Full Length Research Paper

Induction of biochemical active constituents of Jojoba (Simmondsia chinensis (Link) Schneider) callus affected by hormones

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The present study aimed to identify some metabolites products obtained from different jojoba callus tissue extracts using gas chromatography/mass spectrometry analysis (GC-MS). It is known that Jojoba, a medicinal and oil-yielding, has multi-purpose uses. In addition, it produces toxins, fatty acids, phenolic compounds and other secondary metabolites from callus identified using GC-MS. Despite the direct effect of 2,4-D on directing the explant towards callus induction, it has interaction effect with Kin and achieves hormonal balance inside the explant. There is a significant interaction effect between Kin and 2,4-D. The increased concentration of both Kin and 2,4-D raises the extent of nodal segments response towards proliferation, development, and callus induction. Our results showed that the interaction effect between Kin and 2,4-D is the best results for phytochemical active constituents formation achieved from callus cultured. Qualitative results revealed that increasing cytokinins Kin concentration leads to slightly double increase in active constituents from the explants for Triodecanoic and Methoxyacetic acids. While, the gradual increase of 2,4-D to 2.0 mg l\(^{-1}\) caused a single increase in active constituents for Triodecanoic, Methoxyacetic and Octadecanoic acid, respectively. On the other hand, Decanoic acid has no effect by adding both hormones (Kin and 2,4-D) and also Octadecanoic acid when kin is added. Also, there is a direct relation between the development of callus and reproduction of active phytochemical constituents, which increase qualitatively by increasing callus development and 2,4-D hormone concentration at 2 mg l\(^{-1}\) for 1-Hexadecanol, Nonadecatriene, Aristolene and Tricosene, respectively compared to free hormone treatment.

Key words: Active constituents, callus, fatty acids, jojoba, Simmondsia chinensis.

INTRODUCTION

Jojoba [Simmondsia chinensis, family Simmondsiaceae] is a shrub with an oil seed. It has male and female sexual organs, and grows in semi and desert regions. Its native country is Southwestern US. Jojoba is used for medicinal
purposes and is a folk medication for cold, obesity, wounds, sore throat, warts, dysuria, parturition, and cancer (Kolodziejczyk et al., 2000; Mohasseb et al., 2009a). Jojoba belongs to order Caryophyllales, which contains simmondsin and three non-ferulated compounds in different organs of tissue cultured plants which cause reduction in food intake.

Jojoba is a crop that bears harsh environment and thrives in areas where other crops cannot be cultivated. It consumes less water and therefore can be farmed in new areas. Conventional breeding, which aims to improve tolerance to drought and salinity, takes a long time and does not succeed in producing sustainable resistant plants. These circumstances necessitate the use of genetic engineering and biotechnology, as one of the most promising new technologies to improve jojoba (Mohasseb et al., 2009a, b).

Jojoba oil has many usages depending on the site where the modification is being done. Virtually, it has no traces of glycerine, making it a unique plant of oil along with the fact that it can be modified via hydrogenation, sulfurization, halogenation and many other techniques. With its uses in industries like cosmetic, pharmaceutical, lubricant and petrochemicals, the importance of jojoba oil in the market is high (Arya and Khan, 2016).

Jojoba seed has a fluid wax (jojoba oil) used in pharmaceutical products, cosmetics, and mechanical lubricants. Jojoba meal, a waste from jojoba seeds, is the remaining product after extracting of oil and constitutes over half of the seed. Jojoba meal composed of 25 to 30% protein is rich in dietary fiber and may be used in livestock feed fortification. The defatted meal holds sugars and 11 to 15% of natural products, constantly on structurally identified simmondsin. Jojoba is clonally propagated by nodes and the rate of propagation is very limited because the nodes are hard to roots, so that the only solution to solve this problem is through rapid mass production by tissue culture technique (Mohasseb et al., 2009b).

Jojoba plants have many woody stems that commonly grow between 0.6 and 2 meters in height, and over 3 m have been observed in the wild. In full light, it branches near the base. Plants can vary from almost prostrating, with the branches growing laterally. The wood from mature plants is hard and heavy, lemon yellow and without distinctive scent or taste. The natural life span appears to be over 100 years and may exceed 200 years (Mohasseb et al., 2009a).

