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# Investigation of the Biological Activity of Some Gymnosperm Plants Belong to Cycadales Order

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**ABSTRACT**

People are known to utilize plants for the treatment of many diseases. Cycadales are one of these ancient plants that contain different active constituents. Due to the lack of adequate research on Cycadales, we conducted this study. The current study investigated and compared different biological activities for four different plants. *Cycas thouarsii*, *Cycas pectinata*, *Dioon spinulosum*, and *Encephalartos laurentians* were tested for the first time for cytotoxic, antioxidant, and antimicrobial activities. *Dioon spinulosum* displayed significant cytotoxic activity on the African green monkey kidney (VERO) cell line that was higher than 5-Fluorouracil. *D. spinulosum* possessed the highest radical scavenging activity followed by *Cycas thouarsii*. *D. spinulosum* showed the highest protective activity against DNA damage which was higher than the positive control. *D. spinulosum* showed the highest antihemolytic antioxidant activity followed by *Encephalartos laurentians*. *D. spinulosum* presented the highest antimicrobial activity among the tested plants. Most Cycadales tested plants especially *D. spinulosum* displayed significant biological activities that may promising for future drug discovery.

**Keywords:** Antimicrobial, Antioxidant, Cytotoxicity, Cycadales, Dioon.

## 1. INTRODUCTION

Medicinal plants establish an indispensable source of traditional and modern medicine. Many people depend on medicinal plant products to maintain their health or cure diseases. Available evidence suggests that medicinal plant consumption will continue to grow in the short to medium term.[1] Gymnosperm is one of the plant kingdom divisions which is naked seed-producing plants. Cycadales order as a one living representative of gymnosperms is classified into 11 genera in 3 families Zamiaceae, Cycadaceae, and Stangeriaceae distributed all over the world.[2, 3] Cycadaceae and Zamiaceae were chosen to be the target of this study as literature enumerate the presence of a number of active constituents.[4-14]

From Cycadaceae, *Cycas thouarsii* and *Cycas pectinata* were selected while *Dioon spinulosum* and *Encephalartos laurentians* were chosen from Zamiaceae. These plants are dark fields in plant biology, so it was important for us to conduct this investigation and discover the benefits of these ambiguous plants. It is worth mentioning that there are few phytochemical and biological investigations for Cycadales.[15] So, the lack of biological research on these plants is what motivates us to undertake the present study. Here we report the biological activity of four plants from Cycadales different species as a first report.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

**Department of Pharmacognosy, Faculty of Pharmacy, Tanta University, Tanta, Egypt, 31527, Tel: (202) 040-3336007; fax: (202) 040-3335466.*
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Leaves of plants were collected from El Abd garden in Giza city from November 2016 to January 2017. It was kindly identified by Dr. Esraa Ammar, plant ecology lecturer, Botany Department, Faculty of Science, Tanta University, and Rabea Sharawy agronomist and palm researcher. Voucher samples were deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Tanta University. Voucher samples are No. PGG-002 (*Dioon spinulosum*), No. PGG-003 (*Encephalartos laurentians*), No. PGG-004 (*Cycas thouarsii*), and No. PGG-005 (*Cycas pectinata*).

### *2.1.2. Extraction*

The shade-dried and powdered leaves of *Cycas thouarsii*, *Cycas pectinata*, *Dioon spinulosum*, and *Encephalartos laurentians* (50 g of each) were extracted separately with 500 ml MeOH for 72 hours then evaporated to dryness under reduced pressure to give crude extracts that were used for biological investigations. The yield of crude extract was *Cycas thouarsii* (4.9 g), *Cycas pectinata* (4.6 g), *Dioon spinulosum* (5.2 g), and *Encephalartos laurentians* (4.9 g).

## 2.2. Materials for Biological Activity

### *2.2.1. Chemicals*

Fetal bovine serum and RPMI-1640 medium were obtained from GIBCO, UK. Dimethyl sulfoxide (DMSO), 3-(4,5 dimethylthiazole-2-yl)-2,5diphenyltetrazoliumbromide (MTT), sodium dodecyl-sulfonate and 5-fluorouracil were obtained from Sigma Co., St. Louis, MO, USA. Antioxidant activity was performed using different reagents: 2,2`-azino-bis-(3-ethylbenzothiazoline-6-sulfonicacid) ABTS, phosphate buffer solution (pH=7), DNA (Calf thymus type1), bleomycin sulfate, thiobarbituric acid (TBA), $MnO_2$, ethylenediaminetetraacetic acid (EDTA), L-ascorbic acid, 2,2`-azo-bis-(2-amidinopropane) dihydrochloride (AAPH), HCl and $FeCl_3$. All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

