World Journal of
Pharmacy and Medical Science

ISSN: 3049-3501
Impact Factor: 5.157
CODEN (USA): WJPMAA

Research Article
2026 Volume 2, Issue 8
Page No.: 122-143

SCREENING FOR PHYTOCHEMICALS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES DETERMINATION AND CHEMICAL ANALYSIS BY FOURIER TRANSFORME INFRARED SPECTROSCOPY (FTIR) OF FRESHLY PREPARED ETHANOLIC EXTRACT FOR THE FRUIT OF BLUEBERRIES

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Article Received: 18 June 2026 Article Review: 09 July 2026 Article Accepted: 30 July 2026

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DOI: https://doi.org/10.5281/zenodo.21991849

How to cite this Article: Stephanie Yeap Zi Hui, Nabila Perveen, Naeem Hasan Khan (2026). SCREENING FOR PHYTOCHEMICALS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES DETERMINATION AND CHEMICAL ANALYSIS BY FOURIER TRANSFORME INFRARED SPECTROSCOPY (FTIR) OF FRESHLY PREPARED ETHANOLIC EXTRACT FOR THE FRUIT OF BLUEBERRIES. World Journal of Pharmacy and Medical Science, 2(8): 122-143.

INTRODUCTION

Stepping into 21st century, people now is more care about their health because they understand prevention is better than cure. However, they are more prefer taking natural products to obtain the necessary nutrients than taking the synthetic or dietary supplement. One of the fruits which have been discussed the most recently is blueberry.

According to statistics from National Institutes of Health, principal cause of death is Ischaemic Heart Disease (IHD), stroke and followed by lower respiratory disease.

(Alias et al., n.d.) First of two disease is due to oxidative stress. IHD happened mainly due to atherosclerosis and untreated IHD may develop into stroke. Reactive Oxygen Species (ROS) mainly comes from the mitochondrial electron transport chain when produced, ATP and the NADPH oxidase during defense against bacteria. In normal condition, our body have superoxide dismutase and endogenous antioxidant to neutralize free radicals. However, when the ROS in body exceed the limit, it may cause lipid peroxidation and lead to dyslipidemia, protein oxidation lead to enzyme inactivation and DNA strand oxidation lead to DNA damage. (Golenia & Olejnik, 2025; Li & Yang, 2016)

Throughout this research, fruits of blueberries will be used for the phytochemical screening, antioxidant, antibacterial activities and FTIR chemical analysis.

Blueberries are the fruits which widely grow in North America. It is consumed directly and it is an excellent source of Vitamin C, Vitamin K, antioxidant, manganese

and iron. Blueberries are closely related to cranberries and bilberries due to they are under the same genus.(Britannica Editors, 2025) Blueberry plants have simple elliptical leaves that arranged on the dotted stems.

Flowers range in color from white to pale pink. Ideal growth environment of blueberries is highly acidic, well drained, moist soils and cool climates.(Britannica Editors, 2025) Its taxonomical classification has shown in **Table 1 and Figures 2 and 3, respectively**.(PubChem, n.d.)

Table 1: Taxonomical classification of blueberry (PubChem, n.d.).

Taxanomic	Classification
Kingdom	Plantae
Phylum	Magnoliaphyta
Class	Magnoliopsida
Order	Ericales
Family	Ericaceae
Genus	*Vaccinium*

Blueberries contain a lot of phytochemicals which provide various health benefits. The most abundant phytochemicals found in blueberries is polyphenols which include flavonoids and phenolic compound.

Flavonoids play an important role in anti-inflammatory and antioxidant effect. (Ashique et al., 2024)When the blueberry in unripe condition, it contain a lot of flavonoids due to the protective barrier of the fruits from the external environment during the maturity state. Thus, it has optimal level of antioxidant and antibacterial during unripe state.

However, the color of blueberries become more purple when reach the ripe state. This means that the anthocyanin which give color to the blueberries increase and provide antioxidant effect. (Amin Salehi et al., 2023)Besides that, blueberries have the antibacterial activity to both gram positive and gram negative bacteria. (Zimmer et al., 2014)Thus, blueberries also have natural bactericidal activity. Thus, it is important to discuss the antioxidant and antibacterial ability in blueberries to discover more health benefits in human.

Figure 1: Blueberry flowers(Britannica Editors, 2025).

Figure 2: Fruits of blueberry (Britannica Editors, 2025).

