Enrichment of Therapeutically Significant Flavonolignans of *Silybum Marianum* in Vegetative Parts by Applying Fungal Elicitors, Methyl Jasmonate and Silver Nanoparticles as Elicitor in Hydroponic Culture

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

**Introduction**: Medicinal plants are being used to treat several diseases for many decades and this is an ancient method to treat the patients. Herbal plant *Silybum marianum* found most effective one to cure liver disorders. This plant produces silymarin which is a secondary metabolite and have hepatoprotective properties. Silymarin is a mixture of flavonolignans (silybin A, silybin B, isosilybin A isosilybin B, silychristin, silydianin apigenin 7-D glucose and taxifolin) that has antiviral, antibacterial, antifungal and antiallergenic properties. Therefore, the excessive production of silymarin is necessary to cure different types liver disorders, so the present study executed to boost up production of *Silymarin flavonolignans* in vegetative parts of the *Silybum marianum* plant by using different elicitors.

**Materials and Methods**: In the present study, elicitation technique in hydroponics system was used to enhance the production of pharmacologically active flavonolignans in *Silybum marianum*. Fungal elicitors prepared from lyophilized *Aspergillus niger* biomass, methyl jasmonate, silver
nanoparticles and combination of silver nanoparticles and methyl jasmonate were added (0.2g/l), (100µM/l), (1 ppm) and (100µM/lppm) in hydroponics with Hoagland’s solution in hydroponics to enhance the production of flavonolignans of *Silybum marianum*. Controls were also set for each treatment. Plants were harvested after 72 hours of introduction of elicitors. High performance liquid chromatography technique was used for analytical purpose. Four solvents (methanol, acetonitrile, chloroform and 2% Trifloro acetic acid) were used in HPLC. Column was C18 and run time of sample was 1 hour. *Silybum marianum*’s seed extract was used as a standard. Extract of control and treated plants were run on the same polarity in HPLC.

**Results:** Results showed that after elicitation significant increase was observed in production of silymarin’s flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B and apigenin 7-D glucose) in vegetative part of the plant but rate of production was different for each elicitor, fungal elicitors prepared from lyophilized *Aspergillus niger* biomass proved best among all treatments.

| Keywords: Elicitation; Aspergillus niger; methyl jasmonate; silver nanoparticles; flavonolignans; HPLC. |

### 1. INTRODUCTION

*Silybum marianum* is an annual and biennial herbaceous broadleaf plant which belongs to family *Astraceae* [1,2] This herbaceous plant has its origin at Mediterranean and North African areas [3] Silymarin, the active constituent of *Silybum marianum* plant contains 70-80% silymarin flavonolignans (silybin A and B, isosilybin A and B, silychristin and silydianin and flavonoids, taxifolin and quercetin), and the remaining 20-30% comprising of a chemically uncertain portion that involving oxidized and polymeric polyphenolic mixtures [4] Silybin is considered the main and best effective constituent in silymarin [5] Silymarin keeps an extensive range of pharmacological and biological effects, containing anti apoptotic and antioxidant activity [6,7] It is used as effective antiviral treatment for hepatitis C virus [8] Furthermore, its antidiabetic action [9] cardio protection [10] hypolipidemic, anti-inflammatory, neuroprotective, immune modulation, antifibrotic and neurotropic effects are well recognised [11] It is also beneficial in case of harmful liver injury, liver cirrhosis and chronic inflammatory liver infections [12] It modifies a diversity of cell-signalling pathways, which can be resulting in the decline of pro-inflammatory facilitators [13] It plays significant role in initiation of protein synthesis [2] and regeneration of cell [14]). Anti-cancer activity is one of the greatest auspicious actions of silybin, furthermore in chemotherapeutics silybin has shown possible use as an adjuvant in the cancerostatic treatment [15] Silymarin has been verified to defend kidney and liver cells from poisonous impacts of drugs containing radiotherapy and chemotherapy [16,17]. Silymarin has been shown among the greatest examined extracts of plant that have identified mechanisms [1] The main cause for the extensive growing or farming of this plant is because of its wide range of effectiveness in treating biliary and liver diseases and inhibiting liver cancer [16] *Silybum marianum* plants are very violent and modest to agricultural yields, mainly in nutrient-rich places [17] In various kinds of *Astraceae*, the productivity of seed propagation and seedling development is very low, unpredictable and is extremely reliant on several biotic and environmental aspects [1,18].

