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ORGANIC AMENDMENT DEPENDENT MODULATION OF PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT CAPACITY AND CARBOHYDRATE-HYDROLYZING ENZYME INHIBITORY ACTIVITY IN BRASSICA NIGRA AND TRIGONELLA FOENUM GRAECUM

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ABSTRACT
The development and growth of the plant, synthesis of secondary metabolites and production of biologically active substances could be associated with fertilization process. The application of organic amendment for nutrient management as an alternative to chemical fertilizers becomes more and more popular.The aim of the current study was the analysis of phytochemical composition and certain biological activities of Brassica nigra and Trigonella foenum graecum in organic amendment and chemical fertilizer cultivation. The plants were cultivated with two different fertilizers, collected after 15 days cultivation period, dried and powdered. Then the methanolic extracts were prepared, and the analysis of phytochemicals, total phenols, total flavonoids, tannins content, DPPH radical scavenging activity, FRAP assay, α-amylase and α-glucosidase inhibition activities was performed. Furthermore, the analysis of volatile and semi-volatile compounds was carried out by GC-MS.The highest content of total polyphenols (TPC – 57.3 mg/g), total flavonoids (TFC – 58.5 mg/g) and total tannins (53.6 mg/g) were revealed for B. nigra plants fertilized by organic amendment, while the lowest content (29.3, 28.9 and 28.4 mg/g) for B. nigra plants fertilized chemically. T. foenum-graecum plants contained higher amounts of TPC, TFC and TTC during fertilization by organics comparing with those fertilized by chemical. During the antioxidant experiments, the differences according to the type of fertilization were found, when plants fertilized inorganically had lower IC_{50} value of DPPH in both types of plants. Also, the α-amylase and α-glucosidase inhibitory experiments confirmed the inhibition of these plants. As it can be seen from the results, the fertilization affects the phytochemicals and biological activity of both plants.

KEYWORDS: Brassica nigra; Trigonella foenum graecum; biofertilizer; chemical fertilizer; phenolics; flavonoids; antioxidant activity; α-amylase; α-glucosidase; GC–MS.

1. INTRODUCTION

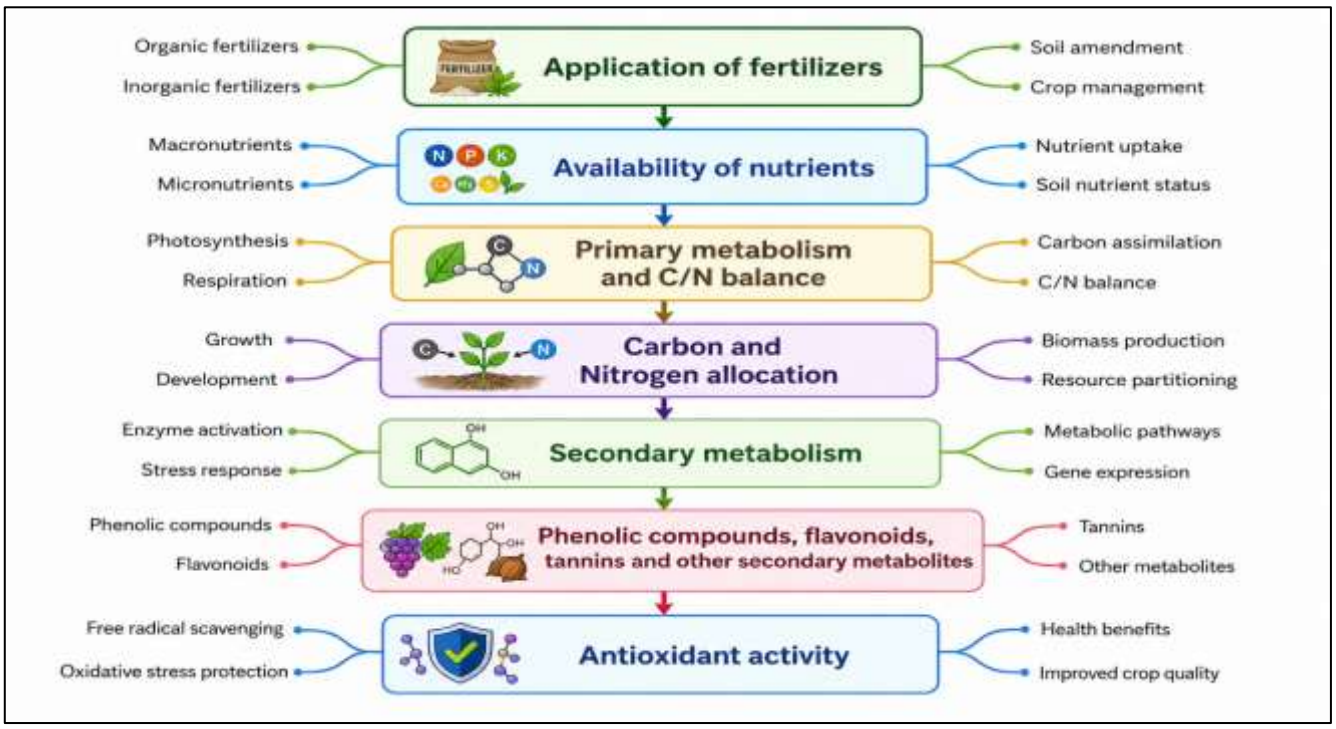
1.1 Nutrient sustainable management in crop production

The current model of crop production is characterized by addressing two significant issues at once increasing the efficiency of crop production and decreasing the negative effect of the nutrients use on the environment. Mineral fertilizers are applied in order to solve the above-mentioned problems. However, the improper use of mineral fertilizers leads to the decrease of the effectiveness of the nutrients because of the loss of the nutrients during the leaching, volatilization, runoff and other processes. This results in such consequences as soil degradation, water pollution, eutrophication and emissions of greenhouse gases. Consequently, the improvement of nutrient use efficiency (NUE) becomes the way of sustainable crop production (Lu et al., 2024).The effects of nutrient acquisition, physiological state, stress tolerance and plant quality may be influenced by plant biostimulants, although the nature of these effects may vary depending on the type of input used, crop type, the way of its application and environment (Lu et al., 2024).There are several options available for influencing nutrient availability with the help of microorganisms. The plant growth promoting rhizobacteria are capable of increasing nutrient availability through nitrogen fixation, phosphate solubilization, siderophore production and stimulation of root growth due to phytohormones release. Thus, the application of microbes changes the plant-soil interactions rather than nutrient availability itself. However, modern scientific research focuses on the interaction between plants and microbes, which increases the availability of nutrients and improves the quality of agricultural plants (Ramírez-Cariño et al., 2024). Thus, sustainable nutrient management requires not only maximization of biomass, but also high productivity and quality of crops. This factor is especially relevant for the crops grown for consumption, including food, spices, medicinal plants and bioactive compounds. Nutrient availability influences metabolic processes that take part in

the production of phenolic compounds, flavonoids, alkaloids and other biochemical responses. Thus, the management of fertilizer affects not only plant growth, but also the biochemical properties of its products (Lu et al., 2024). In the present study, the nutrient management concept provides the basis for comparing the biochemical responses of Brassica nigra and Trigonella foenum graecum cultivated under contrasting organic and chemical amendment treatment.

1.2 Fertilization and plant secondary metabolism

In addition to increased biomass production and growth, some of the physiological effects of fertilizer usage include alterations in substrate availability, enzyme activities, carbon and nitrogen allocation, oxidation, signal transduction and consequently secondary metabolite production. The reason behind this effect is related to the fact that secondary metabolites are part of the defense mechanism of the plant and serve to adapt to stress as antioxidants as well as participate in plant-environment interactions. The connection between the above factors can be represented as follows:



Fertilization and plant secondary metabolism

Phenolic compounds and flavonoids are just two examples of very sensitive secondary metabolites. They are created through interconnected biosynthesis pathways as antioxidant and defense compounds for plants. Therefore, synthesis of flavonoids can be regulated on different biochemical and transcriptional levels, depending on different environmental and nutritional factors (Jiang et al., 2023). There is no linear relationship between secondary metabolism and nutrient availability. Increase in nutrient availability can lead to stimulation of growth and primary metabolism and, as a consequence, decrease in carbon allocation for some defensive metabolites. However, limitation or imbalance of nutrient availability can play a role of physiological stress factors that stimulate accumulation of some secondary metabolites. In other words, the response depends on the type of nutrients, their availability, plant species, its development stage, and the environment.

This needs to be considered particularly in case of comparison of biological and chemical sources of nutrients from this point of view. Biological source can affect nutrient availability in a special way and, in addition, can change rhizosphere properties, whereas chemical source can provide nutrients in easily available form.

Taking into consideration the results of the last research works related to the issue of biostimulatory effect of plants, the involvement of plant biostimulants in the processes of nutrient uptake, photosynthesis, stress response, and quality of products is based on physiological and biochemical principles (Lu et al., 2024). According to the review on stress biology of Brassica, phenolics and flavonoids participate in plant stress responses and act as antioxidants (Muthusamy & Lee, 2024). The analysis of the impact of the fertilizers on the process of phytochemicals production will provide an opportunity to determine whether the usage of fertilizer techniques can affect biochemical processes instead of the growth and yield.

1.3 Brassica nigra as a Source of Phytochemicals

Brassica nigra (L.) W.D.J. Koch is an important plant from the Brassicaceae family; this plant is called black mustard. The biological significance of B. nigra consists in its high content of phytochemicals and glucosinolates. Glucosinolates belong to unique metabolites that contain sulfur and nitrogen atoms and are characteristic only for Brassicaceae genus. The glucosinolate specific for B. nigra is called sinigrin. Glucosinolates are destroyed under tissue damage conditions and can be hydrolyzed with myrosinase enzyme resulting in the formation of isothiocyanates – the active substances responsible for the distinctive smell of mustard and involved in plant defense (Sikorska-Zimny & Beneduce, 2020). Apart from glucosinolates, the phytochemical content of B. nigra includes phenols and flavonoids, which play an essential role in antioxidative activity of the plant. There are some latest studies related to Brassica species where phenols and flavonoids

are included in oxidative stress response (Muthusamy & Lee, 2024). The biological significance of the metabolites generated as a result of glucosinolate hydrolysis process has also become another exciting field of study apart from its importance in protection of the plants from pathogens. There have been a number of scientific researches carried out in this area which involve metabolic and biological aspects of glucosinolate hydrolysis metabolites including their involvement in glucose metabolism. While handling such kind of information, one must be careful because the biological activity of pure compounds cannot be correlated directly with the plant extracts (Bhat et al., 2025). The nature of B. nigra can be considered as an ideal starting point for carrying out experiments related to the influence of nutrients on the phytochemicals. The influence of nutrients on the phytochemicals may vary depending upon development and environment.

