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Research Article
Glucosinolates Constituents and Cytotoxic Activities of *Lepidium sativum* L. Callus Cultures

[1]Mona M. Ibrahim, [2]Marwa M. Mounier and [1]S.A. Bekheet

[1]Department of Plant Biotechnology, Genetic Engineering and Biotechnology Division, National Research Centre, 12622 Cairo, Egypt
[2]Department of Pharmacognosy, Pharmaceutical and Drug Industries Research Division, National Research Centre, 12622 Cairo, Egypt

Abstract

Background and Objectives: *Lepidium sativum* has been widely used to treat a number of ailments in traditional system of medicine such as; antidiabetic, hypocholesterolemic, antihypertensive, antidiarrheal, antispasmodic and laxative activities. The aim of the present study was to develop an efficient protocol for *in vitro* production the callus cultures from different explants of *L. sativum*. Detection the glucosinolates compounds in the different callus extracts. Also the cytotoxic potential of different callus extracts on human breast carcinoma (MCF-7 cell line), human lung carcinoma (A-549 cell line) and colon carcinoma (HCT-116 cell line). Followed by lipid peroxidation efficiency of promising extract. **Materials and Methods:** Different explants separated from *L. sativum* seedling were cultured on MS-medium supplemented with different 2,4-D and Kin combinations and then the production of calli was investigated on the media containing different growth regulators. Glucosinolates compounds from different callus cultures and seeds were detected by GC-MS analysis. Cell viability assay was assessed according to the mitochondrial-dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide) to purple formazan. Callus root extract was further investigated of their effect on lipid peroxidation inside breast tumor cell line through measure MDA (malondialdehyde) as the end product of lipid peroxidation. **Results:** The medium which contained 1.0 mg L^{-1} NAA+0.5 mg L^{-1} BAP achieved maximum callus fresh weights in all studied explants (cotyledon, hypocotyls, root and leaf). The GC-MS analysis revealed 13 compounds of glucosinolate hydrolysis products which were detected from *in vitro* callus tissues and seeds of *L. sativum*. The major of them was N-Cyano carbazole detected in the callus of hypocotyls extract. Callus root extract possessed the most potent cytotoxic activity against human breast carcinoma (MCF-7) cell line and moderate activity on Lung carcinoma (A-549). Other callus extracts showed week activities against all studied carcinoma cell lines. Callus root extract induce lipid peroxidation and increase the concentration of MDA. **Conclusion:** It could be concluded that, the medium which contained 1.0 mg NAA+0.5 mg L^{-1} BAP was more suitable for callus production from different explants. Glucosinolate hydrolysis products were detected in all studied callus cultures and seeds of *L. sativum*. Callus root extract induced most cytotoxic activity against human breast carcinoma (MCF-7) cell line.

Key words: *Lepidium sativum*, callus cultures, glucosinolates, anti-carcinogenic

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Corresponding Author: Mona M. Ibrahim, Department of Plant Biotechnology, Genetic Engineering and Biotechnology Division, National Research Centre, 12622 Cairo, Egypt Tel: 00201014046556

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lepidium sativum L. known Garden cress a medicinal plant belonging to the Cruciferae family and genus *Lepidium*. The genus *Lepidium* includes several species growing in moist temperature and warm climate[1]. The plant is native to Egypt and south west Asia and grown in India, Europe and US[2]. The plant contains several important compounds such as; phenolic compounds, alkaloids, cardiac glycosides, anthraquinone glycosides, tannins, steroids and flavonoids[3,4]. Seeds, leaves and root of *L. sativum* have economic value. Seed contains volatile essential aromatic oils, fatty oils, carbohydrates, proteins, fatty acids, vitamin C, vitamin B-complex, flavonoids and isothiocynates[3]. The plant used in the treatment of bacterial and fungal infections[5]. The seeds paste is beneficial in relieving pain in tumefaction rheumatic joints, is also helpful in dysentery, diarrhoea and skin disease[6]. The leaves of the plant are used in salads and cooked with other vegetables or used in food garnish and are utilized as stimulant and diuretic[7,8]. The roots of the plant are bitter, utilized as a condiment and used in the treatment of secondary syphilis and tenesmus[9]. The plant generally is beneficial to treat asthma, cough with expectoration and bleeding piles[10,11].

The *L. sativum* is an immensely rich source of antioxidants[12]. It contains glucosinolates, which are the precursors of isothiocyanates[13]. It can be believe that the it inhibit the evolution of cancer cells and decrease the several forms of cancer by inhibiting carcinogen activating enzymes, enhancing carcinogen detoxifying enzymes and inducing cell cycle arrest and apoptosis[14,15].