Seeds of this plant have flavonolignans (silymarin) in high concentration that are responsible for its beneficial effects against diseases but their yield is very low. It has been shown that there are some factors such as information of biosynthesis, passageways of signal transduction, gentle rising flora of various species, random changeability in accumulation of these secondary metabolites and low harvest found in nature which is involved in improving the trials for the source of medicinal values [19] In plant cell culture, with the aim of extensively rise the generation of secondary metabolites numerous plans can be beneficial for instance, enhancing the culture conditions, nourishing of originators and elicitation [20] Silymarin mixtures are commonly extracted from the desiccated fruits of ground developed plants that sometimes need several times such as months to years to be attained compounds. Cell cultures of *Silybum marianum* are able to enhance production of flavonolignans, and the amounts that are produced are lesser than those compounds which are formed in the ground developed plants [1] To get better quantity in less time, silymarin production could be enhanced by using different elicitation techniques. In culture medium, synthesis and assembly of secondary metabolites could be initiated by using elicitors [21,22] Elicitors are the molecules that can
induce or activate defence mechanism of plant to boost accumulation of secondary metabolites [23] Elicitors can be distributed into two sets. Abiotic elicitors which are chemicals similar to inorganic salts, heavy metals that can disrupt membrane integrity and the biotic elicitors are polysaccharides that can be derived from cell walls of plant (cellulose or pectin), microorganisms (glucans or chitin) and glycoproteins [24].

Fungal elicitors have ability to induce plant secondary metabolites and cause plant hypersensitivity or protection reaction throughout the physiological processes of disease resistance [25]. The utilization of fungal elicitors has been according to one in all the primary effective ways for rise the output of helpful secondary metabolites in plants [26]. Literature showed that *Aspergillus niger* is one of best fungal elicitors [27]. The cell cultures of periwinkle intracellularly when treated with *Trichoderma viride* and *A. niger* showed increase in the production of tryptamine [28]. In present investigation, the elicitors used to improve the production of secondary metabolites in *Silybum marianum*, were well examined. For instance, association of Shikonin content has been increased by *Aspergillus niger* [29] In *Silybum marianum* cultures, whole silymarin produce endurance which shows that there is several information that are based on the effect of dissimilar abiotic elicitation techniques in vitro culture [30-32]. This study was designed to enhance the production of pharmacologically important flavonolignans of *silybum marianum* in vegetative parts of the plant by using methyl jasmonate, nanoparticles and fungal elicitors.

Effects of nanoparticles on several plant species have been investigated to create complete toxicity level for nanoparticles. The nine types of nanoparticles, mono and bimetallic alloy metal including Gold (Au), copper (Cu), Silver (Ag), Silver Cu (1:3), Silver Cu (3:1), Silver Cu (1:3), Silver Cu (3:1), Silver Au (1:3), Silver Au (3:1) showed results on growth of root and shoot; seed growth and also biochemical level of plant *Silybum marianum*. Seed growth was remarkably increased and improved when treated with nanoparticle suspension and was observed best with Ag nanoparticle suspension. Effect of Ag nanoparticle was more significant among all the nanoparticles were being used, the results gathered are fruitful in mapping the results of dissimilar mono and bi metallic NPs on a species of medicinal plant [33].

Methyl jasmonate is a plant hormone which used for plant defense. Methyl jasmonate and JA is usually found naturally in plants. It's separated from essential oils of Jasminium grandiflorum. It's a stress hormone forms when attacked by insects and naturally producing regulators of greater plant development, gene expression and responses to external stimuli [23]. Its methyl ester, JA, and methyl jasmonate are considered to be very essential in signalling compounds in elicitation process which furthermore exceeding to hyperproduction of several secondary metabolites [4]. Different cultures are used for production of secondary metabolites such as hydroponic culture and tissue culture [21]. Hydroponic cultures have been declared the significant technique to enhance the production of secondary metabolites in various plants Furthermore the treatment of plants with different elicitors for the more better production of secondary metabolites have become a useful scheme in plants [11, 34]. Environmental factors can be controlled in the hydroponics such as temperature, nutrients and light by this way optimal growth can be achieved [16]. This process was identical to fields in which plants were producing naturally but that process was time-taking process and prolonged as well. It resembles with soil which was exposed to long time period of saturation of water which correlates with the faulty system of inadequate irrigation and drainage [17].