This study aimed to produce more amounts of biochemically active constituents and simmondsin by increasing the cell numbers of seeds that produce biochemical active constituents in it. So, callus was produced from seeds or leaf and root by in vitro culture technique. After that, a lot of biochemically active constituents used for many purposes can be extracted and also biochemical active constituents can be produced from callus and hairy roots of jojoba. Thus, there are two sources to produce commercial amounts of biochemical active constituents.

MATERIALS AND METHODS

This study was carried out at the Department of Agricultural Biotechnology, College of Agricultural and Food Sciences, King Faisal University, Al-Hassa, Saudi Arabia.

Explant preparation and sterilization process

Plant materials were taken from semi-hardwood stems of female jojoba adult shrub provided by Mohasseb et al. (2009a).

1. Each inter node (2 cm) explant was excised from a 5-years-old shrub.
2. The cuttings were thoroughly washed by 1% savlon solution for 20 min
3. They were rinsed twice in sterile distilled water (SDW).
4. All subsequent operations were carried out inside a laminar air-flow cabinet.
5. The clean cuttings were given a quick (30 s) rinse in 70% ethanol, followed by two washings in SDW.
6. These cuttings were then surface-sterilized in 0.15% mercuric chloride (HgCl2) solution for 13 min and rinsed thrice with SDW.

Establishment stage and callus induction

1. The cuttings were slightly trimmed at both ends to expose the fresh.
2. Tissues were exposed with inorganic salts, supplemented with (in mg l-1): 100 Myoinositol; 30000 sucrose; 7000 agar (Sigma Chem. Co.)
3. They were planted in MS culture medium (Murashige and Skoog, 1962).
4. The pH value was adjusted at 5.7±0.1 before adding agar.
5. The medium was autoclaved at 121°C for 15 min, while, the changes in the nutrient medium are as follows (Table 1).

| Experiments               | Result          |
|--------------------------|-----------------|
| 1. Callus was dried at 40°C for 24 h in an oven using a vacuum. |
| 2. Samples were ground into powder using a mortar under steam |
off of liquid nitrogen; after that it was extracted.
3. A quantity of 3.0 g dry base of callus powder was extracted with 15 ml of ethanol (70%) at room temperature, protected from sunlight and mixed several times with a sterile glass rod.
4. Mixture was then filtered through Wattman No.1 filter paper. The extracted liquid was subjected to rotary evaporation to dryness in order to remove the ethanol (Akueshi et al., 2002).
5. After that, the extract was stored at 4°C in refrigerator for future use (Harborne, 1984).

Gas chromatography/mass spectrometry analysis (GC-MS)
The adapted samples were chosen based on their total jojoba extract and those with high total biochemical active constituents in callus tissues, considered as promising treatments. They were subjected to GC-MS analysis after filtering with 0.22 µm pore size syringe filter.

GC-MS analysis was carried out in College of Agricultural and Food Sciences, King Faisal University, Al-Hassa, Saudi Arabia, using GC-MS apparatuses, under the following conditions: a HP-5MS capillary column (Agilent Technologies, Santa Clara, CA) operating at electron impact mode at 70 eV. Pure helium gas with built-in purifier was used at a constant flow rate of 1.0 ml/min employed in a split less mode with injector temperature of 250°C and ion source of 280°C. The stepped temperature program was as follows: initial temperature oven was started at 220°C and held for 5 minutes and followed by a ramp to 300°C at 5°C/min held for another 15 min. A post-run of 5 minutes at 300°C was sufficient for the next sample injection.

Biochemical active compounds identification
Mass analyzer was used in full scan mode scanning from 40 to 550 m/z and mass spectra were taken at 70 eV. For the compound identification, manual spectral matching was ascertained by using the mass spectral library of National Institute Standard and Technology (NIST) version 2.0 and with the aid of Automated Mass Spectral Deconvolution and Identification (AMDIS) software version 2.70 by deconvoluting the chromatography peak at the corresponding retention time (Cheong et al., 2016).

It should be noted that the chromatograms not only belong to saponifiable but also some bioactive compounds in the unsaponifiable fraction in the jojoba callus extracts of each treatment.

