert Bosch, University of Barcelona, Spain) previously cultured in 96 multi well plates (Greiner-Bio one, Germany) to estimate the non toxic dose of the tested sample. Cytotoxicity assay was done using cell morphology evaluation by inverted light microscope and cell viability test applying trypan blue dye exclusion method.

### Cell morphology evaluation by inverted light microscopy

Hep-2, BGM, MA104, and CaCo-2 cell cultures ($2\times10^5$ cells/ml) were prepared separately in 96- well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation at 37 ˚C in a humidified 5% (v/v) $CO_2$ atmosphere cell monolayers were confluent, the medium was removed from each well and replenished with 100 µl of bi-fold dilutions of the sample tested prepared in DMEM (GIBCO BRL). For cell controls 100 µl of DMEM without samples was added. All cultures were incubated at 37 ºC in a humidified 5% (v/v) $CO_2$ atmosphere for 72 h. Cell morphology was observed daily for microscopically detectable morphological alterations, such as loss of confluence, cell rounding and shrinking, and cytoplasm granulation and vacuolization. Morphological changes were scored (Simoes *et al.*, 1999).

### Cell viability assay

It was done by trypan blue dye exclusion method (Walum *et al*.,1990). Hep-2, BGM, MA104, and CaCo-2 cell cultures ($2\times10^5$ cells/ml) were grown in 12-well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation, the same assay described above for tested sample was followed by applying 100 µl of tested sample dilutions (bi-fold dilutions) per well. After 72 h the medium was removed, cells were trypsinized and an equal volume of 0.4% (w/v) trypan blue dye aqueous solution was added to cell suspension. Viable cells were counted under the phase contrast microscope.

### Determination of adenovirus type 7, Coxsackievirus B4, rotavirus Wa strain Titers Using plaque assay

Non toxic dilutions were mixed (100 µl) with 100 µl of different doses of adenovirus type 7 Coxsackievirus B4, and rotavirus Wa strain ($1X10^5$, $1X10^6$, $1X10^7$) Plaque-Forming Units per milliliter (PFU/ml). The infectivity of the rotavirus stocks was activated with 10 µg/ml trypsin for 30 min at 37°C. The mixture was incubated for 1/2 hr in 37°C. The inoculation of (100 µl) 10 fold dilutions of treated and untreated Adenovirus type 7, Coxsackievirus B4, and rotavirus Wa strain was carried out separately into Hep 2, BGM, and MA104 cell lines for adenovirus type 7 Coxsackievirus B4, and rotavirus Wa strain respectively in 12 multi well- plates. After 1 hr of incubation for adsorption at 37°C in a 5% CO2-water vapor atmosphere without constant rocking. The plates were rocked intermittently to keep the cells from drying. After adsorption, 1 ml of 2X media (Dulbecco´s Modified Eagle Medium, Gibco- BRL (DMEM) plus 1ml 1% agarose was added to each well, 0.5 µg/ml trypsin was added to the media-agarose mixture in the case of rotavirus Wa strain and the plates were incubated at 37°C in a 5% CO2-water vapor atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formaline fixation, and the number of plaques were counted. The viral titers were then calculated, and expressed as PFU/ml. (Schmidtke *et al.*, 1998).