The *L. sativum* contains benzyl glucosinolate which is the parent compound of benzyl isothiocyanate (BITC)[1]. The activity of BITC as anticarcinogenic compound has been studied widely in garden cress as a chemopreventive agent towards different types of carcinogens[16]. Suppression of pancreatic tumor growth[17], also inhibition of human breast cancer cells by BITC[18].

The glucosinolate profile is usually rely on the genetic background of the individuals and on the environmental conditions[19]. Also, glucosinolate can alter based on the developmental stages of the plant as reported in *Arabidopsis thaliana* the model plant of the Brassicaceae family[20] and in kale[21].

Plant tissue culture techniques achieved great outcomes in micropropagation, conservation and raise natural level of plant secondary products without any climate constraints. Several reports were conducted on *in vitro* callus induction using different explants such as leaves, hypocotyls and roots[22], hypocotyls and nodal explants[23]. Also using different media to induce calli from different explants then determined the shoots and roots regeneration capacity of formed calli[24]. Other investigations achieved multiple shoots production on the different media[23,25,26]. Of these studies, there is no investigation is found to explore the existence of glucosinolates in the *in vitro* callus cultures or the *in vitro* plants. So, in this study, callus cultures of *Lepidium sativum* were induced from different explants and on MS-medium containing different growth regulators. Also, the production of callus was studied to obtain the mass production of callus cultures. Then the presence of glucosinolates in the extracts of the *in vitro* callus tissues was investigated using GC-MS. Also, study of the biological effect of these extracts on three human carcinoma cell lines was achieved.

MATERIALS AND METHODS

Plant material and explants preparation: Seeds of *L. sativum* were immersed in 70% ethanol for 2-3 min then, rinsed three times in sterile distilled water. The seeds were then sterilized for 30 min in 20% commercial Clorox (5% NaOCl) containing 0.5% Tween 20. After rinsing three times with sterile distilled water, seeds were cultured on MS medium[27] containing 3% (w/v) sucrose and solidified with 0.7% (w/v) agar. Culture medium was adjusted to pH 5.8. The seeds were incubated in a culture room at 26±2°C and were kept under 16 h photoperiod of fluorescent, 45 µmol cool white light tubes and 8 h dark.

***In vitro* callus culture induction:** Explants of cotyledon and hypocotyls were separated from 15 days old seedlings, while leaf and root explants were taken from 30 days old seedlings. All separated explants were cultured on MS-solid medium containing different combinations of 2,4-Dichlorophenoxy acetic acid (2,4-D)+Kinetin (Kin). The concentrations of two growth regulators were 2,4-D (0.1, 0.2 and 0.3 mg L^{-1})+Kin (0.1 and 0.3 mg L^{-1}). After 30 days of culturing, callus initiation frequency percentage were recorded and calculated based on the following equation:

$$\text{Callus initiation frequency (\%)} = \frac{\text{No. of initiated calli}}{\text{No. of inculated explants}} \times 100$$

***In vitro* callus maintenance and production:** This step aim to establish a protocol for callus production to utilized in getting the active components from callus cultures. About

0.5 g of induced calli from different explants were weighted and transferred to the media containing different growth regulators, which are A (1.0 mg L^{-1} Naphthalene acetic acid, NAA), B (1.0 mg L^{-1} NAA+0.5 mg L^{-1} Benzyl amino purine, BAP) and C (1 mg L^{-1} NAA+1.0 mg L^{-1} BAP), D (1.0 mg L^{-1} 2,4-D), E (1.0 mg L^{-1} 2,4-D+0.5 mg L^{-1} kin) and F (1.0 mg L^{-1} 2,4-D+1.0 mg L^{-1} kin). After 30 days of culturing three for each treatment (callus from different explants on the different media) were weighted and recorded to detect the best medium for callus production.

Callus growth index: The growth characterization of callus culture and their optimal time of sub-culturing were studied. Therefore, 0.2 g of callus from different explants which formed in the previous experiment were transferred to the best combination medium (1.0 mg L^{-1} NAA+0.5 mg L^{-1} BAP). Average fresh weight (g) was recorded weekly for 4 weeks, then growth index was calculated according to the following equation[28]:

$$\text{Growth index} = \frac{\text{Final callus fresh weight} - \text{Initial callus fresh weight}}{\text{Initial callus fresh weight}}$$

