CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

PHYTOCHEMICAL SCREENING, GC–MS AND FTIR ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN VEGETABLES AND FRUITS

Niaz Ali Malghani[1]✉, Sarfaraz Ahmed Mahesar[1], Jameel Ahmed Baig[1], Farah Naz Talpur[1], Samina Sohu[2], Syed Tufail Hussain Sherazi[1]

[1]National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro-76080, Pakistan
[2]Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro-76080, Pakistan
✉niazalimalghani12@gmail.com

https://doi.org/10.34302/crpjfst/2023.15.1.3

Article history:
Received:
15 July 2022
Accepted:
15 December 2022

Keywords:
Phytonutrients;
Food, Fruits;
Vegetables;
Health.

ABSTRACT

Vegetables and fruits are among the most regularly consumed foods because of their physiological effects. This study aimed to check the potential phytochemical substances in the vegetables and fruits of Larkana, Sindh, Pakistan, using qualitative and quantitative analysis. The vegetables and fruits extract screening analysis showed important phytochemicals such as phenols, proteins, quinones, alkaloids, flavonoids, tannins, terpenoids, and carbohydrates. The GC-MS identified different phytochemical compounds. The major components present in vegetables and fruits were Benzoic acid (7.73%), Lupeol (13.44%), 1-Eicosene (11.49%), N-Tetratetracontane (8.41%), 2-Pentadecanone, 6,10,14-trimethyl (9.33 %), Hexadecanoic acid methyl ester (12.89%), Nonadecane (12.19%), 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (9.14%), Ethyl benzoate (14.43%) and 5-Hydroxymethylfurfural (15.06%). FTIR spectroscopy was also used to identify typical functional groups in freeze-dried materials. Data revealed the strong absorption around 3600-3200 cm^{-1} due to the O-H stretching vibrations and C-H stretching vibration at 3000-2800 cm^{-1}. The representing C=O and C-O stretching vibrations appeared at 1700-1750 cm^{-1} and 1200-1000 cm^{-1}. The C-N stretching vibration was observed at 1300-1200 cm^{-1}.

1. Introduction

Phytochemicals, commonly known as phytonutrients, are non-nutritive plant compounds with disease-preventing or protective properties. Most food, especially fruits and vegetables, contain these complex compounds. Fruits and vegetables have shown several beneficial features, mostly in terms of preserving excellent health and nutritional potential (Aman, Schieber, & Carle, 2005). Vegetables and Fruits contain high concentrations of dietary fibre, vitamins, minerals, electrolytes, antioxidants, and phytochemicals to consider these for nutritional recommendations (Slavin & Lloyd, 2012). Large numbers of phytochemicals have been known in vegetables and fruits. They have been divided into several classes based on biological function and their chemical structure like Phenols, proteins, quinones, alkaloids, flavonoids, tannins, and terpenoids are examples of these phytochemical compounds (Shahidi, McDonald, Chandrasekara, & Zhong, 2008). These substances are produced by live organisms or secondary metabolism. Secondary metabolites are a taxonomic and chemically varied group of molecules with a mysterious purpose. They are active in human therapy, veterinary medicine, agriculture, scientific research, and a variety of other fields (Goud, Suryam, & Charya, 2009). Vitamins and minerals have long been thought to play an important role in disease prevention; however, current research suggests that phytochemicals may contribute more to vitamins or other nutrients. Phytochemicals have been linked to

preventing chronic illnesses such as heart disease, cancer, diabetes, osteoporosis, and eyesight problems. Many forms of cancer, including stomach cancer, lungs cancer, breast cancer, and colon prostate cancers, are adversely connected to fruit and vegetable consumption (Kris-Etherton et al., 2002; Temple & Gladwin, 2003). Fruits and vegetables include phytochemicals that have a preventive impact against certain illnesses. Phytochemicals can also help limit the spread of cancer by reducing the multiplication of cancer cells (Hirsch et al., 2000; Schneider et al., 2000). As a result, there is a need to assess the potential of local vegetables in providing essential nutrients and phytochemicals, which will aid in selecting appropriate green leafy vegetables by food processors, nutritionists, dieticians, and consumers. Biochemicals are typically referred to as secondary metabolites in this environment, and these biochemicals are valuable for the traditional medical system these can be identified by using Gas chromatography-mass spectrometry (GC-MS) technique (Prasain, Wang, & Barnes, 2004). GC-MS has established itself as a fundamental technical platform for analyzing secondary metabolites profiling in vegetables and fruits in recent years (Ganesan & Raja, 2021; Kell et al., 2005; Robertson, 2005).