Phytochemical is secondary metabolites of the plants. Question may come to your mind that what is primary metabolites and secondary metabolites? It's no surprise that all of the living organisms have their own metabolism, same goes to plants which include blueberries (*Vaccinium* spp.). When the plants undergo photosynthesis, major of the carbon, nitrogen and energy will produce carbohydrates, sugars, amino acids and nuclei acid in the plants to allow plants to carry out normal function. This is known as primary metabolites. Secondary metabolites, also known as phytochemicals, which are the assimilation of carbon and energy to form organic molecules in the plants which do not cause any direct function in the plants. Biosynthesis of secondary metabolites usually happen during environmental stress or at the specific stages of plants development. (Fais & Era, 2024) Phytochemicals are classified into three major groups, which are polyphenols, alkaloids and terpenoids. (Mendoza et al., 2018) In **Figure 3 and Table 2** is showing that phenolic compounds and alkaloids are biosynthesized from Shikimic acid pathway while terpenoids is synthesized from Mevalonic acid pathway.

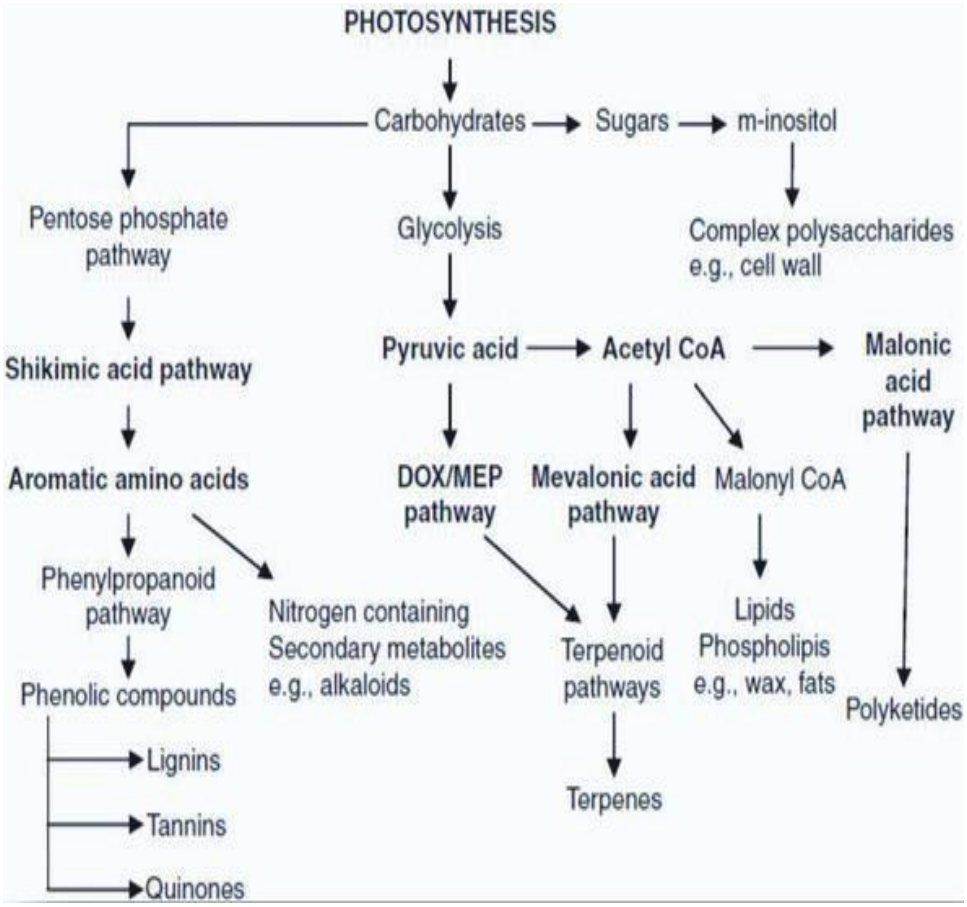


Figure 3: Metabolism of plants. Image adapted from (Mendoza et al., 2018).

The primary phytochemical found in *Vaccinium* spp. is polyphenols.(Ashique et al., 2024; Felgus-Lavefve et al., 2021) Dietary polyphenols found in blueberries consists of flavonoids and phenolic compounds. Example of nonflavonoids or phenolic compound in *Vaccinium* spp. is chlorogenic acids (Kalt et al., 2020; Stevenson & Scalzo, 2012).

Example of flavonoids is flavones, flavanones, flavanols, anthocyanins and chalcones.

