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Antimicrobial and *In vitro* Cytotoxic Efficacy of Betulinic Acid from the Callus, Regeneration, and Mother Plant of *Dillenia indica* L.

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IN THIS STUDY, we established of an application protocol for the micropropagation of *Dillenia indica* with an estimate of betulinic acid from callus, leaf, and regeneration plant. Moreover, the antimicrobial and cytotoxic efficacy of the crude extract and pure compound was compared. The highest number of shoots (3.667 shoots), leaf number per jar (27 leaves) and shoot length (3.667cm) were recorded from the MS medium containing 2.0mg L^{-1} of benzyl amino purine (BAP). Moreover, different physical elicitors (ultraviolet irradiation, microwave radiation, and light quality) were used on the production and increased the concentration of betulinic acid in callus of *D. indica*. The data revealed that the highest betulinic acid content (341.775mg/ 100g fresh weight) was recorded in callus microwaved with 200 watts for 10s. Which was three times more than the betulinic acid content recorded in the mother plant. In addition, different crude extracts of *D. indica* were used to determine their biological activity against six microorganisms. The data showed that the highest diameter of inhibition zones (17.0 mm) was recorded in callus culture extractions incubated under blue light with *Candida albicans*. The cytotoxic efficacy of betulinic acid and extract of callus, mother plant leaf or in vitro regeneration plants of *D. indica* were investigated against the Vero cell line by MTT. The results revealed that The IC_{50} values were 115.7 ± 9.17, 114.98 ± 5.2, 59.45 ± 3.06, and 72.76 ± 6.93µgmL^{-1} of betulinic acid, callus extract, leaf, and regeneration plant, respectively.

Keywords: Antimicrobial activity, Betulinic acid, Callus culture, *Dillenia indica*, Vero cell.

Introduction

Plant-derived medicinal compounds (PDMC), which are also known as secondary metabolites of medicinal plants, have gained much interest in the past decade (Cardoso et al., 2019; Fouda et al., 2015). There are many plant species which have pharmacognostic and phytopharmacological importance, and there are a small number of plants that belong to the Dilleniaceae family which are not well-known but have a very good medicinal value (Dickison, 1979). *Dillenia indica* L. (elephant apple) has many pharmacological activities, and it is broadly used for nourishment and pharmaceutical purposes (Kumar et al., 2010).

In plant cell cultures, lighting is one of the most important factors that affect the global metabolites (Ramakrishna & Ravishankar, 2011). Light is not only an essential abiotic factor for growth and organogenesis, but it is also indispensable for the synthesis of primary and secondary plant metabolites.

In general, metabolites, such as gingerol and zingiberene, produced by *Z. officinale* callus culture are stimulated by light (Anasori & Asghari, 2009). Moreover, a positive correlation has been reported between increased light intensity and phenolate levels (Mahajan & Tuteja, 2005).

Betulinic acid or 3*β*-hydroxy-lup-20(29)-

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en-28-oic acid is represented as plant natural product have various biological and medicinal activities. Pentacyclic lupine-type triterpenoid is a promising plant metabolite exhibits different activities against pathogenic bacteria (Chandramu et al., 2003), malarial vector (Bringmann et al., 1997), anti-inflammatory (Alakurtti et al., 2006), anthelmintic (Enwerem et al., 2001), antinociceptive (Kinoshita et al., 1998), anti-HSV-1 (Ryu et al., 1993), and anti-cancer activities (Zuco et al., 2002).

Betulin oxidation is commonly used to obtain betulinic acid, which is also found abundantly in nature (Hordyjewska et al., 2019). Betulinic acid and its derivatives exhibit varied pharmacological and biological activities, such as antimicrobial, anti-HIV, anti-inflammatory, antitumor, and anti-cancer because betulin acid play an important role in apoptosis pathway via early development to eliminate unwanted cells (Fulda, 2008).

Betulin and betulinic acid are the most important constituents present in almost all parts of plants; these have a good potential in curing various diseases (Mehta, 2013). Betulinic acid, isolated from the methanolic extract of *D. indica* L. fruit, exhibit anti-leukemic activity against human leukemic cell lines U937, HL60, and K562 (Kumar et al., 2010). Vargas et al. (2020) concluded that betulinic acid was present in all organs analyzed *in vitro* plantlets of *Souroubea sympetala*, but the largest amount was detected in stems.

This study aimed to conserve *D. indica*, which is a rare species from the family Dilleniaceae, through micropropagation. Production of betulinic acid from different sources such as extract of callus, mother plant leaf and fruits and in vitro regenerated plants. Study the antimicrobial activity and in vitro cytotoxicity properties of betulinic acid.

Materials and Methods

This investigation was conducted at the Tissue Culture Res. Lab., Faculty of Science, Botany and Microbiology Department, Al-Azhar University, and T. C. & Germplasm Conservation Laboratory, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

Callus initiation and growth

The callus initiated from the sterilized leaf explants of *D. indica* were cut into 1-cm segments and used as explants. The MS medium (Murashige & Skoog, 1962) supplemented with different concentrations of auxin, cytokinin, and combinations of them used for callus culture. Different physical factors were used to induce callus formation, including light quality, microwave irradiation, and ultraviolet irradiation. The data for this part was previously published in accordance with (Abd El-Kadder et al., 2014).

Micropropagation procedures

Regeneration stage

This experiment aimed to study the effect of benzyl amino purine (BAP) as a cytokinin on shoot number, shootlength, and leaf number per shoot. Leaves calli (6.0 weeks old) were transferred to solid MS medium with BAP (0.5, 1.0, and 2.0mg L^{-1}) for shoot proliferation. All cultures were examined after six weeks of incubation at 25± 1.0°C under light/dark condition of 16/8h respectively by cool fluorescent light intensity of 1500 lux. Ten replicates were applied for each treatment.

Rooting stage

The proliferated microshoots (2-4cm length) produced after the shoot formations were transferred to MS medium with different compositions Table 1. Four weeks after culturing the rooting percentage, average root number, and root length (cm) of the initiated roots per micro-shoot were recorded.

Acclimatization stage

In this stage, the plantlets from the rooting stage (2–2.5cm) were transferred into plastic pots (0.2L) supplied with a soil mixture of sand and peat moss (1:1 v/v).

Determination of Betulinic acid from methanolic extract of D. indica by HPLC method.

Betulinic acid standards obtained from SIGMA-ALDRICH were used as standards for the calibration of the high-performance liquid chromatography (HPLC) system. The HPLC method to detect betulinic acid was done at Food Technology Res. Institute. Ministry of Agriculture and Land Reclamation. Agricultural Res. Center.

