

CALLUS PRODUCTION OF GLOBE ARTICHOKE AND MILK THISTLE: *IN VITRO* HYPOLIPIDEMIC AND ANTIOXIDANT ACTIVITIES

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ABSTRACT

The goals of this study were to develop an *in vitro* callus growth system of globe artichoke and milk thistle plants and investigate their hypolipidemic and antioxidant activities. For callus induction, leaf explants were cultured on MS medium supplemented with 5 mg/l NAA + 2 mg/l Kin + 0.1 mg/l GA₃. For solidifying culture medium, gelrite showed high growth of callus in comparison to agar. The effect of picloram added to culture medium on development and growth of callus was examined. Addition of 3 mg/l picloram to culture medium registered the best results of calli growth of the two plants presented as fresh weight and growth value. The influence of salicylic acid (SA) on

growth parameters of calli of the two plants was investigated. The most effective level of SA was 75 μ M in which the highest callus fresh weight and dry matter were registered. Otherwise, effect of the different concentrations of jasmonic acid (JA) on fresh mass and dry matter of both two plants species was tested. Among the tested concentrations of JA, 50 μ M was more suitable for callus growth. However, it was noticed that, callus of milk thistle was more positive responding for exogenous application of both SA and JA compared with callus of globe artichoke. *In vitro* hypolipidemic effects of extracts of globe artichokes and Milk thistle callus cultures were studied. Extracts of calli of both plants showed hypolipidemic viability effects in a dose-dependent manner. In addition, globe artichoke and milk thistle extracts recorded potent antioxidant effects as compared to their standard. There were significant differences between extracts of calli and standards at the low levels groups (0.01, 0.1, 1.0 mg/l). Moreover, globe artichoke extract showed more potent hypolipidaemic and antioxidant effects than the milk thistles extract.

Keyword: globe artichoke; milk thistle; hypolipidemic; antioxidant.

INTRODUCTION

Globe artichoke (*Cynara scolymus* L.) and milk thistle (*Silybum marianum* L.) plants are considered important medicinal herbs producing active ingredients agents treat liver diseases. Extensive studies on the chemical components of the artichoke have revealed it to be a rich source of the polyphenol compounds, with mono- and dicaffeoylquinic acids and flavonoids as the major chemical components [1] Adzet & Puigmacia [2] Wagenbreth [3]. Cynarin (1, 5-di-O-caffeoylquinic acid), the main active compounds of artichoke has several pharmaceutical actions such as liver protection and re-growth and promotion of liver cell [4,5]. Moreover, synthetic cynarin preparations were used as a drug to stimulate the liver and gallbladder. Clinical trials investigating the use of globe artichoke powder and cynarin in treatment of hyperlipidaemia generally reports positive results; the benefits of such hepatoprotective and hepatoregenerating activity have been documented to cynarin *in vitro* and in animals [6]. Otherwise, milk thistle contains isomeric mixture of flavonolignans, including silychristin, silydianin, silybin and isosilybin, collectively known as silymarin [7]. Silymarin of milk thistle was commonly used to treat hepatitis and liver damage [8]. In this concern, *in vitro* and animal studies have demonstrated the hepatoprotective properties of silymarin or silybin [9,10]

Drugs that decrease cholesterol, such as fibrates and bile acid sequestrants, have been used for several decades, but their adverse effects led to the introduction of statins; β -hydroxy- β -methylglutaryl coenzyme A reductase (HMG CoA) inhibitors [11]. In view of the adverse effects associated with synthetic lipid-lowering drugs, the quest for natural products with lipid-lowering potential and minimal or no side effects is warranted [12]. The production of the useful natural components from globe artichoke and milk thistle by the conventional agricultural methods are met with several problems. The seasonal production, diseases, handling and storage prevent offering such demand compounds to pharmaceutical factories. In the search for alternatives to production of the desirable medicinal compounds from globe artichoke and milk thistle, biotechnological approaches, specifically plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production [13]. Tissue and cell cultures are used for the large scale culturing of plant cells from which biologically active agents are extracted. The principal advantage of this technology is that it ultimately provides a continuous, reliable source of active agent year-round. Additional

advantages of such processes include controlled production according to demand and a reduced requirement. Moreover, several products were found to be accumulating in cultured cells at a higher level than those in native plants through optimization of cultural conditions. Manipulation of physical aspects and nutritional elements in a culture is perhaps the most fundamental approach for optimization of culture productivity.