**Antiviral Effect of Algal Extracts on infectious Adenovirus Type 40 using Cell culture-PCR (CC-PCR) technique**

It was done according to Esawy *et al*, 2011, Abdo *et al*, 2012, Briefly, Non toxic dilutions were mixed (100 µl) with 100 µl of different doses of adenovirus type 40 $1X10^4$, $1X10^5$, $1X10^6$ Cell Culture-PCR units/ milliliter (CC-PCR units/ml). The mixture was incubated for 1/2 hr in 37°C. The inoculation of (100 µl) 10 fold dilutions of treated and untreated adenovirus was carried out into Hep 2 cell line in 12 multi well- plates. After 1 hr of incubation for adsorption at 37°C, 1 ml of media (Dulbecco´s Modified Eagle Medium, Gibco- BRL (DMEM) was added to each well. After one week incubation at 37°C, three times freezing and thawing for tested plates were done. Nested PCR was performed for confirmation of adenovirus (presence/ absence) in each well According to Puig *et al.*, 1994. The external primers hexAA 1885 (5-GCCGCAGTGGTCTTACATGCACATC-3) and hexAA1913 (5-CAGCACGCCGCGGATGTCAAAGT-3) were used in the first 30 cycles of amplification, and 1 µl was further added to a new batch of 50 µl of PCR mixture containing each nested primer pair, nehexAA1893 (5-GCCACCGAGACGTACTTCAGCCTG-3) and nehexAA1905 (5-TTGTACGAGTACGCGGTATCCTCGCGGTC-3) at 0.16 µM in a new 30 cycles amplification. An adenovirus CC-PCR unit is defined as the reciprocal endpoint dilution detectable by CC- PCR. The detection limit of this assay is $1 \times 10^1$ CC- PCR units/ml.

**Antiviral Effect of Algal Extracts on infectious astrovirus Type 1 using Cell culture-RT-PCR (CC-RT-PCR) technique**

Non toxic dilutions were mixed (100 µl) with 100 µl of different doses of astrovirus type 1 $1X10^5$, $1X10^6$, $1X10^7$ Cell Culture RT-PCR units/ milliliter (CC-RT-PCR units/ml). The mixture was incubated for 1/2 hr in 37°C. The inoculation of (100 µl) 10 fold dilutions of treated and untreated astrovirus was carried out into CaCo-2 cell line in 12 multi well- plates. Astrovirus copy numbers for treated and untreated samples were calculated as numbers of cell culture RT-PCR units by following a previously described procedure based on combined infection of cultured CaCo-2 cell monolayers and RT-PCR with sets of primers A1/A2 for astrovirus genogroups A. An astrovirus CC-RT-PCR unit is defined as the reciprocal endpoint dilution detectable by CC-RT-PCR. The detection limit of this assay is $1 \times 10^1$ CC-RT-PCR units/ml (Abad *et al.*, 1997, El-Senousy *et al.*, 2007).

**Antimicrobial susceptibility testing methods**

It was done according to disc diffusion method (CLSI, 2008, Walker, 2007) to study the antimicrobial effect against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterococcus faecalis,* and *Candida albicans.*

**RESULTS AND DISCUSSION**

In this study the different types of tested cell lines showed different sensitivities to toxic materials in the ethanol extract of *Spirulina platensis*. The non toxic dose ranged from 1.6 to 1.9 mg/ml (Table 1) which means no big difference between the different cell lines. Different nature of different cell lines buffalo green monkey kidney cells (BGM), Hep-2 derived from human, epidermoid larynx carcinoma, MA104 , derived from embryonic Rhesus Monkey kidney tissue. and CaCo-2 heterogeneous human epithelial colorectal adenocarcinoma cells may explain the different capabilities against toxic materials. In the study of Abdo and Co-workers (2012), the non toxic concentration for methanol and water extracts of *Spirulina platensis*, *Anabaena sphaerica, Chroococcus turgidus*, *Oscillatoria limnetica*, and *Cosmarium leave* in Hep-2 cell line was 2mg/ml.

**Table. 1:** Non toxic doses of *Spirulina platensis* extract on Hep2, BGM, CaCo2, and MA104 cell lines..

| Non toxic dose (mg/ml) | Cell line |
|---|---|
| 1.8 | Hep-2 |
| 1.9 | BGM |
| 1.6 | CaCo2 |
| 1.6 | MA104 |

**Table 2:** Antiviral effect of non toxic doses from *Spirulina platensis.*

| Tested virus | Initial Viral titre | Final viral titre | % of reduction | Mean % of reduction |
|---|---|---|---|---|
| Adenovirus type 7* | $1X10^5$ | $4X10^4$ | 60% | 53.3% |
| | $1X10^6$ | $5X10^5$ | 50% | |
| | $1x10^7$ | $5x10^6$ | 50% | |
| Coxsackievirus B4* | $1X10^5$ | $4X10^4$ | 60% | 66.7% |
| | $1X10^6$ | $4X10^5$ | 60% | |
| | $1x10^7$ | $3x10^6$ | 70% | |
| Astrovirus type 1** | $1X10^5$ | $2X10^4$ | 80% | 76.7% |
| | $1X10^6$ | $2X10^5$ | 80% | |
| | $1x10^7$ | $3x10^6$ | 70% | |
| Rotavirus Wa strain* | $1X10^5$ | $4X10^4$ | 60% | 56.7% |
| | $1X10^6$ | $4X10^5$ | 60% | |
| | $1x10^7$ | $5x10^6$ | 50% | |
| Adenovirus type 40*** | $1X10^4$ | $5X10^3$ | 50% | 50% |
| | $1X10^5$ | $5X10^4$ | 50% | |
| | $1x10^6$ | $5x10^5$ | 50% | |