1.4 Trigonella foenum graecum as a functional crop

Trigonella foenum-graecum L. (fenugreek) serves as one of the key examples of the leguminous crop plants that are cultivated for purposes of being used as food, spices, and medicinal plants. Fenugreek seeds, along with other parts of the plant, contain many different types of primary and secondary metabolites.The key phytochemicals that have been identified in the plant include polyphenols, flavonoids, saponins, alkaloids, steroidal sapogenins, and galactomannans. Some of the major chemical substances that are found in the plant are trigonelline, 4-hydroxyisoleucine, and diosgenin. Some of the reviews on the phytochemistry, as well as biological activity, including the antioxidant and metabolic properties of fenugreek include (Akhtar et al., 2025). Trigonelline is one of the key alkaloids present in the fenugreek. The substance has been studied due to its influence on glucose metabolism and nervous system. The mechanism of the neuroprotective and antidiabetic effects of the substance has been described in one of the latest review articles (Liang et al., 2023). Apart from that, steroidal sapogenins like diosgenin are found in fenugreek. The main reason for conducting various studies on diosgenin in laboratory conditions is because of its effect on biological processes such as lipid metabolism and many others (Sun et al., 2021). One more substance found in fenugreek is galactomannans that are significant soluble polysaccharides which lead to the functional and nutritive properties of fenugreek. Based on the studies conducted on active substances found in fenugreek, it was discovered that galactomannans, saponins, alkaloids, and polyphenols are responsible for the functional properties of fenugreek (Gavahian et al., 2023). Besides, the amount and composition of these substances may differ according to genotype, part of the plant, development stage, environmental conditions, and cultivation process. It is for this reason that fertilizer application turns into one of the most important but least studied factors in affecting the biochemical quality of fenugreek. This is the reason T. foenum-graecum is a perfect subject for studying the influence of different nutrients on phytochemicals and antioxidant/ inhibitor activity.

1.5 Research gap

Although there were many research works of scientific literature devoted to the phytochemical composition and biological activity of B. nigra and T. foenum-graecum, most of them were focused on the chemical composition of the plants under investigation, the processes of extraction and medical usage of the plants. However, no investigations were conducted considering the effect of different nutrition systems on the phytochemical composition of the plants under study.

In addition, although most of the research studies of influence of fertilizers were focused on traditional aspects of agricultural technology such as seed germination, growth and productivity of the plants, only few of them studied the quality of the secondary metabolites of the plants, their influence on the biological activities. Among the questions considered in the current research:Is there any difference in phytochemical composition and the antioxidant and carbohydrate hydrolyzing enzyme inhibition activities of B. nigra and T. foenum-graecum because of the differences in the nutrient management strategies used?This is an important question, as any change in the total content of phenolic compounds, flavonoids and its derivatives is the first evidence of change in the functional quality of the plant. Nevertheless, it does not provide sufficient information on the mechanisms of actions of the active ingredients. Therefore, it is necessary to conduct GC-MS and functional analyses to receive more important data.This question is important for sustainable agriculture, as the main task of the current research is not only to identify which fertilizer increases the biomass of the plants, but also how the nutrient management influences the biochemical and functional qualities of the plants.

1.6 Hypothesis

In view of the relationship between the nutritional value, metabolism and secondary metabolites production, it can be hypothesized that, there is an alteration in the content of secondary metabolites in Brassica nigra and Trigonella foenum graecum as a result of organic amendment leading to different levels of antioxidant activity and enzymes inhibition. Another hypothesis which is based on a comparison of these two plants is: Species is important due to different metabolism in Brassica nigra and Trigonella foenum graecum.The hypothesis will be useful for you since you have to deal with two plants and not one.

2. MATERIALS AND METHODS

2.1. Sample Collection And Preparation

Fresh leaf litter and seeds of Brassica nigra (mustard) and Trigonella foenum graecum (fenugreek) were obtained from a flower market in Koyambedu, Chennai, while black soil, seed trays, and di-ammonium phosphate (DAP) were obtained from a nursery in Virugambakam; cow dung was sourced from cattle near Broadway, and domestic waste was collected from household sources, with all samples transported in sterile zip-lock bags to avoid contamination. The biofertilizer was made by homogenising 250 g of black soil, 250 g of cow dung, 400 g of domestic waste, and 100 g of leaf litter in a covered container and allowing it to shade-dry for 15 days before use. For the pot culture experiment, two seed trays were

used, one filled with the prepared biofertilizer and the other with di-ammonium phosphate (DAP), each divided into two equal halves to accommodate mustard and fenugreek seeds separately, allowing for a comparison of organic versus inorganic growth conditions. Irrigation was done in stages over the 15-day growth period: 25 mL of water was sprayed twice daily during days 1-5, once daily during days 6-10, and on alternate days during days 11-15, after which the seedlings were harvested, thoroughly rinsed under running tap water to remove adhering fertiliser residues, and shade-dried for three days. The dried plant material was then crushed up with a mortar and pestle to produce a fine powder, which was stored in labelled zip-lock bags under ambient conditions for further phytochemical and analytical investigations.

2.2. Extraction of Plant

Extraction must be done in a 1:10 ratio. In four 50ml conical flasks, place 3g of organic Brassica nigra (mustard seeds) and Trigonella foenum graecum (fenugreek seeds) and 30ml of inorganic Brassica nigra (mustard seeds) and Trigonella foenum graecum (fenugreek seeds). Cover with foil paper and leave for 48 hours. After 48 hours, filter the sample with filter paper and heat it in a hot plate for solvent extraction. The resulting plant extract is used for further processing.

2.3. Phytochemical analysis

The crude extract was subjected to a battery of standard colour-reaction tests to screen for the presence of key phytoconstituents. Each test was performed in duplicate to confirm consistency of results.

Table 1 : Analysis of Phytochemicals

NO	PHYTOCONSTITUENT	TEST PROCEDURE	POSITIVE OBSERVATION
1	Carbohydrates	2 mL of extract was treated with 1 mL of alpha-naphthol reagent, followed by 2 mL of ethanol and dropwise addition of 1 mL of concentrated sulphuric acid along the tube wall.	Appearance of a violet ring at the liquid interface
2	Steroids	0.5 mL of extract was mixed with 2 mL of chloroform, and 1 mL of concentrated sulphuric acid was added carefully along the side of the tube	Formation of a reddish-brown ring at the interface
3	Saponins	2 mL of extract was diluted with 2 mL of distilled water and shaken vigorously	Persistent honeycomb-like froth lasting about 10 minutes
4	Flavonoids	1 mL of extract was treated with 1 mL of 2N sodium hydroxide solution	Immediate appearance of a yellow colouration
5	Quinones	1 mL of extract was treated with 1 mL of concentrated sulphuric acid	Development of a red colouration
6	Amino acids (Ninhydrin test)	2 mL of extract was treated with a few drops of 0.2% ninhydrin solution and heated for 5 minutes	Formation of a blue colouration
7	Terpenoids	0.5 mL of extract was mixed with 2 mL of chloroform, and concentrated sulphuric acid was layered carefully beneath the mixture	Reddish-brown colouration at the layer interface
8	Coumarins	1 mL of extract was mixed with 1 mL of 1N sodium hydroxide and heated in a boiling water bath for a few minutes with intermittent shaking	Appearance of a yellow colouration

2.4. Determination of Phenolic content

Using Gallic Acid as a standard (10 mg/10 ml), the Folin-Ciocalteu colorimetric method was used to calculate the amount of phenolic compounds in the extracts. Carbinolic extract of Brassica nigra (mustard seeds) and Trigonella foenum graecum (fenugreek seeds) were taken in different concentrations such as 25µg, 50µg, 75µg and 100µg. Reaction mixture was made by taking 0.5ml of plant extract and mixed with 2.5ml of 10% water dissolved Folin-Ciocalteu's reagent and finally add 2.5ml of 7.5% NaHCO3.The samples prepared for analysis were then incubated in a thermostat at 45ºC for 45 min. The absorbance was noted at 765nm. The samples were prepared in triplicates and the mean value of absorbance was obtained. The procedure was repeated for Standard Positive Control Gallic acid and Calibration curve was developed. Total Phenolic Content was expressed in terms of mg of GAE/gm of extract (Singleton et al 1999).

2.5. Estimation of total flavonoid content

Total flavonoid content was estimated by the aluminium chloride colorimetric method (Lee Wei Har et al., 2012), using quercetin as the standard. Methanolic extracts were prepared at concentrations of 25, 50, 75, and 100 µg. To 1 mL of extract, 4 mL of distilled water, 0.3 mL of 5% sodium nitrite, and 0.3 mL of 10% aluminium chloride were added sequentially at 5-minute intervals, followed by 2 mL of 1M sodium hydroxide, and the volume was made up to 10 mL

with distilled water. Quercetin standards (25–100 µg) were treated identically. Absorbance was measured at 510 nm using a UV-Visible spectrophotometer, and TFC was expressed as mg quercetin equivalent per gram of extract (mg QE/g).

2.6. Determination of Total Tannin Content

Total tannin content was estimated by the Folin-Ciocalteu method (Rajeev Singh *et al.*, 2012), using gallic acid as the standard. Extracts (25–100 µg) were mixed with 7.5 mL distilled water, 0.5 mL Folin-Ciocalteu reagent, and 1 mL 35% Na_2CO_3, made up to 10 mL, and incubated at 35°C for 30 min. Gallic acid standards received the same treatment. Absorbance was measured at 725 nm, and TTC was expressed as mg gallic acid equivalent per gram of extract (GAE/g).

2.7. Antioxidant Activity Study

Antioxidant Activity of Trigonella foenum graecum and Brassica nigra Antioxidant activity of organically and inorganically fertilized Trigonella foenum graecum and Brassica nigra was assessed by DPPH and FRAP assays, with results expressed as IC_{50} (Mean ± SD, ANOVA).

2.8. DPPH Radical Scavenging Activity

DPPH Radical Scavenging Activity DPPH activity was assessed at concentrations of 25, 50, 75, and 100 µg using carbinol as solvent, with BHT as the standard. IC_{50} values for organically and inorganically fertilized T. foenum-graecum were 52.39 µg/mL and 32.02 µg/mL, respectively, while those for B. nigra were 66.24 µg/mL and 27.21 µg/mL, respectively. The standard BHT showed an IC_{50} of 63.98 µg/mL.