Statistical analysis
Data were statistically analyzed by using a randomized complete block design (RCBD) (Snedecore and Cochran, 1990). Mean separations were done by using a MSTAT-C computer program v.4 (Duncan, 1955).

RESULTS AND DISCUSSION
The interaction effect of hormones and their concentrations on callus induction
Table 2 shows the significant effect of Kin on the extent of response of jojoba nodal segment to in vitro callus formation. These data show that the more Kin was used the more the explant responded to callus formation. Increased use of Kin concentration raises the extent of explants' response towards proliferation and development and the best results were achieved at the concentration of 2.00 mg l-1. It can be noted that nodal segments

### Table 1. Composition of nutrient media for in vitro callus induction from jojoba.

| Media code | Kin (mg l⁻¹) | 2,4-D (mg l⁻¹) | Pro² (No) | CP² (No) | FW² (g) | DW² (g) |
|------------|--------------|---------------|-----------|----------|--------|--------|
| A          | 0.0          | 0.9100ᵃ       | 0.0000    | 3.2500ᵇ  | 0.9615ᵇ |        |
| B          | 0.5          | 0.8900ᵃ       | 0.0000    | 1.8960ᵇ  | 0.9116ᵇ |        |
| C          | 1.0          | 0.9200ᵃ       | 0.0000    | 4.1927ᵇ  | 0.6754ᵇ |        |
| D          | 2.0          | 0.8900ᵃ       | 0.0000    | 4.3200ᵇ  | 1.1666ᵇ |        |
| Means      |              | 0.9025        | 0.0000    | 3.4147    | 0.9288  |        |

¹*Symbol of media used in the form of letters A, B, C, ... P.  
²*media code.

### Table 2. Specific effect of Kin on callus induction from nodal segments jojoba.

| Media code | Kin (mg l⁻¹) | Pro² (No) | CP² (No) | FW² (g) | DW² (g) |
|------------|--------------|-----------|----------|--------|--------|
| A          | 0.0          | 0.9100ᵃ   | 0.0000   | 3.2500ᵇ | 0.9615ᵇ |
| B          | 0.5          | 0.8900ᵃ   | 0.0000   | 1.8960ᵇ | 0.9116ᵇ |
| C          | 1.0          | 0.9200ᵃ   | 0.0000   | 4.1927ᵇ | 0.6754ᵇ |
| D          | 2.0          | 0.8900ᵃ   | 0.0000   | 4.3200ᵇ | 1.1666ᵇ |
| Means      |              | 0.9025    | 0.0000   | 3.4147   | 0.9288   |

¹*Symbol of media used in the form of letters A, B, C, ... P.  
²*media code.
cultured on hormone free medium (control) showed slight response almost as weak as that achieved by those cultured on 0.5 mg l⁻¹ Kin medium. Thus, it can be concluded that Kin has indirect effect on callus formation. While, the interaction effect with 2,4-D auxin shows that it has indirect effect on the development and the proliferation of the explant for callus formation and production. These results are in agreement with those reported by Hamama et al. (2001) and Mohasseb et al. (2009b).

Results in Table 3 show that 2,4-D auxin has a clear effective role in the proliferation and development of the explant for callus induction, as it can be noted that 2,4-D containing media were better than auxin free control medium (A) for callus induction from the explant. It can be noted also, that increasing 2,4-D auxin concentration in the nutrient medium revises the extent of explant response towards proliferation and development for callus formation and production as the best results were achieved at the concentration of 2.00 mg l⁻¹ 2,4-D. This was confirmed by Gaber et al. (2007).

Despite the direct effect of 2,4-D on directing the explant towards callus induction, its interaction effect with Kin (Table 4) shows the importance of adding cytokinins into the medium to achieve hormonal balance inside the explant. Table 4 shows that there is a significant interaction effect between Kin and 2,4-D, as the increased concentration of both Kin and 2,4-D raises the extent of nodal segments response towards proliferation, development, and callus induction. The data show that the best result for callus formation was achieved from the nutrient medium that contains the highest concentration of both 2,4-D (2.00 mg l⁻¹) and Kin (2.00 mg l⁻¹). The present results agreed with that of Abu (2000).