The purpose of present study was to enhance flavonolignans of *S. marianum* by applying different biotic and abiotic elicitor and determine their production by HPLC.

2. MATERIALS AND METHODS

2.1 Experimental Materials and Sterilization

This study was conducted at the Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore, Lahore, Pakistan. Seeds of *Silybum marianum* were taken from the oil seed department of NARC (National Agriculture Research Centre) Islamabad, Pakistan. It have been confirmed that the experimental research and lab studies on *Silybum marianum* plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation with appropriate permissions from oil seed department of NARC (National Agriculture Research Centre) Islamabad, Pakistan, and
Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore, Lahore, Pakistan for collection of plant specimens. The seeds were rinsed with distilled water several times and washed with 70% ethanol for 1 minute and then again rinsed with distilled water three times to eliminate all the traces of ethanol. Then seeds were washed with 0.01% mercuric chloride solution for 2 minutes and again rinsed with distilled water for 4 to 5 times. Now, seeds were ready to use.

Sterilized sand was used in present experiments which were rinsed several times with distilled water. After rinsing it was treated sodium hypochlorite (NaClO) for 5 min and washed 3 to 5 times with distilled water to exterminate all the traces of sodium hypochlorite. Sand was then placed in oven at 70°C or drying. Now, sand was ready to use.

2.2 Germination of Seeds

Seeds were placed in the sterile sand at optimum distance from each other and irrigated with half strength Hoagland’s Solution and allowed to germinate. Hoagland’s solution was prepared by using protocol for Hoagland preparation. First of all first stock solution was prepared which contains macronutrients. Dissolved all four chemicals of macronutrients (Ca(NO₃)₂.4H₂O, KNO₃, KH₂PO₄, MgSO₄.7H₂O) in a separate 1 litre of H₂O in a separate glass bottle. After that second stock solution was prepared which contains micronutrients, trace elements and dissolved all five chemicals of micronutrients (H₃BO₃, MgCl₂.4H₂O, ZnSO₄.7H₂O, CuSO₄.5H₂O and NaMoO₄), trace elements in another separate 1 litre glass bottle. Then third stock solution was prepared, 1 litre of KOH was made and its pH was adjusted approximately 5.5 using H₂SO₄. Then EDTA.2Na and FeSO₄.7H₂O were added in it.

2.3 Preparation of Fungal Elicitors

2.3.1 Preparation of inoculum in culture

Sabouraud dextrose agar (SDA) media was prepared based on the instruction given by biomark manufacturers. The media was sterilized in an autoclaved at 121°C and 15 Pa for 1 hour. Petri plates were also autoclaved to prevent the contamination. After autoclaving the media poured into the plates under sterile condition in laminar air-flow hood and allowed all plates to solidify. One plate was used as control and others for inoculating fungus (Aspergillus niger). Fungus was inoculated on media in laminar flow hood with sterile loop and incubated at 28°C for few days to get standardized inoculum. After getting fungal growth on culture media in Petri plates, broth culture media for fungal production was prepared by using glucose, NH₄NO₃, KH₂PO₄, MgSO₄.7H₂O and ZnSO₄. 7H₂O in 1 litre distilled water into the conical flask. Media was autoclaved and the Aspergillus niger was transferred with the help of sterile cutter into the broth media. After inoculation of fungus into culturing media, flasks were placed in an incubator (in darkness) running at 28°C for 7 days, allowed the cells to grow till the completion of log phase.

2.3.2 Preparation of fungal elicitor

For the preparation of elicitor media was centrifuged for 15 min at 5,000 rpm and fungal cells were collected. Fungal biomass was washed a number of times with sterilized distilled water. Fungal cells were filtered through whatman filter paper. The cells were crushed in the pestle mortar. The resulting fungal cell fragments were dried by lyophilisation and then autoclaved. These fungal elicitors were saved at 4°C for further use.

