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Pharmacognostical and biological investigation of *Dioon holmgrenii* De Luca, Sabato & Vázq.Torres, *Dioon mejiae* Standl. & L.O.Williams, and *Dioon meorlae* De Luca, Sabato & Vázq.Torres cultivated in Egypt

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ABSTRACT

Dioon is a genus in the family Zamiaceae. Species in this genus are dioecious and palm like shrubs. Many of them are chemically and biologically unexplored. The macroscopical and microscopical characters of three *Dioon* species that are cultivated in Egypt, *D. holmgrenii* De Luca, Sabato & Vázq.Torres, *D. mejiae* Standl. & L.O. Williams, and *D. meorlae* De Luca, Sabato & Vázq. Torres were investigated. Their methanol extracts were biologically examined via cytotoxic activity against seven cell lines using MTT assay method, and antioxidant effects using bleomycin dependent DNA damage and ABTS antioxidant assay methods. The antimicrobial against Gram-positive, Gram-negative bacteria and fungi were also tested. Phenolic and flavonoid contents were analysed by Folin-Denis and aluminium chloride assays, respectively using gallic acid and rutin as standards. Results from this study revealed that there are some structural differences between the three species. *D. holmgrenii* and *D. mejiae* displayed high cytotoxic activity against number of tested cell lines, while *D. meorlae* showed less cytotoxicity. *D. holmgrenii* exhibited the highest antioxidant activity followed by *D. mejiae* then *D. meorlae*. Regarding the antimicrobial activity, *D. holmgrenii* was the most active species, particularly against Aspergillus flavus and Bacillus Subtilis followed by *D. mejiae*. Methanol extract of *D. holmgrenii* had the highest phenolic and flavonoid contents then D. mejia, while *D. meorlae* possessed the lowest contents. The two Dioon species, *D. holmgrenii* and *D. mejiae* displayed high biological activity.

Keywords: Antimicrobial, Antioxidant, Cytotoxic, *Dioon*, Macroscopic, Microscopic.

1. INTRODUCTION

The cycad genus *Dioon* (Zamiaceae), is a tropical group that includes species occurring from humid forests to arid zones.[1] The genus *Dioon* consists of 15 species.[2] Like many gymnosperms, they are grown as ornamental plants,[3] and are used as food.[4] There are some reports about the chemical constituents of *Dioon* species, indicating that they are rich in biflavones.[5] Biflavones were found to have anticancer [6] and antioxidant [7] activities.

The members of genus *Dioon* are characterized by aerial trunk, clothed with persistent frond bases. The leaf is compound, paripinnate, and exstipulate. Leaflets are numerous, simply pinnate, and longitudinal ptyxis erect.[8] There is little work on anatomical features of leaflets of the selected species and these features are important in identification of plants. The biological activities of the extracts of these *Dioon* species are poorly investigated; this motivated the authors to perform this study.

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2. MATERIALS AND METHODS

2.1. Plant materials

The leaflets of *Dioon holmgrenii* De Luca, Sabato & Vázq. Torres, *D. mejiae* Standl. & L.O.Williams, and *D. meorlae* De Luca, Sabato & Vázq. Torres (Zamiaceae) were collected from Cairo-Alexandria Desert Road, Rabea garden 2017. They were identified by Rabea Sharawy, agronomist and palm researcher and Dr. Esraa Ammar, plant ecology lecturer, Botany Department, Faculty of Science, Tanta University. Voucher specimens of leaflets (No. PGG-006, PGG-007 & PGG-008 for *D. mejiae* , *D. meorlae* & *D. holmgrenii,* respectively) were deposited in Pharmacognosy Department, Faculty of Pharmacy, Tanta University.

2.2. Extraction

The shade-dried powdered leaflets (100 g) of each *Dioon* were extracted by cold maceration with methanol until exhaustion. The solvent was evaporated under reduced pressure at 40°C to yield crude methanolic extracts. The yield was 3.6%, 4.3%, 3.8% for *D. holmgrenii*, *D. mejiae* and *D. meorlae* respectively.