TABLE 1. Composition of the medium used for the rooting of *Dillenia indica*

No. of media	MS-media composition
1	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 0.5mg L^{-1} IBA
2	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) +0.75mg L^{-1} IBA
3	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1})+1mg L^{-1} IBA
4	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) +1mg L^{-1} IBA
5	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 0.5mg L^{-1} IBA +10mg L^{-1} $AgNO_3$
6	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 0.75mg L^{-1} IBA+10mg L^{-1} $AgNO_3$
7	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 1.0mg L^{-1} IBA +10mg L^{-1} $AgNO_3$
8	Full MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 1.5mg L^{-1} IBA + 10mg L^{-1} $AgNO_3$
9	Half MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 0.5mg L^{-1} IBA + 10mg $L^{-1}AgNO_3$
10	Half MS + Sucrose (30g L^{-1}) +Agar (7g L^{-1}) + 0.75mg L^{-1} IBA + 10mg L^{-1} $AgNO_3$
11	Half MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 1.0mg L^{-1} IBA +10mg L^{-1} $AgNO_3$
12	Half MS + Sucrose (30g L^{-1}) + Agar (7g L^{-1}) + 1.5mg L^{-1} IBA + 10mg L^{-1} $AgNO_3$

Apparatus and chromatographic conditions

Betulinic acid was dissolved in methyl alcohol to prepare a standard, and all unknown samples of methanolic extract were analyzed with an HPLC system of Waters' (USA). The system consists of pump Waters HPLC 600, injector Rheodyne 7725i with 5-µL sample loop, on-line de-gasser AF (Waters, USA), absorbance of UV detector Waters 2487 Dual λ (at λ= 210nm, for betulinic acid) with 0.05 aufs sensitivity. A symmetry C-18 (4.6 × 250mm, 5µm) column equipped with an automatic temperature (±0.1°C) controller module was used to analyzed betulinic acid. Acetonitrile: methanol (80:20 v/v) with an elution volume of 0.5 mL/min was selected to identify both acids. The temperature of the column was maintained at 35°C (± 0.1°C).

Preparation and analysis of an extract of samples

About 3g of dry and fresh samples (particle size 0.42 to 0.60mm) were subjected to a fully baffled 150-mL stirred borosilicate glass vessel (5cm ID, and 9.5cm height), along with methanol (75mL) as a solvent. A four-blade turbine type agitator (2cm diameter) was used for stirring at 500 rpm to ensure that all particles remain in a suspended condition. Betulinic acid extraction was carried out at 28°C (±1°C) for 2h. The schematic experimental setup is shown in Fig. 7. The samples were collected at the same time. The collected samples were filtered through a 0.2-µm membrane filter and analyzed in the HPLC system to determine the recoverable betulinic acid from the methanolic extract.

LC-MS analysis of betulinic acid standard and methanolic extract

The methanolic solutions of betulinic acid standards were analyzed with LC-MS to confirm the peaks and their positions. The methanolic extract of the samples was also analyzed with LC-MS for the identification of betulinic acid. Waters LC-MS system, Waters TQD Mass detector, and Waters Masslynx 4.1 software were used, along with Symmetry C-18 column (4.6×250mm, 5µm) with same mobile phase mentioned earlier (Taralkar & Chattopadhyay, 2012).

Antimicrobial activity

In this study, the efficiency of the extract of *in vitro* callus tissues as influenced by light quality, ultraviolet, and microwave treatments, mother plant leaf, fruits and regeneration plants was examined against some pathogenic microorganisms, including *Bacillus subtilis* NCTC 10400, *Staphylococcus aureus*, NCTC 7447 *Escherichia coli*, NCTC 10416, *Pseudomonas aeruginosa*, ATCC 10145, *Candida albicans*, IMRU 3669, and *Aspergillus niger* ATCC 16404 by agar well diffusion method (Fouda et al., 2021b).

Thirteen treatments were executed to obtain the extracts from callus and organ tissues for antimicrobial activity studies. These thirteen treatments include light quality, ultraviolet, and microwave treatments. In addition to these treatments, three types of plant organ tissues, including *in vitro* shoots, *in vivo* mature tree leaves, and *in vivo* mature fruits were used as control treatments.

To examine the antimicrobial activity, Muller-Hinton agar for bacteria and yeast and Malt extract agar for filamentous fungi were prepared and seeded with one of the tested organisms

(100µL of bacterial culture in 20mL medium and 100µL of fungal spore suspension in 25mL broth medium). Approximately, 100µL of each treatment was added to well (0.8mm in diameter) previously prepared in seeded media. The plates were preserved in fridge for one hour before incubation for 24h (bacteria and unicellular fungi) at 35±2°C or 48h (multicellular fungi) at 28± 2°C. At the end of incubation period, the diameter of clear zone around each well was recorded by mm (Hassan et al., 2021). The experiment was achieved in triplicate.

In-vitro cytotoxic efficacy against normal cell line

The normal Vero cell line (kidney of African green monkey) was obtained from the American type of culture collection (ATCC). The efficacy of betulinic acid and different extracts of callus culture, leaf of mother plant, and regeneration plants were investigated using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay method (Fouda et al., 2021a). The morphological cell changes due to different treatment by different concentrations were assessed after 48 h of incubation period at 37°C by inverted light microscope (Nikon, Japan). The Vero cell with a density 1 x 10^5 was inoculated in a 96-well plate and treated with double-fold concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.6, and 7.8ppm) in each treatment. After the incubation period, MTT (5mg mL^{-1} in phosphate-buffered saline) was added to each inoculated well and incubated for 1–5h at 37°C and 5% CO_2. Later, purple formazan crystals were developed, which were further dissolved by adding DMSO (10%). The plates were agitated using a plate shaker for 30 min in dark conditions. Eventually, the color intensity of the samples was measured at 560 nm using a multi-well ELISA plate reader (El-Belely et al., 2021). The cell viability percentage was calculated as follows:

Cell viability(%)= (Absorbance of treated sample/ absorbance of control)× 100

Statistical analysis

The results were represented by means of three independent replicates and characterized by the mean values and standard deviations (SD). The ANOVA analysis (one-way) was used to analyze the obtained data. Also, Fisher LSD was used to detect the significant between the mean values. Posterior multiple comparisons were done using Duncan's range tests at $P \leq 0.05$.

Results and Discussions

Callus induction

Callus formation wasinduced by exposure to various physical factors including light quality, microwave irradiation, and ultraviolet irradiation (Abd El-Kadder et al., 2014). The data showed that leaf disks cultured on MS medium containing 2.0 mgL-1 of BAP and NAA had the highest callus formation. The highest significant fresh weights (5.96 and 5.58g) were attained when the callus was exposed to ultraviolet at 30 watts for 1h.

Shooting regeneration stage

Based on Table 2, the examined plant growth regulators had a significant effect on the shoot regeneration behaviors of explants *in vitro* (i.e., number of shoots formed per explants, developed shoot length, and initiated leaf number per jar). According to the data illustrated in Table 2 and Fig. 1, the highest number of shoots (3.667 shoots), leaf number per jar (27 leaves), and the highest shoot length (3.667cm) were recorded from the MS medium containing 2.0mg L^{-1} of BAP. It also noticed that the average shoot formation was increased with increasing BAP. These data due to BAP was involved in the regulation of cell division and adventitious shoot formation. This may be due to its ability to induce the metabolism and production of natural endogenous hormones for morphogenesis (Ahmed & Anis, 2014). These findings were in agreement with those reported by Abd El-Kadder & Hammad (2012) on *D. indica* L. They showed that the maximum shoot multiplication rate (7.1 shoots per explant) was obtained at 2.0mg L^{-1} of BAP. Islam et al. (2017) reported that the efficiency of shoot bud formation was relied upon and enhanced by supplementing the medium with BA. Similarly, Miri (2020) reported that the increase in BA concentration boosted the induction of axillary shoots of *Zingiber officinale* Rosc Furthermore, (Zhang et al., 2020) concluded that stem explant which grows on an MS medium containing 1.0mg L^{-1} BA without NAA giving high shoot multiplication to *Malus sieversii*.