2.3.3 Hydroponic container setup and culturing of plants

Self-designed local made hydroponic system was used in this investigation plant holder was made and a cut was made in the foam which was exceeding through its length with the usage of razor blade and one plug was prepared per length. These liquid-autoclave foam tube plugs were rinsed with deionized water. An incision in the foam panel into little smaller board was made to ensure the length and width of foam boards are 0.5-1.0 cm smaller than size of the container. Holes were made on the foam board by using a cork borer at a proper distance maintained between the holes to avoid higher density of the plants. It was confirmed that the size of holes was according to the size of the plugs. Filled the containers with nutrient solution and confirmed that root development having sufficient depth to solution, after that placed the foam boards on the solution surface with utmost care. To provide the oxygen in to the solution an air-pump system was installed. Filled the hydroponic container with nutrient solution and seedlings were also transplanted from sand pots to the hydroponics at the same day. Silybum marianum plants have...
specific nutritional requirements which had been successfully fulfilled and plants were grown in Hoagland’s solution. One hydroponic culture was used as control and the other was used for treatments.

2.3.4 Addition of elicitors

Methyl jasmonate, silver nanoparticles, combination of silver nanoparticles and methyl jasmonate and elicitors of *Aspergillus niger* were added in separate hydroponic containers 5ml per, 0.86g, 0.86g + 5ml and 4 g respectively in 20 litre of distilled water with Hoagland’s solution in hydroponics under same temperature and conditions. After six days plants were separated from hydroponic culture.

2.4 Extraction

For extraction of active chemical compounds, leaves were rinsed in distilled water and were dried under shadow before grinding in to fine powder. Leaf powder 100 mg was homogenized with 5 ml of methanol which was relatively best solvent as compared to ethanol and then the homogenized material was sonicated for 15 minutes and extracted for 24 hours to release intracellular product. The extract was centrifuged at 5000 rpm for 10 minutes and collected the supernatant and allowed methanol to vaporize at room temperature for 20 hours. Dissolved this extract again in HPLC grade methanol and filtered it over 0.22 mm filter.

2.5 Identification and Characterisation

High performance liquid chromatography (HPLC) Hitachi primade model was used for analytical purpose. Four solvents i.e., methanol, acetonitrile, water and H2O+ 0.2% trifloroacetic acid (TFA), were used in HPLC technique as elute. Column C18 was used and running time of sample was 1 hour. The quantitative analysis of extracts was done by relating the retention time of the peaks with respect to those of the standardised extract of seed. Seeds extract of *Silybum marianum* was run as a standard to get the peaks of flavonolignans of *Silymarin*. Extract of control and extract of treated plants were also run on the same polarity of HPLC.

3. RESULTS

3.1 Growth of the Fungus

The growth of *Aspergillus niger* was observed through naked eye which showed that the initial growth of fungus after 24 hour was white, which later on turned to black giving white salt to black pepper like in appearance after 1 to 2 days that resulted from pigmented conidia born in huge amount on conidiophores which back turned yellow after 6 to 7 days.

![Fungus plate with control](image-url)
3.2 Identification and Characterization of Fungus

The growth of *Aspergillus niger* was examined under microscope and conidia formation was found in single or group form that look like yeast cells (Fig. 1).

3.3 HPLC Analysis

The HPLC analysis showed that in control (without elicitor) production of flavonolignans in *Silybum marianum* was zero because no peak on chromatogram resulted with control (Fig. 2). Seed extract of *Silybum marianum* was used as standard which showed the presence of flavonolignans of *Silybum marianum* such as silybin A, silybin B, isosilybin A, isosilybin B and apigenin 7 D-glucose. Plants treated with fungal elicitors showed same peaks of flavonolignans as observed in seed extract (silybin A, silybin B, isosilybin A, isosilybin B and apigenin 7 D-glucose) but, peaks were higher than seed’s extract which shows that all elicitors influenced significant enhanced concentrations of these important flavonolignans (Fig. 3). Plants treated with methyl jasmonate also showed production of silybin A, silybin B, isosilybin A, isosilybin B and apigenin 7 D-glucose (Fig. 4) whereas plants which were treated with silver nanoparticles showed production of isosilybin B and apigenin 7 D-glucose only (Fig. 5), same in the case of plants which were treated with both methyl jasmonate and silver nanoparticles showed only the production of apigenin 7 D-glucose and isosilybin B (Fig. 6) (Table 1).