Extraction method and determination analysis

Extracts preparation: Extraction method of glucosinolates derivatives were carried out according to Al-Gendy and Lockwood[29] with slight modification as follow: different callus tissues from different explants were oven-dried for 24 h at 65°C. After drying, 2.0 g of each callus and seeds of *L. sativum* were grounded into the powdered materials. All samples were hydrolyzed by 100 mL of 2N HCL in the soxhlet apparatus for 4 h. The extracts were collected after filtration using whatman No.1 filter paper and transferred to 500 mL conical flasks. About 200 mL of dichloromethane (CH_2Cl_2) were added to the aqueous layer (HCL layer). The CH_2Cl_2 and aqueous layers were separated by separate funnel. The CH_2Cl_2 layer for each sample was collected and evaporated by rotary evaporator, then subjected to Gas Chromatography-Mass spectrometry (GC-MS) analysis.

Gas chromatography-mass spectrometric analysis: The dichloromethane extracts of seeds and different callus cultures were analyzed in National Research Center using Gas Chromatography-Mass Spectrometry with the following specifications. For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The injector and MS transfer line temperature was set at 280°C. The quantification of all the identified components was investigated using a relative peak area (%). A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC-MS system.

Cell lines: Human breast carcinoma (MCF-7 cell line), human lung carcinoma (A-549 cell line), colon carcinoma (HCT-116 cell line) were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden.

Cell culture: The procedure was done in a sterile area using a laminar air flow cabinet biosafety class II level. Culture was maintained in DMEM medium with 1% antibiotic-antimycotic mixture (10,000 U mL^{-1} potassium penicillin, 10,000 µg mL^{-1} streptomycin sulfate and 25 µg mL^{-1} amphotericin B), 1% L-glutamine and supplemented with 10% heat inactivated fetal bovine serum. Culturing and sub-culturing were carried out according to Thabrew *et al.*[30]. Doxorubicin was used as a positive control. A negative control composed of DMSO was also used.

Cell viability assay: This was done according to Selim *et al.*[31] as described by Ohkawa *et al.*[32]. The cells were seeded at concentration of 10×10^3 cells per well in case of MCF-7, 20×10^3 cells/well in a fresh complete growth medium in case of HCT-116 and A-549. Extracts added to give a final concentration of 100 µg mL^{-1}. After 48 h incubation, the medium was aspirated and then 40 µL MTT salt (2.5 mg mL^{-1}) were added and incubated for 4 h at 37°C under 5% CO_2. About 10% sodium dodecyl sulphate (SDS) was added to terminate the reaction. The absorbance was measured at 595 nm and a reference wavelength of 690 nm. Cell viability was assessed according to the mitochondrial-dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) to purple formazan.

Determination of thiobarbituric acid-reactive substances (TBARS): Lipid peroxidation was assessed by the thiobarbituric acid reactive substance (TBARS) assay, which detects mainly malondialdehyde (MDA), an end product of the peroxidation of polyunsaturated fatty acids and related esters. The MCF-7 cells were plated into a 96 well plate at a density of 1.0×10^5 cells/well. After 24 h exposure to CRE the cells

were washed with ice-cold PBS and homogenized. The cell homogenates were used in the TBARS assay. Briefly, the MDA-TBA adduct formed by the reaction of MDA with TBA under high temperature (90-100°C) and acidic condition. After cooling to room temperature the absorbance was measured calorimetry[33] at 532 nm.

Statistical analysis: The experiments were carried out in triplicate and the results were expressed as means±standard errors.

RESULTS AND DISCUSSION

Callus induction: Different explants (leaf, root, cotyledon and hypocotyls) from germinated seeds were examined for callus induction under the effect of different concentrations of 2,4-D and kin. Callus formation from different explants was observed after 4 weeks of inoculation in all tested media with green yellowish color (Fig. 1).

Data in Table 1 elucidated that, in all tested media, leaf and root explants gave the highest callus initiation frequency

Table 1: Effect of different concentration of 2,4-D and kin on callus initiation frequency of different explants from *L. sativum* L. after 4 weeks of cultivation

	Growth regulators concentrations (mg L^{-1})			
Medium	2,4-D	Kin	Explants	Callus initiation frequency (%)
T1	0.1	0.1	Cotyledon	80
			Hypocotyls	70
			Root	100
			Leaf	100
T2	0.2	0.1	Cotyledon	60
			Hypocotyls	70
			Root	100
			Leaf	100
T3	0.3	0.1	Cotyledon	60
			Hypocotyls	70
			Root	100
			Leaf	100
T4	0.3	0.3	Cotyledon	50
			Hypocotyls	60
			Root	80
			Leaf	80