Rooting stage

The groups of the trial, including culture media strength, IBA concentration, and silver nitrate treatments, have been achieved in order to examine their effects on the rooting ability of shoot explants (i.e., rooting percentage, initiated root number per shoot, and formed root). The

data in Table 3 show that the various strengths of MS media, IBA concentration, and sliver nitrate treatments caused a significant change in rooting percentage, initiated root number per shoot, and formed root length. Culturing the micro-shoots into a full and a half strength of MS medium and silver nitrate treatment resulted in a significant difference in rooting percentages, which ranged from 16.667% to 66.667%. The highest percentage of rooted shootlets was noted on MS medium containing 0.75mg L^{-1} IBA and 10 mg L^{-1} sliver nitrate. These findings were in consistent with Tien and co-author who showed that the highest number of roots/shoots appeared on a medium containing 1.0mg L^{-1} IBA (or 1mg L^{-1} IAA) and 1.0mg L^{-1} silver nitrate, containing 5.33 or 5.6 primary roots and 19.54 or 14.76 secondary roots on *Lantana camara* L. (Tien et al., 2019). In addition, Steephen et al. (2010) tested different IBA concentrations for the rooting of *Vitex negundo* and found that 0.5mg L^{-1} was more efficient in inducing roots *in vitro*.

Acclimatization stage

Nearly 92 to 98% of the acclimatized plantlets survived after one month of transferring into a peat moss: sand mixture (1:1 v/v). All the *in vitro*-derived transplants demonstrated the normal development of mother plants (Figs. 2, 3). Sand is an inert material that provides density, increases drainage, and favors air exchange in substrate mixes. Analogous findings were reviewed by numerous scientists. Sayed et al. (2004) reported that the survival ability of *ex vitro* acclimatized plantlets were obtained after using sand, peat moss, or sand plus peat moss (1:1, v/v) for *Cereus peruvianus*. In the same context, Taha et al. (2018) succeeded in acclimatizeding the highly survived plants of *D. indica* on peat moss with sand and perlite. Likewise, the survival ratio of *Gynerium sagittatum* Aubl. under *ex vitro* conditions was 90% for plants transplanted in peat mixed with sand (Suárez et al., 2020).

TABLE 2. Effect of BA concentrations on pre-shooting on calli derived from *D. indica* leaves

Treatment (mg L^{-1})	Shoot number	Leaves number per jar	Shoot length (cm)
Control	1.333±0.333^{b}	12.333±1.453^{c}	0.567±0.176^{b}
0.5mg L^{-1} BA	2.333±0.882^{b}	17.000±1.528^{b}	1.833±0.882^{b}
1mg L^{-1} BA	2.667±0.667^{b}	18.667±4.096^{b}	1.500±0.289^{b}
2mg L^{-1} BA	3.667±0.33^{3}a	27.000±4.726^{a}	3.667±0.441^{a}
F Ratio	2.564	3.438	6.212
P-value	NS	NS	**

The values are the means ± standard error of three independent replicates. The different letters indicate the significantly different (P≤ 0.05).

Fig. 1. Images of micro-shoot of *Dillenia indica* regenerated from callus explants after culturing on MS medium containing of 2.0mg L^{-1} BAP for six weeks

TABLE 3. Means of rooting percentage, root number/ shootlet, and root length (cm) of *D. indica* as affected by different IBA and $AgNO_3$ concentrations

IBA concentration (mg L^{-1})	Strength MS medium	$AgNO_3$ concentration (mg L^{-1})	Rooting percentage %	No. of roots/ shootlet	Root length/ cm
Control	Full strength	0	0	0	0
0.5	Full strength	0	16.667±3.333^{e}	1.667±0.333^{f}	1.223±0.047^{e}
0.75	Full strength	0	23.333±3.333^{de}	2.667±0.333^{def}	1.595±0.108^{cde}
1	Full strength	0	33.333±3.333^{bcd}	3.333±0.333^{cde}	1.712±0.186^{bcde}
1.5	Full strength	0	30.000±5.774^{bcde}	4.000±0.577^{bcd}	1.513±0.150^{de}
0. 5	Full strength	10	26.667±6.667^{cde}	3.333±0.882^{cde}	1.936±0.094^{bcd}
0.75	Full strength	10	30.000±5.774^{bcde}	4.333±0.333^{abc}	2.081±0.021^{bc}
1	Full strength	10	36.667±3.333^{bcd}	2.000±0.577^{ef}	1.789±0.288^{bcd}
1.5	Full strength	10	30.000±5.774^{bcde}	4.333±0.882^{abc}	2.110±0.082^{b}
0. 5	Half strength	10	23.333±8.819^{de}	1.667±0.333^{f}	1.921±0.268^{bcd}
0.75	Half strength	10	66.667±3.333^{a}	5.667±0.333^{a}	2.755±0.347^{a}
1	Half strength	10	40.000±5.774^{bc}	4.333±0.667^{abc}	2.064±0.065^{bc}
1.5	Half strength	10	43.333±3.333^{b}	5.333±0.333^{ab}	2.210±0.083^{b}
F Ratio			6.169	6.358	4.912
P-value			***	***	***

The values are the means ± standard error of three independent replicates. The different letters indicate the significantly different ($P \leq 0.05$).

Fig. 2. Roots formation on half strength MS medium containing 0.75: 10mg L^{-1} of IBA and sliver nitrate respectively (A); *In vitro* acclimatized plantlets survived of *D. indica* after four weeks of transferring into the peat moss: sand mixture (1:1 v/v) (B)