2. Materials and Methods

2.1. Chemicals and Reagents

The chemicals and reagents used in this study were obtained from Merck (Darmstadt, Germany) for the analysis of phytochemicals such as methanol, ferric chloride, sodium hydroxide, copper sulphate, ammonia solution, sulphuric acid, Wagner's reagent, chloroform, Benedict's reagent and distilled water.

2.2. Sample collection

For the present study, three leafy vegetables and two fruits samples were collected from different areas of Larkana, Sindh, Pakistan, such as Spinach (Spinacia oleracea), Coriander leaves (Coriandrum sativum), Fenugreek leaves (Trigonella Foenum-graefum), Jujuba (Ziziphus Jujube) and Guava (Psidium Guajava). In the lab, samples of vegetables and fruits were placed through a three-step washing process that included agitating and rinsing with distilled water first, then three successive washings with ultra-pure water. The freeze-drying process was used to dry the clean vegetable and fruit samples (Shah, Rasapalli, Mello, Singh, & Cai, 2012). In an Agate mortar, the freeze-dried fruit and vegetable samples were powdered and sieved through a nylon sieve with a mesh size of 7 mm.

2.3. Preparation of vegetables and fruits

The collected vegetables and fruits samples were dried and crushed to powder form. Each vegetable and fruit sample was steeped in 50 mL of water, ethanol, and hexane individually. For 48 h, the whole combination was incubated at 4°C. After the incubation time, the mixture was filtered and centrifuged at 4°C for 10 min at 10,000 rpm. In a rotary evaporator (IKA-RV 10 Control), the extracts were concentrated to dryness and kept at 4°C until further analysis.

2.4. Phytochemical analysis tests

2.4.1. Test for Phenols or Ferric chloride test

For analysis of phenols content in vegetables and fruits, the procedure was used as reported earlier (Jigna & Sumitra, 2008). In the test tube, 2 mL of each sample extract was added, followed by 4 mL of distilled water and a few drops of aqueous ferric chloride solution. The presence of phenols is indicated by the production of a blue or green color.

2.4.2. Detection of proteins or Biuret test

The method described by (Jigna & Sumitra, 2008) was used to determine the proteins in vegetables and fruits. 2 mL of each extract were taken into the test tube and reacted with 5% NaOH and 1% (w/v) $CuSO_4$ formed violet colored complex indicates that protein is present.

2.4.3. Detection of quinones

A procedure reported earlier by (Raja & Ravindranadh, 2014) was used to detect quinones in vegetables and fruits. In short, 2 mL of each extract were put into the test tube and reacted with dilute NaOH, which formed the red or blue-green color, indicating quinones' presence.

2.4.4. Alkaloids test or (Wagner's reagent)

Wagner's reagent was used to analyze alkaloids test as reported by (Jigna & Sumitra, 2008). In brief, 2 mL of each extract were put into the test tube and added 2 mL of Wagner's reagent (2 g of KI and 1.27 g of I2 in 100 mL of (H_2O). After mixing the solution, it left for a few min to appear reddish-brown color, indicating alkaloids presence.

2.4.5. Flavonoids test

For confirmation of flavonoids in vegetables and fruits, 2 mL of each extract were put into the test tube, added 5 mL of a dilute solution of ammonia and concentrated H_2SO_4. The yellow color formation confirms the presence of flavonoids, as described by (Jigna & Sumitra, 2008).

2.4.6 Tannins test or Braymer's test

For analysis of tannins, 2 mL of each extract of vegetables and fruits were taken into a test tube and reacted with 0.1% $FeCl_3$ solution. The blue color confirms the presence of tannins, as described by (Jigna & Sumitra, 2008).

2.4.7. Terpenoids

The method applied for the analysis of terpenoids in vegetables and fruits was reported by (Jigna & Sumitra, 2008). In the test tube, 2 mL of each extract of vegetables and fruits were taken, added 4 mL of chloroform and 5 mL concentrated (H_2SO_4). After mixing, the presence of reddish-brown color in the solution confirms the presence of terpenoids.

2.4.8. Carbohydrates (Benedict's test)

The method reported by (Jigna & Sumitra, 2008) was used to analyze carbohydrates in vegetables and fruits. Briefly, 2 mL of each extract were taken into a test tube and mixed with Benedict's reagent. The solution was heated gently in a water bath for 5 min and observed the formation of orange-red precipitate that indicates the presence of carbohydrates.