From the above, it can be concluded that hormonal balance between auxins and cytokinins supports the cultured explant for better development and growth than being cultured alone in one of them (Al-Ani et al., 2008; Mohasseb et al., 2009b).

Using balanced concentrations of auxins and cytokinins leads to achieving the best results and the most optimum growth rate of the explant, especially if the main goal of the research is to produce natural secondary products from the resultant callus. The interaction effect between Kin and 2,4-D, is the best results for phytochemical active constituents formation achieved from callus cultured on

### Table 3. Specific effect of 2,4-D on callus induction from nodal segments jojoba.

| Media code | 2,4-D (mg l⁻¹) | Nodal segments |
|------------|---------------|----------------|
|            | Pro¹ (No)     | CP² (No)       | FW³ (g)       | DW⁴ (g)       |
| A          | 0.0           | 0.9100ᵃ        | 0.0000ᵇ       | 3.2500ᶜ       | 0.9615ᶜ       |
| E          | 0.5           | 0.9300ᵃ        | 0.0000ᵇ       | 2.7013ᵈ       | 1.1212ᵇ       |
| I          | 1.0           | 0.8900ᵃ        | 1.0000ᵇ       | 4.2760ᵇ       | 2.0017ᵃ       |
| M          | 2.0           | 1.0000ᵃ        | 3.0000ᵇ       | 5.2980ᵃ       | 2.1492ᵇ       |
| Means      |               | 0.9325         | 1.0000        | 3.8813        | 1.5584        |

¹- Pro = Proliferation; 2- CP = callus induction; 3- FW = fresh weight; 4- DW = dry weight.

### Table 4. The interaction effect of 2,4-D, Kin and their concentrations on callus induction from nodal segments jojoba.

| Media code | Treatments (mg l⁻¹) | Nodal segments |
|------------|---------------------|----------------|
|            | 2,4-D | Kin | Pro¹ (No) | CP² (No) | FW³ (g) | DW⁴ (g) |
| A          | 0.0   | 0.0  | 0.9100ᵃ | 0.0000ᵇ | 3.2500ᶜ | 0.9615ᶜ |
| F          | 0.5   | 0.5  | 0.6700ᵇ | 0.6700ᶜ | 1.8960ᵇ | 0.9116ᶜ |
| G          | 0.5   | 1.0  | 0.6700ᵇ | 2.0000ᵇ | 1.3580ᵈ | 0.7203ᵈ |
| H          | 2.0   | 2.0  | 0.9300ᵃ | 2.0000ᵇ | 1.9033¹ | 1.0350ᵇ |
| J          | 0.5   | 0.5  | 0.7600ᵇ | 1.0000ᶜ | 3.1627ᶜ | 1.2117ᵇ |
| K          | 1.0   | 1.0  | 0.9000ᵇ | 1.0000ᶜ | 2.9073ᵈ | 1.2025ᵇ |
| L          | 2.0   | 2.0  | 0.6700ᵇ | 2.0000ᵇ | 1.8320¹ | 0.9095ᶜ |
| N          | 0.5   | 0.5  | 0.9200ᵈ | 2.0000ᵇ | 3.3067ᵇ | 0.9677ᶜ |
| O          | 2.0   | 1.0  | 0.9000ᵇ | 2.0000ᵇ | 2.1953ᵃ | 1.0959ᵇ |
| P          | 2.0   | 2.0  | 1.0000ᵃ | 3.0000ᵃ | 3.8573ᵃ | 2.1213ᵃ |
| Means      |       |      | 0.8330 | 1.5670   | 2.5669   | 1.1137   |

¹- Pro = Proliferation; 2- CP = callus induction; 3- FW = fresh weight; 4- DW = dry weight.
medium (P). The balanced concentration of Kin and 2,4-D used for producing these natural products was 2.00 mg l⁻¹, as it produced triple the quantity produced from the callus cultured on control medium (A).

Phytochemical qualitative analysis by GC-MS

Induction of biochemical active constituents from jojoba callus

Data illustrated in Table 5a and b showed that there are two major functional divisions produced by induction of jojoba callus from different doses of plant growth regulator (PGR) [Kin and 2,4-D]. This can be called phytochemical active constituent.