2.3. Chemicals

For anatomical study, Safranin O, ethanol, aniline blue and xylene. For cytotoxic activity: Dimethyl sulfoxide (DMSO), 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), RPMI-1640 medium, penicillin, streptomycin and 5-fluorouracil were used. For antioxidant activity, 2,2`-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ABTS, DNA (Calf Thymus type1), bleomycin, $FeCl_3$, magnesium chloride, KH_2PO_4- KOH buffer (pH 7.0), L-ascorbic acid, methanol, ethylenediaminetetraacetic acid (EDTA), thiobarbituric acid (TBA), hydrochloric acid (HCl), butan-l-ol and MnO_2 were used. For quantitative assay, Folin-Denis (FD) reagent, gallic acid, 20% sodium carbonate solution, aluminium chloride, rutin and 5% aqueous solution of copper acetate were employed. All chemicals were purchased from Sigma-Aldrich Co.

2.4. Cell lines

Human epithelial colorectal adenocarcinoma (Caco-2), human cervical cancer (Hela), human prostate cancer (PC3), human liver cancer (HePG2), human colon cancer (HCT-116), human breast cancer (MCF-7) and monkey kidney epithelial cancer cells (VERO) were obtained from the American Type Culture Collection (ATCC) via Holding Company for biological products and vaccines (VACSERA), Cairo, Egypt.

2.5. Investigated microorganisms and media

Two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), Muller Hinton agar medium (Oxoid), two fungi (*Candida albicans* and *Aspergillus flavus*) and Sabouraud dextrose agar medium (Oxoid) were obtained from ATCC.

2.6. Method used for anatomical study

By modification from Jensen method,[9] staining of paraffin sections obtained from rotary microtome was done as follows: Dewax in xylene (2×5 min), rehydrate to 50% ethanol (100%, 96%, 70%, 50%, 5 min. each), cover sections with safranin O staining solution, 30 min. to 1 hour, wash with distilled water, drain sections, then cover with aniline blue staining solution, stain for 3 min, wash with distilled water, dehydrate in ethanol series, pass quickly through 30%, 70%, 96%, 100% ethanol (maximum 3 min. for all four steps), then xylene (2×5 min), finally mount coverslip with a permanent mounting medium.

2.7. Cytotoxic Assay

The methanol extracts of *D. holmgrenii, D. mejiae* and *D. meorlae* were screened for their cytotoxic effects on: Caco-2, Hela, PC3, HePG2, HCT-116, MCF-7 and VERO. The cytotoxic activity was studied according to the procedures reported by Mosmann.[10] The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100µg/mL streptomycin at 37°C in a 5% CO_2 incubator. The cells were seeded in a 96-well plate at a density of 1.0×10^4 cells/well at 37°C for 48 h under 5% CO_2. The extracts were dissolved in dimethyl sulfoxide (DMSO), serial dilutions using RPMI-1640 medium were prepared to give final concentrations of 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 and 200 µg/mL After incubation the cells were treated with the prepared concentrations and incubated for 24 h. After 24 h of treatment, 20 µL of MTT solution at 5mg/mL was added and incubated for 4 h. DMSO in volume of 100 µL was added into each well to dissolve the purple formazan formed. Then the absorbance was measured at 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A of treated samples/A of untreated sample) ×100.

2.8. Antioxidant evaluation

Antioxidant activity was determined using two different methods:

2.8.1. Bleomycin dependent DNA damage

The procedure was carried out according to the method reported by Aeschlach et al.[11] The reaction mixtures contained in a final volume of 1 mL. Each mixture composed of the following reagents at the final concentrations stated: DNA (0.2 mg/mL), bleomycin (0.05 mg/mL), $FeCl_3$ (0.025 mM), magnesium chloride (5 mM), KH_2PO_4- KOH buffer

