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THE EFFECT OF SUGARS ON THE PRODUCTION OF SECONDARY COMPOUNDS FROM CALLUS INDUCED FROM LEAVES (MATRICARIA CHAMOMILLA) AND IDENTIFICATION OF SOME REDUCING SUGARS USING THE FOURIER TRANSFORMATION OF INFRA-RED (FTIR) TECHNIQUE

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Abstract:

The effect of sucrose on the induction of callus induced by chamomile leaves was studied for the period from April 2023 to January 2024. The concentrations of sugars added to the medium were 20, 40, 60, or 80 g/L. Polysaccharides were added to MS medium individually. Callus was induced after culturing true leaves on MS medium prepared with 1.0 mg/L D2,4- and 0.2 mg/L BA. The results showed that adding sucrose at a concentration of 20 g/l led to a positive effect, and the highest value was for thujone and borneol compounds, reaching 135 and 154 micrograms/g dry weight of callus, respectively, while the concentration reached 40 g/g. L led to an increase in the production of Cineol1-8 121 µg/g, and the high concentration of sucrose (80 g/L) is the most effective in stimulating the production of callus. It is known that reducing sugars are aldehyde or ketone monosaccharides, the most important of which are glucose, mannose, galactose, and fructose, sugars play a vital role in metabolism, and its content in chamomile flowers means that it can be transported to carbohydrate levels in food and biological systems. The percentage of reducing sugars was determined Infrared radiation to identify the active groups of the compounds fructose and glucose (FTIR) and it appeared that they contain some aliphatic, hydroxyl and carboxyl groups.

Keywords: Tissue Culture, *Matricaria Chamomilla*, Secondary Compounds Callus, Fourier Transformation of Infra-Red (FTIR)

INTRODUCTION: Chamomile is a herbaceous plant about 15-50 cm high, with a fast-growing stem. It has many branches and blooms 6-8 weeks after germination, and its leaves are alternating, feathery, divided into small, elongated, threadlike sections. The plant has a distinctive refreshing scent [1]. The inflorescence and surrounding flowers are white, and the inner flowers are tubular in color yellow, it lives in fields, on the edges of valleys, and in home gardens chamomile is known scientifically as *chamomilla matricaria,* which is a species wild, the part used is the open flowers, and it contains chemicals contains up to 1.5% volatile oil from dried flowers and substances [2]. The active ingredient in this oil is the blue chamazulene compound. The health effect returns chamomile is a healer due to the presence of azulene, one of whose properties is like olive oil which contains unsaturated fatty acids with high chemical affinity bonding with other materials to create useful materials [3].

The medicinal uses of chamomile have been known since ancient times. It has been used to relieve stress and insomnia relax muscles when using chamomile oil with massage, or drinking chamomile tea treating lower back pain and menstrual pain. Treating mouth ulcers caused by medications used to treat cancer when used in the form of mouthwash, relieving diarrhea in children, relieving nausea and vomiting, and treating children's colic. The oil extracted from the plant is used to relieve digestive problems such as diarrhea, nausea, indigestion, feeling bloated, and colic. It also works to enhance the digestion process by relaxing the intestinal muscles [4].

Plants produce complex organic compounds that do not have a direct function in growth, called secondary metabolic compounds are produced from primary metabolic compounds (carbohydrates, proteins, and fats) that are of great importance in the processes of plant growth and development. The role of secondary compounds in plant defense is evident as a preventive means against pathogens. Studies indicate the great importance of secondary metabolite compounds in the pharmaceutical and medicinal drug industry, and that more than a quarter of the medicines produced in the world during the past three decades are derived from secondary plant compounds, despite the fact that modern chemical compounds, medicines, and drugs manufactured and produced in the laboratory are highly effective in treating many diseases with It is cheap and widely produced, but it has serious side effects on human health[5]. In two ways, it may benefit and harm at the same time if the treatment continues for a long time. Therefore, started returning to nature, herbs, and plant therapy due to their great benefits, including the nutritional elements they contain, in addition to the natural active substances they contain, which help in preventing and healing diseases, and have no harmful effects on humans or their health [6]. A medicinal plant is defined as containing in one or more of its various organs one or more chemical substances at a low or high concentration that has the physiological ability to treat a specific disease or at least reduce the symptoms of this disease if it is given to the patient either in its pure form after being extracted from the plant material or if it was used while it was still in its original state as a fresh, dried or partially extracted herb [7].