Fig. 3. Images of *in vivo* acclimatization of *D. indica*

Determination of betulinic acid by HPLC

Betulinic acid is one of the by-products found in many plant cell that has a wide-range of pharmacological and biological properties (Hordyjewska et al., 2019). Despite its importance, the low amount of BA in plants makes it insufficient to meet the demand for this compound (Jin et al., 2019). Thus, the aim of this study was to increase the amount of BA in callus culture using different factors. The data in Table 4 and Fig. 4 exhibit the BA content in callus subjected to physical elicitation compared with leaves and fruits of the mother tree and *in vitro*-grown micro-shoots. The data revealed that the highest BA content (341.775mg/100g fresh weight) was noted in callus microwaved with 200w for 10s which was three times more than three-fold BA content was recorded in the mother plant. Gandhi et al. (2019) used microwave power at 100w to obtain the good extraction yield of betulinic acid from *Dillenia indica*. Hu et al. (2008) and Xiao et al. (2008), found that the microwave power ruptures the plant cells easily which releases the phytocontents faster and result in shorter extraction time. In this regard, Hayashi et al. (2005), reported that BA accumulation was examined in cultured cells of *Glycyrrhiza glabra* L. (licorice). The time course of their accumulation was different in cultured cells. Yeast extract promoted BA accumulation. Moreover, BA, oleanolic acid (OA), pentacyclic triterpenoids, and ursolic acid (UA) were accumulated in leaf disk explant of *Lantana camara* (Srivastava et al., 2010). The influence of light quality on growth, production of industrially important secondary metabolites, and antioxidant activity in callus cultures of *Rhodiola imbricata* was investigated (Kapoor et al., 2018). Additionally, Chen et al. (2020) used an ultrasonic-assisted ethanol method to extract and increase the botulin from *Betula platyphylla* suk. Bark. Pandey et al. (2015) used methyl jasmonate on growth media to increase the amount of BA in the callus culture of *S. mammosum*. Currently, the synthesis of secondary metabolites from plant using *in vitro* culture techniques was studied by changing the culturing media composition (Alenizi et al., 2020). Moreover, Anfal et al. (2020) used different types and levels of chemical elicitors to enhance the production of BA in *Lantana camara* L. callus. Coelho et al. (2021) showed that the nodal segments of *Urtica dioica* L. which grow in MS media with different wavelength and light intensity enhanced their growth, and phytochemical contents.

TABLE 4. Effect of light quality, ultraviolet, and microwave irradiation treatments on betulinic acid content (mg/100g F.W.) of *D. indica* callus cultures, leaves, and fruits of the mother tree and *in vitro*-grown micro-shoots

Explant	Treatment	Betulinic acid mg/100g fresh callus
Callus exposure to different light quality	3000 lux	89.925
	Dark	30.225
	Blue light	93.5
	Greenlight	211.325
	Red light	114.725
Callus exposure to different microwave irradiation	100w for 10s	147.275
	100w for 20s	315.9
	200w for 10s	341.775
	200w for 20s	134.775
Callus exposure to different ultraviolet irradiation	15w for 120min	294.725
	15w for 60min	173.625
	30w for 120min	66.725
	30w for 60min	61.9
Mother plant(fruit)	Fruits of the mother plant	99.675
Mother plant(leaves)	Leaves of the mother plant	108.325
Regeneration plant	Micro-shoot of regeneration plant	47.65

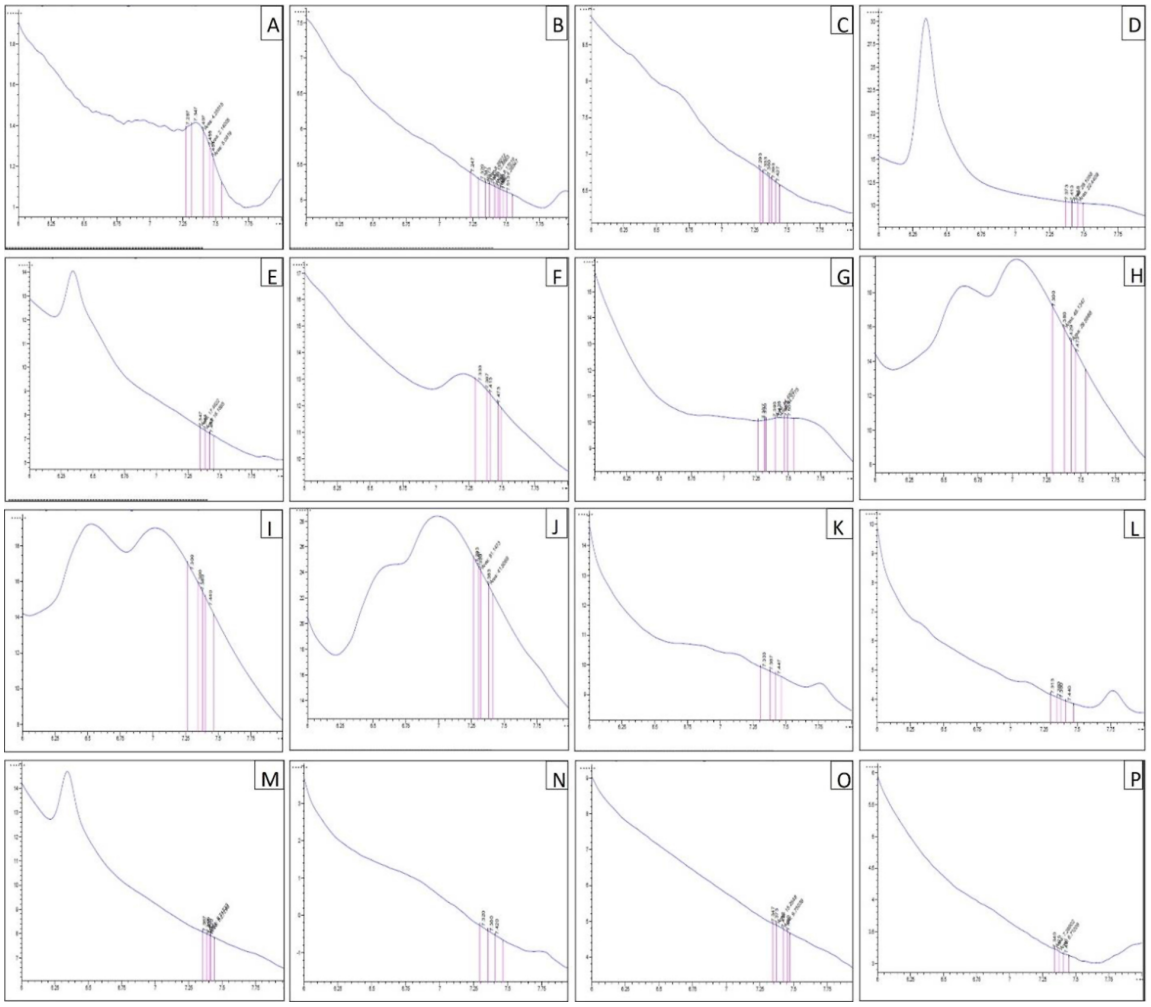

Fig. 4. HPLC chromatogram representing Betulinic acid content of *Dillenia indica* [(A) Callus incubated at dark, (B) Callus incubated at 3000 lux., (C) Callus incubated at the blue light, (D) Callus incubated at a green light, (E) Callus incubated at a red light, (F) Callus exposure to microwave for 100w for 10s, (G) Callus exposure to microwave for 100w for 20s, (H) Callus exposure to microwave for 200w for 10s, (I) Callus exposure to microwave for 200w for 20s, (J) Callus exposure to UV at 15w for 120min.,(K) Callus exposure to UV at 15w for 60min, (L) Callus exposure to UV at 30w for 120min, (M) Callus exposure to UV at 30w for 60min, (N) Fruits of the mother plant, (O) Leaves of the mother plant, (P) Microshoot]