### *2.2.2. Cell lines*

Breast carcinoma cell line (MCF-7) and liver carcinoma cell line (HepG2), African green monkey kidney (VERO) cancer cell live and colorectal carcinoma cell line (HCT-116) were obtained from the American Type Culture Collection (ATTC) via holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

### *2.2.3. Investigated microorganisms*

For the antimicrobial study, all tested organisms were obtained from (ATCC) including the bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 19659), Escherichia coli (ATCC 10536) and *Pseudomonas aeuroginosa* (ATCC 27853) as well as the fungi *Candida albicans* (ATCC 90028) and *Aspergillus flavus* (ATCC 46283).

## 2.3. Cytotoxic Assay

Potential cytotoxicity of the different plant extracts was investigated using MTT assay according to the method reported by Skehan et al. and the obtained data classified according to Ayyad et al.[16, 17] MCF-7, HepG2, VERO, and HCT-116 cell lines were used. 5-Fluorouracil was used as a positive control. ELISA processor II microplate reader was used for cytotoxic activity assessment.

## 2.4. Antioxidant Assay

Antioxidant activity was investigated using three different methods; ABTS [2,2`-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)], bleomycin-dependent DNA damage, and erythrocyte hemolysis method.

### *2.4.1 ABTS method*

Antioxidant activity determination was evaluated according to the method reported by Lissi et al.[18] The radical cation derived from ABTS [2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] was obtained from the reaction of ABTS (60 mL) with $MnO_2$ (3 mL, 25 mg/mL) in phosphate buffer (pH 7, 0.1 M). The % inhibition for each extract is calculated from the following equation: % inhibition= [Ac-Aa/Ac]×100 Where Ac is the absorbance of ABTS radical + MeOH/phosphate buffer (1:1); Aa is the absorbance of ABTS radical plus sample or standard. The ascorbic acid solution was used as a standard antioxidant (positive control). The solvent blank sample was run using MeOH/phosphate buffer (1:1) without ABTS.[18] All measurements were performed using total methanol extract for all tested plants. The absorbance of the antioxidant assay was measured using a UNICO spectrophotometer (UV/Vis) UV-2000 (UNICO Instrument Co. LTD, USA).

### *2.4.2 Bleomycin-dependent DNA damage*

The procedures were carried out according to the method reported by Aeschlach et al.[19]The extent of DNA damage was measured by increase in absorbance at 532 nm All measurements were performed using total methanol extracts for the tested plants.

### *2.4.3 Erythrocyte hemolysis method*

The procedures were carried out according to the method reported by Malagoli et al.[20] Hemolytic levels were expressed by percentage of hemolysis, calculated with the ratio between the value measured for each sample and that registered for the total hemolysis. The data were expressed as mean±standard deviation. Ascorbic acid was used as a positive control.

## 2.5. Antimicrobial Assay

All the plant extracts were individually evaluated for in vitro antibacterial activity against a panel of two Gram-negative bacteria (*Pseudomonas aeuroginosa* and *Escherichia coli*)

and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) using Muller Hinton agar medium (Oxoid). The antifungal activity was tested against two fungi (*Aspergillus flavus* and *Candida albicans*) by using Sabouraud dextrose agar medium (Oxoid) and conventional broth dilution method.[21] Clotrimazole and Ampicillin were used as reference drugs. The results of each tested total extracts were recorded as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm.

## 2.6. Statistical Analysis

This analysis was carried out by the Graph Pad Prism 5 Program. All measurements were performed in triplicate(n=3), shown as mean±standard deviation (SD) and significant difference at P≤ 0.05 by Student's test.

## 3. RESULTS AND DISCUSSION

Cytotoxic activity of methanol extracts of examined plants was carried out using MTT assay. Breast cancer cell (MCF7), liver cancer (HepG2), African green monkey kidney (VERO) cancer cell line, and colorectal carcinoma (HCT-116) cell lines are used. According to Ayyad et al.[17], *D. spinulosum* showed very strong activity against MCF7, HCT-116, VERO and HepG2 cell lines with IC50 of 9.55±0.8, 7.53±0.9, 8.35±0.7 and 11.27±1.0 µg/mL, respectively. *D. spinulosum* cytotoxic activity (IC50=8.35±0.7) exceeded the positive control (IC50=9.01±0.9) against the VERO cell line. *Cycas thouarsii* exhibited strong activity against MCF7, HCT-116, VERO and HepG2 cell lines with IC50 of 15.74±1.3, 14.6±1.4, 10.8±1.1 and 17.88±1.5 µg/mL, respectively, whereas *Encephalartos laurentians* showed strong activity against MCF7, HCT-116 and HepG2 cell lines with IC50 of 20.34±1.8, 12.54±1.3 and 13.38±1.2 µg/mL, respectively, and moderate activity (IC50=25.85±1.9) against VERO cell line. *Cycas pectinata* showed moderate activity against MCF7, HepG2 and VERO cell lines with IC50 of 37.39±2.2, 42.54±2.3 and 30.45±2.5 µg/mL, respectively and weak activity (IC50=53.31±3.1) against HCT-116 cell line (Table 1 and Figure 1).