Firstly, anthocyanins is a flavonoid which contribute 60% of phenolic compounds (Kalt et al., 2020) and occur predominantly in the outer cell layers of *Vaccinium* spp. and give the colors in the fruits. (Felgus-Lavefve et al., 2021) Anthocyanins is under the group of glycosides as it is made up of aglycone and glycine. Anthocyanidins such as pelargonidin (Pg), cyanidin (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt) and malvidin (Mv) are the aglycones of anthocyanins(Ashique et al., 2024).

Glycoses are sugars such as galactose, glucose and arabinose (Felgus-Lavefve et al., 2021), they joined with the aglycones which use the flavylium ion skeleton by O-glycosidic bonds.

Next, flavanols are also found abundantly in *Vaccinium* spp. in the form of flavan-3-ols as the hydroxy group is normally bound to position 2 of the C rings. Example of flavanols which found in blueberries are myricetin, quercetin and kaempferol (Wang et al., 2019) and sugar like glucose, galactose and robinose can substitute on them. Flavanols provide a high antioxidant and high anti-inflammatory properties. (Ashique et al., 2024)Furthermore, hydrolysable tannins which is known as ellagitannins (Ashique et al., 2024) primarily in the form of lambertianin C and sanguine H-6 are found in *Vaccinium* spp.(Bader Ul Ain et al., 2022). This plays an important role in reducing systolic blood pressure of hypertensive patients(Looi et al., 2022).

Proanthocyanidins (condensed tannins) are also found in blueberries in B-type which containing exclusively single inter-flavan linkages (Wang et al., 2019) and lacking of A-type which help in prevention urinary tract infection. However, proanthocyanidins proved to help in metabolic health especially in obesity patients.(American Physiological Society, n.d.).

Terpenes such as limonene, α-terpinolene and α-terpineol also detected in blueberries to provide aroma and responsible for its odour due to its volatile properties.(Farneti et al., 2017) Alkaloids are not expected to present in blueberries as it normally present in only medicinal plants.

Table 2: Mean content of individual phenolic and anthocyanins over 80 blueberry genotypes from the Plant & Food Research (PFR) (Stevenson & Scalzo, 2012).

Compound	Mean content (mg/100 g)
Total anthocyanin content	199.0
Total malvidin	79.4
Mv-Gal / Pn-Glu	39.1
Chlorogenic acid	36.9
Mv-Glu / Pn-Ara	28.1
Dp-Glu / Cy-Gal	27.5
Dp-Gal	25.6
Quercetin-glycosides	22.7
Pet-Gal / Cy-Ara	21.9
Mv-Ara	19.8
Pet-Glu	16.6
Cy-Glu / Dp-Ara	15.8
Pet-Ara / Pn-Gal	14.8
Acetylated anthocyanin	11.6

Antioxidant activities of blueberries

High phenolic content and anthocyanins in blueberries contribute to antioxidant activity of blueberries.(Siddiq et al., 2018) Anthocyanins able to reduce or prevent the formation of superoxide. It able to neutralize the superoxide anion by donating the hydrogen atom to it due to the weak dissociation energy of O-H group.(Zhou et al., 2020) According to (Felgus-Lavefve et al., 2021), phenolic components in blueberries play an important role in scavenging reactive oxygen species (ROS), improving cellular health and enhancing the action of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase (SOD) and catalase (Superoxide dismutase (SOD) and catalase are two essential antioxidant enzymes that work together to protect cells from oxidative damage. Various research applied the DPPH assay to determine the scavenging capacity of blueberry extract by measuring its absorbance at 515nm.

Ascorbic acid is used as the positive control. (Gonçalves et al., 2023) DPPH is a stable free radical thus it is suitable for the assay to measure antioxidant of blueberry. If the sample contain antioxidant, it will donate hydrogen to DPPH to neutralize it, and color will change from purple to yellow. Thus, the greater the antioxidant present, the greater the changes in color, the lower the absorbance at 515nm. According to DPPH• assay performed in(Gonçalves et al., 2023), the blueberries showed IC_{50} scores of 30.87 ± 0.67 µg/mL of dw which is 4.3 times less effective than ascorbic acid control.