2.9. FRAP Assay

Quercetin was used as the standard to measure FRAP activity in a similar manner. T. foenum graecum fertilized both organically and inorganically had IC_{20} values of 44.31 µg/mL and 22.13 µg/mL, respectively, while B. nigra had values of 56.23 µg/mL and 31.02 µg/mL. The IC^{21} of the standard quercetin was 58.26 µg/mL.

2.10. Antidiabetic Activity

2.10.1. Inhibition Assay for Alpha Amylase Activity

The α-amylase enzyme and 0.1% starch solution were used to measure the α-amylase inhibitory activity, and DNSA reagent was used for colorimetric detection. Plant extracts (25–100 µg) were incubated with starch for 10 minutes, then the enzyme was added for 10 minutes at 25°C. The process was then stopped with DNSA reagent, boiled for 5 minutes, cooled, diluted, then measured at 540 nm. In triplicate, acarbose was used as the standard and DMSO as the blank. % Inhibition is equal to [(Abs control − Abs sample)/Abs control] × 100.

2.10.2. Inhibition assay for alpha glucosidase activity

A starch (maltose/sucrose) substrate was used to measure the inhibitory activity of α-glucosidase. The extracts were treated with Tris buffer (pH 8, 5 min, 37°C), then α-glucosidase (40 min, 37°C), and finally 6N HCl. In triplicate, absorbance was measured at 540 nm using acarbose as the standard and DMSO as a blank. Inhibition was computed using the following formula

% Inhibition = (Absorbance of control- Absorbance of sample) × 100 Absorbance of Control

2.10.3. Inhibition assay for alpha glucosidase activity

α-Glucosidase inhibitory activity was measured using a 2% starch (maltose/sucrose) substrate. Plant extracts were incubated with 1 mL of 0.2M Tris buffer (pH 8) for 5 min at 37°C, followed by 1 mL α-glucosidase (1 U/mL) and incubation for 40 min at 37°C. The reaction was terminated with 2 mL of 6N HCl, and absorbance was determined at 540 nm. In triplicate, acarbose was used as the standard and DMSO as the blank. % Inhibition is equal to [(Abs control − Abs sample)/Abs control] × 100.

2.11. Characterization

2.11.1. Gas Chromatography–Mass Spectrometry (GC-MS)

Using a Perkin Elmer Clarus 500 GC-MS system equipped with an Elite-5MS capillary column (30 × 0.25 mm × 0.25 µm), the mass spectra were recorded at 70 eV over a 45–450 Da range and identified using the NIST library. The oven temperature was programd from 110°C (10°C/min to 200°C, then 5°C/min to 280°C, held for 9 minutes.

2.11.2. Compounds Identification

Molecular structure, molecular mass, and fragmentation pattern were used to identify compounds; spectral interpretation was carried out using the NIST library, which has over 62,000 reference patterns; each detected component's name, molecular weight, and structure were ascertained by comparing its mass spectrum with those kept in the NIST database; the relative percentage of each component was computed from its average peak area relative to the total peak area.

3. RESULTS

3.1. Compost Preparation

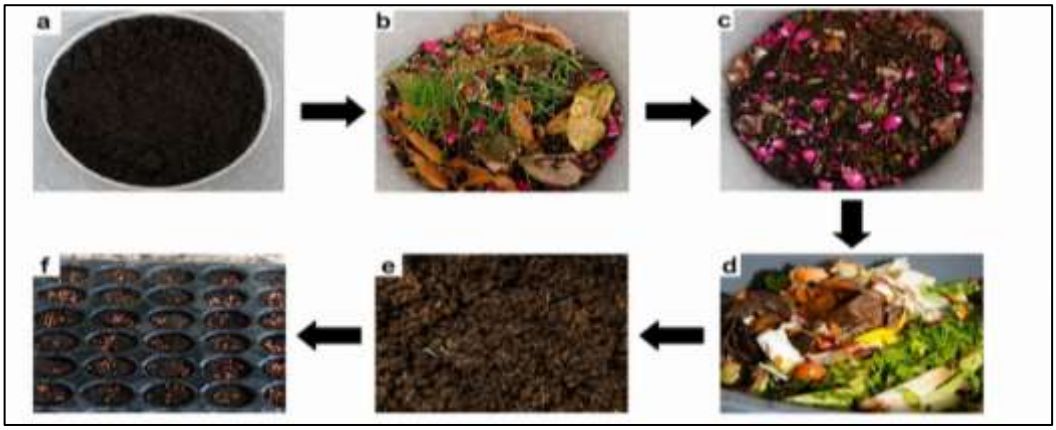


Figure 1 : Raw materials and stages involved in compost preparation: (a) black soil, (b) leaf litter, (c) cow dung mixed with soil, (d) domestic organic waste, (e) prepared compost, and (f) compost-filled nursery trays

3.2. Extraction and Processing of Plant Samples

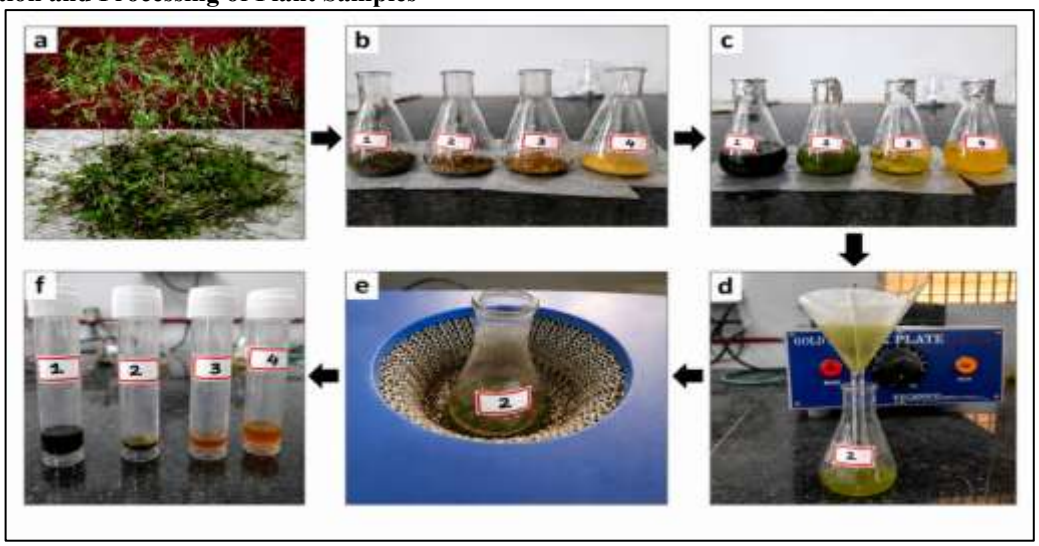


Figure 2 : Extraction and processing of plant samples: (a) harvested plant material, (b) powdered plant samples in extraction flasks, (c) solvent extraction of the plant samples, (d) filtration of the extracts, (e) concentration of the filtered extract, and (f) extracted samples stored in centrifuge tubes for further processing.

3.3. Biological Studies of Trigonella foenum-graecum and Brassica nigra

Carbinol was used as the solvent system to extract Trigonella foenum-graecum and Brassica nigra, which were cultivated utilizing both organic and inorganic fertilizers, in order to calculate the percentage yield. T. foenum graecum yielded 51.3% and 40.1% were cultivated organically and inorganically, respectively, while B. nigra yielded 56.9% and 33.7%. The samples that were grown organically showed the highest percentage yield of Brassica nigra.

Table 2 : Yield percentage of carbinolic plant extracts of Trigonella foenum -graecum and Brassica nigra

S. No	Parameters	Trigonella foenum - graecum		Brassica nigra	
		Organic	Inorganic	Organic	Inorganic
1	Total weight of plant extract (g)	3.9	7.8	4.3	6.2
2	Yield percentage of plant extract(%)	51.3	40.1	56.9	33.7

3.4. Preliminary Phytochemical Analysis

Table 3. Preliminary phytochemical analysis of Trigonella foenum-graecum and Brassica nigra; ++ indicates a higher amount of the compound, + indicates the presence of the compound, and − indicates its absence

S. No	Test	Sample-A	Sample-B	Sample-C	Sample-D
1.	Carbohydrate	-	-	-	-
2.	Steroid	+	+	+	+
3.	Saponins	-	+	-	-
4.	Flavonoid	++	++	+	++
5.	Quinones	-	-	+	-
6.	Amino acid	-	-	-	-
7.	Terpinoids	-	+	+	+
8.	Coumarins	++	++	++	++
9.	Tanin	++	++	++	++
10.	Phenol	++	++	++	++

3.5. Estimation of samples

3.5.1. Total Phenolic Content (TPC)

Trigonella foenum graecum and Brassica nigra were selected for biological studies where carbinol was used as a solvent system for estimating total Phenolic content (TPC). The values are expressed in terms of mg/g using ANNOVA method and are entered in terms of Mean ± SD. Gallic acid was used as a standard positive control. Total Phenolic Content (TPC) Trigonella foenum graecum and Brassica nigra grown using organic and in organic fertilizers were estimated as 48.8mg/g, 37.5mg/g, 57.3mg/g and 29.3mg/g respectively whereas standard positive control Gallic acid showed 36.5mg/g of total Phenolic content.

3.5.2. Total Flavonoid Content (TFC)

Trigonella foenum graecum and Brassica nigra were selected for biological studies where carbinol was used as a solvent system for estimating total flavonoid content (TFC). The values are expressed in terms of mg/g using ANNOVA method and are entered in terms of Mean ± SD. Gallic acid was used as a standard positive control. Total Flavonoid Content (TPC) Trigonella foenum graecum and Brassica nigra grown using organic and in organic fertilizers were estimated as 40.8mg/g, 21.2mg/g, 58.5mg/g and 28.9mg/g respectively whereas standard positive control Gallic acid showed 36.5mg/g of total flavonoid content.

3.5.3. Total Tannin Content (TTC)

Trigonella foenum graecum and Brassica nigra were selected for biological studies where carbinol was used as a solvent system for estimating total tannin content (TPC). The values are expressed in terms of mg/g using ANNOVA method and are entered in terms of Mean ± SD. Gallic acid was used as a standard positive control. Total Tannin Content (TPC) Trigonella foenum graecum and Brassica nigra grown using organic and in organic fertilizers were estimated as 48.7mg/g, 21.3mg/g, 53.6mg/g, 28.4mg/g respectively whereas standard positive control Gallic acid showed 36.5mg/g of total tannin content.