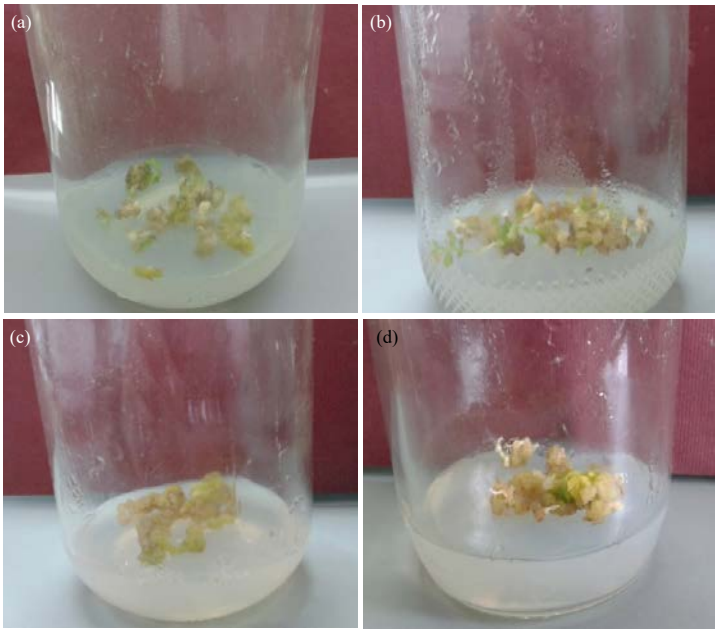

Fig. 1(a-d): Callus initiation of *L. sativum* L. from, (a) Cotyledon, (b) Hypocotyls, (c) Root and (d) Leaf explants on MS-medium containing 0.1 mg L^{-1} 2,4-D+0.1 mg L^{-1} kin

compared with other explants, they recorded 100% in all tested media except T4 medium decreased to 80%. While cotyledon explant was more responsive in the T1 medium (80%) and less responsive in other tested media. With hypocotyls explants, callus initiation frequency also was highest in all tested media except T4 medium it was declined to 60%. Otherwise, it was found that, when kin concentration was increased to 0.3 mg L^{-1} in T4 medium, there was a decrease in the percentage of callus formation in all tested explants, other three tested media can be considered suitable for callus formation. These results can be summarized as follows, leaf and root explants were the most responsive in all tested media. Also T1 medium achieved maximum callus initiation frequency (%) for all explants compared with the other medium.

The effects of plant growth regulators and explants type were studied for achieved and development a protocol for callus induction of garden cress (*L. sativum* L.)[22]. They used three explants (leaf, hypocotyls and root) to induce callus from *L. sativum*, cultured on MS-medium containing different concentrations of 2,4-D and NAA. Leaf explants were the most responsive on MS medium containing 2.0 mg L^{-1} 2,4D. This finding to some extent agreement with present studies, while leaf and root explants were more responsive in all used media.

Shoot tip, cotyledonary leaf and internodes explants were used for shoots proliferation of *L. sativum*[25]. They reported that 100% callus formation occurred on the medium containing 2,4-D and kin by using shoot tip and cotyledonary leaves.

Identical results were obtained by Sandhya *et al.*[26], where callus formation was observed on the medium containing 0.1 mg L^{-1} 2,4-D and 0.1 mg L^{-1} kin with the percentage of 87.23% by using shoot tip explants.

Calls of *L. sativum* L. was also produced by using other growth regulators (NAA and BAP) from shoot apex[23].

Callus production and maintenance: In this experiment, different media with various growth regulators were tested to find the best medium that gives highest callus fresh weight from different explants after 4 weeks of cultivation. The NAA alone and combination with BAP as well as 2,4-D alone and combination with kin were used to achieve this goal. Data in Table 2 revealed that, when 0.5 mg L^{-1} BAP was added to 1.0 mg L^{-1} NAA, the callus fresh weights gave the maximum values from different explants compared to other used media. This medium (1.0 mg L^{-1} NAA+0.5 mg L^{-1} BAP) achieved callus fresh weights from different explants higher than the medium which contained 1.0 mg L^{-1} NAA alone and when BAP concentration was increased to 1.0 with 1.0 mg L^{-1} NAA addition, the callus fresh weights from different explants were decreased. This is possible a means that, the addition of BAP to NAA is important for callus production obtaining but not more than 0.5 mg L^{-1}. Also, it was found that, the media which contain 2,4-D alone or combination with kin gave callus fresh weights less than the supplemented

Table 2: Effect of different callus production media on callus weights resulted from different explants of *L. sativum* L. after 4 weeks