2.5. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical compositions of ethanol extract were investigated through GC-MS. The GC-MS 6890 N from Agilent Technology was interfaced to a Mass Spectrometer equipped with an HP-5MS capillary column (30mx 0.25mm x 0.25µm film thickness). The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–550 m/z. The GC-MS was controlled using Agilent Chem Station 6890 Scale Mode software. Helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. 1 µL each sample was inserted as a split mode ratio of 10:1. The starting oven temperature was 110°C (isothermal for 2 min) and increased to 200°C with an increase of 10°C /min, then 5°C/min to 280°C and stayed for 10 min. The injector temperature was selected at 250°C, for detector temperature at 270°C. All analysis was performed in triplicate and the relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

2.6. Fourier transform infrared (FTIR*)*

For qualitative analysis, FTIR was used to identify the typical groups (functional) in the dried vegetable and fruits powders. The spectra of vegetable and fruits samples were collected using Nicolet iS10 (Thermo) spectrometer equipped with a DTGS detector and controlled with OMINIC software (version 9). Other parameters such as resolution 4 cm^{-1}, scan 16, range 4000-650 $cm^{-1,}$ and diamond SB-ATR accessory was used. Before each analysis, the crystal was cleaned with a solvent, and a background spectrum was collected (Sandosh, Peter, & Raj, 2013; Talukdar, Choudhury, Chakraborty, & Dutta, 2010).

3. Results and Discussion

3.1. Analysis of phytochemicals

The phytochemicals are found in vegetables possess various therapeutic applications (Okwu, 2001). Phenols are widely distributed in vegetables. They contribute color, flavor, and astringency to vegetables. These phenols are considered secondary metabolites of plant metabolism, which contributes to physiological or ecological functions (Oyeyinka & Afolayan, 2020). Isoprenoids are another name for terpenoids. Many vegetables contain these abundant and architecturally varied natural compounds. They are a diverse category of

natural compounds that may be found in all types of living organisms.

Alkaloids in the vegetables reveal their beneficial importance. Alkaloids in the diet may help in the healing of wounds, ulcers, haemorrhoids, and burns. These alkaloids have antiviral and cytotoxic properties, as well as a variety of other physiological effects (Ding et al., 2017). Multiple viruses have been shown to be inhibited by flavonoid compounds. These are water-soluble antioxidants and free radical scavengers that protect cells from oxidative damage and have potent anticancer properties (Duraipandiyan, Ayyanar, & Ignacimuthu, 2006). Dietary flavonoids provide several health benefits, including antioxidant and antiproliferative activities that may protect the human body from a variety of illnesses (Duraipandiyan et al., 2006). Phytochemical analysis of leafy vegetables and fruit extracts gave information which type of phytochemical compounds is present in vegetables and fruits. For qualitative analysis of all vegetables and fruits extract, a total of eight phytochemicals were analysed, such as phenols, proteins, quinones, alkaloids, flavonoids, tannins, terpenoids, carbohydrates. The results of the qualitative analysis of vegetables and fruits are shown in Table 1, where (+) represents the phytochemical is present and (-) indicates the absence of phytochemicals.

Table 1. Screening analysis of vegetables and fruits

Compounds	Spinach	Coriander Leaves	Fenugreek leaves	Jujube	Guava
Phenols	+	+	+	+	+
Proteins	+	+	+	+	+
Quinones	+	+	+	+	-
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	-
Tannins	+	+	-	-	+
Terpenoids	+	+	-	-	+
Carbohydrates	+	+	+	+	+

Proteins and carbohydrates were present in all the vegetables and fruits. Spinach and coriander leaves among the analyzed samples were the rich sources of phytochemicals and showed presence of all eight phytochemicals (Aborisade, Olagoke, & Abdulhakeem Dapo, 2018) also studied for spinach leaves is rich source of flavonoids, phenol, and tannins, whereas other phytochemicals were absent. (Al-Marzoqi, Hameed, & Idan, 2015) was reported Alkaloids, Flavonoids were absence in coriander leaves Phenols Tannins, Carbohydrate and Proteins were presence. Tannins and phenols were absent in fenugreek leaves, whereas all the studied phytochemicals were present. The tannins, terpenoids were absent in jujube, whereas phenols, proteins, quinones, alkaloids, flavonoids, terpenoids, and carbohydrates were present. In a study (Roughani & Miri, 2019) reported a similar trend of phytochemicals for jujube where terpenoids and tannins were absent, on the other hand, phenol, flavonoids, and alkaloids were present in the extract. In guava extract, except quinines and flavonoids, all other phytochemicals such as phenols, proteins, alkaloids, tannins, terpenoids, and carbohydrates were present.