Studies indicate the possibility of stimulating cultivated plant parts to produce compounds secondary education when exposed to stress, which is represented by the addition of some compounds to the food environment, including sugars. Increasing the concentration of sucrose added to the medium from 2% to 4% led to an increase in the production of the compound polyphenol in callus of the shrub rose plant Hybrida Rosa. While others indicated that raising the percentage of sucrose from 2% to 5% in the growing medium of rosemary callus, which led to an increase in the concentration of rosmaric acid. In a study, it was proven that adding sucrose at a concentration of 30, 60, 90, or 120 g/L to the culture medium of callus *somniferumpPapaver*, adding 90 g/L to the MS medium prepared with 30 mg/L of amino acid tyrosine gave the highest amount of morphine and codeine alkaloids, reaching 2.9 and 2.8 mg/g[8].

Fresh weight of callus respectively adding 6% sucrose to the medium led to an increase in alkaloid production morphine for poppy plants. By studying the effect of different types of sugars, including sucrose and glucose, fructose, and maltose in concentrations of 30, 50, 70, or 90 g/L. Adding 30 g/L of maltose was the best in producing cardiac glycosides from the purpure digitalis plant and that's an addition sucrose at a concentration of 40, 60, 80, or 120 g/L into the callus culture medium of *rosa vinca* plant. High concentrations of sucrose led to increased production of *vincristine alkaloids* [9,10]. This study aims to determine the effect of adding different types and concentrations of sugars in stimulating the production of some compounds secondary infection of chamomile plant callus induced by leaves.

2. MATERIAL AND METHODOLOGY:

This study was carried out in the Tissue Culture Laboratory of the Ministry of Science and Technology from April 2023 to January 2024, laboratory analyzes were conducted to determine the active ingredients. The seeds were sterilized after soaking them with a concentration of 4% of the commercial minor, which contains 6% of sodium hypochlorate NaOCl for (10) minutes according [11]. Then it was washed with sterile distilled water three times to remove any traces for the sterile substance, seeds were planted on a solid medium free of growth regulators and with a quarter strength of salt. The seeds were placed in the growth room with a light intensity of 1000 lux, an illumination period of 16 hours of light, and a temperature of 25 ± 2. Four weeks after the seeds germinated, the true leaves were removed and planted in cultivation tubes measuring 25 x 150 mm each tube contains 10 ml of MS medium supplemented with 1.0 mg/L of D2,4- and 0.2 mg/L of BA and a number of sugars, including the sugars sucrose, glucose, fructose, sorbitol, or mannitol in concentrations of 20, 40, 60, or 80 g/L of each type of sugar separately and was running the process of replanting on new media every three weeks until the required callus weight is reached[12].

2-1 Extraction, quantitative and qualitative estimation of active substances:

Dry the callus at 40°C for 24 hours, then grind it and take 1.5 grams of powder. 7.5 ml of 96% pure ethyl alcohol was added to it, and the samples were placed on a shaking device for 24 hours. Then the solution was filtered using filter paper in a picrate in an electric oven at a temperature of 40°C for 24 hours to turn it into a powder. Dissolve the powder in 3 ml of pure ethyl alcohol. The active ingredients in the solution, including Thujone, Borneol, Camphor, and Cineol1-8, were determined using an HPLC mass device. Inject all of the solution The standard and the sample in the HPLC device type 2010 LC Chromatography al Shimadzuja to detention time and rotation , sample beam height area for both the standard solution and the sample where the extract is injected in a d.L mm2-6×50 column, and a mobile phase consisting of:

methanol deionized water with a ratio of 90:10 V/V, and the flow speed of the device was 0.8 ml/min, and the readings were measured on wavelength of 280 nm and a temperature of 30°C. The concentration of each sample was calculated according to the following equation according [13]:

Area of the standard solution x concentration of the standard solution x number of dilutions Area of the sample = concentration of the sample

2-2 Fourier Transformation of Infra Red (FTIR):

Spectroscopic organic diagnosis for the determination of total and reducing sugars using a method ready-made sheets of glass made of silica foil (20 cm and 0.2 mm thick) were used. They were dried and activated in an oven at 110°C for an hour and a half, with dimensions of 10 gel G to eliminate moisture, sample liquid samples at a concentration of mg/cm3 were placed with standard samples at a distance of 1 cm from 10. The process of direct drying of the stains was carried out using a thermal dryer. µl(the edge of the board in equal-sized patches. A mixture of ethyl acetate: propional alcohol: distilled water was used in a ratio of: 1:5, respectively, as a mobile phase to separate sugars, saturate the separation vessel with the solvent mixture before transferring the plate to it. Allow the solvent to rise 15 cm from the place where the models were placed. Naphthoresor cinol % dissolved in water and dried the plate at laboratory temperature. The chips were then sprayed with a mixture of 0.2 and phosphoric acid, 85%, with a volume ratio of (9:1), then heated at 120°C for 5 minutes to reveal the stains.