Media	Explants	Callus weights (g)
1.0 mg L^{-1} NAA	Root	0.92±0.10
	Leaf	0.69±0.30
	Cotyledon	1.05±0.05
	Hypocotyls	1.20±0.04
1.0 mg L^{-1} NAA+0.5 mg L^{-1} BAP	Root	1.35±0.10
	Leaf	1.31±0.10
	Cotyledon	1.56±0.30
	Hypocotyls	1.32±0.04
1.0 mg L^{-1} NAA+1.0 mg L^{-1} BAP	Root	0.73±0.09
	Leaf	0.76±0.03
	Cotyledon	0.82±0.04
	Hypocotyls	0.85±0.01
1.0 mg L^{-1} 2,4-D	Root	0.51±0.10
	Leaf	0.61±0.04
	Cotyledon	0.72±0.04
	Hypocotyls	0.69±0.04
1.0 mg L^{-1} 2,4-D+0.5 mg L^{-1} kin	Root	0.61±0.06
	Leaf	0.65±0.06
	Cotyledon	0.70±0.04
	Hypocotyls	0.69±0.02
1.0 mg L^{-1} 2,4-D+1.0 mg L^{-1} kin	Root	0.54±0.07
	Leaf	0.54±0.04
	Cotyledon	0.63±0.02
	Hypocotyls	0.61±0.02

Mean±SD

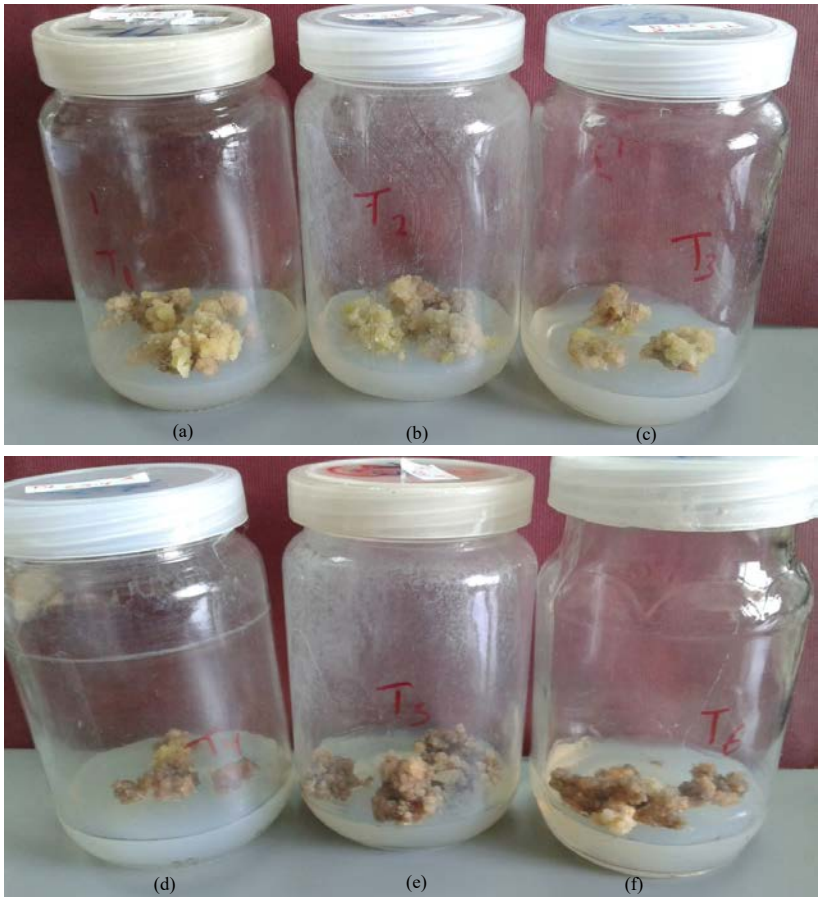

Fig. 2(a-f): Callus cultures from root explants of *L. sativum* grown on the media, (a) 1.0 mg L^{-1} NAA, (b) 1.0 mg L^{-1} NAA+0.5 mg L^{-1} BAP, (c) 1.0 mg L^{-1} NAA+1.0 mg L^{-1} BAP, (d) 1.0 mg L^{-1} 2,4-D, (e) 1.0 mg L^{-1} 2,4-D+0.5 mg L^{-1} kin and (f) 1.0 mg L^{-1} 2,4-D+1.0 mg L^{-1} kin

media with NAA alone or combination with BAP. It is also worth noting that, the media which contained NAA alone or combination with BAP produced friable callus with yellowish color, whereas browning callus was observed with the media which contained 2,4-D alone or combination with kin (Fig. 2 a-f). From these result, it can be concluded that, the medium which contained 1.0 mg L^{-1} NAA+0.5 mg L^{-1} BAP was the most convenient medium for callus growth from different explants and it used for callus production and maintenance.