Tissue culture systems have been developed for micropropagation of globe artichoke varieties for mass propagation and virus elimination purposes [14-16]. Otherwise, callus formation in globe artichoke has been reported [17-19]. On other side, conditions of *in vitro* culture for callus production of milk thistle has been studied [20, 21]. Recently, an applicable protocol for *in vitro* morphogenesis and hairy root cultures of milk thistle was recognized [22]. The goals of the current study were optimize calli tissue culture systems for globe artichoke and milk thistle through investigate the addition of picloram, salicylic and jasmonic acids to culture medium and examine the hypolipidaemic and antioxidant effects of extracts through *in vitro* inhibition of β -hydroxy- β -methylglutaryl coenzyme A reductase and DPPH free radicals scavenging activity.

MATERIALS AND METHODS

Establishment of *in vitro* cultures

Seeds of globe artichoke and milk thistle (local varieties) were washed with distilled water and then immersed in 70 % ethanol for 1 min followed by 50 % Clorox (5.25 % sodium hypochlorite) for 20 min and finally washed three times with sterilized distilled water. The disinfected seeds were placed in jars containing 50 ml of MS-basal medium [23]. For callus induction, leaf explants were excised from aseptically grown seedlings and cultured on MS medium supplemented with 5 mg/l NAA + 2 mg/l Kin + 0.1 mg/l GA₃ according to Bekheet et al. [22]. Leaf derived calli of the two plant species were subcultured twice onto same freshly prepared medium (callus induction medium) to obtain stock of calli.

Effect of gelling agent on callus growth

In this experiment, two types of gelling agents (agar and gelrite) in two levels were investigated. Agar was added to culture medium in 6 and 7 g/l while gelrite was 2 and 3 g/l. Fresh weight and growth value of callus were determined after five weeks of sub-culturing.

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

Effect of picloram on callus growth

To assess the effect of picloram (4-amino-3,5,6-trichloropicolinic acid) on callus growth of globe artichoke and milk thistle, about 250 mg of callus tissue were sub-cultured on callus growth medium (MS + 5 mg/l NAA + 2 mg/l Kin + 0.1 mg/l GA₃) supplemented with 1, 2, 3 and 4 mg/l of picloram. Fresh weight and growth value were determined after five weeks of sub-culturing.

Effect of salicylic acid on callus growth

Salicylic acid (SA) was dissolved in distilled water and sterilized by autoclaving at 120°C and 1 for 20 min and used as an elicitor solution. SA was added to culture medium in final concentrations of 0, 25, 50, 75 and 100 µM. Fresh weights and dry matter of callus were recorded after five weeks of sub-culturing.

$$\text{Dry matter} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Effect of jasmonic acid on callus growth

Jasmonic acid (JA) was prepared in ethanol and stock was completed with distilled water. JA was added to culture medium in final concentrations of 0, 25, 50, 75 and 100 µM. Fresh weights and dry matter of callus were recorded after five weeks of sub-culturing.

Extraction of phenolic compounds

Air dried samples of both *in vitro* and *in vivo* grown globe artichoke and milk thistle (5g each) were used for phenolic compounds extraction. The samples were homogenized with 15 ml of 80% (v/v) methanol as the traditional method and then grinded in a mill with sieve holes of 1 mm diameter. Pulverized plant materials were extracted by percolation with 80% methanol for three successive times. The dry residue was resuspended in 3 ml of distilled water, extracted twice with methanol, filtered and desiccated under reduced pressure at 40°C. After filtration, the solvent was evaporated and the total methanolic extracts were concentrated under reduced pressure using rotavapour at 45°C.