Besides DPPH, methods that are widely used to determine the antioxidant activity of blueberries included (Fluorescence Recovery After Photobleaching (FRAP) is a microscopy technique used to study molecular mobility and binding kinetics within living cells. FRAP analysis and ABTS (An ABTS assay measures the total antioxidant capacity of a substance) +(Fuentes et al., 2019; Tobar-Bolaños et al., 2021). FRAP analysis involved the reduction of Fe^{3+}-TPTZ complex to a blue-colored Fe (II) TPTZ by antioxidant in the extract due to the donation of electron and the absorbance measured at 595nm. Trolox which is a Vitamin E analogue was used as a positive control.(Dróżdż et al., 2017) The total antioxidant capacity values for blueberries for FRAP were found to be between 454.93-3632.96 µ/mol Trolox 100 g(Okan et al., 2018)

ABTS method is based on the deactivation radical cation ABTS•+ by the donation of electron or hydrogen from blueberry extract and the absorbance measured at 734nm.(Huang et al., 2012; Rodrigues et al., 2011)The total antioxidant activity of the blueberry, in fresh weight, was expressed in µMol.100 g-1 of TEAC (Trolox-equivalent antioxidant capacity). The total antioxidant activity for blueberries for ABTS were found to be between 1238.48 to 2445.96 µmol TEAC.$100g^{-1}$FW. (Rodrigues et al., 2011) In ABTS assays, blueberries are well-documented as highly potent sources of antioxidants. Trolox-equivalent antioxidant capacity (TEAC) values for blueberries generally range from ~8.11 to 38.29 µM Trolox/g fresh weight, or roughly 14.98 mmol Trolox/100 g dry weight in comparative studies.

(Trolox equivalents per 100 g dry weight is the standard unit used to measure total antioxidant capacity (TAC) in foods, plants, and biological samples. It indicates how well a sample scavenges free radicals, typically utilizing assays like ABTS, DPPH, or FRAP. Trolox equivalents per 100 g dry weight is the standard unit used to measure total antioxidant capacity (TAC) in foods, plants, and biological samples. It indicates how well a sample scavenges free radicals, typically utilizing assays like ABTS, DPPH, or FRAP.

Thus, all of the tests that were carried out proved the antioxidant ability of blueberries. Most of the chronic illness caused by oxidative stress and the formation of ROS will be accelerated by the environmental factor and lifestyle habits. This will further cause protein, nuclei acid and cellular oxidation and lead to increase in the pro-inflammatory marker which maintain oxidative and inflammatory stress. Consequences of the cellular oxidation may cause various cardiovascular disease, cancer or neurodegenerative disease(Leyane et al., 2022).

Although there is antioxidant enzymes present in our body, however it unable to suppress the formation of ROS by only itself(Ashique et al., 2024). Thus, antioxidant in blueberries do wonders in scavenging the ROS as described in **Figure 4.**