Plant growth regulators play an important role in the *in vitro* callus induction as well as callus production, because each step required different combination and concentration of plant growth regulators.

In this area, callus production of *L. sativum* observed on the media which contained NAA and BAP by using various explants[23]. They reported that, there were a significant differences in the callus production due to the different concentrations of NAA and BAP. This finding in a certain degree with our results. In the other finding, the effect of different growth regulators on callus fresh weights of *L. sativum* were studied by Varma *et al.*[24]. It was found that, callus fresh weight was less in the media which contained 2,4-D alone or 2,4-D + kinetin compared to the medium that contain 2,4-D+kinetin+BAP. Also, the highest callus fresh weight from leaf explants of *L. sativum* was obtained in the medium containing[22] 2.0 mg L^{-1} 2,4-D.

Callus growth index: Callus growth dynamic is an efficacious method enable us to know the average time of sub-culturing. Cells have high ability for growth and division for a specified period. After this time, they enter the stationary phase in which its ability to divide is reduced and then entre the cell differentiation stage[34]. Therefore, this tool enables us to keep the cells in an active state of division.

From the previous experiment, the following was concluded, the medium which contained 1.0 mg L^{-1} NAA+

Table 3: Growth index of *L. sativum* callus resulted from different explants on medium contained 1.0 mg L^{-1} NAA+0.5 mg L^{-1} BA for 4 weeks

Callus from different explants	Weeks	Callus fresh weights	Callus growth index
Root	0	0.20	
	1	0.56±0.01	1.80
	2	0.70±0.05	2.50
	3	1.29±0.03	5.45
	4	0.92±0.04	3.60
Leaf	1	0.52±0.05	1.60
	2	0.68±0.10	2.40
	3	1.07±0.10	4.35
	4	0.98±0.01	3.90
Cotyledon	1	0.49±0.01	1.45
	2	0.61±0.03	2.05
	3	1.20±0.10	5.00
	4	0.52±0.04	1.60
Hypocotyls	1	0.44±0.05	1.20
	2	0.97±0.01	3.85
	3	1.09±0.05	4.45
	4	0.62±0.09	2.10

Mean±SD

Table 4: Total relative percentage of sulfur compound, nitrogen compounds and sulfur-nitrogen compounds in the seeds and different callus extracts of *L. sativum*

	Total relative (%)				
Chemicals	SE	CRE	CHE	CCE	CLE
Sulfur compounds	6.73	0.55	1.84	2.74	1.48
Nitrogen compounds	22.53	6.41	2.95	8.58	30.00
Sulfur nitrogen compounds			2.32	1.00	1.22

SE: Seeds extract, CRE: Callus root extract, CHE: Callus hypocotyls extract, CCE: Callus cotyledon extract, CLE: Callus leaf extract

0.5 mg L^{-1} BAP was the best to obtain high callus fresh weights from all studied explants. Therefore, this media was used for callus production as well as callus growth index evaluation.

Table 3 indicated that, callus fresh weights from all explants increased gradually until the 3rd week then decreased in the 4th week of cultivation. Thus, the callus of *L. sativum* sub-cultured every 3 week intervals to maintain the cells in the state of division and obtain mass production. Maximum callus fresh weight (1.29 g) and callus growth index (5.45) recorded from root explants followed by callus from cotyledon explant recorded 1.20 g and 5.00, respectively, then come the next callus from hypocotyls and leaf explants.

GC-MS of glucosinolates (GLS) compounds: This part of study was conducted to investigate the possibility of synthesis glucosinolates compounds in the *in vitro* callus tissue of *L. sativum*.

Identification of GLS hydrolysis products was based on Wiley and Nist library databases Glucosinolates are sulfur and nitrogen-containing plant secondary metabolites common in the *Brassicaceae* and related plant families[35]. Thus knowing the proportion of these components is essential to the formation of glucosinolates compounds.

The data in Table 4 shows that, maximum total relative percentage of sulfur compounds (6.73%) was detected in the seeds extract, while the minimum was in the callus of root extract (0.55%). On the other hands maximum total relative (%) of nitrogen compounds was found in the callus leaf extract (30%) followed by seeds extracts (22.53%). Whilst the minimum was in hypocotyls callus extract (2.95%). Sulfur-nitrogen compounds found only in the three extracts (hypocotyls callus extract, cotyledon callus extract and leaf callus extract) with small percentage.