In vitro hypolipidemic activity

Extracts were evaluated for their hypolipidaemic activity by estimation of β -hydroxy- β -methylglutaryl Coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34. [24]. The reaction mixture consisted of 40 unit HMG-CoA reductase, 0.15 µmol HMG-CoA substrate, 0.1 mL of the tested plant samples and 0.1 M potassium phosphate buffer (3.5 mM EDTA, 10 mM

dithiothreitol, 0.1 g/l bovine serum albumin and 0.30 μmol NADPH). Incubation at 37 °C for 5 min took place and the decrease in absorbance due to the oxidation of NADPH to NADP was measured at 340 nm after 1–2 min.

Enzyme activity $\mu\text{mol}/\text{mg protein} =$

$$\frac{\Delta A}{E} \times \frac{1}{\text{mg dried extract}}$$

ΔA is the difference between absorbance measurements.

E = extinction coefficient of NADPH ($6.22 \times 10^{-3} \times \mu\text{mol}^{-1} \text{cm}^{-1}$).

In vitro antioxidant activity

Serial extract concentrations (10:100 g) were estimated by the method of Chen et al. [25], where DPPH free radicals react with plant antioxidants and the decrease in absorbance (A) of DPPH- was calculated in relation to absorbance of control as follows:

Percentage inhibition (IP) = $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$.

Incubation conditions and experimental analysis

Cultures were normally maintained at 25°C and 16 hr photoperiod provided by white fluorescent tubes (3000 Lux). Each experiment was set up as a separate completely randomized design. Data of tissue culture were statistically analyzed using standard error (SE) according to the method described by Snedecor and Cochran[26]. However, Data of hypolipidemic and antioxidant activity were expressed as mean \pm SD of six reading. Statistical analysis was carried out by one-way analysis of variance (ANOVA), Costat Software Computer Program. Significance difference between groups was at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of gelling agent on callus growth

Gelling agents are used for the *in vitro* cultures as a support whereby nutrient media are solidified. In this experiment, two types of gelling agents i.e., agar and gelrite in two different concentrations were examined for their effects on callus growth of both globe artichoke and milk thistle plants. The callus grown on medium gelled with gelrite generally showed higher growth and good quality in comparison to that grown on the same medium gelled with agar. The highest fresh weights (1.10, 1.15) and growth values (3.4, 3.6) of globe artichoke and milk thistle respectively were observed with 3 g/l gelrite (Table 1 & Fig. 1-A). It is noted that growth values of the two plant species were higher on the low concentration (6 g/l) of agar

compared with the high concentration (7 g/l). In general, it was remarkable that, milk thistle callus showed relatively higher growth values (fresh weight and growth value) compared with globe artichoke.

Most of published media were gelled with agar, but gelrite may be a better alternative. Our results indicated that the treatments in which the calli were grown with gelrite as gelling agent in the culture medium showed a higher occurrence of growth. This may be due to the diffusion of nutrients to callus in gelrite was higher resulting in higher nutrient availability and consequently, an increasing in growth compared with agar. The decrease in water potential resulting from the matric potential of agar limits uptake, since nutrient uptake is closely associated with the rate of water influx into tissues. The present results are parallel with those reported by Pravin et al. [27]. They mentioned that medium contained 3.0 g/l gelrite increased callus induction over the agar medium in *indica* rice varieties. In this respect, Cimino et al. [21] stated that the best culture medium for callus formation for biomass production of milk thistle as a potential source of milk clotting peptidases was B5 medium solidified with 2.5 g/l of phytigel and supplemented with 0.05 mg/l BA and 0.5 mg/l 2,4-D.