Table 3 : Estimation of Total Phenolic Content of Trigonella foenum-graecum and Brassica nigra

S.No	Concentration (µg)	Trigonella foenum - graecum		Brassica nigra		Gallic Acid (Mean ± SD)
		Organic (Mean ± SD)	Inorganic (Mean ± SD)	Organic (Mean ± SD)	Inorganic (Mean ± SD)	
1.	25	0.447± 0.008	0.524± 0.007	0.267± 0.007	0.131± 0.007	0.154± 0.006
2.	50	0.631 ± 0.006	0.762± 0.006	0.421± 0.008	0.371± 0.006	0.297± 0.007
3.	75	0.824 ± 0.009	1.124± 0.007	0.623± 0.007	0.424± 0.008	0.353± 0.008
4.	100	1.064 ± 0.007	1.358± 0.008	0.857± 0.009	0.610± 0.009	0.431± 0.007

Total Phenol Content (mg/g)	48.8	37.5	57.3	29.3	36.5

Table 4 : Estimation of Total Flavonoid Content of Trigonella foenum-graecum and Brassica nigra

S.No	Concentration (µg)	Trigonella foenum - graecum		Brassica nigra		Gallic Acid (Mean ± SD)
		Organ ic (Mean ± SD)	Inorganic (Mean ± SD)	Organic (Mean ± SD)	Inorganic (Mean ± SD)	
1.	25	0.658± 0.007	0.790± 0.006	0.438± 0.007	0.344± 0.008	0.151± 0.006
2.	50	0.765 ± 0.006	0.836± 0.007	0.562± 0.008	0.492± 0.007	0.296± 0.007
3.	75	0.833 ± 0.008	0.965± 0.009	0.605± 0.007	0.581± 0.009	0.352± 0.008
4.	100	0.974 ± 0.008	1.027± 0.007	0.791± 0.009	0.626± 0.007	0.431± 0.007
Total Flavonoid Content (mg/g)		40.8	21.2	58.5	28.9	36.5

Table 5 : Estimation of Total Tanin Content of Trigonella foenum-graecum and Brassica nigra

S.No	Concentration (µg)	Trigonella foenum - graecum		Brassica nigra		GallicAcid (Mean ± SD)
		Organic (Mean ± SD)	Inorganic (Mean ± SD)	Organic (Mean ± SD)	Inorganic (Mean ± SD)	
1.	25	0.530± 0.007	0.603± 0.007	0.354± 0.008	0.254± 0.008	0.153± 0.006
2.	50	0.742 ± 0.006	0.829± 0.008	0.518± 0.007	0.487± 0.007	0.298± 0.007
3.	75	0.904 ± 0.008	1.069± 0.008	0.724± 0.006	0.631± 0.009	0.354± 0.008
4.	100	1.091 ± 0.008	1.201± 0.009	0.943± 0.007	0.841± 0.007	0.432± 0.007
Total Tannin Content(mg/g)		48.7	21.3	53.6	28.4	36.5

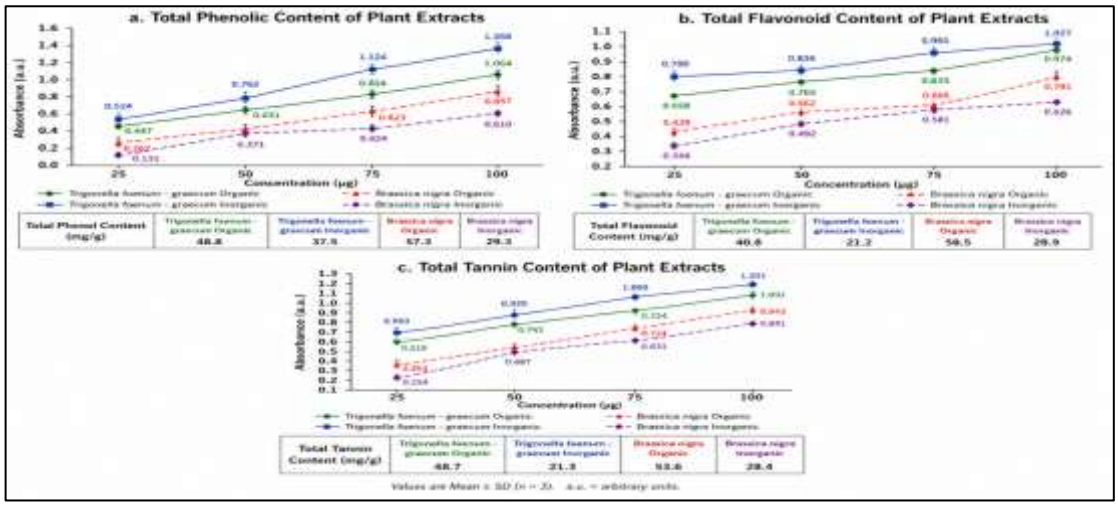


Figure 3 : Comparative analysis of total phenolic, flavonoid, and tannin contents of Trigonella foenum-graecum and Brassica nigra extracts at different concentrations: (a) total phenolic content,(b) total flavonoid content, and (c) total tannin content, showing organic and inorganic extracts with mean ± SD values.

3.6. Antioxidant Activity Study

Antioxidant activity of Trigonella foenum - graecum and Brassica nigra grown using organic and inorganic fertilizers was studied using two important assays such as DPPH and FRAP. The values were observed and are entered in terms of IC50.

Table 6 : DPPH Assay of Trigonella foenum-graecum and Brassica nigra

S. No	Concentration (µg)	Trigonella foenum – graecum		Brassica nigra		BHT (Mean ± SD)
		Organic (Mean ± SD)	Inorganic (Mean ± SD)	Organic (Mean ± SD)	Inorganic (Mean ± SD)	
1	25	46.32 ± 1.01	49.17 ± 0.82	41.71 ± 0.72	26.23 ± 0.63	47.82 ± 1.21
2	50	65.21 ± 0.68	53.91 ± 1.12	52.53 ± 0.54	31.28± 0.52	51.31 ± 0.32
3	75	75.62 ± 0.81	61.02 ± 0.69	60.73 ± 0.86	38.19 ± 0.72	62.98 ± 0.48
4	100	83.43 ± 0.53	70.51 ± 0.89	69.09 ± 0.73	42.63 ± 0.68	67.38 ± 0.43
IC50 Value		52.39µg/ml	32.02µg/ml	66.24µg/ml	27.21µg/ml	63.98µg/ml
P – Value		0	0	0	0	0

Table 7 : FRAP Assay of Trigonella foenum-graecum and Brassica nigra

S. No	Concentration (µg)	Trigonella foenum – graecum		Brassica nigra		Quercitin (Mean ± SD)
		Organic (Mean ± SD)	Inorganic (Mean ± SD)	Organic (Mean ± SD)	Inorganic (Mean ± SD)	
1	25	50.18 ± 0.76	45.51 ± 0.58	46.21 ± 0.49	40.35 ± 0.52	15.81 ± 0.07
2	50	54.43 ± 0.52	51.60 ± 0.72	49.70 ± 0.62	44.13 ± 0.56	17.13 ± 0.08

3	75	60.21 ± 0.81	54.88 ± 0.68	55.31 ± 0.58	48.27 ± 0.48	21.31 ± 0.08
4	100	64.69 ± 0.61	60.27 ± 0.56	61.45 ± 0.51	50.87 ± 0.61	21.95 ± 0.07
IC50 Value		44.31 µg/ml	22.13µg /ml	56.23µ g/ml	31.02µ g/ml	58.26µ g/ml
P – Value		0	0	0	0	0

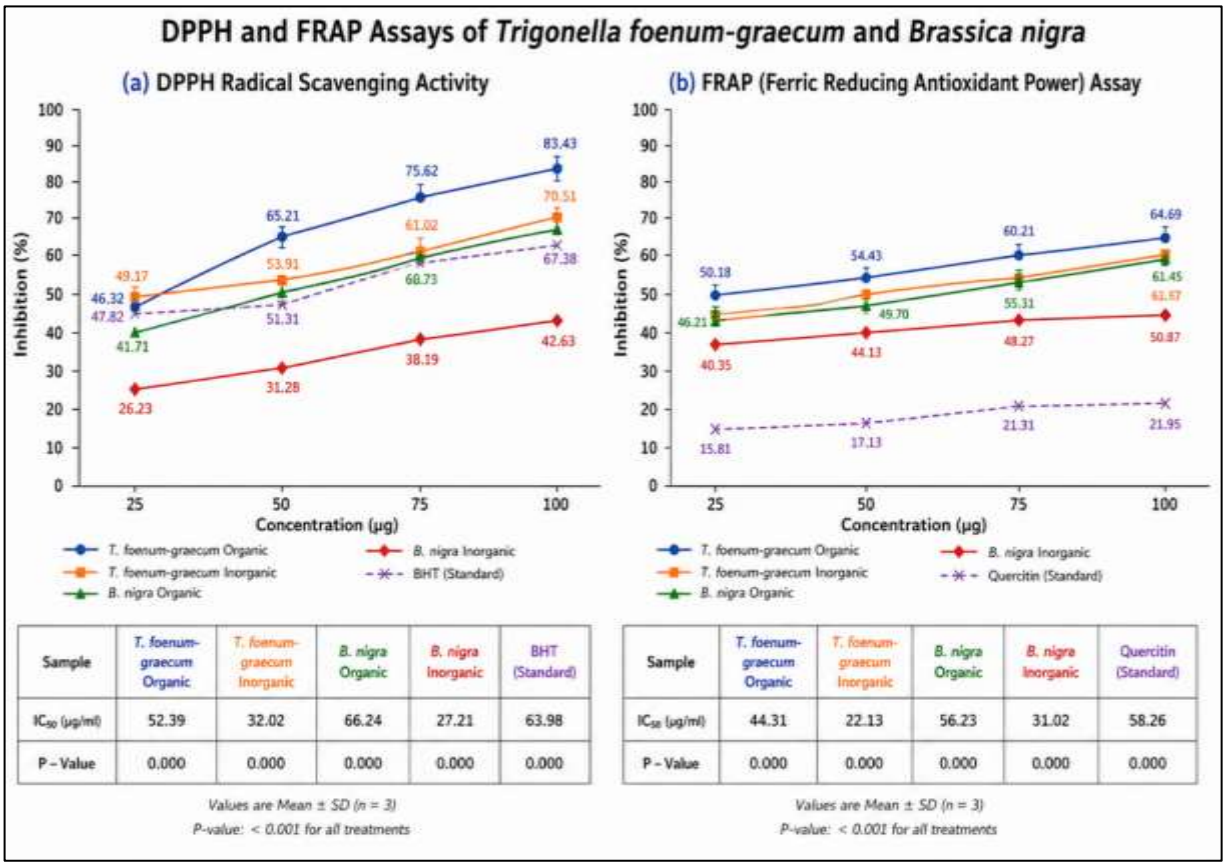


Figure 4 : DPPH radical scavenging and FRAP assays of Trigonella foenum-graecum and Brassica nigra extracts. (a) DPPH radical scavenging activity of organic and inorganic extracts compared with BHT, and (b) FRAP assay of the corresponding extracts compared with quercetin at different concentrations (25–100 µg). Values are expressed as mean ± SD (n = 3).