3.2. Characterization of GC-MS analysis

In general, GC-MS is utilised to investigate compounds found in vegetables and fruits directly. In recent years, GC-MS studies for the analysis of vegetables and fruit have become more common since this technique has proven to be a useful tool for the examination of phytochemicals (Marston, 2007; Sridharan, Vaidyanathan, Venkatesh, & Nayagam, 2011). The molecular formula and peak area are used to confirm the phytochemical compounds' identity. The name of phytochemical compounds and concentration (%) is given in Table 2, which is important for regulating several significant functions like lipid levels, blood pressure, immune response, and inflammatory response to injuries. The GC-MS analysis in the vegetables and fruits described the presence of different phytochemical compounds that could contribute to the antioxidant and therapeutic properties of vegetables and fruits.

Table. 2 GC-MS analysis of spinach leaves

S.no	Compounds	Area (%)
1	3,4-dimethyl-1H-Pyrazole	2.03
2	Thymol	0.12
3	N-Methyl propionic acid amide	1.05
4	(S)-(+)-Glutamic acid	3.06
5	3-Methyl-3-pyrazolin-5-one	5.12
6	Glycine betaine	4.05
7	Propanoic acid, 2-(hydroxyimino)-, methyl ester	1.11
8	Thiole	6.50
9	Benzeneacetaldehyde	0.91
10	3-Hydroxypyrrolidine	1.19
11	Phenol, 2-methoxy-4-vinyl-	7.13
12	Benzoic acid	7.73
13	Ephedrin	5.62
14	Pyrollidine, 2,5-bis(imino)-	3.27
15	N-methyl piperazine	3.16
16	2-Pyrrolidinone	5.36
17	2,5-Diethylphenol	2.23
18	D-Valine	1.53
19	Monoethylaminoethanol	0.20
20	Thiophene	2.09
21	Succinic acid, diethyl ester	4.76
22	1-Eicosanol	1.94
23	Pyrrolidin-1-acetic acid	3.73
24	Palmitic acid ethyl ester	2.39
25	1-Methyl-5-D1-1,2,4-Triazole	1.33
26	L-Proline, 5-oxo-	2.21
27	Phthalic acid, dipropyl ester	5.22
28	Lauric acid	4.22

3.2.1. GC-MS analysis of spinach leaves

The GC-MS analysis shows different phytochemical compounds in the methanol extract of spinach leaves, as shown in Table 2. These compounds include 3,4-dimethyl-1H-Pyrazole (2.03%), Thymol (0.12%), N-Methyl propionic acid amide (1.05%), (S)-(+)-Glutamic acid (3.06%), 3-Methyl-3-pyrazolin-5-one (5.12%), Glycine betaine (4.05%), Propanoic acid,2-(hydroxyimino)-, methyl ester (1.11%),

Thiole (6.50%), Benzeneacetaldehyde (0.91%), 3-Hydroxypyrrolidine (1.19%), Phenol, 2-methoxy-4-vinyl- (7.13%), Benzoic acid (7.73%), Ephedrin (5.62%), Pyrollidine, 2,5-bis(imino) (3.27%), N-methyl piperazine (3.16%), 2-Pyrrolidinone (5.36%), 2,5-Diethylphenol (2.23%), D-Valine (1.53%), Monoethylaminoethanol (0.20%), Thiophene (2.09%), Succinic acid diethyl ester, (4.76%), 1-Eicosanol (1.94%), Pyrrolidin-1-acetic acid (3.73%), Palmitic acid ethyl ester (1.33%), L-Proline, 5-oxo- (2.21%), Phthalic acid dipropyl ester (5.22%), Lauric acid (4.22%). These phytochemical compounds indicate that spinach leaves can reduce the risk of different diseases.

3.2.2. GC-MS analysis of coriander leaves

The GC-MS analysis shows different phytochemical compounds in the methanol extract of coriander leaves (Table 3). The most phytochemical compounds present in the extract are n-Tetracosane (2.71%), Glycerin (2.99%), Benzenesulfonyl chloride (5.11%), N-Tetratetracontane (8.41%), 2-Methoxy-4-vinylphenol (6.22%), Tetradecanoic acid (2.66%), L(+)-isoleucine (3.85%), Cyclohexadecane (1.97%), 2-Isopropyl-5-methyl 1heptanol (2.51%), Cyclohexadecane (1.88%), Octadecane (1.72%), 9,12-Octadecadienoic acid (Z, Z)-methyl ester (3.29%), 1-Mercapto-4-methylbicyclo[2.2.2]octane (3.48%), Hexadecanoic acid (4.19%), Hexadecanoic acid (4.19), 1-Eicosene (13.44%), Squalene (6.31%).