(pH 7.0, 30 mM) and L-ascorbic acid (0.24 mM), which was used as a positive control. The tested extracts were dissolved in MeOH to give a concentration of 0.1 mg/mL. The reaction mixtures were incubated in a water bath at 37°C for 1 h. At the end of the incubation period, 0.1 mL of 0.1 M ethylenediaminetetraacetic acid (EDTA) was added to stop the reaction (the iron-EDTA complex is unreactive in the bleomycin assay). DNA damage was assessed by adding 1 mL 1% (w/v) thiobarbituric acid (TBA) and 1 mL 25% (v/v) HCl followed by heating in a water bath maintained at 80°C for 15 min. The chromogen formed was extracted into butan-l-ol and the absorbance was measured at 532 nm. The extent of DNA damage was assessed by the increase in absorbance at 532 nm.

2.8.2. ABTS antioxidant assay

The method reported by Lissi et al.[12] was adopted for the determination of ABTS activity of different total methanolic extracts. Briefly, the method was performed as follows: For each of the investigated sample, 2 mL of ABTS solution (60 μM) was added to 3 mL MnO_2 solution (25 mg/mL), all prepared in 5 mL aqueous phosphate buffer solution (pH 7; 0.1 M). The mixture was shaken, centrifuged, filtered and adjusted to approximately 0.5 mL, the absorbance of the resulting green-blue solution (ABTS radical solution) at λ_{max} 734 nm. was measured. Then, 50 μL of solution of the tested compound dissolved in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in colour intensity was expressed as % inhibition. L-ascorbic acid was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1). Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only. Each sample was measured in triplicate and averaged.

The activity was calculated using the following equation:

% inhibition = [Ac – As / Ac] × 100

Where Ac is the absorbance value of the negative control and As is the absorbance value of the ABTS + sample or standard. Values are means of 3 replicates ± standard deviation (SD) and significant difference at $P < 0.05$ by Student's test.

2.9. Antimicrobial activity

The methanolic extracts of D. mejiae, D. holmgrenii, and D. meorlae were individually tested against a panel of two Gram-positive bacteria (S. aureus and B. subtilis) and two Gram-negative bacteria (E. coli and P.aeruginosa) using Muller Hinton agar medium (Oxoid). The antifungal activities of the compounds were tested against two fungi (*C. albicans* and *A. flavus*) using Sabouraud dextrose agar medium (Oxoid).[13] Each of the extracts was dissolved in DMSO and solution of the concentration 1 mg /mL was prepared separately. Paper discs of Whatman filter paper were prepared with standard size (5mm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the extract solution were placed aseptically in the petri dishes containing Muller Hinton agar medium (Oxoid) seeded with tested bacteria or fungi using Sabouraud dextrose agar medium (Oxoid). The petri dishes were incubated at 36°C and the inhibition zones were recorded after 24 h of incubation. The antibacterial activity of a common standard antibiotic ampicillin and antifungal clotrimazole was recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the following formula:

% Activity Index = A/B × 100

A: Zone of inhibition by test (diameter by mm)

B: Zone of inhibition by standard (diameter by mm)

2.10. Quantitative determination of phenolic content in *Dioon* species

The total phenolic content in the three *Dioon* species was performed according to the procedure mentioned by Mechikova et al.[14], the method was based on measuring the intensity of the color developed when phenolic compounds react with Folin-Denis (FD) reagent. The percentage was calculated as Gallic Acid Equivalent (GAE), from the pre-established standard calibration curve of gallic acid.

2.10.1. Preparation of calibration curve of gallic acid

I. Different aliquots equivalent to 1-10 μg of ethanolic solution of gallic acid (0.005%), were separately introduced into 50 mL measuring flasks.

II. Then each was treated with 20.0 mL of Folin-Denis (FD) reagent and 10.0 mL of 20% sodium carbonate solution. The mixture was shaken for 3–5 min, the volume was completed to the mark with ethanol, and the content was thoroughly stirred, then kept for 30 min at 80°C on a water bath.

III. The absorbance was measured at 765 nm in a 10 mm thick cell, three determinations for each concentration of standard solution were carried out, and the average calculated absorbance was plotted versus concentrations (**Figure 5**).