4.7. Antidiabetic Activity

Two key enzyme inhibition tests, α-amylase and α-glucosidase inhibition tests, were used to assess the antidiabetic efficacy of Trigonella foenum-graecum and Brassica nigra grown using organic and inorganic fertilizers. The organic and inorganic extracts' inhibitory action was assessed at various concentrations, and the findings were reported as IC₂₀ values and % inhibition.

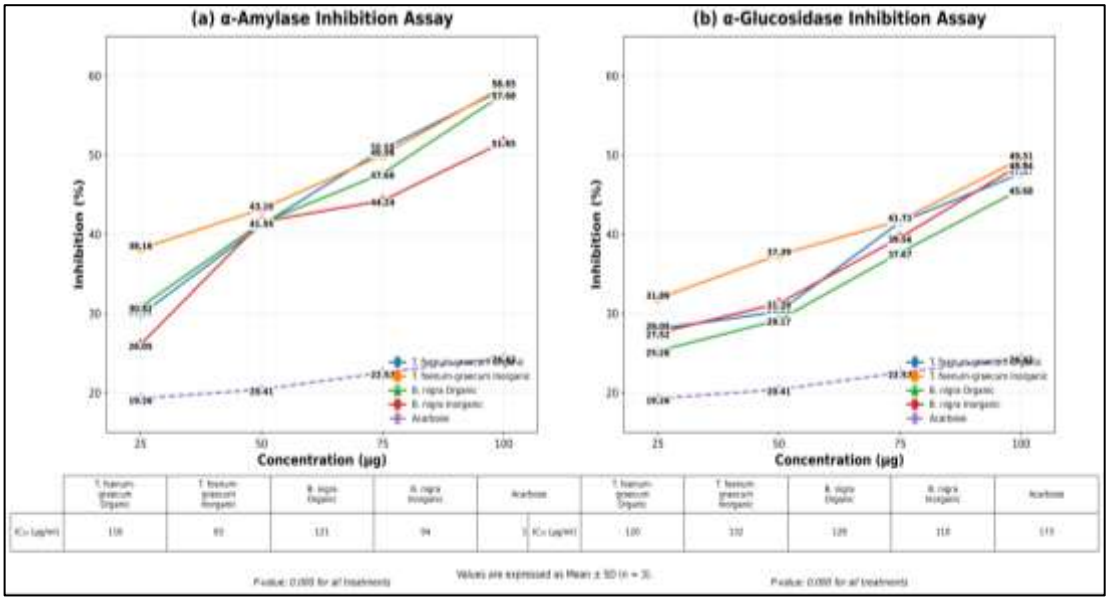
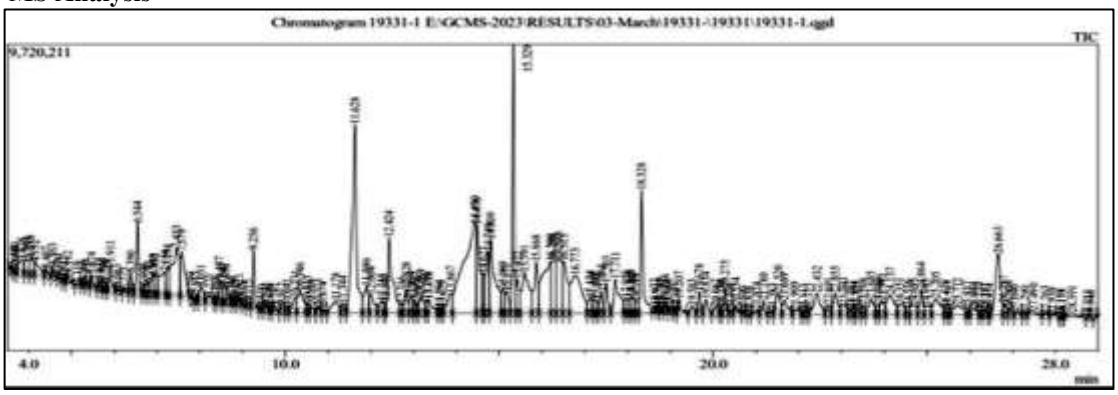


Figure 4 : α-Amylase and α-Glucosidase inhibition activities of organic and inorganic extracts of Trigonella foenum-graecum and Brassica nigra at different concentrations, with acarbose as the standard. (a) α-Amylase inhibition assay and (b) α-Glucosidase inhibition assay. Values are expressed as Mean ± SD (n = 3).

4.8. GC MS Analysis



Peak#	R.Time	Area	Area%	Height	Name
1	3.603	179044	0.01	80658	OXIRANE, (METHOXYMETHYL)-
2	3.657	195781	0.01	62316	N,N-DIMETHYL-1,3-BUTADIEN-1-AMINE
3	3.851	811333	0.04	361536	PYRIDINE
4	4.047	9782154	0.47	1252181	Pyrazine, methyl-
5	4.151	6673650	0.32	1182331	1-Propanamine, 2-methyl-N-(2-methylpropylidene)-
6	4.256	4044777	0.19	1075480	4-CYCLOHEXENE-1,2-DIOL
7	4.311	8475083	0.41	1259275	METHANE, SULFINYLBIS-
8	4.436	3714076	0.18	975508	1H-Pyrrole, 2,5-dimethyl-
9	4.510	5813437	0.28	1074295	2-Furanmethanol
10	4.657	14118401	0.68	1728065	2-Propenamide
11	4.814	7084541	0.34	1204943	1-AZABICYCLO[2.2.2]OCTANE
12	4.966	8119681	0.39	1028411	1,5-DIMETHYL-2,3-DIHYDRO-1H-PYRROLE #
13	5.029	1486703	0.07	992307	THIOPHENE
14	5.114	11695113	0.56	1058164	2-PROPENAMIDE
15	5.255	3319176	0.16	936482	1,4-PENTADIENE, 2,3,3-TRIMETHYL-
16	5.353	6749305	0.32	1658562	PYRAZINE, 2,5-DIMETHYL-
17	5.422	17864849	0.86	2678813	Ethanamine, N-ethyl-N-[(1-methylethoxy)methyl]-
18	5.618	12118284	0.58	1615934	2-Heptanol, 6-methyl-
19	5.735	10614555	0.51	1355116	2-HYDROXY-2,5,5-TRIMETHYL-3-MORPHOLINONE #
20	5.913	9587313	0.46	1159878	2(3H)-FURANONE, 5-METHYL-
21	6.044	10170543	0.49	1130806	2-Furanmethanol, 5-methyl-
22	6.254	7395950	0.35	884591	2-FURANCARBOXALDEHYDE, 5-METHYL-
23	6.401	4691109	0.23	1164838	1H-Indole, octahydro-3-methyl-
24	6.472	3285118	0.16	867501	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
25	6.536	3400205	0.16	731766	1-Butene, 4-isothiocyanato-
26	6.639	1970308	0.09	575142	1,6-HEPTADIENE, 5-DEUTERO-3,3,6-TRIMETHYL-
27	6.864	20028966	0.96	2300109	2(3H)-FURANONE, DIHYDRO-4,5-DIMETHYL-, (4R-CIS)-
28	6.941	6624786	0.32	1335056	PYRIDINE, 3,4-DIMETHYL-
29	7.075	8972554	0.43	1397429	2(3H)-FURANONE, DIHYDRO-4,5-DIMETHYL-, (4R-CIS)-
30	7.197	13649359	0.65	1544570	1,3-OXAZOLIDINE, 2-METHYL-3-NITROSO-
31	7.411	15070595	0.72	1639705	3-(1-Piperidinylmethyl)phenol
32	7.489	10416351	0.50	1931719	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (+/-)-
33	7.587	24883041	1.19	2745267	4H-1,2,4-TRIAZOLE-3,4,5-TRIAMINE #
34	7.781	2124954	0.10	897214	Pantolactone
35	7.840	3472024	0.17	1152334	3-BUTEN-2-OL, 2-METHYL-
36	7.896	6156996	0.30	1562082	2,5-ANHYDRO-1,6-DIDEOXYHEXO-3,4-DIULOSE
37	7.966	7815149	0.38	1114461	2,5-ANHYDRO-1,6-DIDEOXYHEXO-3,4-DIULOSE
38	8.149	11728051	0.56	1239694	2-PYRROLIDINONE
39	8.281	4597806	0.22	905520	2,3-DIHYDRO-5-HYDROXY-6-METHYL-4H-PYRAN-4-ONE
40	8.381	1886803	0.09	703865	N-(3-Methyl-2,5-dioxo-imidazolidin-4-yl)-acetamide
41	8.500	5259045	0.25	828593	KETONE, ISOPROPYLIDENECYCLOPROPYL METHYL
42	8.577	4372886	0.21	1078826	Glutaraldehyde
43	8.640	8386933	0.40	1999970	3-HYDROXY-4,5-DIMETHYL-2(5H)-FURANONE DIT SOTOLON
44	8.771	23678190	1.14	5640499	2-Methylpyrrolidine
45	8.868	2687553	0.13	842524	2-METHYLHEPTANAL
46	8.952	4423097	0.21	805790	5,5-DIMETHYL-3-HEXANONE
47	9.074	2418180	0.12	492048	Piperazine, 1,2,4-trimethyl-
48	9.173	2631302	0.13	714516	.alpha.-Amino-.gamma.-butyrolactone
49	9.302	37674034	1.81	5702361	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
50	9.514	40912	0.00	23337	2-(2,2,4-TRIMETHYL-1,3-DIOXOLAN-4-YL)ETHYL ACETATE

Figure 5 : GC MS Analysis of Brassica nigra (Organic)