Data presented in Table 5a revealed that, there is a direct relation between the development of callus and re-induction of phytochemical active constituents, which increase qualitatively by increasing callus development and 2,4-D hormone concentration at 2 mg l⁻¹ for 1-hexadecanol, nonadecatriene, aristolene and tricosene, respectively as compared to free hormone treatment (Abu, 2000).

In addition, there is fluctuation in qualitative measurements in Kin treatment; it decreased under initial limit and then increased with increased concentration of Kin hormone in nonadecatriene and aristolene. There is a remarkable decrease in qualitative measurements of 7-hexadecenal and tricosene as compared to free hormone treatment (Agoramoorthy et al., 2007).

Both Kin and 2,4-D have the same significant effect on induced active constituents as they gave better results than those cultured on hormone free (control) medium (Table 6). It was noticed that increased Kin and 2,4-D concentration leads to increased active constituents induction from the explants at 2.00 mg l⁻¹, especially for 1-hexadecanol, nonadecatriene, aristolene and 7-hexadecenal. Also, the same trend of increasing value was observed in 1-hexadecanol constituent by increasing both hormones gradually from 0.5 mg l⁻¹ (2,4-D) and 1.0 mg l⁻¹ (Kin) up to 2.0 mg l⁻¹. On the other hand, the fraction of tricosane was not affected by increasing the concentration of both hormones. This was confirmed by Cheong et al. (2016) and Arya and Khan (2016).

From results in Table 5a, it can be concluded that there is a direct positive relationship between growth and development of callus and the resultant active constituents enhanced by PGR stress. These major active constituents are divided into five fractions: 1. 1-Hexadecanol which has the following biological activity: a composite medium used in the synthesis of other compounds (Turgumbayeva et al., 2015), has flavors and smells like composite simmondsin (Scrivner et al., 1984) and has luminous bacteria (Shimomura et al., 1974); 2. nonadecatriene compound acting as pheromone that attracts male insects (Nilsson, 2009); 3. Aristolene with the following biological activity: has a smell that attracts predators and wolves (Scrivner et al., 1984), is an antibiotic bacterium, Bacillus, and is used for throat congestion (Zour), it is anti-inflammatory, the cradle of the nerves (Tian-Shung et al., 2004), pain reliever and has luminous bacteria (Shimomura et al., 1974); 4. 7-Hexadecenal with the following biological activity: used for treating patients with diabetes, by increasing the secretion of insulin in the body (Turgumbayeva et al., 2015), it is found in the liver of sheep and thus attractive to wolves and used in hunting wild animals (Scrivner et al., 1984), has Luminous Bacteria “Aldehydes” Dark Mutant (Shimomura et al., 1974); 5. Tricosene which has kairomone effect (Ananthakrishnan et al., 1991).

From the data obtained in Table 5b and 6, it can be concluded that there is a direct positive relation between growth and development of callus and the resultant active constituents. Whereas using both Kin and 2,4-D has the same significant effect on active constituents induction as they gave better results than those cultured on hormone free (control medium).

Table 5b revealed that increasing cytokinins Kin concentration leads to slightly double increase in the active constituents from the explants clearly in case of triodecanoic acid and methoxyacetic. While, gradual increase of 2,4-D up to 2.0 mg l⁻¹ caused increase in active constituents singly in case of triodecanoic acid, methoxyacetic and octadecanoic acid, respectively. On the other hand, decanoic acid has no effect by adding both plant growth regulators (Kin and 2,4-D, PGR) and also octadecanoic acid by adding Kin. While, data obtained from Table 6 showed that there is a significant effect in interaction between both PGR in growth medium from 0.5 up to 2.0 mg l⁻¹. Also, it can be mentioned from the data presented in Table 6, that all the fractionated active constituents decreased by half at 0.5 mg l⁻¹ in both PGR.