Biological activity

In this study, different crude extracts of *D. indica* were used to assess their biological activity against six pathogenic microorganisms. The data in Table 5 showed that the callus crude extracts of *D. indica* displayed inhibition zones (mm) against *Staphylococcus aureus* NCTC 7447, *Bacillus subtilis* NCTC 10400, *Escherichia coli* NCTC 10416, *Pseudomonas aeruginosa* ATCC 10145, and *Candida albicans* IMRU 3669. Conversely, the extractions of callus culture of *D. indica*, mother plant, and regeneration plant, showed no response against *Aspergillus niger* AT CC 16404. This result may be due to an increase in BA content in callus subjected to physical elicitation, such as light quality, ultraviolet, and microwave irradiation treatments. The highest diameter of inhibition zones (17.0mm) was recorded with extractions of callus culture incubated under blue light with *Candida albicans* IMRU 3669 (Table 5). Moreover, *Bacillus subtilis* NCTC 10400 showed the diameter of inhibition zones (15.0 and 14.0mm) with callus culture exposure to UV 15w for 60min., 30w for 120min. respectively. In addition, the extract of callus culture was exposed to microwave irradiation of 200w for 10s. The treatment showed the inhibition zones (mm) against all microorganisms except *Aspergillus niger*. In contrast, in the extractions of micro-shoots, fruits, and leaves of the mother

plant, no response have been recorded against previous pathogenic microorganisms (Table 5). In this regard, Maslova et al., (2020) found that all dilution extracts from the callus of *Bollota nigra* had greater antimicrobial activity against *E. coli* than the extract of flower and leaves of the plant. In contrast, Nkop & Zudonu (2020) observed that the inhibitory effect of the leaf and stem bark extracts of *D. indica* against pathogenic bacterial strains (*B. subtilis, B. cereus, S. typhi, P. aeruginosa,* and *P. mirabilis*) increases with an increased in concentration.

TABLE 5. Antimicrobial activities of plant organ tissues and callus extracts of Dillenia indica influenced by light quality, ultraviolet, and microwave treatments.

Treatment		*B. subtilis*	*S. typhi*	E. coil	*P. aeruginosa*	*C. albicans*	*A. niger*
Control		0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Callus exposure to different light quality	3000 lux	13 ± 1.6^{b}	11 ± 2.5^{cde}	0^{a}	0^{a}	16 ± 0.4^{ab}	0^{a}
	Dark	0^{a}	10 ± 1.3^{de}	0^{a}	0^{a}	0	0^{a}
	Blue light	14 ± 1.6^{c}	0^{a}	0^{a}	0^{a}	17 ± 0.3^{ab}	0^{a}
	Green light	0^{a}	0^{a}	12 ± 0.9^{bc}	13 ± 1.5^{c}	16 ± 0.3^{ab}	0^{a}
	Red light	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Callus exposure to different microwave irradiation	100w for 10s	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	100w for 20s	0^{a}	12 ± 1.8^{cde}	0^{a}	0^{a}	0^{a}	0^{a}
	200w for 10s	12 ± 1.2^{b}	12 ± 1.2^{b}	12 ± 0.9^{bc}	12 ± 1.0^{b}	11 ± 1.7^{bc}	0^{a}
	200w for 20s	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Callus exposure to different ultraviolet irradiation	15w for 120min	14 ± 1.2^{b}	13 ± 0.5^{b}	0^{a}	0^{a}	0^{a}	0^{a}
	15w for 60min	15 ± 0.8^{ab}	13 ± 2.1^{bcd}	14 ± 1.7^{bc}	0^{a}	0^{a}	0^{a}
	30w for 120min	15 ± 1.1^{ab}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	30w for 60min	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Mother plant (fruit)	Fruits of mother plant	12 ± 1.6^{cde}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Mother plant (leaves)	Leaves of mother plant	10 ± 1.9^{cde}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
New plant	Micro shoot of new plant	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Control, callus extract not exposed to any physical elicitation.
The values are the means ± standard error of three independent replicates..
The different letters indicate the significantly different ($P\leq 0.05$).

In vitro cytotoxicity

The cytotoxic efficacy of BA, callus culture extract, leaf of mother plant extract, and extract of *in vitro* shoots of *D. indica* was investigated against Vero cell line (normal cell line) using MTT assay method. This method is colorimetric, highly accurate, and highly sensitive assay method used to measure the cell viability and cell proliferations due to exposure to different compounds (Lashin et al., 2021; Mohamed et al., 2019). Betulinic acid is found in many plant kingdom species. In recent decades, the biological activities of BA have been recognized, such as anti-cancer, anti-inflammatory, and anti-malarial properties (Hordyjewska et al., 2019). In this study, the double-fold concentrations of the extracts of calli, plant leaf, and regeneration plants of *D. indica* and BA were investigated against Vero cells. Data analysis showed that the activity of different treatment was dose-dependent; the activity increased with decreasing concentration (Fig. 5). The morphological characteristics of Vero cell treated with different concentrations were altered, with complete or partial monolayer loss. Some treated cells were shrank or granulated at high concentrations as compared with untreated cell. These morphological changes were decreased or disappeared by lowering the concentrations. Recently published studies confirmed this finding, that the activity of the Vero cell detected by MTT assay method was dependent on the concentration of external substances (Eid et al., 2020; Fouda et al., 2020; Salem et al., 2020; Shaheen et al., 2021). Arifianti et al. (2020) also examined the safety of BA and callus extract of *S. mammosum* against normal cell Vero. The results presented those concentrations up to 500µg/mL to both samples were more than 60% cell viability, which indicates that the sample is safe for normal cells. The IC_{50} values (the concentration that inhibit 50% of cell viability) were 115.7±9.17, 114.98± 5.2, 59.45± 3.06, and 72.76± 6.93µg mL^{-1} for BA, callus extract, leaf, and regeneration plant, respectively (Fig. 6). The main callus extract and BA have efficacy against normal Vero cell at higher concentration than those from mother plant and regeneration plant. Thus, we strongly recommended utilizing the callus extract with normal cell than the extract of leaf and regeneration plant.