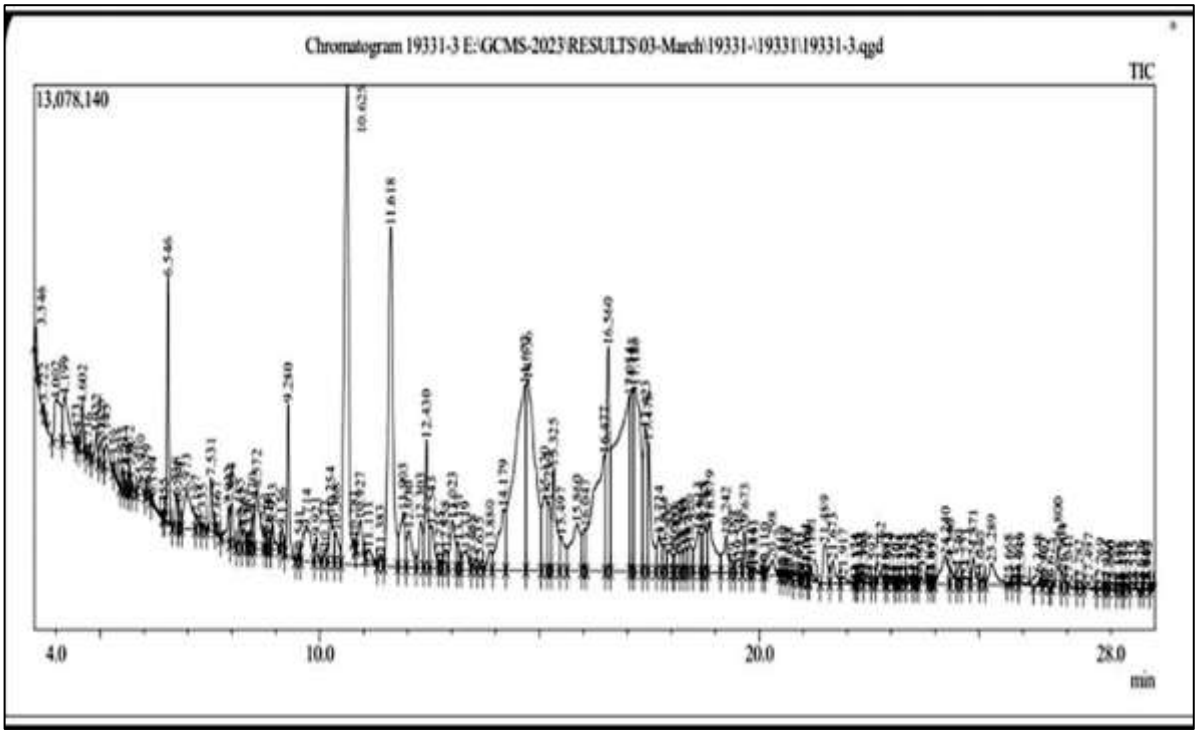
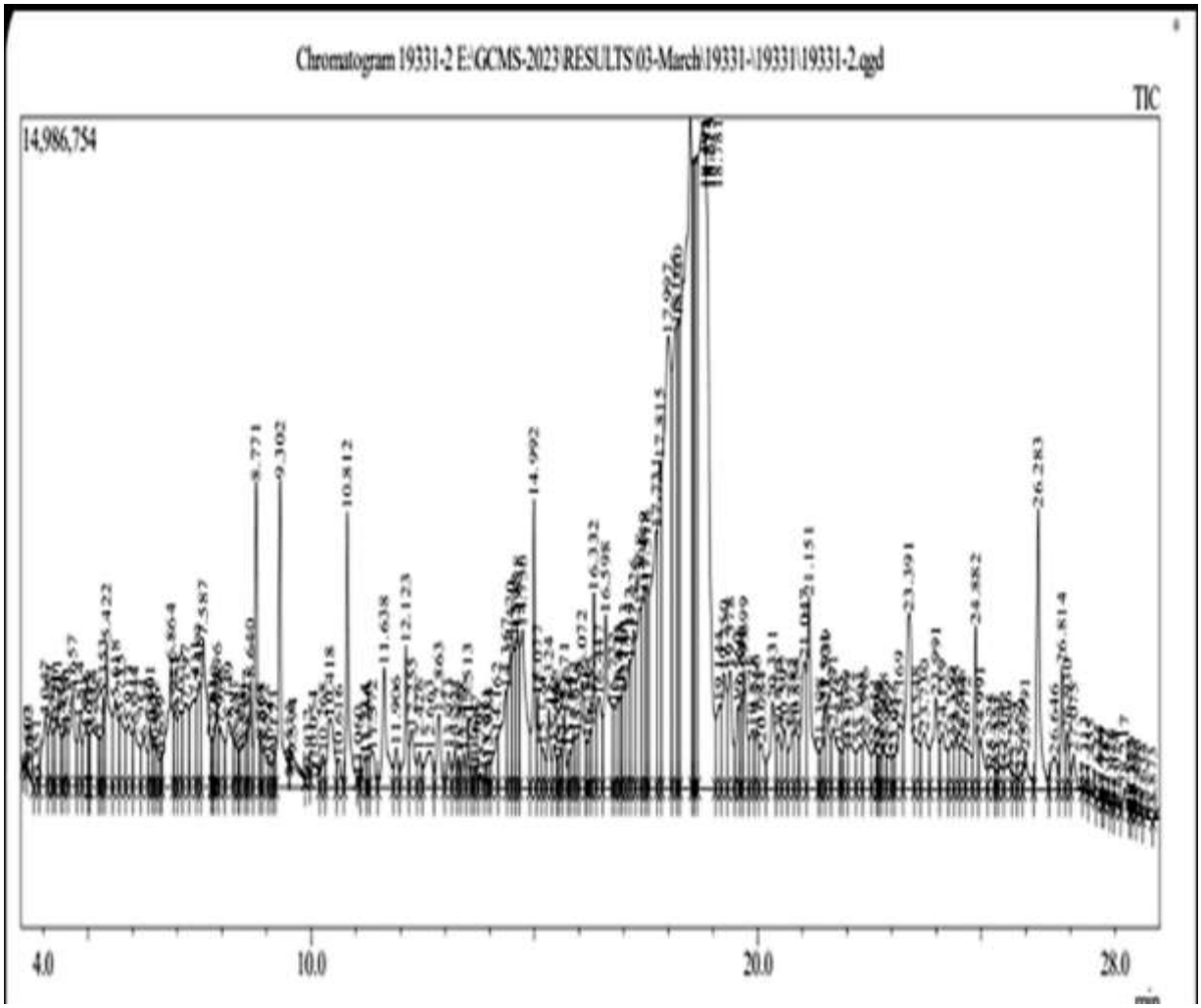


Figure 6 : GC MS Analysis of Brassica nigra (Inorganic)

Peak#	R.Time	Area	Area%	Height	Name
1	3.546	2091317	0.20	899734	METHYL 3-BUTENYL-1-D2 ETHER
2	3.722	633653	0.06	246434	METHYL 2-OXOPROPANOATE
3	4.002	10696073	1.04	947340	N,N-Dimethylaminoethanol
4	4.199	9510403	0.92	1103229	2-FURALDEHYDE
5	4.473	115009	0.01	59933	Dimethylsulfoxonium formylmethylide
6	4.602	4010023	0.39	1148161	2-Furanmethanol
7	4.736	438784	0.04	96683	OXIRANE, 2-METHYL-2-(2-METHYLPROPYL)-
8	4.922	2869811	0.28	670703	Allyl Isothiocyanate
9	5.025	3081373	0.30	600760	PROPANENITRILE, 3-METHOXY-
10	5.145	4097523	0.40	574418	dl-Glyceraldehyde dimer
11	5.339	376278	0.04	148182	Ethanone, 1-(2-furanyl)-
12	5.515	1483875	0.14	559603	2-OXEPANONE
13	5.576	663475	0.06	264624	6,7-Dioxabicyclo[3.2.2]non-8-ene
14	5.672	1576076	0.15	635150	2-Cyclopenten-1-one, 2-hydroxy-
15	5.728	843410	0.08	269854	Acetic acid, 2-(dimethylamino)ethyl ester
16	5.930	2450542	0.24	466988	HEPTANE, 4-PROPYL-
17	6.079	1045349	0.10	324320	N,N-DIMETHYL-3-BUTEN-1-AMINE #
18	6.194	808775	0.08	173454	2-METHYL-3-OXO-BUTYRONITRILE
19	6.455	23319	0.00	16916	2-FURANCARBOXALDEHYDE, 5-METHYL-
20	6.546	17211354	1.67	5885401	1-Butene, 4-isothiocyanato-
21	6.738	2880487	0.28	754837	2-Hydroxy-gamma-butyrolactone
22	6.784	2019370	0.20	640126	2H-Pyran-2,6(3H)-dsone
23	6.973	11101337	1.08	855120	2-Hydroxy-gamma-butyrolactone
24	7.197	1645748	0.16	348390	FORMIC ACID, PROPYL ESTER
25	7.335	588664	0.06	171279	2-CYCLOPENTEN-1-ONE, 2-HYDROXY-3-METHYL-
26	7.531	4636140	0.45	1264942	2-BUTENOIC ACID, 4-OXO-4-(2-PROPENYLAMINO)-, (Z)-
27	7.667	851283	0.08	280893	PENTANOIC ACID, 4-OXO-
28	7.932	4212159	0.41	904451	t-Butyl-n-pentamethylenetrithiopercarbamate
29	7.994	3673138	0.36	998634	TETRAHYDRO-2-FURANYLMETHANOL
30	8.185	2626150	0.26	739465	1,3,5-TRIAZINE-2,4,6-TRIAMINE
31	8.262	2273615	0.22	483373	2-Furancarboxylic acid, hydrazide
32	8.376	785345	0.08	221650	6,8-DIOXABICYCLO(3.2.1)OCTAN-3.BETA.-OL-3-DI
33	8.470	3412300	0.33	855686	PENTANAL
34	8.572	11468264	1.11	1430804	CYCLOPROPYLMETHANOL
35	8.763	1365528	0.13	379988	Maltol
36	8.824	1284075	0.12	293399	6,8-Dioxabicyclo[3.2.1]octan-4-one, oxime
37	8.933	2799676	0.27	620890	Cyano-3,4-epithiobutane
38	9.136	1931495	0.19	571250	2-ACETYL-2-HYDROXY-.GAMMA.-BUTYROLACTONE
39	9.280	12593500	1.22	3652047	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE
40	9.541	815156	0.08	261942	Benzoic acid
41	9.714	10418003	1.01	781864	1,2,3-PROPANETRIOL
42	9.921	2421814	0.24	603775	2-METHYL-2H-PYRAN-3,4,5(6H)-TRIONE
43	10.122	1505825	0.15	313378	Pentanoic acid, 2-isopropoxyphenyl ester
44	10.254	7829160	0.76	1135637	1,2-BENZENEDIOL
45	10.368	4345011	0.42	739113	Dianhydromannitol
46	10.625	85868539	8.34	11497263	5-Hydroxymethylfurfural
47	10.781	332045	0.03	167571	BENZENEPROPANENITRILE
48	10.927	4979869	0.48	808460	2,3-DIHYDROXYPROPYLACETATE
49	11.111	1971042	0.19	252751	1,2-Benzenediol, 4-methyl-
50	11.383	1536532	0.15	308831	1-[2-(ACETYLOXY)ETHYL]-3-OXOOCTYLACETATE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10.812
14.992
26.283
4.0
10.0
20.0
28.0