From results in Table 5b, it can be concluded that there is a direct positive relationship between growth and development of callus and the resultant fatty acids activity enhanced by PGR stress. Therefore, we noticed that these major active constituents are divided into four fractions: 1. Triodecanoic acid, which has the following biological activity: it prevents the effect of bacteria and fungi fat, and has a role in getting rid of harmful pollutants, tolerant to salinity and so is grown on the banks of canals, used to get rid of contaminants (Agoramoorthy et al., 2007), used for the analysis of fat, and stimulates the working of lipase enzyme, which analyzes the cell walls of microbes (Enig, 2004); 2. Methoxyacetic acid with the following acid: it is used against harmful animals like mice (toxic baits), that inhibit fertilization processes and thus prevent the breeding and spread of these dangerous animals (Shannon et al., 1983); 3. Decanoic acid which is used in the manufacture of insect repellents; 4. Octadecanoic acid used for the treatment of bulges (Rampage stomach), aids digestion
Table 5a. Qualitative phytochemical analysis for active constituents from Jojoba (*Simmondsia chinensis* (Link) Schneider) callus under the specific effect of Kin and 2,4-D.

| Active constituents | Hormone (mg l⁻¹) | Media code | Properties of active constituents | Reference no. |
|---------------------|------------------|------------|-----------------------------------|---------------|
|                     | Kin              | 2,4-D      | Chemical Structure               |               |
| 1-Hexadecanol       | ++               | ++         | C₁₆H₃₄O                         | - Composite medial enters in the synthesis of other compounds. |
|                     | ++               | ++         |                                 | - Enters the Flavors and smell like simmondsin Composite. |
|                     | ++               | +++        |                                 | - Luminous bacteria. |
|                     | ++               | ++         |                                 | Turgumbayeva et al., 2015 |
|                     | ++               | ++         |                                 | Scrivner et al., 1984 |
|                     | ++               | ++         |                                 | Shimomura et al., 1974 |
| Nonadecatriene      | ++               | ++         | C₁₉H₃₄O₂                        | - Pheromone attracts male insects (Dynamic combat). |
|                     | ++               | ++         |                                 | Nilsson, 2009 |
|                     | ++               | ++         |                                 | Turgumbayeva et al., 2015 |
|                     | ++               | ++         |                                 | Scrivner et al., 1984 |
|                     | ++               | ++         |                                 | Tian-Shung et al., 2004 |
|                     | ++               | ++         |                                 | Tian-Shung et al., 2004 |
|                     | ++               | ++         |                                 | Shimomura et al., 1974 |
| Aristolene          | ++               | ++         | C₁₅H₂₄                           | - Has a smell attractant for predators, where the sheep so they are attractive to the wolves and predators. |
|                     | ++               | ++         |                                 | - Antibiotic bacteria Bacillus, and is used for congestion-Throat (Zour). |
|                     | ++               | ++         |                                 | - Anti-inflammatory, and the cradle of the nerves, and pain reliever. |
|                     | ++               | ++         |                                 | - Luminous bacteria. |
|                     | ++               | ++         |                                 | Scrivner et al., 1984 |
|                     | ++               | ++         |                                 | Tian-Shung et al., 2004 |
|                     | ++               | ++         |                                 | Tian-Shung et al., 2004 |
|                     | ++               | ++         |                                 | Shimomura et al., 1974 |
| 7-Hexadecenal       | ++++             | ++         | C₁₆H₃₅O                         | - The treatment of patients with diabetes, where increases the secretion of insulin in the body (Nutritional supplement). |
|                     | ++++             | ++         |                                 | - There in the liver of sheep and attractive to the wolves, so it can be used in hunting wild animals. |
|                     | ++++             | ++         |                                 | - Luminous Bacteria “Aldehydeless” Dark Mutant. |
|                     | ++++             | ++         |                                 | Turgumbayeva et al., 2015 |
|                     | ++++             | ++         |                                 | Scrivner et al., 1984 |
|                     | ++++             | ++         |                                 | Shimomura et al., 1974 |
| Tricosene           | ++++             | ++++       | C₂₃H₄₆O                        | - Kairomone Effect. |
|                     | ++++             | ++++       |                                 | Ananthakrishnan et al., 1991 |

- (+) = One portion, (++) = two portion, (++++) = six portion of constituents.
Table 5b. Qualitative phytochemical analysis for active constituents from jojoba (S. chinensis [Link] Schneider) callus under the specific effect of Kin and 2,4-D.