* Initial and final viral titre values are expressed as PFU/ml.
** Initial and final viral titre values are expressed as CC-RT-PCR units/ml
*** Initial and final viral titre values are expressed as CC-PCR units/ml

This is the first Egyptian report concerns with the antiviral activity of ethanol extract of *Spirulina platensis* against enteric viruses. The highest percentage of reduction was observed with astrovirus type 1 76.7% followed by Coxsackievirus B4 66.7%, rotavirus Wa strain 56.7%, adenovirus type 7 53.3%, and adenovirus type 40 50% (Table 2). This indicates the promising antiviral results of ethanol extract of *Spirulina platensis* against RNA and DNA enteric viruses. It also indicates the virucidal effect of the ehanol extract of *Spirulina platensis* on the tested viruses. Higher reduction of the infectious units of RNA viruses after treatment with *Spirulina platensis* ethanol extract than DNA viruses was observed.

The method used to test the antiviral effect of the ehanol extract of *Spirulina platensis* which depends on mixing the virus and the extract and inoculating them on cell cultures after an

incubation period and calculate the loss of infectious units of the tested viruses may indicate a virucidal effect of the tested materials on the viruses. In the study concerned with the effect of methanol extract of *Spirulina platensis* on adenovirus type 40, Considerable reduction of adenovirus type 40 titre *in vitro* (50%) was observed with methanol extract of *Spirulina platensis* (Abdo *et al.*, 2012). They explained the antiviral effect by the presence of highly polar compounds in the methanol extract of *Spirulina platensis*. This explanation may be the reason of the virucidal effect of the ethanol extract of *Spirulina platensis* on enteric viruses in the present study. It may return to the presence of highly polar compounds in the ethanol extract of *Spirulina platensis*. The antiviral effect of the highly polar compounds was confirmed in the study of Corona *et al.*, (2002), who reported that methanol extract of *Spirulina maxima* exhibited antiviral activity against HSV-2 with EC50 6.9 mg/ml, and IC50 0.13 mg/ml. *Spirulina maxima* methanol extract did not have a virucidal effect on herpes virus, inhibited herpes virus infection was explained by blocking the adsorption and penetration events of t7he viral replication cycle. According to the results obtained with the extracts from *Spirulina maxima* using solvents with different polarity, They suggested that the antiviral activity could be due to highly polar compounds present in methanol extract.

The work of Ayehunie *et al.*, (1998), showed that water extract of *Spirulina platensis* inhibited human immunodeficiency virus type 1 (HIV-1) replication in human T-cell lines, peripheral blood mononuclear cells (PBMC). Extract concentrations ranging between 0.3 and 1.2 µg/ml reduced viral production by approximately 50% in PBMCs. The 50% inhibitory concentration (EC50) of extract for PBMC growth ranged between 0.8 and 3.1 mg/ml. Corona *et al.*, (2002) also mentioned that hot water extract of *Spirulina maxima* inhibited the infection for adenovirus type 3 with a percentage less than 20%, with an IC50 5.2 mg/ml. They also reported that the hot water extract of *Spirulina maxima* showed no cell growth inhibition at concentrations below 2 mg/ml.

In this study we did not compare the effect of the ehanol extract of *Spirulina platensis* on enteric viruses with the effect of control drug because till now there is no drug against enteric viruses worldwide. So, it gives a great importance to the studies of natural products and synthetic compounds as antiviral candidate compounds.