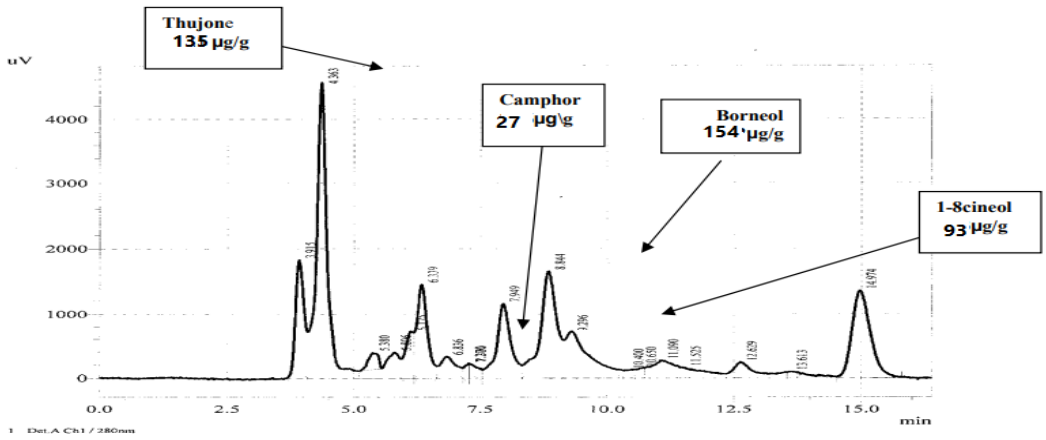
3-STATISTICAL ANALYSIS:

A completely randomized design (CRD) was used with ten replicates, and each test tube represents a replicate. The means were compared to show the statistical differences between the coefficients at the probability level of 0.05 (20). 2 Using T and x scan[14].

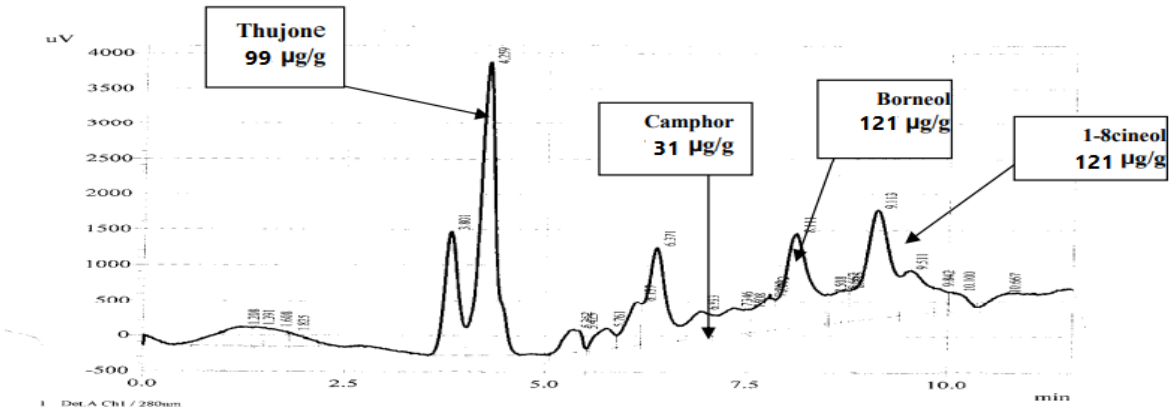
4-RESULTS AND DISCUSSION:

4-1 The effect of sucrose on the production of secondary compounds:

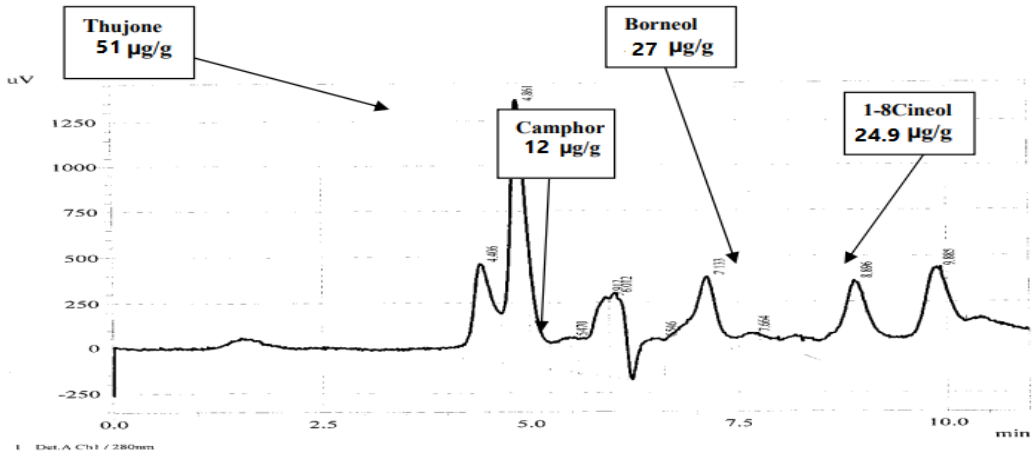
It is noted from the results of the table below that there are significant differences between the concentrations of the studied secondary compounds when different concentrations of sucrose are added to the nutrient medium. The concentration of 20 g/L gave the highest amount of Thujone, amounting to 134.1 micrograms/g dry weight of the callus, while increasing the concentration low (41.9 µg/g dry weight of calluses). However, the high concentration of sucrose (80 g/L) was most effective in stimulating the callus to produce Camphor, as its value reached 37.0 micrograms/g dry weight of callus adding low concentrations of sucrose (20 or 40 g/L) to the nutritional medium led to an increase in the concentrations of Borneol and Cineol1-8 reached 151.2, 115.5 and 78.4 and 109.9 micrograms/g dry weight, respectively.

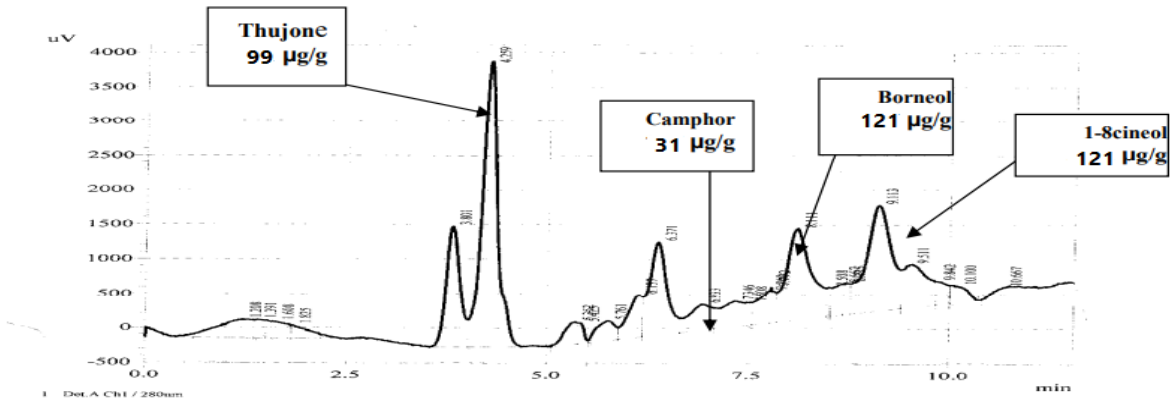


The effect of sucrose at a concentration of 20 g/L on the production of secondary compounds from the callus tissue of chamomile plants

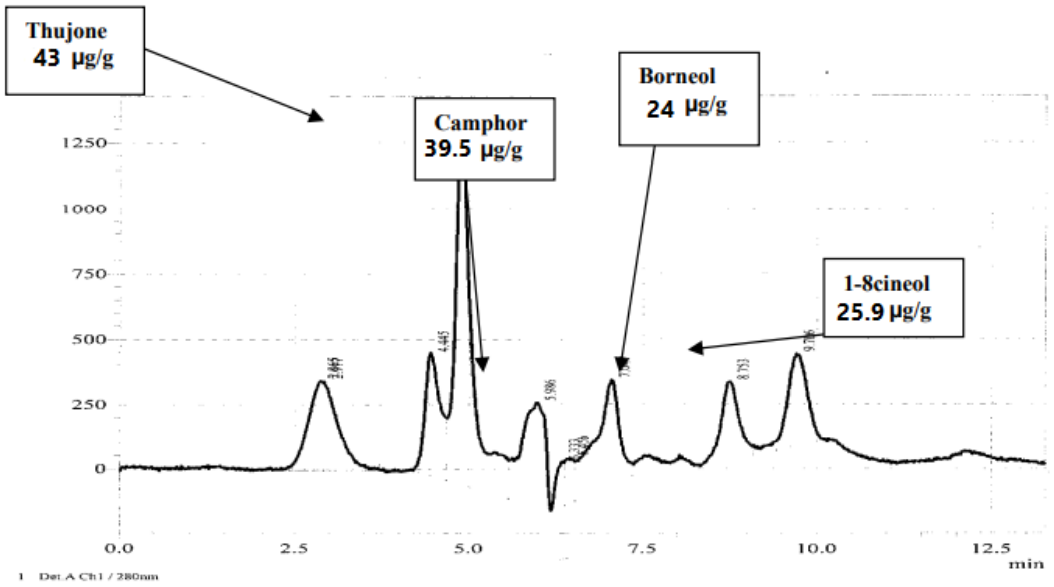


The effect of sucrose at a concentration of 40 g/L on the production of secondary compounds from the callus tissue of chamomile plants