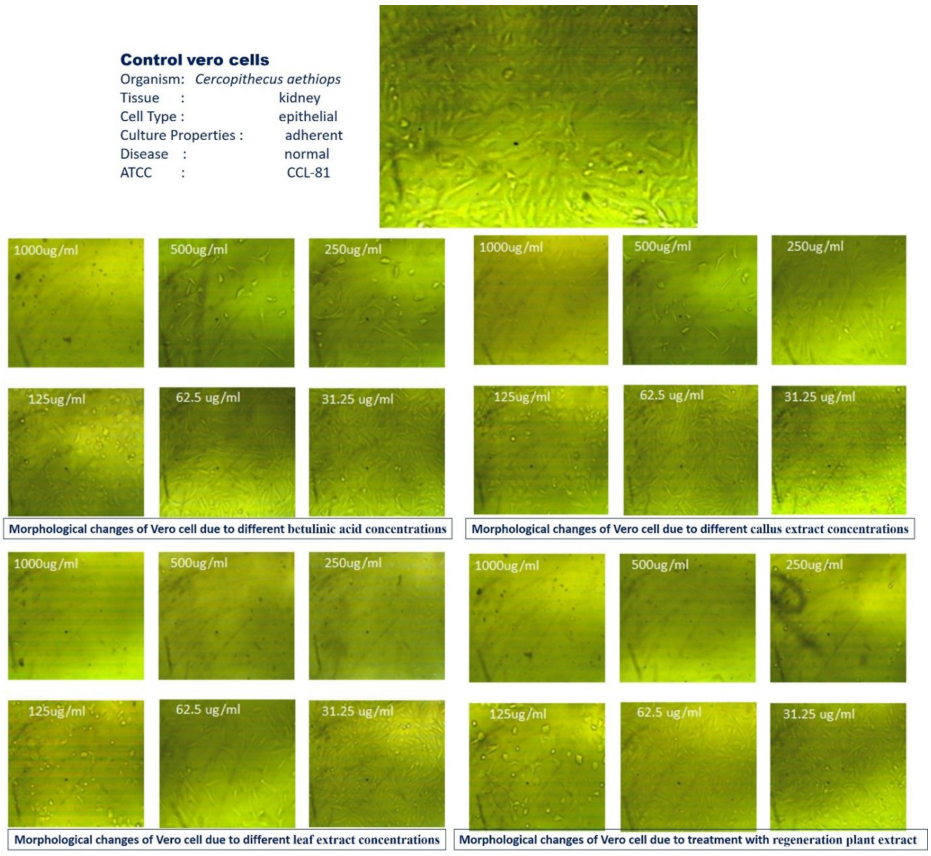

Fig. 5. Morphological change of Vero cell line due to treatment with different concentration of betulinic acid, callus culture extract, leaf of mother plant extract, and regeneration plant extract of *Dillenia indica*

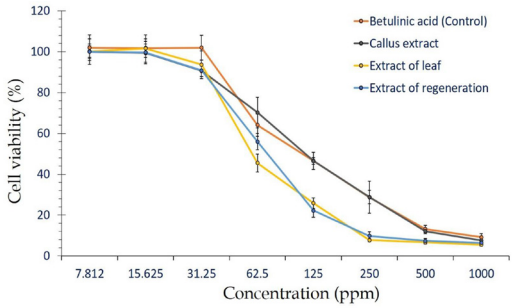

Fig. 6. *In vitro* cytotoxic effects of different concentration of betulinic acid, callus culture extract, leaf of mother plant extract, and regeneration plant extract of Dillenia indica.against Vero cell line. The data are statistically different at P≤ 0.05, (n= 3); error bars are means ± SE (standard error).

Conclusion

In this study, using BA at 2mg L^{-1} enhanced the leaf formation, shoot maximum length, and the greatest shoot number of per explant. Moreover, the highest significant rooting response of micro-shoot explants, the root formation in the greatest number, and root elongation produced on medium containing 0.75 mg L^{-1} IBA, with half-strength MS medium and 10 mg L^{-1} $AgNO_3$. The highest survival rates of *ex vitro* rooted plantlets could result when the acclimatization procedures were achieved by a mixture of sand plus peat moss (1:1 v/v). Furthermore, the exposure of the callus culture of *D. indica* to some physical elicitors giving BA higher than the mother plant. Additionally, the crude extracts of the callus culture of *D. indica* showed inhibitory action against some pathogenic bacteria and fungi than the extract of mother plants. Notably, the effect of high concentrations of callus extract and BA on normal Vero cell line at (IC50= 114.98± 5.2 and 115.7± 9.17µg mL^{-1}, respectively). Based on the obtained data, it can be concluded that the callus extract can be used to produce BA with high antimicrobial activity.

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Author contribution: Islam Lashin: Contributed to define the study idea and write the manuscript. Mohamed Aref: Contributed to plant the practical experiment. Esam Adb El-Kader: Plane the practical experiment and data collection. Emad El-Din Ewais and Esam Hussein: Review and editing.

*Data availability:*The data used to support the findings of this study are available from the corresponding author upon request.

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References

Abd El-Kadder, E.M., Hammad, H. (2012) *In vitro* propagation of Dillenia indica. *Australian Journal of Basic and Applied Sciences*, **6**(7), 452-457.

Abd El-Kadder, E.M., Lashin, I., Aref, M.S., Hussian, E.A., Ewais, E.A. (2014) Physical elicitation of *Dillenia indica* callus for production of secondary metabolites. *New York Science Journal*, **7**, 48-57.

Ahmed, R., Anis, M. (2014) Rapid *in vitro* propagation system through shoot tip cultures of Vitex trifolia L.-an important multipurpose plant of the Pacific traditional Medicine. *Physiology and Molecular Biology of Plants,* **20**(3), 385-92.

Alakurtti, S., Mäkelä, T., Koskimies, S., Yli-Kauhaluoma, J. (2006) Pharmacological properties of the ubiquitous natural product betulin. *European Journal of Pharmaceutical Sciences*, **29**(1), 1-13.

Alenizi, A., Shibli, R., Tahtamouni, R., Qudah, T., Remeilh, B. (2020) *In vitro* propagation and enhancement of Quercetins and Isorhamnetin production in wild *Paronychia argentea* L. *Jordan Journal of Pharmaceutical Sciences*, **13**, 2020-65.

Anasori, P., Asghari, G. (2009) Effects of light and differentiation on gingerol and zingiberene production in callus culture of *Zingiber officinale* Rosc. *Research in Pharmaceutical Sciences*, **3**(1), 59-63.

Anfal, A.-A., Rida, S., Reham, T., Tamara, A.-Q., Bashaer, A.-I. (2020) Enhancement of pentacyclic triterpenoids (betulinic and oleanolic acids) production from callus cultures of *Lantana camara* L. *Advances in Horticultural Science*, **34**(3), 255-263.

Arifianti, L., Elsafira, A., Ilmiah, L., Suciati, S. (2020) *In vitro* anticancer property of *Solanum mammosum* callus culture against HeLa and Vero cell lines. *Journal of Research in Pharmacy*, **24**, 218-224.

Bringmann, G., Saeb, W., Assi, L.A., Francois, G., Narayanan, A.S., Peters, K., Peters, E.-M. (1997) Betulinic acid: Isolation from *Triphyophyllum peltatum* and *Ancistrocladus heyneanus*, antimalarial activity, and crystal structure of the benzyl ester. *Planta Medica*, **63**(03), 255-257.

Cardoso, J.C., Oliveira, M.E.B., Cardoso, F.d.C. (2019) Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. *Horticultura Brasileira*, **37**, 124-132.

Chandramu, C., Manohar, R.D., Krupadanam, D.G., Dashavantha, R.V. (2003) Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **17**(2), 129-134.

Chen, H., Xiao, H., Pang, J. (2020) Parameter optimization and potential bioactivity evaluation of a betulin extract from White Birch Bark. *Plants*, **9**(3), 392.