Effect of picloram on callus growth

The effect of picloram added to culture medium on development and growth of callus proliferated from leaf segments of globe artichoke and milk thistle are illustrated in Table (2). Results indicated that supplementation of culture medium with picloram generally enhanced callus growth values of the two plant species compared to the control (0.0 picloram). The data pointed out that, addition of 3 mg/l picloram to culture medium registered the best results of callus growth presented as fresh weight and growth value (Table 2 & Fig. 1-B). At this medium the growth value of milk thistle callus was 7.2, however, it was 6.8 with callus of globe artichoke. It was noticed that significant differences in callus growth within the two plant species have been observed and the growth dynamic of milk thistle callus was higher than that of globe artichoke.

Picloram (4-amino-3,5,6-trichloropicolinic acid) supports growth of tissue explants in culture, inhibits root growth, induces cell wall loosening, produces stem curvature and other formative effects, promotes loss of chlorophyll, and regulates abscission and rooting responses. Using picloram in plant tissue culture was rather neglected. In the present study, it was found that supplementation of culture medium with picloram generally enhanced callus

growth of both globe artichoke and milk thistle. It gave rise to the best responses towards callus growth, especially at the concentration of 3 m/l. These results seem to agree with the results of Hasanloo et al.[28] who reported that picloram had positive effects on growth and flavonolignan production in calli of milk thistle. In this connection, Bach & Pawlowska [29] proved that picloram could be used to induce embryogenic callus from leaf explants of *Gentiana pneumonanthe* in the medium containing 8 μ M. Also, picloram has been reported to be successful in Arecanut palm tissue culture in terms of callus and somatic embryo production.[30]. Moreover, Ahmed et al. [31] mentioned that the best embryo callus induction of *Phyla nodiflora* (L.) Greene was obtained using medium contained 0.1 mg/l picloram. Furthermore, Castellar et al. [32] in their study on establishment of callus and cell suspension cultures of *Petiveria alliacea* L reported that cells grown in the presence of picloram entered the exponential phase after a lag phase of four weeks, with a 4-fold increase in fresh weight associated to approximately 2-fold increase in dry weight by the end of the 5th week.

Effect of salicylic acid on callus growth

The effect of salicylic acid (SA) on growth parameters of calli derived from leaf explants of both globe artichoke and milk thistle was examined. Our observations reveal that changes in callus growth and development were associated with the presence of SA in culture media. Callus appeared as a mass of cells with significant proliferative capacity, having a lax texture, not very compact (Fig. 1-C). The most effective level of SA was 75 μ M. At this level the highest callus fresh weights of globe artichoke (2.55 g) and milk thistle (2.85 g) as well as callus dry matters (10.58, 11.22) were registered (Table 3). It was noticed that, callus of milk thistle was generally more positive responding for exogenous application of SA compared with callus of globe artichoke.

Salicylic acid (SA) is a hormone- like substance that has important role in the regulation of plant growth and development [33]. Moreover, SA is essential in systemic acquired resistance induced by pathogen attack and leads to the synthesis of pathogenesis-related proteins. In the current study, significant increase of callus growth of the two plants presented as fresh weight and dry matter was observed with media supplemented with the different concentrations of SA. These positive results are probably associated to the fact that SA is a signaling molecule that plays an essential role in growth of plant cells. In this respect, Orenes et al [34] elucidated that the exogenous application of SA not only protects plants against stress, but

also enhances their growth and productivity. Moreover,[35] El-Mergawi & Abdel-Wahed [35] mentioned that the effect of SA on the physiological processes is variable depending on its concentration, plant species, developmental stages and environmental conditions. On other side, Khalili et al. [36] stated that elicitation with SA can be regulated the jasmonate pathway that may mediate the elicitor- induced accumulation of silymarin.