The effect of sucrose at a concentration of 60 g/L on the production of secondary compounds from the callus tissue of chamomile plants



The effect of sucrose at a concentration of 80 g/L on the production of secondary compounds from the callus tissue of chamomile plants

Increasing the concentration of sucrose added to the nutrient medium reduces the callus production of secondary compounds, with the exception of camphor, the results of which showed that it is different, as its concentration increases with the increase in the amount of sucrose according [15]. This may be due to the fact that sucrose is a carbohydrate substance that is added to tissue culture media at concentrations ranging between 20-30 g/L and the concentration increases, and the added substance may have led to changes in the water relations of the soil. cells due to an increase in the voltage applied to the cells, which requires the cells to reorganize their osmotic potential in a way that ensures their ability to adapt to the new conditions to which the cells are exposed, which leads to a decrease in the availability of water and thus the solubility of nutrients in the medium in which the cells grow agree with [10]. The effect of explants and various experimental concentrations working to maximize the benefit of callus induction and cell proliferation in cell suspension culture is the first step to production of large quantities of active compounds, first step in plant tissue culture is the development of culture callus from the whole plant, callus is widespread cluster of cells without any significant differentiation, callus can be obtained from any part of the whole plant containing dividing cells, which indicates plant hormones or growth regulators necessary to induce callus tissue and strengthen growth of multiple cell lines. Since all plant species it requires different types and levels of plant hormones for callus induction, growth and metabolites production, it is important to specify more suitable growth regulators and their identification optimal concentrations, the goals determination of undifferentiated cell mass in vitro. It served as a support for plant reproduction and maintenance for a long time in the laboratory for a long time and be suitable for genetics transmission and genetic engineering techniques to preserve genetic material successful results have been obtained by other researchers from planting explants stem, leaves according to [15,16]. The medium contains NAA and Kinetin which produced the results our experiments are consistent with the results in reports of callus induction by addition of kinetin at lower or equal concentrations are necessary for callus growth that was consistent with the results obtained in this study[17] . The results showed that the percentage of reducing sugars reached 22%. It is known that reducing sugars are monosaccharides, aldehydes or ketones, the most important of which are glucose, mannose, galactose, and fructose, sugars play a vital role in metabolism, and their content in chamomile flowers means they can be transported to carbohydrate systems. The presence of these sugars is confirmed by chromatographic analysis of the thin layer of chamomile flowers.

Tabl1 FTIR Infrared vibration frequencies of active aggregates in chamomile

Assignment	Wavenumber (cm-1)	
O-H stretching	3048	Strong and broad
C-H stretching	2920	Strong, sharp
C=C stretching	1354	Medium
C-O stretching	1001	Weak
C=O stretching	1743	Strong and broad

Since sugars are polar compounds, they contain multiple hydroxyl groups Aldehyde or ketogenic, this method showed that the aldehyde sugars C=O carbonyl Glucose gives a blue spot, while the ketone sugar

fructose gives a dark red spot. For these components, compared to a standard model, they were 0.6 and 0.75, respectively. It was observed that there was a convergence in the values that belong to the O-H carboxylic acids, some of them, and the appearance of effective sites for absorption bands represented by composition, which goes back to esters, carboxylic acids, aldehydes, and ketones, C=O, alcohol or water, and groups that go back to the aliphatic or aromatic structures found in chamomile, C=C.

5- CONCLUSION :

In general, it was found that by increasing the concentration of sucrose added to the nutrient medium reduces the callus production of secondary compounds, except for Camphor, which showed results, it s different, as its concentration increased with the increase in the amount of sucrose added, and this may be due to the fact that sucrose is a carbohydrate which is added to the tissue culture medium at concentrations ranging between 20-30 g/L and the concentration increases the additive may have led to changes in the water relations of the cells due to the increased voltage applied to the cells, which requires the cells to reorganize their osmotic potential in a way that ensures they can adapt to the new conditions to which the cells are exposed which causes a decrease in the availability of water and therefore dissolved nutrients in the medium in which the cells grow.

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