Although, there was no inhibition zones with *Escherichia coli* and *Salmonella typhi* (Gram negative bacteria of family Enterobacteriaceae) and *Staphylococcus aureus* (Gram positive bacteria, member of the Firmicutes) there were clear inhibition zones in case of *Enterococcus faecalis* and *Candida albicans* (Table 3). Both *Escherichia coli* and *Salmonella typhi*, are Gram negative bacteria of family Enterobacteriaceae which both of them were not affected by the ethanol extract of *Spirulina platensis*. Both *Staphylococcus aureus* and *Enterococcus faecalis* are Gram positive bacteria, member of phylum Firmicutes. Only *Enterococcus faecalis* was affected by the ethanol extract of *Spirulina platensis*. *Candida albicans* also was affected by the ethanol extract of *Spirulina platensis*. It is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans (Denfert and Hube, 2007). This confirms the biological activity of the ethanol extract of *Spirulina platensis*.

**Table. 3:** Antibacterial effect of non toxic doses from *Spirulina platensis*.

| Tested bacteria | Zone inhibition +/- |
|---|---|
| *Escherichia coli* | - |
| *Candida albicans* | + |
| *Staphylococcus aureus* | - |
| *Salmonella typhi* | - |
| *Enterococcus faecalis* | + |

## CONCLUSION

This study confirmed the biological activity of the ethanol extract of *Spirulina platensis* against non-enveloped RNA and DNA enteric viruses and also, *Enterococcus faecalis* and *Candida albicans*. Further studies are needed to confirm the biological activity of different fractions of *Spirulina platensis*.

## REFERENCES

Abad FX, Pintó RM, Villena C, Gajardo R, Bosch A. Astrovirus survival in drinking water. Appl. Environ. Microbiol. 1997; 63: 3119–3122.

Abdo SM, Hetta MH, El-Senousy WM, Salah El Din RA, Ali GH. Antiviral activity of freshwater algae. Journal of Applied Pharmaceutical Science. 2012; 2: 21-25.

Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, Graham DY. Norwalk virus shedding after experimental human infection. Emerging Infectious Diseases. 2008; 14: 1553–1557.

Ayehunie S, Belay A, Baba T, Ruprecht R. Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthrospira platensis). J. Acqui. Immune Deficin. Syndr and Human Retrovirol. 1998; 18: 7-12.

Bassyouni FA, Saleh TS, El Hefnawi M, Abd El-Moez SI, El- Senousy WM, Abdel-Rehim M. Synthesis, Pharmacological Activity Evaluation and Molecular Modeling of New Polynuclear Heterocyclic Compounds Containing Benzimidazole derivatives. Archive of pharmacal Research ARPR. 2012; 35: 2063-2075.

Belay A, Ota Y, Miyakawa K, Shimamatsu H. 1994. Production of high quality *Spirulina* at Earthrise Farms. In: Phang *et al*., eds. *Algal Biotechnology in the Asia-Pacific Region*. University of Malaya. 92-102.

Blatchley III, Gong WL, Alleman JE, Rose JB, Huffman DE, Otaki M, Lisle JT. Effects of wastewater disinfection on waterborne bacteria and viruses. Water Environment Research. 2007; 79, 81–92.

Bosch A. 2007. Human viruses in water, 1st ed. Elsevier, Amsterdam; Boston.

Bosch, A. Human enteric viruses in the water environment: a minireview. Internatl. Microbiol. 1998; 1: 191-196.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI) 2008. Document M31-A3. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, Approved Standard, Third Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, USA. 19087-1898.

Corona AH, Nieves I, Meckes M, Chamorro M, Barron BL. Antiviral activity of Spirulina maxima against herpes simplex virus type 2. Antiviral Research. 2002; 56: 279-285.

Costafreda MI, Bosch A, Pintó RM. Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis a virus in clinical and shellfish samples. Applied and Environmental Microbiology. 2006; 72: 3846–3855.

Denfert C, Hube B. 2007. Candida: Comparative and Functional Genomics. Caister Academic Press.

El-Senousy WM, Costafreda M, Pinto RM, Bosch A. Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. Int J Food Microbiol. 2013; 167: 74-79.