Coelho, A.D., de Souza, C.K., Bertolucci, S.K.V., de Carvalho, A.A., Santos, G.C., de Oliveira, T., et al. (2021) Wavelength and light intensity enhance growth, phytochemical contents and antioxidant activity in micropropagated plantlets of Urtica dioica L. *Plant Cell, Tissue and Organ Culture (PCTOC)*, **145**(1), 59-74.

Dickison, W.C. (1979) Note on the wood anatomy of Dillenia (Dilleniaceae). *IAWA bulletin-International Association of Wood Anatomists.* New Series.

Eid, A.M., Fouda, A., Niedbała, G., Hassan, S.E., Salem, S.S., Abdo, A.M., et al. (2020) Endophytic *Streptomyces laurentii* mediated green synthesis of Ag-NPs with antibacterial and anticancer properties for developing functional textile fabric properties. *Antibiotics (Basel)*, **9**(10). https://doi.org/10.3390/antibiotics9100641

El-Belely, E.F., Farag, M.M.S., Said, H.A., Amin, A.S., Azab, E., Gobouri, A.A., Fouda, A. (2021) Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using *Arthrospira platensis* (Class: Cyanophyceae) and evaluation of their biomedical activities. *Nanomaterials (Basel)*, **11**(1). https://doi.org/10.3390/nano11010095

Enwerem, N., Okogun, J., Wambebe, C., Okorie, D., Akah, P. (2001) Anthelmintic activity of the stem bark extracts of Berlina grandiflora and one of its active principles, Betulinic acid. *Phytomedicine*, **8**(2), 112-114.

Fouda, A.H., Hassan, S.E.-D., Eid, A.M., Ewais, E.E.-D. (2015) Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss.). *Annals of Agricultural Sciences*, **60**(1), 95-104.

Fouda, A., Hassan, S.E., Abdo, A.M., El-Gamal, M.S. (2020) Antimicrobial, antioxidant and larvicidal activities of spherical silver nanoparticles synthesized by endophytic *Streptomyces* spp. *Biological Trace Element Research*, **195**(2), 707-724.

Fouda, A., Abdel-Maksoud, G., Saad, H.A., Gobouri, A.A., Mohammedsaleh, Z.M., Abdel-Haleem, El-Sadany, M. (2021a) The efficacy of silver nitrate ($AgNO_3$) as a coating agent to protect paper against high deteriorating microbes. *Catalysts*, **11**, 310. https://doi. org/10.3390/catal11030310

Fouda, A., Hassan, S.E.-D., Saied, E., Hamza, M.F. (2021b) Photocatalytic degradation of real textile and tannery effluent using biosynthesized magnesium oxide nanoparticles (MgO-NPs), heavy metal adsorption, phytotoxicity, and antimicrobial activity. *Journal of Environmental Chemical Engineering*, **9**(4), 105346.

Fulda, S. (2008) Betulinic acid for cancer treatment and prevention. *International Journal of Molecular Sciences,* **9**(6), 1096-1107.

Gandhi, D., Patel, H., Patel, N., Mehta, P. (2019) Optimization of microwave-assisted extraction technique for isolation of betulinic acid from *Dillenia indica* Linn. and its quantification using developed HPTLC method. *Journal of Applied Pharmaceutical Science,* **9**(8), 101-110.

Hassan, S.E., Fouda, A., Saied, E., Farag, M.M.S., Eid, A.M., Barghoth, M.G., et al. (2021) Rhizopus oryzae-mediated green synthesis of magnesium oxide nanoparticles (MgO-NPs): A promising tool for antimicrobial, mosquitocidal action, and tanning effluent treatment. *Journal of Fungi*, **7**(5). https://doi.org/10.3390/jof7050372

Hayashi, H., Hiraoka, N., Ikeshiro, Y. (2005)Differential regulation of soyasaponin and betulinic acid production by yeast extract in cultured licorice cells.

Plant Biotechnology, **22**(3), 241-244.

Hordyjewska, A., Ostapiuk, A., Horecka, A., Kurzepa, J. (2019) Betulin and betulinic acid: triterpenoids derivatives with a powerful biological potential. *Phytochemistry Reviews*, **18**(3), 929-951.

Hu, Z., M. Cai, H.-H. (2008) Liang, Desirability function approach for the optimization of microwave-assisted extraction of saikosaponins from Radix Bupleuri. *Separation and Purification Technology*, **61**, 266-275.

Islam, N., Islam, T., Hossain, M., Bhattacharjee, B., Hossain, M., Islam, S.M.S. (2017) Embryogenic callus induction and efficient plant regeneration in three varieties of soybean (*Glycine max*). *Plant Tissue Culture and Biotechnology*, **27**, 41.

Jin, C.-C., Zhang, J.-L., Song, H., Cao, Y.-X. (2019) Boosting the biosynthesis of betulinic acid and related triterpenoids in Yarrowia lipolytica via multimodular metabolic engineering. *Microbial Cell Factories*, **18**(1), 77.

Kapoor, S., Raghuvanshi, R., Bhardwaj, P., Sood, H., Saxena, S., Chaurasia, O.P. (2018) Influence of light quality on growth, secondary metabolites production and antioxidant activity in callus culture of Rhodiola imbricata Edgew. *Journal of Photochemistry and Photobiology B*, **183**, 258-265.

Kinoshita, K., Akiba, M., Saitoh, M., Ye, Y., Koyama, K., Takahashi, K., Kondo, N., et al. (1998) Antinociceptive effect of triterpenes from cacti. *Pharmaceutical Biology*, **36**(1), 50-55.

Kumar, D., Mallick, S., Vedasiromoni, J.R., Pal, B.C. (2010) Anti-leukemic activity of *Dillenia indica* L. fruit extract and quantification of betulinic acid by HPLC. *Phytomedicine*, **17**(6), 431-5.

Lashin, I., Fouda, A., Gobouri, A.A., Azab, E., Mohammedsaleh, Z.M., Makharita, R.R. (2021) Antimicrobial and *in vtro* cytotoxic efficacy of biogenic silver nanoparticles (Ag-NPs) fabricated by callus extract of *Solanum incanum* L. *Biomolecules*, **11**(3). DOI: 10.3390/biom11030341

Mahajan, S., Tuteja, N. (2005) Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics*, **444**(2), 139-158.

Maslova, E.V., Glodik, T.V., Kalashnikova, E.A., Spichak, I.V., Perelygina, T.A. (2020) Anti-bacterial activity of the vallus tissue *Ballota nigra* L. *in vitro*. *Systematic Reviews in Pharmacy*, **11**(11), 752-756.

Mehta, D.G. (2013) *Dillenia indica* Linn. and *Dillenia pentagyna* Roxb.: Pharmacognostic, phytochemical and therapeutic aspects. *Journal of Applied Pharmaceutical Science*, **3**(11), 134-142.

Miri, S.M. (2020) Micropropagation, callus induction and regeneration of ginger (*Zingiber officinale* Rosc.). *Open Agriculture*, **5**(1), 75-84.

Mohamed, A.A., Fouda, A., Abdel-Rahman, M.A., Hassan, S.E.-D., El-Gamal, M.S., et al. (2019) Fungal strain impacts the shape, bioactivity and multifunctional properties of green synthesized zinc oxide nanoparticles. *Biocatalysis and Agricultural Biotechnology*, **19**, 101103.