| Active constituents | Hormone (mg l\(^{-1}\)) | Media code | Chemical structure | Properties of active constituents | Biological activities | References |
|---------------------|--------------------------|------------|--------------------|----------------------------------|-----------------------|------------|
| Triodecanoic acid   | Kin (+)                  | 2,4-D (+)  | \(C_{13}\)H\(_{26}\)O\(_2\) | Saturated with him the perverse effect of bacteria and fungi fat. | - Saturated with him the perverse effect of bacteria and fungi fat. - Has a role in getting rid of harmful pollutants tolerant to salinity (found in mangrove) so is grown on the banks of canals and to get rid of contaminators. - Effective material for the analysis of fat. - Stimulate the working of lipase enzyme, which analyzes the cell walls of microbes. | Agoramoorthy et al., 2007 |
| Methoxyacetic acid  | Kin (+)                  | 2,4-D (+)  | \(C_{10}\)H\(_{20}\)O\(_2\) | Uses anti vital for harmful animals like mice (toxic baits), where he works on the inhibition of fertilization processes and thus prevent the breeding and spread of these dangerous animals. | - Uses anti vital for harmful animals like mice (toxic baits), where he works on the inhibition of fertilization processes and thus prevent the breeding and spread of these dangerous animals. | Shannon et al., 1983 |
| Decanoic acid       | Kin (+)                  | 2,4-D (+)  | \(C_{10}\)H\(_{20}\)O\(_2\) | Used in the manufacture of materials insect repellents. | - Used in the manufacture of materials insect repellents. - Used in organic synthesis and industrially in the manufacture of perfumes, lubricants, greases, rubber, dyes, plastics, food additives and pharmaceuticals. - Enters in the treatment of bulges (Rampage stomach). - Material aid digestion and prevent fermentation (Thrombosis) and clots. - Does not lead to high cholesterol. - Get rid of (LDL) low molecular weight lipids that cause atherosclerosis. | Tvrzicka et al., 2011 |
| Octadecanoic acid   | Kin (+)                  | 2,4-D (+)  | \(C_{10}\)H\(_{20}\)O\(_2\) | - Enters in the treatment of bulges (Rampage stomach). | - Material aid digestion and prevent fermentation (Thrombosis) and clots. - Does not lead to high cholesterol. - Get rid of (LDL) low molecular weight lipids that cause atherosclerosis. | Tvrzicka et al., 2011 |

- (+) = One portion, (++) = two portion, (++++) = six portion of constituents.

and prevents fermentation (Thrombosis), aids clots, does not lead to high cholesterol, and gets rid of (LDL) low molecular weight lipids that cause atherosclerosis (Tvrzicka et al., 2011).

**Active constituents from jojoba (Simmondsia chinensis [Link] Schneider)**

The physical properties of jojoba oil have high stability and low volatility. Its composition is less affected by temperatures up to 300°C. Jojoba oil contains straight-chained C20 and C22 fatty acids and alcohols and two unsaturated bonds, which make the oil susceptible to many different types of chemical manipulations. Fatty Acids present in jojoba oil according to Busson-Breysse et al., (1994), is seen in this site https://en.wikipedia.org/wiki/Jojoba_oil. The fatty acids are Lignoceric acid (C24:0), and Nervonic acid (C24:1). The oil can be used as an antifoam agent in antibiotics production and as a treatment for skin disorders. Other proposed uses include candles, plasticizers, detergents, fire retardants, transformer oil, and for the leather industry (Undersander et al., 1990).

Biogenetically, phenolic compounds proceed from two metabolic pathways: the shikimic acid pathway where, mainly, phenylpropanoids are formed and the acetic acid pathway, in which the main products are the simple phenol (Sánchez-Moreno, 2002). Most plants phenolic compounds are synthesized through the phenylpropanoid pathway (Hollman, 2001). The combination of
Table 6. Evaluate of phytochemical active constituents form by Kin and 2,4-D interaction up to 2 mg l⁻¹ stress in Jojoba (S. chinensis (Link) Schneider) callus.