Table 1. Showing flavonolignans produced under different treatments

| Treatments                              | Flavonolignans of *Silybum marianum* |
|-----------------------------------------|--------------------------------------|
| 1. Control                              | No flavonolignans                    |
| 2. Fungal elicitor                      | Apigenin 7-D Glucose                 |
|                                         | Silymin A                            |
|                                         | Silybin B                            |
|                                         | Isosilybin A                         |
|                                         | Isosilybin B                         |
| 3. Methyl jasmonate                     | Apigenin 7-D Glucose                 |
|                                         | Silymin A                            |
|                                         | Silybin B                            |
|                                         | Isosilybin A                         |
|                                         | Isosilybin B                         |
| 4. Silver nanoparticles                 | Apigenin 7-D Glucose                 |
|                                         | Silybin B                            |
| 5. methyl jasmonate + silver nanoparticles | Apigenin 7-D Glucose               |
|                                         | Silybin B                            |

![Fig. 2. Chromatogram of control](image-url)
Fig. 3. Standard and fungal elicitor treated plant extract’s chromatogram

Fig. 4. Standard and methyl jasmonate treated plant extract’s chromatogram
DISCUSSION

Before the discovery of new drugs, plants have been used for treatment of numerous diseases. Elicitors usually activate chemical defence responses in plants [33]. Literature supported cell culturing techniques as plants developed through this technique have higher capacity to produce silymarin those grown in field [1]. Cell culturing is also necessary because with increase in world population the demand for silymarin supply is also increasing while the plants of *Silybum marianum* in the Mediterranean region are limited and scattered. In present study fungal elicitors, methyl jasmonate and silver nanoparticles were used to improve production of silymarin through hydroponics. This result is supported by Hasanloo et al., [34] who investigated the effects of dissimilar concentrations of fungal elicitors that comprising of *Rhizoctonia solani, Fusarium*.
proliferatum, Aspergillus niger on hairy root cultures of Silybum marianum and observed an increase in production of flavonoids of Silybum marianum. Elicitation of F. proliferatum significantly enhanced the production of taxifolin, silybin, isosilybin and silydianin in the hairy root culture of Silybum marianum but our findings showed an increase in production of flavonoids of silymarin such as silybin A, silybin B, isosilybin A, isosilybin B and apigenin 7-D glucose in the vegetative parts of Silybum marianum. In the same line present study also indicated that fungal species have affected the production of silymarin. So, Proper application of all above applications was effective in enhancing the production of silybin, isosilybin and apigenin 7-D glucose in Silybum marianum.

This study also showed the higher production of the flavonolignans in the vegetative parts of the Silybum marianum as compared to control hydroponic culture (Figs. 2-5). Results showed that elicitation with Aspergillus niger has proved good treatment for the enhancement of secondary metabolites in vegetative parts of the plants (Fig. 3). Plants are the functional source for research point of view. In current researches the plant hormones are considered as important topic. Methyl jasmonate is a plant hormone which functions as a plant stress hormone and human stress hormones. The main focus is on the characteristics of drugs which is taken for the medical treatment. Methyl jasmonate is an elicitor which enhanced the production secondary metabolites which is useful feature for the defense mechanism of plants [35]

Li et al., [36] discussed and observed the effect of methyl jasmonate on different plants and on leaves. They observed that methyl jasmonate can alter the physical and chemical characteristics in plants while it is use in defense mechanism of plants. They use three different plants e.g they apply methyl jasmonate on tomato, sun flower and soybean and treated these plants by 0.1, 0.0, 0.5, 1.0 and 2.5mM. Clear changes are observed in plant trichome, plant heights, plant biomass etc. it decreases the upto 39% and its biomass about 79%. MeJA affect the large increase in the density of leaf trichome in above three species. By applying methyl jamsonate they also observed the changes in the thickness, cuticle composition and in the density of stomata that there is small change occur as compared to the trichome density. The thickness of cuticle is increase in the tomato but it decreases in soybean and sunflower. They concluded that methyl jasmonate has great role in growth and changes in the plant for their defense mechanism.

Mahmood et al. [37] notice the effect of methyl jasmonate of water stress on the growth of banana, they were treated the tips of shoots with methyl jasmonate on different levels, the result was that the growth of tips were increased as they increase the doses of methyl jasmonate, while imposing the 30g L⁻¹ polyethylene glycerol which encouraged the stress of water and it decreases the growth of shoot tips. They concluded that methyl jasmonate had great impact on the plant growth. Li et al.,[36] treated pitaya fruit with methyl jasmonate. They examine that by applying methyl jasmonate wound-induced was repressed and it decreases the organic acids, ascorbic acid. It speed up the utilization of sugars, encouraged activities of enzymes in mechanism of energy but when methyl jasmonate is with wounding stress it decreases the utilization and conversion of sugars which give important ancestor and power for the accumulation of phenolic for wound-induced.