Table 3. GC-MS analysis of coriander leaves

S. no	Compounds	Area (%)
1	n-Tetracosane	2.71
2	Glycerin	2.99
3	Benzenesulfonyl chloride	5.11
4	N-Tetratetracontane	8.41
5	2- Methoxy-4-vinylphenol	6.22
6	Tetradecanoic acid	2.66
7	L(+)-isoleucine	3.85
8	Cyclohexadecane	1.97
9	2-Isopropyl-5-methyl-1-heptanol	2.51
10	Cyclohexadecane	1.88
11	Octadecane	1.72
12	9,12-Octadecadienoic acid (Z, Z)-methyl ester	3.29
13	1-Mercapto-4-methylbicyclo[2.2.2]octane	3.48
14	Hexadecanoic acid	4.19
15	1-Eicosene	11.49
16	Lupeol	13.44
17	Squalene	6.31

3.2.3. GC-MS analysis of fenugreek leaves

The compounds identified by GC-MS in alcoholic extracts of fenugreek leaves are described in (Table 4) as Stigmasterol (2.23%), Hexadecane (4.19%), Diazoprogesterone (3.88%), L-Isoleucine (2.43%), Z-2-Dodecenol (1.81%),2-Pentadecanone, 6,10,14-trimethyl (9.33%), Methyl linolenate-Linolenic acid, methyl ester(5.15%), Hexadecanoic acid, methyl ester (12.89%), Nonadecane (12.19%), Hexadecanoic acid, ethyl ester, (6.31%), 10,13-Octadecadienoic acid, methyl Ester (4.17%), Phytol (8.23%), Glutamic acid (6.72%), Hexadecanoic acid, butyl ester (7.44%), Octadecane (1.11%), Campesterol (1.69%), Isophytol (3.42%),n-Hexadecanoic acid (2.18%), 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (9.14%).

3.2.4. GC-MS analysis of Jujube

GC-MS identified the compound in alcoholic extracts of Jujuba are described in Table 5, these include stigmasterol (1.57%),

formic acid (1.33%), molinate (3.18%), 5-Hydroxymethylfurfural (15.06%), Oleic acid (1.09%), Maltol (1.33%), 2-Propyloctanoic acid (5.89%), Beta-D-Glucopyranoside, methyl (5.97%), Pentanoic acid nonyl ester (1.17%), Thymine (4.09%), D-Allose (4.63%), Polygalitol (6.73%), 3,4-Altrosan (3.91%), Glucose (5.77%), Erucic acid (1.09%), 1,5-Anhydroglucitrol (7.89%), Tetradecanoic acid (2.59%).

3.2.5. GC-MS analysis of Guava

The compounds identified by GC-MS in alcoholic extracts of guava are described in Table 6, such as Pentadecanoic acid, (2.14%), Ethyl butanoate (3.28%), Butyl-2-methylbutyrate (4.69%), 9, 12-octadienoic acid (4.22%), Oleic acid, (6.51%), (+)-Aromadendrene (1.53%), Decyl fluoride (1.79%), Phytol, acetate (1.63%), β-Caryophyllene (3.29%), Arachic acid (3.23%), Acetoin (3.34%), (S)-(−)-Limonene (1.91%), Palmitic acid (1.11%), Nonanal (1.16%), (Z)-β-Farnesene (2.66%), cis-3-Hexenyl butanoate (1.01%), Eicosanoic acid (8.81%), α-Terpineol (3.37%), Methyl 3-propionamidobenzoate (2.13%), cis-3-Hexenyl isobutanoate (7.16%), Ethyl octanoate (6.23%), (−)-α-Copaene (5.29%), Ethyl butanoate (1.18%), (E, Z)-α-Farnesene(2.19%), γ-bisabolene (2.45%).

Table 4. GC-MS analysis of fenugreek leaves

S.no	Compounds	Area (%)
1	Stigmasterol	2.23
2	Hexadecane	4.19
3	Diazoprogesterone	3.88
4	L-Isoleucine	2.43
5	Z-2-Dodecenol	1.81
8	2-Pentadecanone, 6,10,14-trimethyl	9.33
9	Methyl linolenate-Linolenic acid, methyl ester	5.15
10	Hexadecanoic acid, methyl ester	12.89
11	Nonadecane	12.19
12	Hexadecanoic acid, ethyl ester	6.31
13	10,13-Octadecadienoic acid, methyl Ester	4.17
14	Phytol	8.23
15	Glutamic acid	6.72
16	Hexadecanoic acid, butyl ester	7.44
17	Octadecane	1.11
18	Campesterol	1.69
29	Isophytol	3.42
20	n-Hexadecanoic acid	2.18
21	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	9.14

Table 5. GC-MS analysis of Jujube

S.no	Compounds	Area (%)
1	Stigmasterol	1.57
2	Formic acid	1.33
3	Molinate	3.18
3	5-Hydroxymethylfurfural	15.06
5	Oleic acid	1.09
6	Maltol	1.33
7	2-Propyloctanoic acid	5.89
8	Beta-D-Glucopyranoside, methyl	5.97