Effect of jasmonic acid on callus growth

Jasmonic acid (JA) and some of its derivatives possess various physiological activities when applied to plants. In this part of study, fresh weights and dry matter content of globe artichoke and milk thistle calli were determined after five weeks of sub-culturing in response to different concentrations of JA and presented in Table (4). Data reveal that fresh mass and dry matter of calli of both two plants was positively affected by addition of JA to culture medium. Among the tested concentrations of JA, 50 μ M was more suitable concentration for callus growth of both globe artichoke and milk thistle (Table 4 & Fig. 1-D). As in case of SA, callus of milk thistle was more responding to addition of JA compared with callus of globe artichoke. It is important here to mention that SA was more effective on enhancement of callus growth (Fresh mass and dry matter) of both globe artichoke and milk thistle compared with JA.

The effects of jasmonates on plant growth are varied and include storage organ formation, induction of plant defenses against biotic and abiotic stresses, and can interact with other hormone pathways, especially ethylene, to affect growth and development. Our results indicate that addition of JA to culture medium showed significant growth of calli of both globe artichoke and milk thistle. These results are in consistent with Ravinkar et al. [37] who reported that when JA is added to growth medium in concentrations up to 10 μ M, it significantly stimulates the elongation of axillary buds and cell division of stem node and protoplast cultures respectively of potato. In this respect, jasmonates are signal molecules in plant stress responses and are also important regulators of plant growth and development e.g. jasmonic acid stimulates cell division and enlargement [38, 39]. On the other hand, Mizukami et al. [40] elucidated that JA and its derivatives are involved in a part of the signal transduction pathway that induced particular enzymes catalyzing biochemical reactions for the synthesis of secondary metabolites.

In vitro hypolipidemic and antioxidant activity

The aim of this part of study was to investigate the *in vitro* hypolipidaemic and antioxidant effects of extracts of globe artichokes and milk thistle callus cultures. For hypolipidaemic activity, standards and crude extracts were tested at 0.01, 0.1, 1.0, 10 and 100 mg levels. Inhibition of β -hydroxy- β -methylglutaryl CoA (HMG-CoA) reductase was measured. Extracts of calli of both plants showed hypolipidaemic viability effects in a dose-dependent manner (Table 5 & Fig. 2). It was found that there are significant differences between extracts and standards at the low levels groups (0.01, 0.1, 1.0 mg). However at the high levels groups (10 and 100 mg), differences were not significant. Results also reveal that globe artichoke extract inhibited β -hydroxy- β -methylglutaryl coenzyme A reductase enzyme by 85.77% at 100 mg, while its standard recorded inhibition by 50.94%. On the other hand, milk thistles exhibited 69.86% at 100 mg plant concentration, while its standard recorded inhibition by 98.77%. Therefore, we can recommend that globe artichoke callus extract has more potent hypolipidemic effect than milk thistles extract.

For antioxidant activity, various concentrations (100, 200, 300, 400 and 500 μ g/ml of globe artichokes and milk thistle calli extracts and standards of their active ingredients i.e., cynarin and silymarin respectively were used for the scavenging activity of the DPPH free radicals. It was found that the degree of discoloration of the extract indicates the potential for binding free radicals present by the various extracts. Scavenging activity increased as increasing of extracts concentrations (Table 6). Globe artichoke and milk thistle calli extracts recorded potent antioxidant effects as compared to their standard. Moreover, globe artichoke callus extract showed more potent antioxidant effect than the milk thistles extract.