The formation of nitriles and glucosinolate hydrolysis products occurred through plant tissue damage by proteins and therefore; depend on the presence of myrosinase[36,37].

Data in Table 5 revealed 13 compounds of glucosinolate hydrolysis products detected by GC-MS analysis of *in vitro* callus tissues and seeds of *L. sativum*. Maximum total relative percentage of GLS hydrolysis products was detected in hypocotyls callus extract (10.3%) followed by seeds extract (7.91%). While minimum total relative (%) was found with root callus extract (1.23%). N-Cyano carbazole was found in the hypocotyls callus extract with maximum relative percentage represents 6.51% followed by 1,1-dicyano-2-phenyl propene (6.27%) in the seeds extract. While 2, 3-Dicyano-succinonitrilewas found in the hypocotyls callus extract with minimum relative percentage represents 0.16%.

It is worth noting that, detected cyanide (nitrile) components appeared in all studied extracts, unlike thiocyanate compounds which appeared in the callus of root and leaf extracts. Two thiocyanate compounds were identified are Isobornylthio-cyanoacetate in the callus root extract and Androst-4-en-11-ol-3,17-dione, 9-thio-cyanate in the callus leaf extract.

Glucosinolates are the major secondary products of *L. sativum*, which degraded to benzyl thiocyanate and benzyl isocyocyanates[38]. Numerous studies reported that, *L. sativum* plant extracts considered as chemopreventive and chemotherapeutic agents[39].

The glucosinolates hydrolysis and identification of thiocyanate-forming protein in *L. sativum* was studied[37]. They showed that, thiocyanate formation may be modulated by the presence of one or several supplementary protein factors that influence glucosinolate hydrolysis. Also, the ability to form thiocyanate is bound with seedlings, flowers and seeds of *L. sativum*.

Another research studied the glucosinolates of *L. sativum* and their biological activity[1]. They investigated that, two glucosinolates compounds (glucotropaeolin and gluconasturin) were found in the seeds. On the other hand, four glucosinolates were isolated from the fresh herb and were identified as 2-ethyl butyl glucosinolate, methyl glucosinolate (glucocapparin), butyl glucosinolate and glucotropaeolin. Also, the hepatoprotection activity of the herb different extracts was studied using hepatocytes monolayer culture from rat and concluded that both the petroleum ether and the 80% ethanolic extracts had hepatoprotective effect.

Else research studied the effects of cultivar and developmental stage on glucosinolates accumulation of *L. sativum* L. They showed that, the major glucosinolates compound was glucotropaeolin of the benzyl group. The concentration of glucotropaeolin varied between 1.79-4.57 μmol g^{-1} DW according to the cultivar and developmental stage of the plants[40]. The current results demonstrated that, glucosinolates compounds synthesized in the callus tissues of *L. sativum*. In the future research, it should aim to increase these compounds and benefit from them as they are known to be anti-cancer activity.

Cytotoxic activity of different *L. sativum* extracts on human carcinoma cell lines: Regardless of the scientific revolution, cancer still threaten the life of cancer patients worldwide. Searching for a promising candidate to fight this deadly disease still capture the interest of the researcher all over the world.

The present study aims to study the cytotoxic potentiality of different *L. sativum* extracts (seeds and different callus tissues) over 3 human carcinoma cell lines (MCF-7, A-549 and HCT-116). From the results in Fig. 3, it is observed that, callus root extract possessed the most potent cytotoxic activity

Table 5: Glucosinolates hydrolysis products of *in vitro* callus tissues and seeds of *L. sativum* L.

		Relative (%)				
GLS hydrolysis products	Molecular formula	SE	CRE	CHE	CCE	CLE
3-(2-Oxopropyl) 4- phenyl 1,1,2,2-cyclo butane tetracarbonitrile	$C_{17}H_{12}N_4O$	0.76				
3,3-Bis- benzylsulfanyl-2-fluoroacrylonitrile	$C_{17}H_{14}FNS_2$	0.88				
1,1-dicyano-2-phenyl propene	$C_{11}H_8N_2$	6.27				
p-Tolylisocyanide	C_8H_7N		0.80			
Isobornylthiocyanoacetate	$C_{13}H_{19}NO_2S$		0.43			
2-Cyano-4,5-diphenyl-1-methylimidazole	$C_{17}H_{13}N_3$			0.38		
Hexadecanenitrile	$C_{16}H_{29}N$			1.05	3.58	
Oleanitrile	$C_{18}H_{33}N$			2.20	3.48	
N-Cyanocarbazole	$C_{13}H_8N_2$			6.51		
2,3-Dicyano-succinonitrile	$C_6H_2N_4$			0.16		
Dicarbadodecaborane-c,c'-bis-(propanenitrile)	$C_8H_{18}B_{10}N_2$					0.69
Androst-4-en-11-ol-3,17-dione, 9- thiocyanato	$C_{20}H_{25}NO_3S$					0.51
Methyl 3-(3'-cyano methyl) thioureido thiophene-2-carboxylate	$C_9H_9N_3O_2S_2$					0.24
Total relative (%)		7.91	1.23	10.3	7.06	1.44