Peak#	R.Time	Area	Area%	Height	Name
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3	3.851	811333	0.04	161536	PYRIDINE
4	4.047	9782154	0.47	1252181	Pyrazine, methyl-
5	4.151	6673650	0.32	1182321	1-Propanamine, 2-methyl-N-(2-methylpropylidene)-
6	4.256	4044777	0.19	1075480	4-CYCLOHEXENE-1,2-DIOL
7	4.311	8475083	0.41	1259275	METHANE, SULFINYLBIS-
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17	5.422	17864849	0.86	2678815	Ethanamine, N-ethyl-N-[(1-methylethoxy)methyl]-
18	5.618	12118284	0.58	1615934	2-Heptanol, 6-methyl-
19	5.735	10614555	0.51	1355116	2-HYDROXY-2,5,5-TRIMETHYL-3-MORPHOLINONE #
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21	6.044	10170543	0.49	1130008	2-Furanmethanol, 5-methyl-
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23	6.401	4691109	0.23	1164838	1H-Indole, octahydro-3-methyl-
24	6.472	3285118	0.16	867501	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
25	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
26	6.639	1970308	0.09	575142	1,6-HEPTADIENE, 5-DEUTERO-3,3,6-TRIMETHYL-
27	6.864	20028966	0.96	2300109	2(3H)-FURANONE, DIHYDRO-4,5-DIMETHYL-, (4R-CIS)-
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32	7.489	10416351	0.50	1931719	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (+/-)-
33	7.587	24883041	1.19	2745267	4H-1,2,4-TRIAZOLE-3,4,5-TRIAMINE #
34	7.781	2124954	0.10	897214	Pantolactone
35	7.840	3472024	0.17	1152334	3-BUTEN-2-OL, 2-METHYL-
36	7.896	6156996	0.30	1562082	2,5-ANHYDRO-1,6-DIDEOXYHEXO-3,4-DIULOSE
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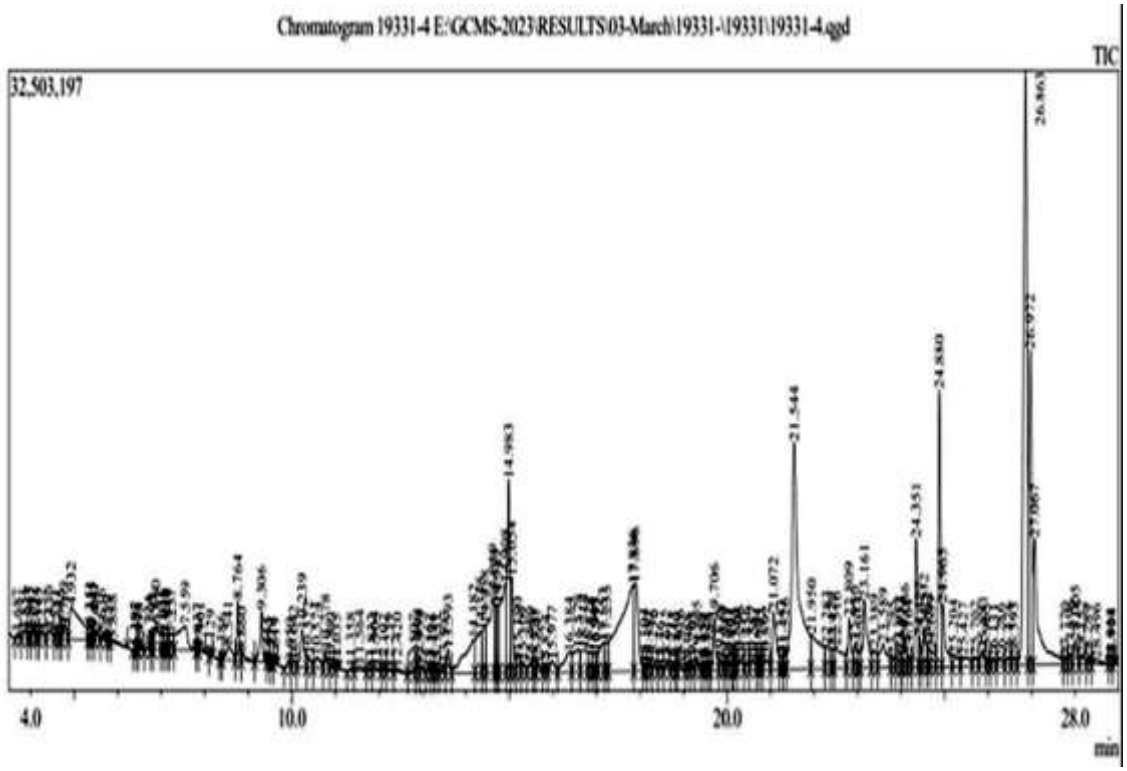
44	8.771	23678190	1.14	5640499	2-Methylpyrrolidine
45	8.868	2687553	0.13	842524	2-METHYLHEPTANAL
46	8.952	4423097	0.21	805790	5,5-DIMETHYL-3-HEXANONE
47	9.074	2418180	0.12	492048	Piperazine, 1,2,4-trimethyl-
48	9.173	2631302	0.13	714516	.alpha.-Amino-.gamma.-butyrolactone
49	9.302	37674034	1.81	5702361	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
50	9.514	40912	0.00	23337	2-(2,2,4-TRIMETHYL-1,3-DIOXOLAN-4-YL)ETHYL ACETATE

Figure 7 : Fig 19. GC MS Analysis of Trigonella foenum-graecum (Organic)



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3	4.002	10696073	1.04	947340	N,N-Dimethylaminoethanol
4	4.199	9510403	0.92	1103229	2-FURALDEHYDE
5	4.473	115009	0.01	59933	Dimethylsulfoxonium formylmethylide
6	4.602	4010023	0.39	1148161	2-Furanmethanol
7	4.736	438784	0.04	96683	OXIRANE, 2-METHYL-2-(2-METHYLPROPYL)-
8	4.922	2869811	0.28	670703	Allyl Isothiocyanate
9	5.025	3081373	0.30	600760	PROPANENITRILE, 3-METHOXY-
10	5.145	4097523	0.40	574418	dl-Glyceraldehyde dimer
11	5.339	376278	0.04	148182	Ethanone, 1-(2-furanyl)-
12	5.515	1483875	0.14	559603	2-OXEPANONE
13	5.576	663475	0.06	264624	6,7-Dioxabicyclo[3.2.2]non-8-ene
14	5.672	1576076	0.15	635150	2-Cyclopenten-1-one, 2-hydroxy-
15	5.728	843410	0.08	269854	Acetic acid, 2-(dimethylamino)ethyl ester
16	5.930	2450542	0.24	466988	HEPTANE, 4-PROPYL-
17	6.079	1045349	0.10	324320	N,N-DIMETHYL-3-BUTEN-1-AMINE #
18	6.194	808775	0.08	173454	2-METHYL-3-OXO-BUTYRONITRILE
19	6.455	23319	0.00	16916	2-FURANCARBOXALDEHYDE, 5-METHYL-
20	6.546	17211354	1.67	5885401	1-Butene, 4-isothiocyanato-
21	6.738	2880487	0.28	754837	2-Hydroxy-gamma-butyrolactone
22	6.784	2019370	0.20	640126	2H-Pyran-2,6(3H)-dione
23	6.973	11101337	1.08	855120	2-Hydroxy-gamma-butyrolactone
24	7.197	1645748	0.16	348390	FORMIC ACID, PROPYL ESTER
25	7.335	588664	0.06	171279	2-CYCLOPENTEN-1-ONE, 2-HYDROXY-3-METHYL-
26	7.531	4636140	0.45	1264942	2-BUTENOIC ACID, 4-OXO-4-(2-PROPENYLAMINO)-, (Z)-
27	7.667	851283	0.08	280893	PENTANOIC ACID, 4-OXO-
28	7.932	4212159	0.41	904451	t-Butyl-n-pentamethylenetrithiopercarbamate
29	7.994	3673138	0.36	998634	TETRAHYDRO-2-FURANYLMETHANOL
30	8.185	2626150	0.26	739465	1,3,5-TRIAZINE-2,4,6-TRIAMINE
31	8.262	2273615	0.22	483373	2-Furancarboxylic acid, hydrazide
32	8.376	785345	0.08	221650	6,8-DIOXABICYCLO(3.2.1)OCTAN-3.BETA.-OL-3-D1
33	8.470	3412300	0.33	855686	PENTANAL
34	8.572	11468264	1.11	1430804	CYCLOPROPYLMETHANOL
35	8.763	1365528	0.13	379988	Maltol
36	8.824	1284075	0.12	293399	6,8-Dioxabicyclo[3.2.1]octan-4-one, oxime
37	8.933	2799676	0.27	620890	Cyano-3,4-epithiobutane
38	9.136	1931495	0.19	571250	2-ACETYL-2-HYDROXY-.GAMMA.-BUTYROLACTONE
39	9.280	12593500	1.22	3652047	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE
40	9.541	815156	0.08	261942	Benzoic acid
41	9.714	10418003	1.01	781864	1,2,3-PROPANETRIOL
42	9.921	2421814	0.24	603775	2-METHYL-2H-PYRAN-3,4,5(6H)-TRIONE
43	10.122	1505825	0.15	313378	Pentanoic acid, 2-isopropoxyphenyl ester
44	10.254	7829160	0.76	1135637	1,2-BENZENEDIOL
45	10.368	4345011	0.42	739113	Dianhydromannitol
46	10.625	85868539	8.34	11497263	5-Hydroxymethylfurfural
47	10.781	332045	0.03	167571	BENZENEPROPANENITRILE
48	10.927	4979869	0.48	808460	2,3-DIHYDROXYPROPYL ACETATE
49	11.111	1971042	0.19	252751	1,2-Benzenediol, 4-methyl-
50	11.383	1536532	0.15	308831	1-[2-(ACETYLOXY)ETHYL]-3-OXOOCTYL ACETATE