El-Senousy WM, Guix S, Abid I, Pintó RM, Bosch A. Removal of Astrovirus from Water and Sewage Treatment Plants, Evaluated by a Competitive Reverse Transcription-PCR. Appl. Environ. Microbiol. 2007; 73: 164-167.

Esawy MA, Ahmed EF, Helmy WA, Mansour NM, El-Senousy WM, El-Safty MM. Production of a halophilic levansucrase from novel honey Bacillus subtilis isolates capable of producing antiviral levans. Carbohydrate Polymers. 2011; 86: 823-830.

Fayzunnessa N, Morshed A, Uddin A, Parvin A, Saifur R. In vivo study on the efficacy of hypoglycemic activity of *Spirulina plantesis* in long evan rats . International Journal of Biomolecules and Biomedicine (IJBB). 2011; 1: 27-33.

Fisher B, Harvey RP, Champe P. 2007. Lippincott's Illustrated Reviews: Microbiology (Lippincott's Illustrated Reviews Series). Hagerstown, MD: Lippincott Williams & Wilkins. 332–353.

Ghazy MM, El-Senousy WM, Abdel-Aatty AM, Hegazy B, Kamel M. Performance evaluation of a waste stabilization pond in a rural area in Egypt. American Journal of Environmental Sciences. 2008; 4: 316-326.

Hamdy NA, El-Senousy WM. Synthesis andf antiviral evaluation of some novel pyrazoles and pyrazolo[3,4-d] pyridazines bearing 5,6,7,8-tetrahydronaphthalene. Acta Poloniae Pharmaceutica n Drug Research. 2013; 70: 99-110.

Hamdy NA, El-Senousy WM, Fakhr IMI. Enaminone as Building Blocks in Organic Chemistry: a Novel Route to Polyfunctionally 2- substituted 5, 6, 7, 8-tetrahydronaphthalenes and their Antiviral Evaluation. Journal of Heterocyclic Chemistry. 2013; 50: 337-343.

Puig M, Jofre J, Lucena F, Allard A, Wadell G, Girones, R. Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. Appl. Environ. Microbiol. 1994; 60: 2963-2970.

Ibrahim LF, El-Senousy WM, Hawas UW. NMR Spectral analysis of flavonoids from Chrysanthemum coronarium. Chemistry of Natural Compounds. 2007; 43: 659-662.

Khan M, Ather A, Thompson K, Gambari R. Extracts and molecules from medicinal plants against herpes simplex viruses. Antiviral Res. 2005; 67: 107-19.

Puig M, Jofre J, Lucena F, Allard A, Wadell G, Girones, R.

Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. Appl. Environ. Microbiol. 1994; 60: 2963-2970.

Raoof B, Kaushik BD, Prasanna R. Formulation of a low-cost medium for mass production of *Spirulina*. Biomass and Bioenergy. 2006; 30: 537-542.

Salem M, Sakr S, El-Senousy WM, Madkour H. Synthesis, Antibacterial, and Antiviral Evaluation of New Heterocycles Containing the Pyridine Moiety. Archiv der Pharmazie. 2013; 346: 766-773.

Schmidtke M, Knorre C, Blei L, Stelzner A, Birch-Hirschfeld E. Penetration and Antiviral Activity of Coxsackievirus B3 (Cvb3)-Specific Phosphorothioate Oligodeoxynucleotides (Ps-Odn). Nucleosides and Nucleotides. 1998; 17: 1557-1566.

Simões CMO, Amoros M, Girre L. Mechanism of antiviral activity of triterpenoid saponins, Phytoth Res. 1999; 21: 317-325.

Steven C, Russell J. 1993. Bioactive natural products: detection, isolation and structural determination. CRC Press.

Walker RD. 2007. Antimicrobial susceptibility testing and interpretation of results. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM eds. Antimicrobial Therapy in Veterinary Medicine, Ames, IA, Blackwell Publishing.

Walum E, Strenberg K, Jenssen D. 1990. Understanding Cell Toxicology: Principles and Pratice. Ellis Howood, NewYork. 97-111.

Zarrouk C. 1966. Contribution a l,etude d,une cyanophycee. Influence de divers facteurs physiques et chimiques sular croissance et la photosynthese de Spirulina maxima. Ph.D. Thesis, Universite de Paris, Paris.

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