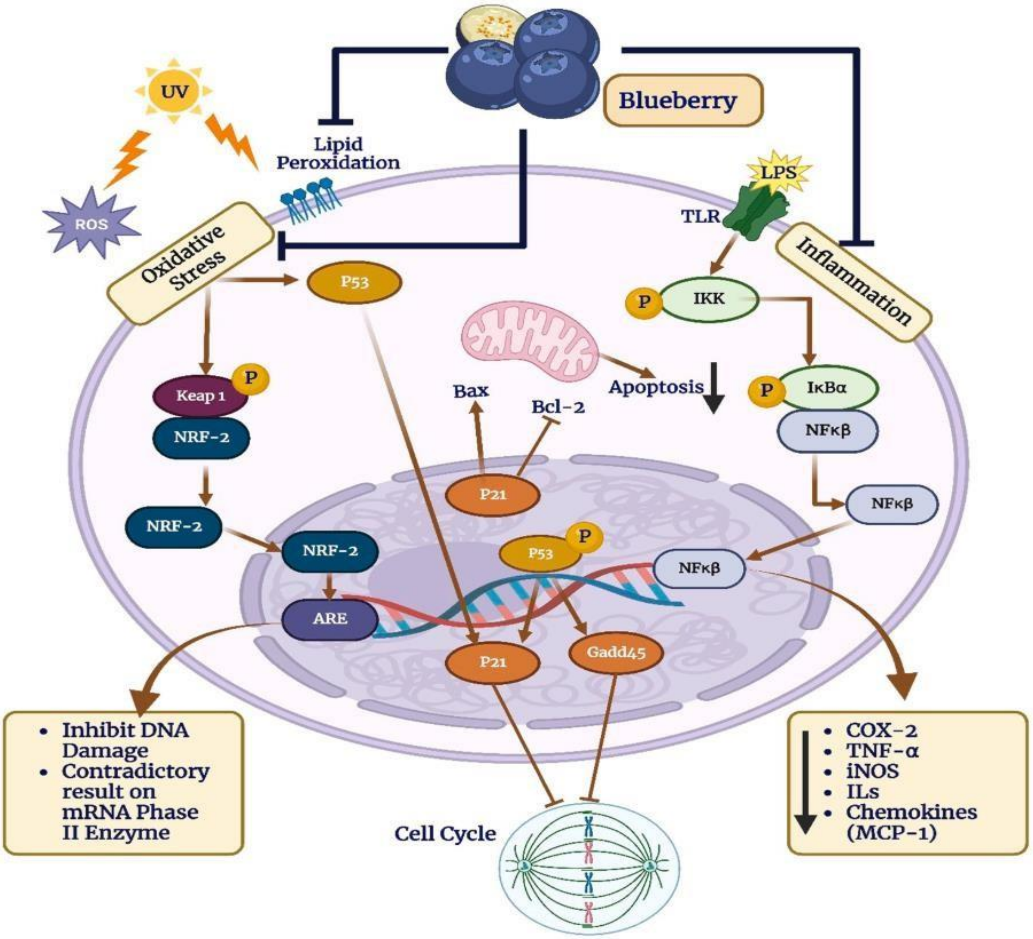


Figure 4: The concise overview of the cellular regulatory mechanisms hypothesized for blueberry extracts in relation to their effects on inflammatory and oxidative stress pathways.

The investigation primarily focuses on cell-based models to elucidate these processes. Image adapted from (Ashique et al., 2024).

High anthocyanin content in blueberries able to prevent cardiovascular disease such as hypertension, myocardial infarction and coronary artery disease. (Kalt et al., 2020) Besides that, it also proved that anthocyanin able to lower 3-9% of body fat and decrease the risk factor of obesity to cause hypertension. Furthermore, blueberry extract able to increase the insulin sensitivity and help in the prevention of T2DM.(Ashique et al., 2024) Moreover, ageing occur due to increase concentration of ROS which cause mitochondrial dysfunction and slow down the cellular function and regeneration (Leyane et al., 2022). Thus, daily intake of blueberries able to reduce the formation of ROS and slow down ageing.

Antioxidants in blueberries able to prevent and reduce the risk of neurodegenerative disease such as Alzheimer's disease and Parkinson disease (Leyane et al., 2022).

Antibacterial activities of blueberries

Secondary metabolites found in blueberries such as phenolic compound and flavonoids has discovered that exhibit antibacterial effect. Studies show that blueberry is used as traditional medicine to inhibit the growth of pathogenic microorganisms. (Borozan et al., 2025) Phenolic compounds exhibit antibacterial activity through mechanisms such as cell membrane disruption, enzyme inhibition, induction of oxidative stress and inhibition of quorum sensing and biofilm formation.

Phenolic compound increase membrane permeability by interacting with lipid bilayer and cause intracellular components to flow out due to cell lysis. Phenolic compounds also can form hydrogen bond with the enzyme and disrupt the bacterial enzyme function. (Dembińska et al., 2025; Kauffmann & Castro, 2023).

Tube dilution test is used to determine minimum inhibitory concentration (MIC) of blueberry extract while agar diffusion is used to determine minimum bactericidal concentration (MBC) of blueberry extract.

The result showed that unripe blueberries have higher antibacterial activities as the MIC is 3.5-3.8 mg/mL and MBC is 5.6-5.7 mg/mL. The bacteria used in this experiment is a foodborne pathogen, *Salmonella typhimurium*.(Amin Salehi et al., 2023) Semi-ripe and ripe fruit also show antibacterial activities but with higher MIC and MBC. This is because unripe state has higher concentration of phenolic components and flavonoids to protect the fruits from microbial attack.

When using the Bacterial Killing Time Measurement assay with the extract-free sample and chloramphenicol sample, microplate well with blueberry extract do not show any bacteria survival after 24 hours like chloramphenicol proved the antibacterial activities of blueberries.(Amin Salehi et al., 2023) Result is shown in Figure 5.