**Table1:** The $IC_{50}$ of total methanol extracts of different plants.

| Sample | In vitro Cytotoxicity $IC_{50}$ (µg/mL) | | | |
|---|---|---|---|---|
| | HePG2 | HCT-116 | VERO | MCF-7 |
| **5-FU** | 6.29±0.5 | 7.19±0.8 | 9.01±0.9 | 4.97±0.3 |
| ***E. laurantianous*** | 13.38±1.2 | 12.54±1.3 | 25.85±1.9 | 20.34±1.8 |
| ***C. thoursaii*** | 17.88±1.5 | 14.60±1.4 | 10.80±1.1 | 15.74±1.3 |
| ***C. pectinata*** | 42.54±2.3 | 53.31±3.1 | 30.45±2.5 | 37.39±2.2 |
| ***D. spinulosum*** | 11.27±1.0 | 7.53±0.9 | 8.35±0.7 | 9.55±0.8 |

$IC_{50}$ (µg/mL): 1-10 (very strong), 11-25 (strong), 26-50 (moderate), 51-100 (weak) and above 100 (non-cytotoxic) according to Ayyad et al. classification.[17] Significant difference was carried out by Student's test at P≤ 0.05.

The results are in accordance with those reported by Rejon et al. concerning the cytotoxic activity of petioles of *D. spinulosum* on Hep2 (laryngeal carcinoma).[22]

ABTS method was carried out to evaluate the antioxidant activity of different plant extracts: This method reported by Lissi et al.[18] *D. spinulosum* presented the highest antioxidant activity (84.2% inhibition) followed by *Cycas thouarsii*, *Encephalartos laurentians* and *Cycas pectinata* (83.2%, 82.6%, and 81.8%, respectively) (Figure 2).

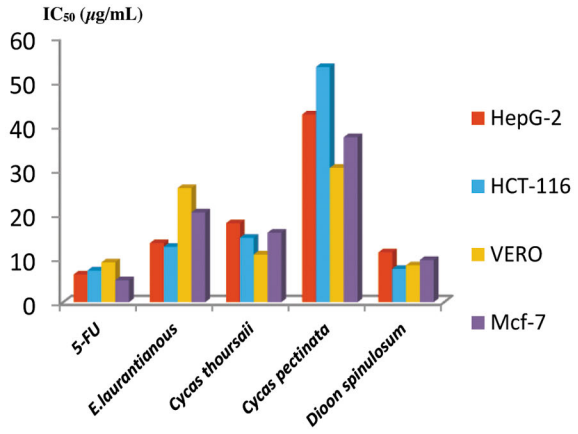


**Figure 1:** The $IC_{50}$ of total methanol extracts of the different plants.

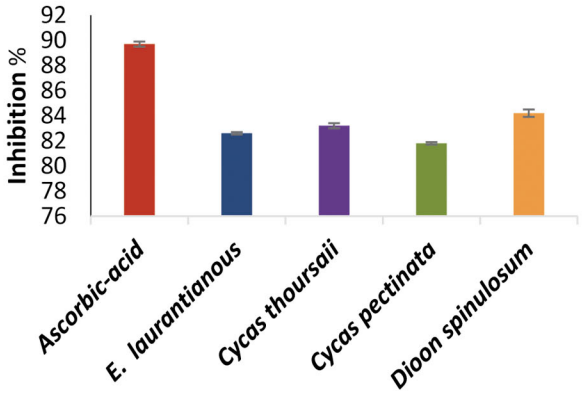


**Figure 2:** Antioxidant activity of total methanol extracts of the different plants using ABTS method.

The DNA protective activity was carried out according to the procedures reported by Aeschlach et al.[19], and the results are displayed in Figure 3. The absorbance of *D. spinulosum* was 0.058 for a sample concentration of 0.1 mg/ml. It showed the highest protective activity against DNA damage which exceeded the positive control (absorbance = 0.063). The activity of *Encephalartos laurentians* (absorbance = 0.064) was comparable to that obtained by ascorbic acid (positive control).