9	Pentanoic acid, nonyl ester	1.17
11	Thymine	4.09
12	D-Allose	4.63
13	Polygalitol	6.73
14	3,4-Altrosan	3.91
15	Glucose	5.77
16	Erucic acid	1.09
17	1,5-Anhydroglucitrol	7.89
19	Tetradecanoic acid	2.59

Table 6. GC-MS analysis of Guava

S. no	Compounds	Area (%)
1	Pentadecanoic acid	2.14
2	Ethyl butanoate	3.28
3	Butyl-2-methylbutyrate	4.69
4	9, 12-octadienoic acid	4.22
5	Oleic acid	6.51
6	(+)-Aromadendrene	1.53
7	Decyl fluoride	1.79
8	Phytol, acetate	1.63
9	β-Caryophyllene	3.29
10	Arachic acid	3.23
11	Acetoin	3.34
12	(S)-(−)-Limonene	1.91
13	Palmitic acid	1.11
14	Nonanal	1.16
15	(Z)-β-Farnesene	2.66
16	Ethyl benzoate	14.43
17	cis-3-Hexenyl butanoate	1.01
18	Eicosanoic acid	8.81
19	α-Terpineol	3.37
20	Methyl 3-propionamidobenzoate	2.13
21	cis-3-Hexenyl isobutanoate	7.16
22	Ethyl octanoate	6.23
23	(−)-α-Copaene	5.29
24	Ethyl butanoate	1.18
25	(E,Z)-α-Farnesene	2.19
26	γ-bisabolene	2.45

3.3. Fourier Transform Infrared Spectrophotometer (FTIR) analysis

The qualitative characteristics of organic substances in freeze-dried vegetables and fruits were identified using FTIR spectroscopy. Chemical components of bioactive molecules are represented by many indicator bands associated with various functional groupings. The fingerprint area has distinct bands in the FT-IR spectrum. The infrared spectrum identifies the major components and shows a spectral variation to find differences among the vegetables and fruits. The typical functional groups observed in the vegetables and fruits are shown in Table 7

Table 7. FTIR peak assignment of vegetables and fruits.

Functional group	Spinach	Coriander Leaves	Fenugreek leaves	Jujube	Guava
O-H stretching vibration presence of alcohols, phenols	3362	-	-	-	-
	3276	3276	3275	3280	3273
C-H stretching vibration presence of alkenes	2959	2920	2918	2930	2920
	2917	-	-	-	-
	2850	2851	2849	2855	2851
C=O s stretching vibration presence of alcohols, carboxylic acids, esters, ethers	-	1733	1732	1724	-
-C=C- stretching vibration presence of alkenes	1635	1601	1616	1634	1626
C-C stretching vibration presence of aromatics	-	-	-	1416	-
N-O stretching nitro compound	1539	1558	1558	-	1557
	-	1540	1539	-	-
O-H bending	1371	-	1334	1338	1378
	1338	1394	1398	-	1320
	1321	-	-	-	-
C-N stretching	1241	1241	1261	1239	1241
stretching of C-O group	1099	-	-	1147	1155
	-	-	-	1051	-
	1046	1014	1027	1027	1028
	1030	-	-	-	-
C=C bending	-	-	893	918	-
	827	-	-	815	-
C-H bending	775	-	-	-	-

3.3.1. FTIR analysis of Spinach

In the spinach**,** a very strong absorption band in the region 3600-3200 cm^{-1} was observed, representing O-H stretching vibrations that may be due to the presence of alcohols. Absorption bands at 2959 cm^{-1}, 2917 cm^{-1}, 2850 cm^{-1} represent the presence of alkanes' C-H stretching vibration. The C=C stretching vibration of alkenes showed an absorption band at 1635 cm^{-1}. The band at 1539 cm^{-1} represents the N-O stretching due to the nitro compound. The absorption bands at 1371cm^{-1}, 1338 cm^{-1}, and 1321 cm^{-1} appeared due to the bending of O-H. The C-N stretching vibration shows an absorption band at 1241 cm^{-1}. The absorption bands at 1099 cm^{-1}, 1046 cm^{-1}, and 1030 cm^{-1} appeared due to the stretching of the C-O group. Two absorption bands appeared at 827 cm^{-1} and 775 $cm^{-1,}$ representing C=C bending and C-H bending.