2.10.2. Determination of phenolic content in the plant samples

An aliquot of each extract (0.1 mL) was transferred separately to 50 mL measuring flask, containing 20.0 mL of Folin-Denis (FD) reagent and 10.0 mL of 20% sodium carbonate solution. The mixture was shaken for 3–5 min, the volume was completed to the mark with ethanol, and the content was thoroughly stirred, then kept for 30 min at 80°C

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on a water bath. Then, the absorbance was measured at 765 nm in a 10 mm thick cell, three determinations for each extract were carried out, and the average absorbance was calculated. The results were calculated from Figure (5) and recorded in Table (6) as micrograms of gallic acid equivalent per milligram of dry extract (µg GAE/mg extract).

2.11. Quantitative determination of flavonoid content of *Dioon* species

The procedure mentioned by Karawya and Aboutabl[15] was adopted. The method was based on measuring the intensity of the color developed, when flavonoids were complexed with Aluminium chloride and the percentage was calculated as rutin equivalent (RE), from the pre-established standard calibration curve of rutin.

2.11.1. Preparation of calibration curve of rutin

Different aliquots equivalent to 1-10 µg of ethanolic solution of rutin (0.004%), were separately introduced into test tubes, evaporated to dryness on hot water bath. Then, the obtained residue from each, was treated with five mL of 0.1 M aluminium chloride reagent, the intensity of the developed yellow color was measured at λmax 420 nm immediately against blank (ethanol) using a UV spectrophotometer. Three determinations for each concentration of standard solution were carried out and the average calculated absorbance was plotted versus concentrations **(Figure 6)**.

2.11.2. Determination of flavonoid content of the plant samples

Known volume (0.5 mL) of each ethanolic extracts was transferred to separate test tubes and evaporated to dryness. To each residue, five mL of 0.1 M aluminium chloride solution were added, and the procedure continued as under the calibration curve. The concentration was calculated from standard calibration curve of rutin and the results were calculated from Figure (6) and recorded in Table (6) as micrograms of rutin equivalent per milligram of dry extract (µg RE/mg extract).

3. RESULTS AND DISCUSSION

3.1.Macroscopical identification of the leaves

The three species possess exstipulate paripinnate compound leaves with numerous leaflets, longitudinal ptyxis erect **(Figure 1, Table 1)**. The leaflets lacking a midrib but with many parallel longitudinal veins, become smaller towards the end. Leaflets are lanceolate, with acute apex, symmetric base, smooth surface and entire margin. Leaflets are dark green in color in the upper surface, with more prominent veins in the lower side with light green color. The venation is usually parallel. The texture is coriaceous. The leaves have no characteristic odor or taste

Figure 1: Macroscopic characteristics of the entire *Dioon* leaves. A: *D. mejiae*, B: *D. holmgrenii* and C: *D. meorlae* whole leaves, D, E & F : show the corresponding leaflets of each Dioon.

Table 1: Macroscopical measurements

Organ	*D. mejiae*	*D. holmgrenii*	*D. meorlae*
Leaves	120-150 cm long, 22-24 cm width in the middle	105-115 cm long, 17-20 cm width in the middle	100-125 cm long, 18-24 cm width in the middle
Petioles	0.5-1 cm long	6-9 cm long	7-23 cm long
Leaflets in the middle	13-15 cm long, and 1-1.2 cm in width	9-10 cm long, and 0.5 cm in width	10-13 cm long, and 0.5-0.7 cm in width

D. mejiae has the longest leaf and relatively largest leaflets, while *D. meorlae* has the longest petiole. *D. holmgrenii* relatively has the smallest leaf and medium petiole.

3.2. Microscopical investigation

3.2.1. T.S. of leaflets

A transverse section in the leaflet lamina shows veins that are slightly more prominent on the lower surface, especially in case of *D. meorlae*. The leaflets have

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dorsiventral structures with one row of palisade and an interrupted second row. The hypodermis is schlerenchymatus. In the vein region, the vascular bundle is collateral **(Figure 2, Table 2)**.