Murashige, T., Skoog, F. (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum,* **15**(3), 473-497.

Nkop, E.J., Zudonu, O.C. (2020) Phytochemical screening and antimicrobial activity of leave and stem bark of *Dillenia indica*. *The Pharmaceutical and Chemical Journal*, **7**(1), 1-4.

Pandey, H., Pandey, P., Singh, S., Gupta, R., Banerjee, S. (2015) Production of anti-cancer triterpene (betulinic acid) from callus cultures of different Ocimum species and its elicitation. *Protoplasma*, **252**(2), 647-55.

Ramakrishna, A., Ravishankar, G.A. (2011) Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav*, **6**(11), 1720-31.

Ryu, S.Y., Lee, C.-K., Ahn, J.W., Lee, S.H., Zee, O.P. (1993) Antiviral activity of triterpenoid derivatives. *Archives of Pharmacal Research*, **16**(4), 339-342.

Salem, S.S., El-Belely, E.F., Niedbała, G., Alnoman, M.M., Hassan, S.E., Eid, A.M., et al. (2020) Bactericidal and *in-vitro* cytotoxic efficacy of silver nanoparticles (Ag-NPs) fabricated by endophytic actinomycetes and their use as coating for the textile fabrics. *Nanomaterials (Basel)*, **10**(10). doi: 10.3390/nano10102082

Sayed, S.S., Abou-Dahab, T., Youssef, E. (2004) In vitro propagation of cactus (*Cereus peruvianus* L.). *Arab Journal of Biotechnology*, **8**, 169-176.

Shaheen, T.I., Fouda, A., Salem, S.S. (2021) Integration of cotton fabrics with biosynthesized CuO nanoparticles for bactericidal activity in the terms of their cytotoxicity assessment. *Industrial & Engineering Chemistry Research*, **60**(4), 1553-1563.

Srivastava, P., Kasoju, N., Bora, U., Chaturvedi, R. (2010) Accumulation of betulinic, oleanolic, and ursolic acids in In vitro cell cultures of *Lantana camara* L. and their significant cytotoxic effects on HeLa cell lines. *Biotechnology and Bioprocess Engineering*, **15**(6), 1038-1046.

Steephen, M., Nagarajan, S., Ganesh, D. (2010) Phloroglucinol and silver nitrate enhances axillary shoot proliferation in nodal explants of *Vitex negundo* L.–an aromatic medicinal plant. *Iranian Journal of Biotechnology,* **8**(2), 82-89

Suárez, I.E., Yépez, J.E., López, C.M. (2020) Effect of different substrates on adaptation of arrow cane (*Gynerium sagitatum* Aubl.) micropropagated plants. *Temas Agrarios*, **25**(1), 77-84.

Taha, L., Ibrahim, E., Youssef, N., El-sayed, I. (2018)*In vitro* culture development and secondary metabolites of *Dillenia indica* Tree. *American Journal of Plant Physiology*, **13**, 44-52.

Taralkar, S., Chattopadhyay, S. (2012) A HPLC method for determination of ursolic acid and betulinic acids from their methanolic extracts of *Vitex negundo* Linn. *Journal of analytical and bioanalytical Techniques*, **3**(3), 1-6.

Tien, N.Q.D., Nhan, T.T.T., Le Thi Anh, T., Loc, N.H. (2019) Effect of silver nitrate in combination with some plant growth regulators on micropropagation Of *Lantana camara* L.-A Valuable Medicinal Plant. Plant Cell Biotechnology and Molecular Biology, **20**(15&16), 634–642.

Vargas, A.R., Gomez, A.H., Liu, R., Rojas, M.O., Vindas, P.S., Durst, T., Arnason, J.T. (2020) *In vitro* culture of the new anxiolytic plant, *Souroubea sympetala*. *Journal of Natural Health Product Research*, **2**(1), 1-10.

Xiao, W., L. Han, Shi, B. (2008) Microwave-assisted extraction of flavonoids from Radix Astragali. *Separation and Purification Technology,* **62**(3), 614-618.

Zhang, Y., Bozorov, T.A., Li, D.X., Zhou, P., Wen, X.J., Ding, Y., Zhang, D.Y. (2020) An efficient in vitro regeneration system from different wild apple (Malus sieversii) explants. *Plant Methods*, **16**(1), 56.

Zuco, V., Supino, R., Righetti, S.C., Cleris, L., Marchesi, E., Gambacorti-Passerini, C., Formelli, F. (2002) Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. *Cancer Letters*, **175**(1), 17-25.

فعالية حمض البيتولينيك من كالس والنباتات النامية معمليا ونبات الديلينا الام كمضاد للميكروبات والسميه الخلويه للخلايا

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تهدف هذة الدراسة إلى وضع بروتوكول للتكاثر الدقيق للديلنيا الهندية وإنتاج أحد مواد الأيض الثانوية معملياً (حمض البيتولينيك) عن طريق مزارع الكالوس وايضا فعالية حمض البيتولينيك من الكالس والنباتات النامية معمليا ونبات الديلينا الام كمضاد للميكروبات والسميه الخلويه للخلايا.وقد أظهرت النتائج ان استخدام بنزيل أمينوبيوريين بتركيز 2 ملليجم/ لتر أعطي أكبر عدد من الأفرع (3.667 فرع) كما أدى إلى زيادة عدد الأوراق المتكونة (27 ورقة). علاوة على ذلك، تم استخدام عوامل فيزيائية مختلفة (الإشعاع فوق البنفسجي، إشعاع الميكروويف، وجودة الضوء) في إنتاج وزيادة تركيز حمض البيتولينيك في الكالس. أظهرت البيانات أن أعلى محتوى من حمض البيتولينيك (341.775 مجم / 100 جم وزن طازج) تم تسجيله في الكالس الذي تم وضعة في الميكروويف 200 وات لمدة 10 ثوان. والتي كانت أكثر بثلاث مرات من محتوى حمض البيتولينيك المسجل في النبات الأم. بالإضافة إلى ذلك، تم استخدام مستخلصات مختلفة من الكالس والنباتات النامية معمليا ونبات الديلينا الام لتحديد نشاطها البيولوجي ضد ستة كائنات دقيقة. أظهرت البيانات تسجيل أعلى قطر لمناطق التثبيط (17.0 مم) مع *Candida albicans* باستخدام مستخلص الكالس والذي تم تحضينة تحت تأثير الضوء الأزرق. أيضا تم فحص فعالية السمية الخلوية لحمض البيتولينيك ومستخلص الكالس ومستخلص أوراق النبات الأم ومستخلص النباتات النامية معمليا ضد Vero cell بواسطة MTT . أظهرت النتائج أن قيم IC50 كانت 115.7 ± 9.17 ، 114.98 ± 5.2 ، 59.45 ± 3.06، 72.76 ± 6.93 ميكروجرام لتر من حمض البيتولينيك ومستخلص الكالس والأوراق للنبات الام والنباتات النامية معمليا على التوالي.