The Erythrocyte hemolysis method was carried out according to the method reported by Malagoli et al.[20], and the results are shown in Figure 4. *D. spinulosum* presented the highest anti-hemolytic activity (5.1% hemolysis) followed by

*Encephalartos laurentians* (7.5%hemolysis), which compared to that of ascorbic acid (4.2% hemolysis).

The antimicrobial activity of different tested extracts was evaluated using the conventional broth dilution method. *D. spinulosum* presented the highest antibacterial activity followed by *Cycas thouarsii*; *D. spinulosum* showed high activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) than Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*). *D. spinulosum* presented the highest antifungal activity followed by *Encephalartos laurentians*, the results are presented in Table 2.

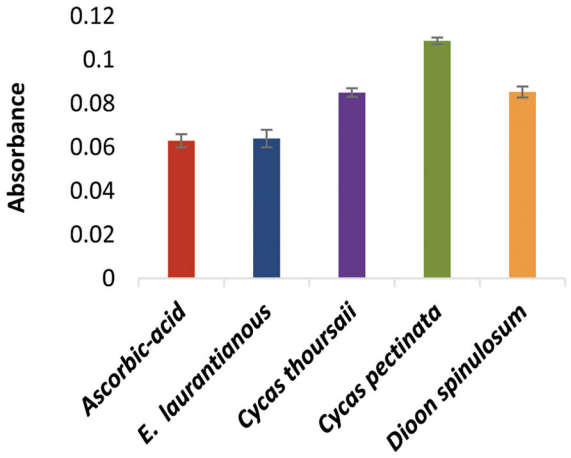


**Figure 3:** Antioxidant activity of total methanol extracts of the different plants using bleomycin dependent-DNA damage method.

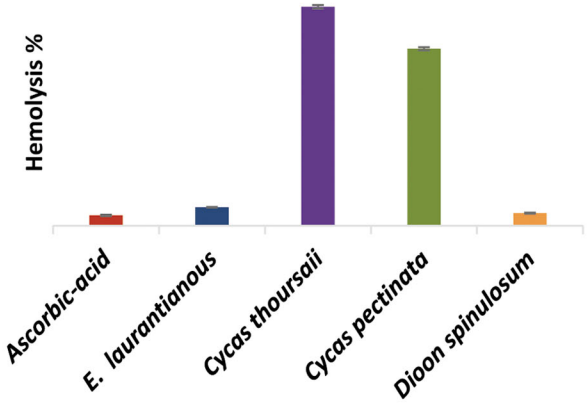


**Figure 4:** Antioxidant activity of total methanol extracts of the different plants using erythrocyte hemolysis method.

## 4. CONCLUSIONS

The investigated plants in the present study showed potent cytotoxic activity. They displayed different antimicrobial activities. Also, the tested plants presented high protective activities against DNA damage which were higher than the positive control in the case of *D. spinulosum* leaves. A promising future awaits these plants especially *D.spinulosum* in drug discovery.

**Table2:** Antimicrobial activity of total methanol extracts of the different plants.

| Extracts | *E. coli* | | *P. aeuroginosa* | | *S. aureus* | | *B. subtilis* | | *C. albicans* | | *A. flavus* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diameter of IZ * | % Activity index | Diameter of IZ * | % Activity index | Diameter of IZ* | % Activity index | Diameter of IZ * | % Activity index | Diameter of IZ * | % Activity index | Diameter of IZ * | % Activity index |
| ***E. laurantianous*** | 9 | 39.1 | 13 | 54.2 | 10 | 45.4 | 18 | 75.0 | 17 | 73.9 | 21 | 84.0 |
| ***C. thoursaii*** | 12 | 52.2 | 17 | 70.8 | 18 | 81.8 | 19 | 79.2 | 14 | 60.9 | 13 | 52.0 |
| ***C. pectinata*** | 6 | 26.1 | 12 | 50.0 | 10 | 45.4 | 9 | 37.5 | 8 | 34.8 | 10 | 40.0 |
| ***D. spinulosum*** | 14 | 60.9 | 20 | 83.3 | 19 | 86.4 | 21 | 87.5 | 21 | 91.3 | 24 | 96.0 |
| **Ampicillin** | 23 | 100 | 24 | 100 | 22 | 100 | 24 | 100 | 0 | ---- | 0 | ---- |
| **Clotrimazole** | 0 | ---- | 0 | ---- | 0 | ---- | 0 | ---- | 23 | 100 | 25 | 100 |

*Diameter of inhibition zone (mm)

 

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGMENT

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