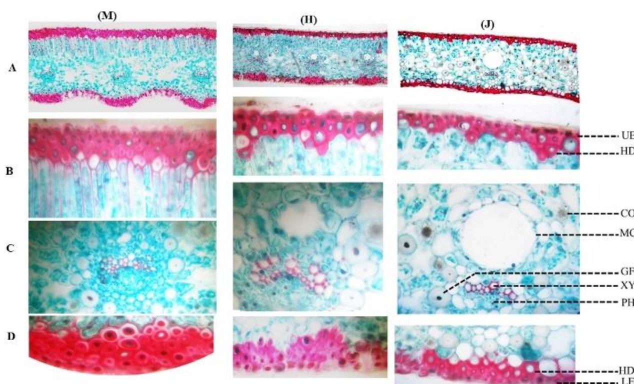

Figure 2: Transverse sections of the three species. (M), (H) and (J) represent sections of *D. meorlae*, *D. holmgrenii* and *D. mejiae* respectively, A: T.S of the whole leaflet (X 50), B: upper, C: middle and D: lower parts (X 100). UE: Upper epidermis, HD: Hypodermis, CO: Calcium oxalate cluster, MC: Mucilage canal, GF: G fiber, XY: Xylem, PH: Phloem, LE: Lower epidermis.

3.2.2. The upper epidermis

The upper epidermis of the three species consists of one row of schlerenchymatus lignified sub-rectangular cells, which appear irregularly elongated and differ in shape from one species to another in surface view, covered with thick smooth cuticle. Stomata and hairs are absent.

3.2.3. The hypodermis

Hypodermis is situated directly below the upper epidermis in the lamina area and vein region and above the lower epidermis. It consists of one row of lignified sclerenchymatous fibres and a discontinuous second layer in case of *D. mejiae*, from one to three rows in case of *D. holmgrenii*, and from two to four continuous rows in case of *D. meorlae*. The discontinuous rows are interrupted with parenchyma some of them contain calcium oxalate (CaOx) clusters.

3.2.4. The lower epidermis

The lower epidermis is nearly similar to the upper epidermis in some characters but contains sunken stomata, and consists of one row of lignified sclerenchymatous cells.

3.2.5. The vein region

In addition to all the tissues presented above, there is a mucilage duct, which is more obvious in case of *D. mejiae* than in *D. holmgrenii* than in *D. meorlae*. The endodermis surrounds the stele; it consists of one single layer of sub-rectangular tangentially elongated cells. The vascular tissue is composed of a collateral vascular bundle, formed of xylem, and phloem. Xylem consists of lignified tracheids, fibres, and non lignified wood parenchyma. The cambium is hardly distinguishable. The phloem tissue is situated under xylem. The pericycle is formed of collenchymatus tissue in the form of small arc below the phloem. At the lower part of the cortical tissue, the endodermis is followed by 2-3 rows of parenchymatous cells, which contain cluster crystals of CaOx. It can be noticed that *D. meorlae* has the most prominent palisade layer. *D. mejiae* has the biggest mucilage canal. G fibers, CaOx, are present in all species with some variation in their sizes **(Table 2)**.

Table 2: Microscopical measurements (in µm)