Hypolipidaemic effects of globe artichokes and milk thistle callus were *in vitro* determined using enzyme activity. Extracts of callus cultures of the two plant species recorded *in vitro* inhibition of HMG-CoA reductase which confirms accumulation of antioxidants in tissue cultures is similar in nature to those found in mother plants. These results are similar to those Fintelmann [41] who observed indirect inhibitory effects of artichoke extract exerted at the level of HMGCoA reductase, a key enzyme in cholesterol biosynthesis. In this concern, hypolipidaemic, hypocholesterolaemic and choleretic activities are well documented for artichoke leaf extracts and particularly for the constituent cynarin. Clinical trials investigating the use of globe artichoke and cynarin in the treatment of hyperlipidaemia report positive results [42, 43] mentioned that the antioxidant activity of artichoke has a close relationship

with the total flavonoid content. On the other side, several studies have shown that silymarin extracted from milk thistle, a flavonoid antioxidant, possesses a hypolipidemic effect [44-46]. The treatment with silymarin decreased the levels of plasma cholesterol and LDL-cholesterol in hyperlipidaemic rats [47]. Otherwise, the presence of different types of phenolic compounds in the callus cultures of milk thistle was reported Ahmed John & Koperuncholan [48].

Table 1: Fresh weights and growth values of globe artichoke and milk thistle calli grown for five weeks on callus induction medium contained two concentrations of agar and of gerlite.

Gelling agent	Globe artichoke		Milk thistle	
	Fresh weight (g)	Growth value	Fresh weight (g)	Growth value
Agar (6 g/l)	0.65 ± 0.04	1.6	0.80 ± 0.03	2.2
Agar (7 g/l)	0.60 ± 0.02	1.4	0.70 ± 0.02	1.8
Gerlite (2 g/l)	0.90 ± 0.04	2.6	0.95 ± 0.04	2.8
Gerlite (3 g/l)	1.10 ± 0.06	3.4	1.15 ± 0.08	3.6

± Standard Error (SE).

Table 2: Fresh weights and growth values of f globe artichoke and milk thistle calli grown for five weeks on callus induction medium contained various concentrations of picloram.

Picloram (mg/l)	Globe artichoke		Milk thistle	
	Fresh weight (g)	Growth value	Fresh weight (g)	Growth value
0.0	1.15 ± 0.22	3.6	1.25 ± 0.09	4.0
0.1	1.60 ± 0.30	5.4	1.65 ± 0.20	5.6
0.2	1.80 ± 0.14	6.2	1.90 ± 0.40	6.6
0.3	1.95 ± 0.50	6.8	2.05 ± 0.15	7.2
0.4	1.70 ± 0.24	5.8	1.85 ± 0.27	6.4

± Standard error (SE)

Table 3: Fresh weight and dry matter of globe artichoke and milk thistle calli in response to different concentrations of salicylic acid, after five weeks of culture.

Salicylic acid (μM)	Globe artichoke		Milk thistle	
	Fresh weight (g)	Dry matter (%)	Fresh weight (g)	Dry matter (%)
0.0	1.05 ± 0.12	10.19	1.10 ± 0.19	10.09
25	1.75 ± 0.20	10.28	1.85 ± 0.20	10.27
50	2.00 ± 0.15	10.50	2.20 ± 0.20	10.68
75	2.55 ± 0.30	10.58	2.85 ± 0.18	11.22
100	2.20 ± 0.20	10.45	2.10 ± 0.17	10.95

± Standard error (SE)

Table 4: Fresh weight and dry matter of globe artichoke and milk thistle calli in response to different concentrations of jasmonic acid, after five weeks of culture.

Jasmonic acid (μM)	Globe artichoke		Milk thistle	
	Fresh weight (g)	Dry matter (%)	Fresh weight (g)	Dry matter (%)
0.0	1.10 ± 0.10	10.09	1.15 ± 0.11	10.16
25	1.50 ± 0.21	10.66	1.60 ± 0.33	10.81
50	1.90 ± 0.15	11.05	1.95 ± 0.14	11.28
75	1.70 ± 0.15	10.58	1.80 ± 0.22	10.72
100	1.55 ± 0.34	10.32	1.65 ± 0.25	10.36

\pm Standard error (SE)

Table 5: *In vitro* hypolipidaemic effects of globe artichoke and milk thistle calli extracts and their standards i.e., cynarin and silymarin respectively.