SE: Seeds extract, CRE: Callus root extract, CHE: Callus hypocotyls extract, CCE: Callus cotyledon extract, CLE: Callus leaf extract

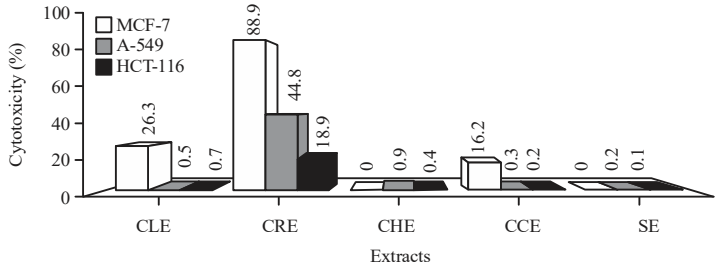

Fig. 3: Percentage of cytotoxic activity of different *L. sativum* extracts on three human carcinoma cell lines (MCF-7, A-549 and HCT-116) at 100 μg mL^{-1}

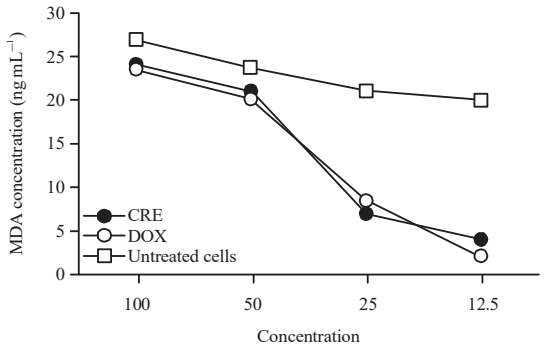

Fig. 4: MDA concentration in MCF-7 treated with callus root extract in comparison with reference drug and untreated drug

over MCF-7 with 88.9% of cytotoxicity and moderate activity on A-549. While callus leaf extract and callus cotyledon extract gave week activity on MCF-7 with 26.3 and 16.2%, respectively. On the other hand, all studied extracts gave week effect on the other two cell lines A-549 and HCT-116.

It was previously mentioned that ROS induced by cisplatin could increase lipid peroxidation, which alters structural proteins and generate apoptotic cascades[41]. Malondialdehyde (MDA) is an aldehyde produced by lipid peroxidation of polyunsaturated fatty acids which consider useful marker for oxidative stress.

Thiobarbituric Acid Reactive Substances (TBARS) is a well-established assay for screening and monitoring lipid peroxidation cells. Where malondialdehyde forms the adduct with two thiobarbituric acid molecules to produce a pink color. Induction of lipid peroxidation inside cells leads to changes in the permeability and fluidity of the membrane lipid which dramatically alter cell integrity[42].

The MDA level in MCF-7 treated with CRE was studied and compare it with untreated MCF-7 and MCF-7 treated with reference drug Doxorubicin.

From the data in Fig. 4, it is clear that, callus root extract induces lipid peroxidation and increase the concentration of MDA in comparison with control cells, suggesting the root extract represent a good candidate as anti-cancer.

CONCLUSION

The data of the present study be able to utilized to develop the protocol of callus culture production and maintenance of *L. sativum*. Glucosinolates hydrolysis products were found in all studied calli derived from different explants. This indicates the possibilities of synthesis these compounds in the callus tissues, enabling us to increase and produce the glucosinolates in callus culture of *L. sativum*. Callus root extract was the most potent selective cytotoxic effect on breast cancer cell lines and weak activity on the other remaining two (colon and lung) cell lines. Also, this extract cause lipid peroxidation inside breast cancer cell line which explains their cytotoxic effect on MCF-7. Further studies should be performed to benefit of the important glucosinolates compounds found in *L. sativum*, which are carried out continuously and quickly using plant tissue culture techniques.

SIGNIFICANCE STATEMENT

The glucosinolates compounds are important as chemoprotective agents and in this study it proved their ability against breast cancer cells. Therefore; find a way to produce them *in vitro* is the best suited alternative and promising method.

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