Layer from T.S.	*D. mejiae*	*D. holmgrenii*	*D. meorlae*
Upper epidermis	15.06 ± 1.36	23.91 ± 3.47	20.64 ± 3.30
Hypoepidermis layer 1	21.17 ± 2.85	19.79 ± 2.26	20.45 ± 1.99
Hypoepidermis layer 2	-	24.12 ± 4.38	23.27 ± 2.24
Mesophyll palaside length	65.41 ± 12.72	92.08 ± 23.43	135.89 ± 19.21
Mesophyll palaside width	29.21 ± 8.03	31.12± 2.01	16.19 ± 4.35
Mesophyll parenchyma diameter	41.91 ± 7.45	38.1 ± 6.69	39.37 ± 10.71
Mucilage cavity diameter	123.83 ± 13.80	68.58 ± 8.36	61.6 ± 10.39
G fibres diameter	38.74 ± 7.60	35.88 ± 5.20	34.61 ± 4.35
CaOx	35.56 ± 6.13	36.83 ± 4.02	34.29 ± 3.28
Endodermis	29.21 ± 4.44	21.59 ± 6.66	25.82 ± 6.81
Xylem trachedis diameter	6.35 ± 1.01	4.45 ± 1.00	5.72 ± 0.95
lower epidermis	19.46 ± 3.35	21.99 ± 1.79	24.75 ± 5.00
Hypoepidermis layer 1	23.16 ± 5.06	19.89 ± 3.82	21.15 ± 4.32
Hypoepidermis layer 2	-	19.69 ± 3.15	21.47 ± 2.64
From surface preparation			
Lower epidermis stomata	88.27 ± 10.13	89.54 ± 7.60	81.28 ± 13.32
Upper epidermis	128.91 ± 53.38	125.73 ± 25.36	118.11 ± 33.63
	15.88 ± 3.35	19.69 ± 4.69	17.15 ± 3.07
Lower epidermis	154.94 ± 22.64	118.75 ± 34.79	121.92 ± 25.54
	16.51 ± 3.28	17.15 ± 3.07	17.15 ± 3.07

Values are expressed as mean ± SD (n=5).

3.2.6. Surface preparation

The upper epidermal (adaxial) cells are arranged in rows that are parallel to the long axis of the leaflet in the three species. They consist of long thick walled irregularly rectangular; some are bone to boot shaped cells.[2] The general arrangement differs from one species to another **(Figure 3)**. Upper epidermis in all species is devoid of stomata. The lower (abaxial) epidermis contains sunken stomata. It has stomatal bands and non stomatal interband regions. The width of these bands differs from one species to another. The interband cells are nearly similar to upper epidermis in *D. mejiae*, the same shape but smaller in *D. meorlae*, while in case of *D. holmgrenii,* it is much different. The cells are irregular rectangular, square and wedge to boot shaped.

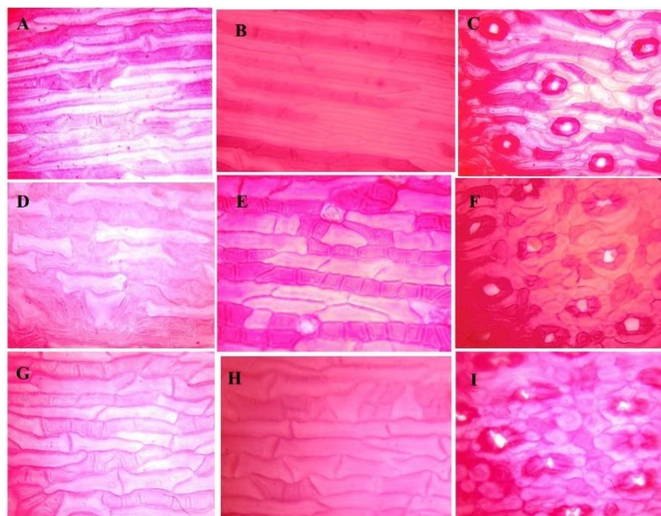

Figure 3: Surface preparations of *Dioon* species. A: Upper, B & C: lower epidermis of *D. mejiae*. D: Upper, E & F: lower epidermis of *D. holmgrenii*. G: Upper, H & I: lower epidermis of *D. meorlae* (X 200).

3.2.7. Powder

The powder of the three species is green in color with no characteristic odor or taste. The differentiation between the powder of the three species can be achieved by the differences in the shape of upper and lower epidermis as shown in surface preparation **(Figure 3)** and in measurements **(Table 2)**. Other elements are the same, which are G-fibers, lignified xylem fibers, trachieds and cluster crystal of CaOx. Figure (4) represents the powder of *D. mejiae* as an example to show these elements.