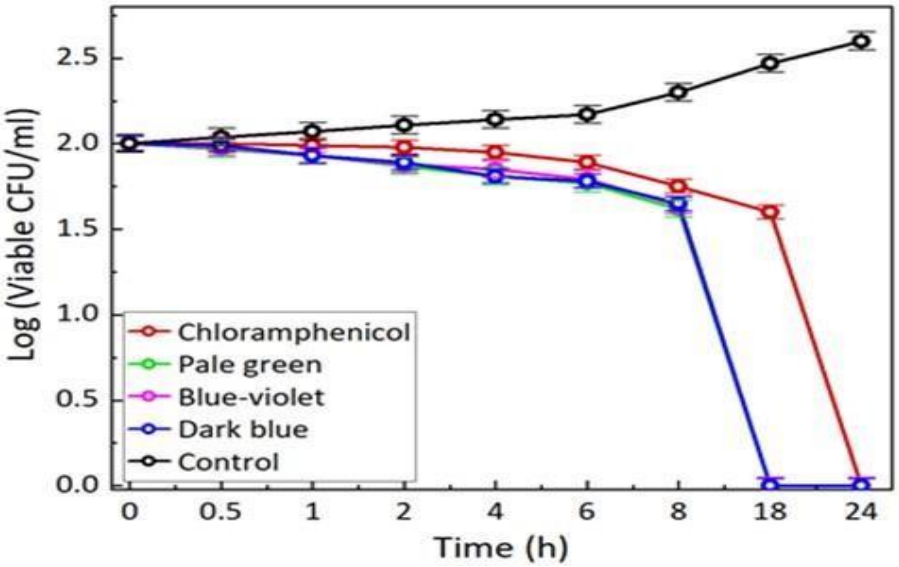


Figure 5: Time-killing curves of blueberry fruit extract (unripe fruit, semi-ripe fruit, and fully ripe fruit) against Salmonella *typhimurium*. (Amin Salehi et al., 2023)

Agar well diffusion method is also used to measure antibacterial activity. Antimicrobial activity was determined by measured the diameter of clear zone around the well which is the inhibition zone where bacterial growth is inhibited.

Experiment is conducted in triplicate with the positive control using antibiotic and distilled water as negative control.(A. Satashia & Pandya, 2025)

According to (Silva et al., 2023), blueberry extract at 1000 $\mu g/mL^{-1}$, was capable of effectively inhibiting the growth of *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella enteritidis.* Biofilm is a community of bacterial enclosed in an extracellular polymeric substance matrix.(Donlan, 2002) Biofilm which made of *Staphylococcus epidermidis* hard to eradicate and cause recurrent nosocomial infection due to the antimicrobial resistance and resistance to the immune defense (Silva et al., 2016; Zimmer et al., 2014).

Chlorogenic acid in the blueberry extract is found to be able to prevent biofilm formation caused by *Staphylococcus epidermidis*. Thus, blueberry extract has antibacterial effects towards gram positive and gram-negative bacteria. As *Bacillus cereus, Salmonella enteritidis* and *Salmonella* typhimurium is gram positive bacteria while *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus epidermidis* is gram negative bacteria.

Fourier Transform Infrared spectroscopy (FTIR) spectroscopy

FTIR is an absorption spectroscopy using the infrared ray radiation (Schuttlefield & Grassian, 2008). It involved molecular vibration to produce a spectrum. IR spectrum is a plot of percentage transmittance and wave number. Wave number is directly proportional to the energy and frequency. This method has been frequently used to determine functional group of a compound and bonding between the atoms. Molecule will undergo stretching vibration which involved changes in bond length and bending vibration which involved changes in bond angle. IR spectrum is separated into three regions which is functional group region (4000-1300cm^{-1}), fingerprint region (1300-910cm^{-1}) and aromatic region (910-400cm^{-1}). ATR technique will be used in this FTIR due to it measure the absorption of light by the sample which placed on the high refractive index diamond and formation of evanescent wave. (Pasieczna-Patkowska et al., 2025).

Table 3: Wave number range of key functional group (Al-Kelani & Buthelezi, 2024; Pasieczna-Patkowska et al., 2025).

Functional Group	Stretching Vibration (cm^{-1})	Bending Vibration (cm^{-1})
O–H (Hydroxyl)	3200-3550	1300-1400
N–H (Amines)	3300-3500	1600-1650
C–H (Alkanes)	2850-3000	1450-1480
C=O (Carbonyl)	1725-1740	1680-1690

C=C (Alkenes)	1600-1680	1450-1600
C≡C (Alkynes)	2100-2260	-
C–N (Amines)	1030-1250	-
C–O (Alcohols)	1000-1300	-
S–H (Thiols)	2550-2600	-
C–S (Thioethers)	600-700	-
Si–H (Silane)	2100-2300	-
N=O (Nitro)	1530-1550	1300-1375
Aromatic Rings	1400-1600	675-900
Phosphate (P=O)	1200-1300	900-1100