Krzyzanowska et al. [38] observed the effect methyl jasmonate and jasmonic acid on the growth of cells and on the growth of rosmarinic acid in culture of cell suspension of Mentha 9 piperita. The growth rosmarinic acid was greatly shown about 117,95mg g-1 DW, when it is treated with 100 1M methyl jasmonate, same concentration was decrease, when it is treated with jasmonic acid of 200 1M, these concentration is higher about 1.5 times, as compared to the control stage of without elicitation they concluded from their research that methyl jasmonate had great role in plants.

In the discussed study scientist stated that when Stemona sp. cultures treated with MeJA and salicylic acid caused to increase the production of Stemona alkaloid. The maximum Stemona alkaloid amassing was established in culture by using 100 µM salicylic acid though this treatment give rise the production of stemofoline in 1.69 fold and 1.61 fold 1′,2′-didehydrostemofoline greater quantities than the control correspondingly. This study supports our results of flavonolignans production by MeJA application.

Nanotechnology is developing technology working in all field of science [39] Nanoparticles are found in atmosphere that can impact on the
medicinal plants of pharmacological characteristic. Effects of nanoparticles on several plant species have been investigated to create complete toxicity level for nanoparticles. Seed growth was remarkably increased and improved when treated with nanoparticle suspension and was observed best with Ag nanoparticle suspension [33] Nanoparticles have special effect on seed growth and hairy root of many plants such as lactuca sativa, Brassica napus, ze a mays, Raphanus sativus Cucumis sativus and lolium multiformur. Lin and Xing, [40] reported that silver NPs have positive effect on Silybum marianum as a result indicates that secondary metabolies have remarkably increased. This study supports our results. Jasim et al., [41] also reported that plant secondary metabolites are enhanced by using the elicitor silver nanoparticles e.g. diosgenin and Zang et al., [25] also concluded same after research on artemisinin. These metabolites are enhanced when the plant is treated with the elicitor, silver nanoparticles because silver nanoparticles have ability of adsorbing metabolites (kurepa et al., 2014). Many articles and researches indicate that silver nanoparticles are able to transformed metabolism of secondary compounds; it is proven that ROS levels are initial stage for the response of silver nanoparticles to the plant, than the influx of calcium ions occur by which the (MAPK) is an enzyme which is up-regulated. This all causes the induction of ROS bursting. This whole process is lead to the production of secondary metabolites [42]

*Silybum marianum* hairy root culture are able to generate all flavonolignans that are found in the part of fruit but also found in the seeds and leave, while in dried fruit the production was generally lower [34,43-47] This study is in agreement with our results as in our study fresh extract was used to check on HPLC that showed production of flavonolignans in leaves of the *S. marianum* plant [48-53].

**CONCLUSION**

*Silybum marianum* (milk thistle) is a medicinal plant which has been used for many of year as a treatment for a variety of diseases. In recent study, *Silybum marianum* were used to show the impact of elicitation on the production and expression of *silymarin flavonolignans*. By present study it can be concluded that fungal elicitors proved best elicitors for enhancement of the production of flavonolignans in *Silybum marianum* as compared to other methyl jasmonate and silver nanoparticles. HPLC results revealed that there is significant increase in the production of silymarin flavonolignans such as silybin A, silybin B, isosilybin A, isosilybin B and apigenin 7-D glucose in vegetative parts of the plant as compared to seed extract. This can help to get useful quantity of medicinally important of silymarin in vegetative portions in some days instead of waiting for seed to get silymarin to make medicines. The use of elicitors to *S. marianum* in hydroponic culture might be a useful tool for reviewing the rule of plant cell metabolism in reactions to several stress factors. Further studies are vital to describe the pathways underlying the intracellular increase of silymarin in elicited *S. marianum* cultures medium, and also to recognise main phases that can be contributing in the signalling network triggered by the elicitor. Such information can be valuable for controlling the biosynthesis of silymarin in *Silybium marianum*.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

It is not applicable.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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