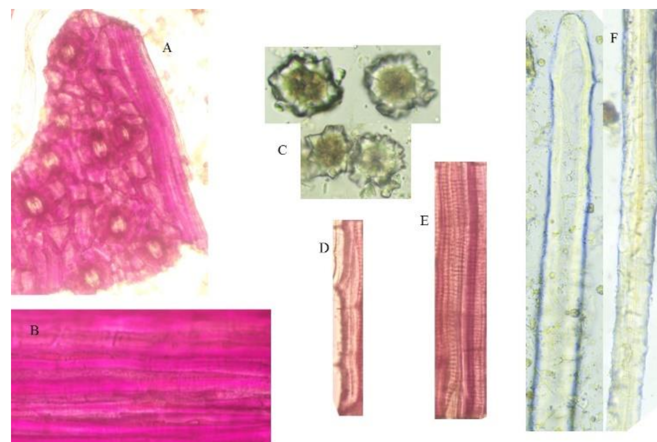

Figure 4: Microscopic characterization of *D. mejiae*.A: Lower epidermis showing stomata, B: Upper epidermis devoid of stomata (X200), C: Calcium oxalate clusters, D: Xylem fibers, E: Xylem tracheids (X400) F: G fibers (X200).

3.3. BIOLOGICAL ACTIVITIES

3.3.1. Cytotoxic activity

All the IC_{50} values of the three species methanol extracts were compared to the IC_{50} values of the positive control; 5-fluorouracil. The three plants demonstrated various cytotoxic effects against the studied cancer cell lines **(Table 3)**. According to Ayyad et al.[16] classification, D. mejiae showed very strong cytotoxicity against Caco-2 and strong cytotoxicity against Hela, VERO and MCF-7 cell lines and moderate cytotoxicity against PC3, HePG2 and HCT-116 cell lines. *D. holmgrenii* represented very strong cytotoxicity against MCF-7 cell lines, strong cytotoxicity against Caco-2 and HePG2 cell lines and moderate cytotoxicity against the rest cell lines. *D. meorlae* displayed the least cytotoxic activity comparing to the other two species.

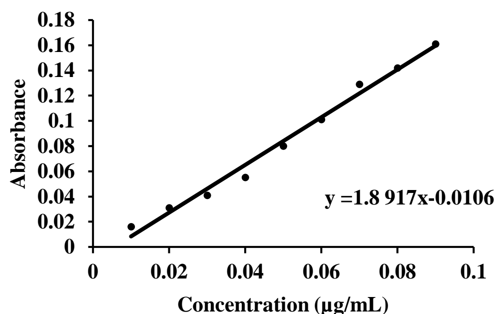

Figure 5: Standard calibration curve of gallic acid.

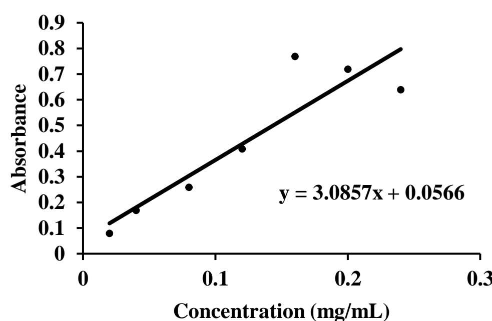

Figure 6: Standard calibration curve of rutin

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Table 3: Cytotoxic activity

Cell lines	*In vitro* Cytotoxicity IC_{50} (µg/ml)*						
Plant	**Caco-2**	**Hela**	**PC3**	**HePG2**	**HCT-116**	**VERO**	**MCF-7**
5-fluorouracil	7.10±0.8	4.81±0.5	8.27±0.7	6.29±0.5	7.19±0.8	9.01±0.9	4.97±0.3
D. mejiae	9.31±1.0	14.06±1.6	24.51±2.1	33.54±1.9	23.50±1.7	16.15±1.4	17.84±1.6
D. holmgrenii	10.49±1.3	25.20±1.6	28.06±1.8	12.18±1.2	33.70±2.1	38.91±2.4	7.13±0.9
D. meorlae	12.41±1.2	30.07±2.3	38.78±2.8	83.68±4.6	66.11±3.2	90.27±4.8	63.52±3.9