Doses of extracts	Globe artichoke	Cynarin	Milk thistle	Silymarin	Control
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
100mg	0.255 ± 0.059^e (-85.77)	1.059 ± 0.178^d (-40.59)	0.540 ± 0.102^e (-69.61)	0.022 ± 0.005^c (-98.79)	1.792 ± 0.131^d (---)
10mg	2.373 ± 0.492^d (-53.81)	2.275 ± 0.371^{cd} (-55.72)	2.44 ± 0.314^d (-52.51)	0.164 ± 0.024^c (-96.80)	5.138 ± 0.508^d (---)
1mg	5.955 ± 2.69^c (-80.50)	3.755 ± 0.574^c (-87.70)	4.91 ± 0.702^c (-83.92)	1.794 ± 0.525^c (-94.13)	30.54 ± 2.51^c (---)
0.1mg	71.682 ± 2.87^b (-42.27)	59.84 ± 1.97^b (-51.81)	16.013 ± 0.91^b (-87.11)	12.968 ± 2.00^b (-89.55)	124.187 ± 2.69^b (---)
0.01mg	181.798 ± 2.24^a (-42.14)	81.82 ± 2.39^a (-73.96)	47.98 ± 1.18^a (-84.73)	112.62 ± 5.74^a (-64.162)	314.252 ± 11.4^a (---)

* Data are mean \pm SD of six values.

*Unshared letters are significance values between groups at $p < 0.0001$.

* Enzyme activity (β -hydroxy- β -methylglutaryl Coenzyme A reductase) is represented by $\mu\text{mole/mg}$ dried extract.

* Values between brackets are percentage of inhibition = $((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$

Table 6: DPPH free radical scavenging activity of globe artichoke and milk thistle calli extracts.

Concentration ($\mu\text{g/ml}$)	Inhibition (%)			
	Globe artichoke	Standard Cynarin	Milk thistle	Standard Silymarin
100	36.67	35.70	13.64	35.7
200	44.80	41.40	20.00	37.04
300	50.00	65.50	28.60	44.40
400	64.30	71.40	33.33	50.00
500	36.67	79.20	42.11	60.00

* Data are percentage of inhibition = $((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$