Application of FTIR in chemical analysis of blueberries

According to the (Gazola et al., 2022), absorption of O-H group is more obvious when the blueberry is extracted via hydrophilic solvent such as ethanol compared to lipophilic solvent. This is due to the secondary metabolites in the blueberry such as phenolic compounds and alcohol group.(Aziz et al., 2025) Besides that, N-H(primary amine), C=C binding stretch, C−H (alkanes), C=O, CH2, C−O and C−X (alkyl halides) are also present. FTIR give a clear chemical and molecular structure analysis of blueberry. FTIR proved the presence of phenols, flavonoids, alkaloids, terpenoids, polysaccharides, aromatic compounds, ethers, esters, carboxylic acids, fatty acids, and more also include halogenated compounds, aromatic compounds, unsaturated alkenes, ethers, esters, carboxylic acids, phenols, primary amines and secondary amines based on **Figure 6.** and **Table 4**.(Aziz et al., 2025)

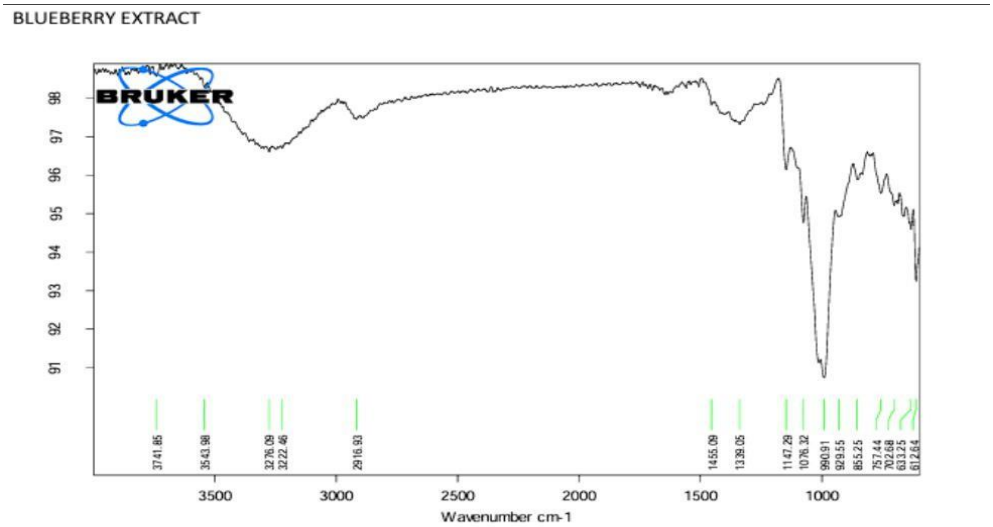


Figure 6: FTIR spectrum of blueberry extract(Aziz et al., 2025).

Table 4: FTIR analysis of blueberry formulation(Aziz et al., 2025)

IR Spectra (cm^{-1})	Intensity	Possible Functional Group	Compound Class	Phytochemicals
612.44	93.3	C-Cl Stretching	Halo compounds	Halogenated compounds or aromatic skeletal vibrations
633.25	94.6	C-Cl Stretching	Halo compounds	Halogenated compounds or aromatic skeletal vibrations
702.68	95.5	C-H bending	Alkane group	Alkane groups or aromatic structures confirming presence of compound with benzene rings
757.44	95.6	C-H bending (aromatic)	Chloride	Aromatic compounds, confirms aromatic ring system
855.25	96.1	C-H bending (aromatic)	Alkenes	Presence of aromatic compounds like flavonoids or phenols
929.55	95.2	C=C bending	Alkenes	Presence of Unsaturated compounds or specific alkenes groups
990.91	90.3	C=C bending	Alkenes	Presence of Unsaturated compounds or specific alkenes groups

1076.32	94.75	C-O stretching	Esters	Secondary alcohols or esters indicates glycosidic linkage. or secondary alcohol presence
1147.29	96.2	C-O stretching	Ether, esters or carboxylic acids	Suggests carbohydrate, glycosides or ester compounds
1339.05	97.4	O-H bending	Phenolic groups	Indicates presence of phenolic groups
1455.09	97.8	C-C stretching	Aromatic or alkene	Aromatic compounds, or unsaturated hydrocarbons, likely phenolic or flavonoids derivatives
2916.93	97.5	Methylene C-H stretch	Alkyl group, alkanes	Presence of aliphatic hydrocarbons likely from fatty acids or long chain organic molecules
3222.46	96.75	N-H (or) O-H stretching	Hydroxyl group	Suggest Presence of Primary or Secondary amines or hydrogen bonded hydroxyl group in alcohol or phenols
3276.09	96.55	N-H (or) O-H stretching	Hydroxyl group	Suggest Presence of Primary or Secondary amines or hydrogen bonded hydroxyl group in alcohol or phenols
3543.98	98.6	O-H stretching	Hydroxyl group	Presence of phenols (or) water molecules involved in H-bonding
3741.85	98.8	O-H stretching	Free Hydroxyl group	Indicates presence of free hydroxyl groups, possibly from alcohols (or) phenols not involved in H-bonding.