3.3.2. FTIR analysis of Coriander leaves

In the coriander leaves, a very strong absorption band in the region 3200 cm^{-1} was observed, representing O-H stretching vibrations that may be due to the presence of alcohols. Absorption bands at 2920 cm^{-1}, 2851 cm^{-1} represent the presence of alkanes' C-H stretching vibration. The C=O stretching vibration presence may be due to alcohols showed an absorption band at 1733 cm^{-1}. Absorption bands at 1601 cm^{-1} C=C stretching vibration presence of alkenes. The absorption band at 1558 cm^{-1},1540 cm^{-1} shows N-O stretching due to nitro compound. The absorption bands at 1394 cm^{-1} appeared due to the bending of O-H. The C-N stretching vibration shows an absorption band at 1241 cm^{-1}. The absorption bands at 1014 cm^{-1} appeared due to the stretching of the C-O group.

3.3.3. FTIR analysis of Fenugreek leaves

In the Fenugreek leaves**,** a very strong absorption band in the region 3275 cm^{-1} was observed, representing O-H stretching vibrations that may be due to the presence of alcohols. Absorption bands at 2918 cm^{-1}, 2849 cm^{-1} represent the presence of alkanes C-H stretching vibration. The C=O stretching vibration that may be due to esters, alcohols,

ethers, carboxylic acids showed an absorption band at 1732 cm^{-1}. The C=C stretching vibration of alkenes showed an absorption band at 1616 cm^{-1}. The band at 1558 cm^{-1} and 1539 cm^{-1} represents the N-O stretching due to the nitro compound. The absorption bands at 1334 cm^{-1} and 1398 cm^{-1} appeared due to O-H bending. The C-N stretching vibration shows an absorption band at 1261 cm^{-1}. The absorption bands at 1027 cm^{-1} appeared due to the stretching of the C-O group. The absorption bands appeared at 893 cm^{-1}, representing C=C bending.

3.3.4. FTIR analysis of Jujuba

In the Jujuba**,** a very strong absorption band in the region 3280 cm^{-1} was observed, representing O-H stretching vibrations that may be due to the presence of alcohols. Absorption bands at 2930 cm^{-1},2855 cm^{-1} represent the presence of alkanes C-H stretching vibration. The C=O stretching vibration that may be due to esters, alcohols, ethers, carboxylic acids showed an absorption band at 1724 cm^{-1}. The C=C stretching vibration of alkenes showed an absorption band at 1634 cm^{-1}. The band at 1416 cm^{-1} represents the C-C stretching vibration presence due to the aromatics compound. The absorption bands at 1338 cm^{-1} appeared due to the bending of O-H. The C-N stretching vibration shows an absorption band at 1239 cm^{-1}. The absorption bands at 1147 cm^{-1}, 1051 cm^{-1}, and 1027 cm^{-1} appeared due to the stretching of the C-O group. Two absorption bands appeared at 918 cm^{-1} and 815 cm^{-1}, representing C=C bending

3.3.5. FTIR analysis of Guava

In the guava**,** a very strong absorption band in the region 3273 cm^{-1} was observed, representing O-H stretching vibrations that may be due to the presence of alcohols. Absorption bands at 2920 cm^{-1}, 2851 cm^{-1} represent the presence of alkanes C-H stretching vibration. The C=C stretching vibration of alkenes showed an absorption band at 1626 cm^{-1}. The band at 1557 cm^{-1} represents the N-O stretching due to the nitro compound. The absorption bands at 1378 cm^{-1} and 1320 cm^{-1} appeared due to O-H bending. The C-N stretching vibration shows an absorption band at 1241 cm^{-1}. The absorption bands at 1155 cm^{-1} and 1028 cm^{-1} appeared due to the stretching of the C-O group.

4. Conclusions

Natural antioxidants are increasingly being used in place of synthetic antioxidants. Vegetables and fruits are common dietary sources that contain many nutritional, functional, antioxidant, and other medicinal characteristics. The results of phytochemical screening, GC-MS, and FTIR indicated the presence of different phytochemical compounds with different compositions in freeze-dried vegetables and fruits. Hence, this study recommends that vegetables and fruits are essential for increasing potential health and safe food since they contain beneficial bioactive substances.

5.References

Aborisade, A., Olagoke, O., & Abdulhakeem Dapo, O. (2018). Phytochemical analysis and antibacterial activities of spinach leaf. *American Journal of Phytomedicine and Clinical Therapeutics 6*, 8.

Al-Marzoqi, A. H., Hameed, I. H., & Idan, S. A. (2015). Analysis of bioactive chemical components of two medicinal plants (Coriandrum sativum and Melia azedarach) leaves using gas chromatography-mass spectrometry (GC-MS). *African Journal of Biotechnology, 14*(40), 2812-2830.

Aman, R., Schieber, A., & Carle, R. (2005). Effects of heating and illumination on trans−cis isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. *Journal of Agricultural and Food Chemistry, 53*(24), 9512-9518.