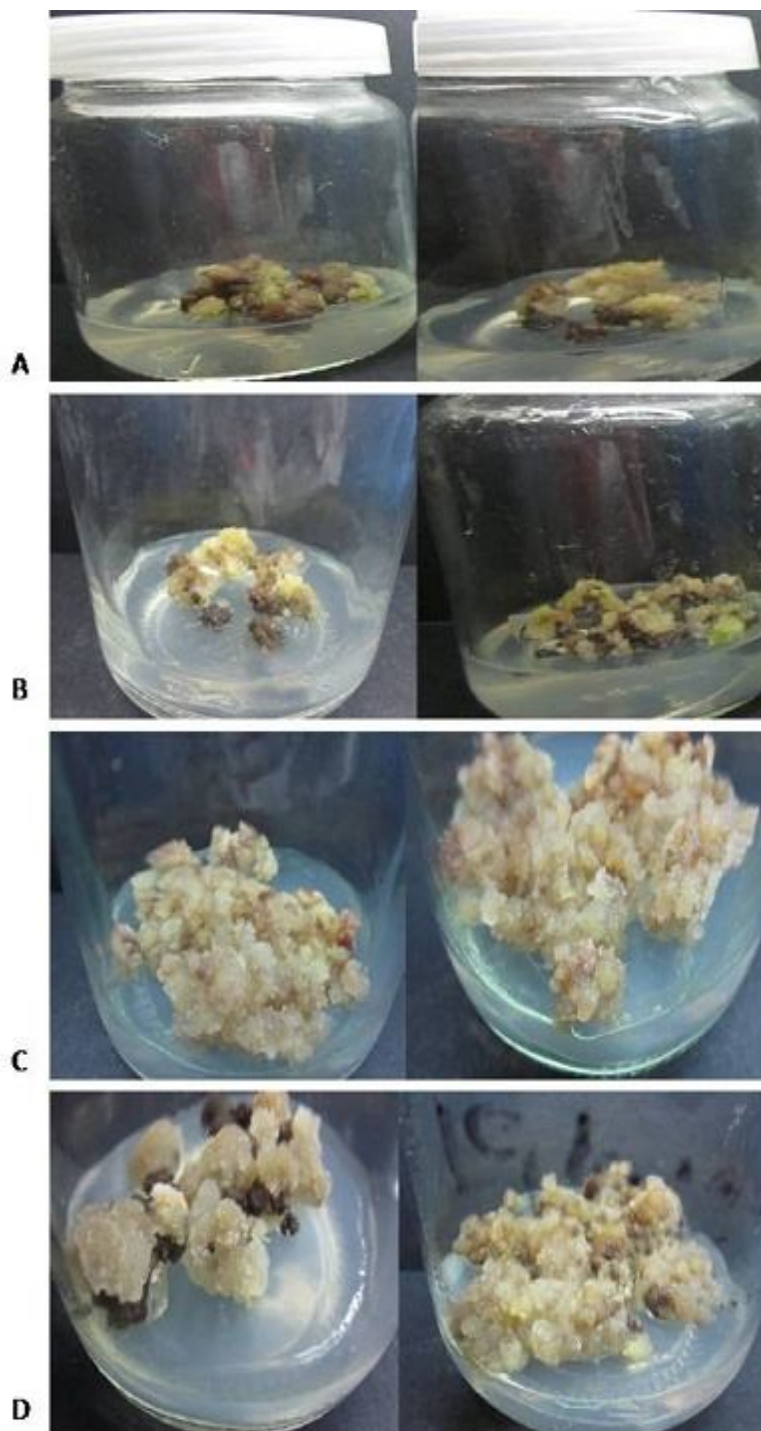


Fig. 1. Callus of globe artichoke (left) and milk thistle (right) grown on MS medium gelled with 3 g/l gerlite (A), contained 3 mg/l picloram (B), supplemented with 75 μ M salicylic acid (C) and 50 μ M jasmonic acid (D).

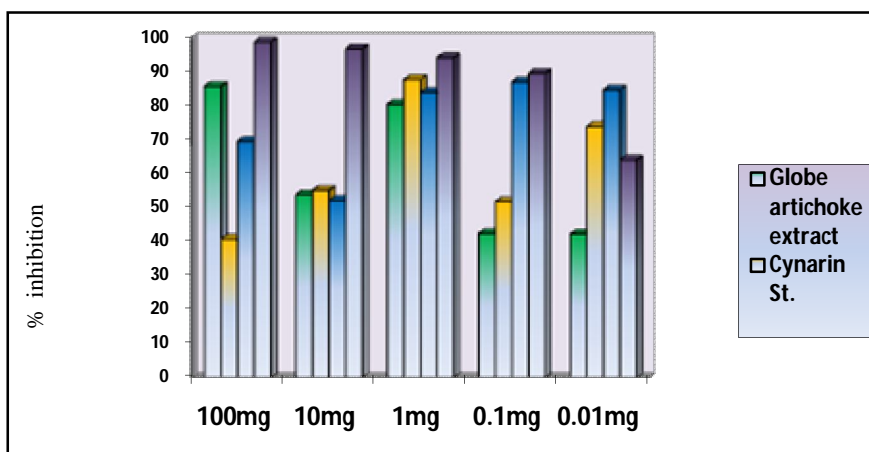


Fig. 2. Inhibition percentages of β -hydroxy- β -methylglutaryl Coenzyme A reductase after incubation with serial plant concentrations of globe artichoke and milk thistle extracts and cynarin and silymarin as standards.

Competing interests

The authors declare that they have no competing interests.

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