Figure 8 : GC MS Analysis of Trigonella foenum-graecum (Inorganic)

4. DISCUSSION

4.1 Effect of the fertilization method on phytochemical content

As can be seen from this experiment, organic amendment method affects not only growth processes of plants but also biochemical properties of Brassica nigra and Trigonella foenum-graecum. The selected comparative method is extremely relevant as the two plants under study belong to different botanical families and, therefore, to different metabolic systems. In this respect, the methodology applied in this work was cultivation of plants under the effect of both organic and inorganic fertilizers followed by methanol extraction and the determination of phytochemicals, antioxidants, enzyme inhibitors and GC-MS. The reason why such difference exists between fertilization methods is related to nutrient supply and carbon allocation. Nutrient substances, especially nitrogen and phosphorus, affect primary metabolism, photosynthesis and biosynthesis of secondary metabolites, which are composed of carbon and nitrogen. Thus, the change in the concentration of nutrients will influence the metabolic pathway, which will determine the carbon partitioning to phenolics, flavonoids and other secondary metabolites. Moreover, biofertilizer may affect plant metabolism through nutrients mobilization, phytohormone synthesis and rhizosphere effects. One recent review views microbial inoculants as one of the key tools for nutrient management, plant growth and sustainable agriculture (Shahwar et al., 2023).

The obtained results show the efficiency of certain fertilizers. In fact, the obtained data demonstrate that the application of fertilizers affects the phytochemicals composition of plants and the degree of this effect may differ depending on various parameters.

4.2 Phenolic compounds and flavonoids as metabolic response indicators

Phenolic compounds and flavonoids are important secondary metabolites possessing antioxidative properties and being able to act as antioxidants in terms of hydrogen or electron transfer as well as reactive oxygen species neutralization. It should be said that the genus Brassica is distinguished by the significant amount of phenolic compounds in addition to glucosinolates (Ayadi et al., 2022). It is not surprising therefore, that the total amount of phenolic and flavonoid compounds changes depending on the type of fertilizer due to its influence on the nutrient availability and consequently, carbon fixation and precursor availability for phenylpropanoid metabolism. It is especially significant while making the comparison between the two plants. Firstly, the secondary-metabolites spectrum of the Brassicaceae from B. nigra

comprises glucosinolates, glucosinolates' hydrolysis derivatives and phenolics. As can be seen from the literature review, B. nigra is among the species of Brassica that contain a lot of glucosinolates. At the moment, literature is interested in the effect of cultivation on the presence of phytochemicals in the Brassica plants (Sikorska-Zimny & Beneduce, 2020). Secondly, the list of metabolites in T. foenum-graecum is different and comprises polyphenols, flavonoids, saponins, alkaloids, trigonelline, diosgenin, galactomannans (Akhtar et al., 2025).

4.3 Antioxidant activity: DPPH and FRAP assays

Data received regarding antioxidant activity may be considered an additional argument which supports the difference between the samples revealed during the phytochemical analysis. The DPPH assay reveals radical scavenging ability of the sample while the FRAP assay measures reducing ability of the sample. Therefore, data received by means of these two tests cannot be compared. The IC_{50} values for the activity of DPPH for the T. foenum-graecum extract of plants grown under conditions of organic and inorganic fertilization were equal to 52.39 μg/mL and 32.02 μg/mL, respectively. For the extract of B. nigra plants, these values were 66.24 μg/mL and 27.21 μg/mL, respectively. For BHT, the IC_{50} value was equal to 63.98 μg/mL. As the lower IC_{50} value means lower concentration and, consequently, high activity of radical scavenging, the plant extract obtained from inorganic fertilizer treated plants demonstrates a higher DPPH activity in the current study. It should be noted that the data obtained cannot be regarded as the evidence of the superiority of antioxidant activity of plants grown using organic/biofertilizers. For the FRAP test, the almost same results were obtained in comparison with DPPH. IC_{50} values are 44.31 and 22.13 μg/mL for T. foenum-graecum with the usage of organic and inorganic fertilizer, respectively, 56.23 and 31.02 μg/mL for B. nigra with the organic and inorganic fertilizer usage, respectively. Quercetin served as a standard. It can be supposed that the cause for the similarities in the results of the DPPH and FRAP tests is the influence of fertilizer usage on the reducing power and antioxidant activity of extracts. But in order to prove this assumption, it is necessary to carry out statistical analysis.

4.4 Inhibition of α-amylase and α-glucosidase

As these enzymes are contained in the extracts of the plants, it may be concluded that the investigated plants are rich in bioactive substances capable of inhibiting carbohydrate-degrading enzymes. Since these enzymes break down carbohydrates, inhibition of their activity may indicate the ability of the plant to influence carbohydrate digestion in vivo. It is necessary to take into account the obtained results carefully. The inhibition of enzyme activity in the crude extract of the plant does not prove the ability of the plant to treat diabetes mellitus in humans. It is preliminary information that requires further investigation. The higher enzyme inhibition activity in some fertilizers can be explained by the alteration in the content and composition of specific phenolic compounds, flavonoids, tannins and other secondary metabolites. Fenugreek is rather interesting in this respect because this plant is rich in active metabolites related to metabolism such as 4-hydroxyisoleucine, trigonelline, galactomannan and steroid compounds (Visuvanathan et al., 2022). As for B. nigra, it is necessary to pay attention to the fact that the content of glucosinolates and their degradation products is rather high in B. nigra and this explains its unique chemistry and biological properties (Sikorska-Zimny & Beneduce, 2020).

4.5 GC-MS profiling and metabolic pathway differences associated with fertilizers

4.6 GC-MS profiling gives additional diversity of chemical compounds in analysis of biochemical samples. In case of B. nigra and T. foenum-graecum in connection with organic and inorganic fertilizers two separate GC-MS analyses of the metabolite profile are performed. If there is any difference in GC-MS spectra of the two fertilizers, it may be considered that the difference is connected with fertilizer management influence on metabolite profile. However, writing the manuscript for Scopus publication, identification of GC-MS peaks cannot be called identification of compounds using library. It is better to identify the peaks along with retention time, retention index, match scores using mass spectrometry and standards, even. It is important since difference in GC-MS spectra indicates difference in biosynthesis, metabolism, extraction yield and metabolites abundance and not the presence of new metabolite. At last, it is impossible to analyze glucosinolates using GC-MS owing to their chemical nature. Therefore, it is necessary to report GC-MS data in GC format, whereas glucosinolates have to be analyzed using LC-MS/MS.

4.7 Responses of the plant species in the experiment

One of the strong sides of the experiment under consideration is the use of two plant species B. nigra and T. foenum-graecum. They have different metabolic processes; B. nigra has secondary metabolism glucosinolates belonging to the family Brassicaceae while T. foenum-graecum has polyphenols, alkaloids, saponins, steroidal sapogenins and soluble dietary polysaccharides belonging to the Fabaceae family (Sikorska-Zimny & Beneduce, 2020). It is impossible to have similar biochemical processes in these two plant species using the same type of fertilizer. It could be explained by the difference in nutrient absorption, nitrogen metabolism, carbon partitioning and specialized metabolism regulation. It once again proves the hypothesis that the fertilization is not only agronomic practice of biomass production but also biochemical one.

4.8 Implications of the research for sustainable agriculture and the study of functional foods

While there could be many other implications of the research not necessarily connected to crop productivity, biofertilizers are quite actively studied nowadays as an alternative and/or supplementary fertilizer in place of chemicals, as inoculation of microorganisms increases crop yield without the use of any synthetic products (Shahwar et al., 2023). But, apart from these, the results of the research could have another interpretation as well as the sustainability of the crops and their phytochemical activity do not correlate directly; according to the obtained results, inorganic fertilizers demonstrated considerably lower IC_{50} values in both DPPH and FRAP tests than organic fertilizers, meaning their antioxidant activity

was higher. Thus, the only significant implication of the conducted research is the fact that the fertilization regime had some effect on phytochemical and biological activity but not that biofertilizers increase the latter. This would be extremely useful for the scientific significance of the research and help prevent any possible criticism from the reviewers.

5. Limitations and future research directions

Several limitations should be addressed. First of all, there are no means for estimation of individual compound content by their total content, that is why all the information about phenolics, flavonoids and tannins provided is the estimative one. Secondly, both DPPH and FRAP assays are purely chemical reactions, not physiological activity measurements. Thirdly, enzyme inhibition assays can only provide preliminary results and are not able to reveal potential effects of the studied compounds on human health. It is important that data obtained from GC-MS has to be validated with the use of standards or LC-MS/MS analysis. In further research the analyses of soil, microflora and plant metabolome will be conducted to determine connection between fertilizer and phytochemicals.Among the most promising directions for further research there are multivariate analysis like PCA, hierarchical clustering and correlation/network analysis.

6. CONCLUSION

From the experiment, it is now evident how crucial the nutrient management system not only is in terms of the growth and development of Brassica nigra and Trigonella foenum graecum but also in the phytochemical content of the plant species. The determination of the total amount of phenolic, flavonoid, and tannin content, along with the use of DPPH and FRAP, could be considered the initial step in the determination of the role played by the nutrient in antioxidant activities. The testing of α-amylase and α-glucosidase could be considered the initial step in the determination of the inhibitory activities of the extract against carbohydrate metabolism enzymes. In this respect, it is important to highlight the fact that the obtained results of the influence of the organic and chemical amendments on the plants B. nigra and T. foenum-graecum have to be analyzed based on the particular biochemical reaction of the plants being researched because of the differences in their metabolic systems. It should be noted that the current research proves the general hypothesis of the influence of the fertilizers on the biochemical characteristics of the plants. In the future, further research may be conducted in order to identify the relation between the nutritional system of the plants, their metabolism and functioning. However, it should be emphasized that the results obtained during the current research have to be regarded as the preliminary results, which do not include the in-vivo tests. It means that the results of the influence of the fertilizers on the plants have to be regarded as the non-therapeutic results. Further research will include the field trials, soil and plant testing for the nutrients, metabolome testing, bioactive compounds identification and in-vivo tests.

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