Ding, Y., Qu, D., Zhang, K.-M., Cang, X.-X., Kou, Z.-N., Xiao, W., & Zhu, J.-B. (2017). Phytochemical and biological investigations of Amaryllidaceae alkaloids: a review. *Journal of Asian natural products research, 19*(1), 53-100.

Duraipandiyan, V., Ayyanar, M., & Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India.

BMC complementary and alternative medicine, 6(1), 1-7.

Ganesan, K., & Raja, A. B. (2021). evaluation of phyto-compounds from dictyota dichotoma extract using gas chromatographic and mass spectroscopic technique. *International Journal of Modern Agriculture, 10*(2), 2544-2548.

Goud, J. V., Suryam, A., & Charya, M. S. (2009). Biomolecular and phytochemical analyses of three aquatic angiosperms. *African journal of microbiology research, 3*(8), 418-421.

Hirsch, K., Danilenko, M., Giat, J., Miron, T., Rabinkov, A., Wilchek, M., . . . Sharoni, Y. (2000). Effect of purified allicin, the major ingredient offreshly crushed garlic, on cancer cell proliferation. *Nutrition and cancer, 38*(2), 245-254.

Jigna, P., & Sumitra, C. (2008). Phytochemical screening of some plants from western region of India. *Plant Archives, 8*(2), 657-662.

Kell, D. B., Brown, M., Davey, H. M., Dunn, W. B., Spasic, I., & Oliver, S. G. (2005). Metabolic footprinting and systems biology: the medium is the message. *Nature reviews microbiology, 3*(7), 557-565.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Etherton, T. D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American journal of medicine, 113*(9), 71-88.

Marston, A. (2007). Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry, 68*(22-24), 2786-2798.

Okwu, D. (2001). Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae.*Pakistan Veterinary Journal, 14*, 160-162.

Oyeyinka, B. O., & Afolayan, A. J. (2020). Comparative and correlational evaluation of the phytochemical constituents and antioxidant activity of Musa sinensis L. and Musa paradisiaca L. fruit compartments (Musaceae). *The Scientific World Journal, 2020*.

Prasain, J. K., Wang, C.-C., & Barnes, S. (2004). Mass spectrometric methods for the determination of flavonoids in biological samples. *Free Radical Biology and Medicine, 37*(9), 1324-1350.

Raja, S., & Ravindranadh, K. (2014). preliminary phytochemical screening and tlc fingerprinting of whole plant extracts of michelia champaca. *World Journal of Pharmaceutical Research, 3*(10), 631-645.

Robertson, D. G. (2005). Metabonomics in toxicology: a review. *Toxicological Sciences, 85*(2), 809-822.

Roughani, A., & Miri, S. (2019). *Spinach: An important green leafy vegetable and medicinal herb.* Paper presented at the The 2nd International Conference on Medicinal Plants, Organic Farming, Natural and Pharmaceutical Ingredients.

Sandosh, T. A., Peter, M. P. J., & Raj, J. Y. (2013). Phytochemical analysis of Stylosanthes fruticosa using UV-VIS, FTIR and GC-MS. *Research Journal of Chemical Sciences ISSN, 2231*, 606X.

Schneider, Y., Vincent, F., Duranton, B. t., Badolo, L., Gossé, F., Bergmann, C., . . . Raul, F. (2000). Anti-proliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. *Cancer letters, 158*(1), 85-91.

Shah, A., Rasapalli, S., Mello, C., Singh, B. R., & Cai, S. (2012). Antibacterial activities of commonly used traditional Chinese medicines as cold and flu remedies. *Journal of Medicinal Plants Research, 6*(2), 234-242.

Shahidi, F., McDonald, J., Chandrasekara, A., & Zhong, Y. (2008). Phytochemicals of foods, beverages and fruit vinegars: chemistry and health effects. *Asia Pacific Journal of Clinical Nutrition, 17*.

Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in nutrition, 3*(4), 506-516.

Sridharan, S., Vaidyanathan, M., Venkatesh, K., & Nayagam, A. A. J. (2011). Available online through www. jpronline. info.

Journal of Pharmacy Research, 4(3), 741-742.

Talukdar, A. D., Choudhury, M. D., Chakraborty, M., & Dutta, B. (2010). Phytochemical screening and TLC profiling of plant extracts of Cyathea gigantea (Wall. Ex. Hook.) Haltt. and Cyathea brunoniana. Wall. ex. Hook (Cl. & Bak.). *Assam University Journal of Science and Technology, 5*(1), 70-74.

Temple, N. J., & Gladwin, K. K. (2003). Fruit, vegetables, and the prevention of cancer: research challenges. *Nutrition, 19*(5), 467-470.