*IC50 (µg/mL): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic) according to Ayyad et al, 2012 classification. Values are expressed as mean ± SD (n=3)

3.3.2. Antioxidant capacity

In the bleomycin dependent DNA damage method, as the extent of DNA damage increases, the absorbance at 532 nm increases. The higher antioxidant produces the lower absorbance, so *D. holmgrenii* showed the highest antioxidant activity **(Table 4)** followed by *D. mejiae* then *D. meorlae.*

Table 4: Antioxidant activity

Compound / Extract	Bleomycin dependent-DNA damage	ABTS method
	Absorbance of samples	**% Inhibition**
Ascorbic acid	0.075 ± 0.002	89.30 ± 3.5
D. mejiae	0.097 ± 0.004	83.00 ± 4.1
D. holmgrenii	0.092 ± 0.003	86.60 ± 5.2
D. meorlae	0.126 ± 0.005	69.10 ± 2.8

Values are expressed as mean ± SD (n=3).

The experimental data of ABTS scavenging reduction indicated that the inhibition percentage of *D. holmgrenii* extract was more than 85 % which is comparable to that of the positive control (ascorbic acid). Therefor *D. holmgrenii* showed strong antioxidant activity. Also *D. mejiae* showed high activity (83 % inhibition), while *D. meorlae* had less activity.

3.4.1. Antimicrobial activity

Plants are important source of potentially antimicrobial agents. Many compounds have been isolated with interesting antibacterial, antiviral, and antifungal activities.[17] The first step is to test the methanol extracts *in vitro* against several microbes. The methanol extracts of *Dioon* species displayed different activities against Gram positive and Gram negative as well as against fungi **(Table 5)**. *D. mejiae* showed antibacterial activity against *E. coli* and *S. aureus* more than *D. holmgrenii*, and *D. meorlae*. *D. holmgrenii* had antibacterial activity against *P. aeruginosa* and *B. subtilis* more than *D. mejiae* and *D. meorlae*. *D. holmgrenii* had antifungal activity against *C. Albicans* and *A. flavus*, more than *D. mejiae* and *D. meorlae*.

Table 5: Antimicrobial activity

Compound	% Activity index					
	E. coli	***P. aeruginosa***	***S. aureus***	***B. subtilis***	***C. Albicans***	***A. flavus***
Ampicillin	100	100	NA	100	-	-
Clotrimazole	-	-	-	-	100	100
D. mejiae	43.5	66.7	59.1	62.5	39.1	48
D. holmgrenii	34.8	70.8	54.5	87.5	65.2	92
D. meorlae	21.7	12.5	27.3	33.3	21.7	28

3.4. Bioactive compounds

Phenolic compounds represent an important group of active constituents of plants with potential antioxidant and free radical scavenging properties.[18] From Table (6) *D. holmgrenii*, showed the highest total phenolic content followed by *D. mejiae. D. meorlae* had the lowest phenolic content.

Flavonoids represent one of the most important groups of phenolic compounds. They have many biological activities as antimicrobial, antioxidant, anti-inflammatory and other effects.[19] The flavonoid content of *D. holmgrenii*, was found to be higher than that of D. *mejiae*. and *D. meorlae.* The flavonoid content was found parallel to total phenolic content.

Table 6: Total phenolic and flavonoid contents of *Dioon* leaves.

Species	Total phenolic content (µg GAE/mg DE)	Flavonoid content (µg RE/mg DE)
D. mejiae	37.14	2.32
D. holmgrenii	56.01	5.56
D. meorlae	28.00	1.16

4. CONCLUSION

This study demonstrated the anatomical features of three *Dioon* species and biological activities of their methanol extracts in addition to estimation of some bioactive compounds. *D. holmgrenii* and D. *mejiae* showed high biological activities, which were parallel to the content of

polyphenols and flavonoids. These two species are promising sources for isolation of biologically active compounds.

5. References

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