| Media code | Treatments (mg l⁻¹) | Phytochemical active constituents |
|------------|---------------------|----------------------------------|
|            | 2,4-D | Kin | 1-Hexadecanol | Nonadecatriene | Aristolene | 7-Hexadecenal | Tricosene | Triodecanoic acid | Methoxycetic acid | Decanoic acid | Octadecanoic acid |
| A          | 0.0   | 0.0 | ++            | ++             | ++         | ++++         | ++++      | ++              | ++              | ++          | ++              |
| F          | 0.5   | 0.5 | +             | +              | +          | +            | ++        | +               | +               | +           | +               |
| G          | 0.5   | 1.0 | ++++          | ++             | ++         | ++          | ++++      | ++              | ++              | ++          | ++++            |
| H          | 2.0   | 1.0 | ++++          | ++             | ++         | ++++        | ++++      | ++              | ++              | ++          | ++++            |
| J          | 0.5   | 1.0 | +++           | ++             | ++         | ++++        | ++++      | ++              | ++              | ++          | ++++            |
| K          | 1.0   | 1.0 | ++++          | ++             | ++         | ++++        | ++++      | ++              | ++              | ++          | ++++            |
| L          | 2.0   | 0.5 | +++           | ++             | ++         | ++++        | ++++      | ++              | ++              | ++          | ++++            |
| N          | 2.0   | 1.0 | ++++          | ++             | ++         | ++++        | ++++      | ++              | ++              | ++          | ++++            |
| O          | 2.0   | 2.0 | ++++          | +++            | +++        | ++++        | ++++      | +++              | +++              | +++         | ++++            |

- (+) = One portion, (++) = two portion, (++++++) = six portion of constituents.

Both pathways leads to the formation of flavonoids, the highest group of phenolic compounds in nature (Sánchez-Moreno, 2002). Additionally, through the biosynthetic pathways to the flavonoids synthesis, among the not well-elucidated condensation and polymerization phases, the condensed tannins or non-hydrolysable tannins and glycosides are formed. Hydrolysable tannins are derivatives of gallic acid or hexahydroxydiphenic acid (Stafford, 1983). The result of the present study further revealed that jojoba simmondsin content increased quantitatively with the increase in callus from main to the fourth weeks of cultivation. The percentage of browning and necrosis is also in conformity with increase in fatty acids content in old culture. The increase in simmondsin content is normally associated with increase in the enzymes that regulate the synthesis of fatty acids compound, while the intensity of browning is related with the hyperactivity of oxidative enzymes (Cochrane, 1994). This compound has also shown a very strong antibacterial activity against bacteria and was detected only in jojoba oil in the present study. The GC-MS analysis showed the presence of forty-eight compounds in the leaves of B. Cylindrica by comparing their retention times and by interpretation of their mass spectra. The compounds identified and their retention time, molecular formula, and concentration (peak area %) are presented in Table 5a and b.

It should be noted that the obtained chromatograms repreent not only the fatty acids and oil but also all the compounds in the extracts of each treatment by gas chromatography-mass spectroscopy (GC-MS) analysis (Table 5a). In this study, various secondary metabolites were detected within jojoba callus. We have described the biological activity of certain metabolites and compared the callus based on the effect of different growth regulators on produced metabolites. Many of the important compounds were produced by jojoba callus. Some of them were not detected in any of the other species, for instance 3-Triodecanoic acid. This compound is a derivative of jojoba oil octane, which has a wide variety of biological activities and has a substantial importance as a structural fragment of a synthetic pharmaceutical compound (Table 5b).

Eventually, it can be concluded that there is a direct proportion between callus growth and active constituents’ induction in general as growth and development of cells and tissues contribute greatly to production, of the active constituents from the explant. In addition, auxins play more role than that of cytokinins for callus induction and subsequent active constituents production from the explants.

Conclusion

Some secondary metabolites are involved in the complex interactions between hormones added in tissue culture media and their growth conditions. Thus, analytical methods for the identification of...
criteria like mass-spectral factor, the application of this method shows a wide variety of major metabolites in jojoba callus from different explants. According to the trait of the compounds described in this study, we suggest the usage of complementary methods for purification of jojoba callus compounds, due to their application in agriculture as biological control agents of plant pathogens, or in medicine as sources of antimicrobial substances.

**Conflict of interests**

The authors have not declared any conflict of interests.

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