METHODOLOGY

Fruit material preparation

750g blueberries were purchased from the hypermarket along with my research supervisor and the selection criteria for the samples will include uniformity in size, ripeness and absence of visible damage or defects. Blueberries were washed with the distilled water to remove the dirt and contaminants, then dried with paper towels at room temperature. This is to ensure that the extraction which is carried out later will produce impurity-free extract and thus more accurate results will be obtained as shown in Figures 7,8.

Figure 7: Washing blueberries with distilled water.

Figure 8: Crushing blueberries with mortar and pestle.

Requirements

Extraction process and phytochemical screening

Chemicals

1. Ethanol, 95% (John Kollin Corporation, USA)
2. Distilled water freshly prepared in the laboratory
3. Sulphuric acid (95-99%) (EMD M.C. Germany)
4. Hydrochloric acid (37%) (Friendemann Schmidt Chemical)
5. Sodium hydroxide (Friendemann Schmidt Chemical)
6. Chloroform (EMD M.C Germany)
7. Ammonia (HmBG Chemicals)
8. 10% Lead acetate solution
9. Ferric chloride (EMD M.C. Germany)
10. Fehling's A and Fehling's B reagent (R&M Chemicals, UK)
11. Dragendorff's reagent (R&M Chemicals, UK)
12. Molisch's reagent (R&M Chemicals, UK)

Equipment

1. Digital analytical weighing balance
2. Rotary evaporator
3. General purpose water bath
4. Water bath sonicator
5. Vortex mixer
6. Hot plate

Glassware and Apparatus

1. Blueberries

Antioxidant activities determination

Chemicals

1. Ethanol, 95% (John Kollin Corporation)
2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent (Sisco Research Laboratories Pvt. Ltd India)
3. Ascorbic acid powder (HmbG Chemicals C1416)
4. Distilled water freshly prepared in the laboratory

Equipment

1. Digital analytical weighing balance
2. Vortex mixer
3. UV-Visible spectrophotometer

Glassware and Apparatus Materials

1. Blueberry extract

Antibacterial activities determination

Chemicals

1. Nutrient agar (HiMedia M001- 500G)
2. Nutrient broth (HiMedia M002-500G)
3. Mueller Hinton broth (HiMedia M391-500G)
4. Mueller Hinton agar (HiMedia M173-500G)
5. Distilled water freshly prepared in the laboratory
6. 70% ethanol

Equipment

1. Digital analytical weighing balance
2. Autoclave machine
3. Incubator
4. Hot air oven
5. Laminar airflow cabinet

Glassware and Apparatus

Bacterial strains

1. *Bacillus subtilis* (ATCC 6633)
2. *Escherichia coli* (ATCC 8739)

Fourier Transform Infrared (FTIR) spectroscopy analysis

Chemicals

1. 70% ethanol
2. Isopropyl alcohol (IPA)

Equipment

1. Fourier Transform Infrared Spectroscopy
2. Computer with FTIR software

Glassware and Apparatus Materials

1. Blueberry extract

Extraction of blueberry juices

Maceration

The extraction of blueberry juices were carried out through the maceration process with the whole blueberry fruit. A 2L round bottom flask was prepared and washed by acetone. 1.5L of 95% ethanol (Liu et al., 2021) was prepared and poured carefully into the round bottom flask. The crushed fruits of blueberries were inserted into the 2L round bottom flask to immerse themselves in the 95% ethanol. The mixture was shaken one time per day to ensure the mixture mixed thoroughly during the process. The shaking process was carried out for around fifteen minutes. The duration needed for maceration process was seven days. After seven days, the solution was poured out from the 2L round bottom flask and the residues were removed from the flask. The residues was put on the surface of clean muslin cloth and the solution remaining the residues were squished out by compressing the muslin cloth. The solution was collected in IL beaker.

Filtration was then carried out to remove any solid residue and impurities by setting up the filter funnel and tripod stand.

The filtrate was then covered by aluminum foil and few holes made on the surface of it. The beakers were kept in dark place with normal room temperature.(Abubakar & Haque, 2020) as shown in Figure 9.

Evaporation

After the extract obtained from the extraction process, the extract was evaporated by using rotary evaporator to remove solvent. The temperature of rotatory evaporator was set to 60°C, below the boiling point of 95% ethanol to avoid overheating. When there were no further changes observed, the extract was removed and transferred into China dish.

The China dish was heated by placing on water bath at 68°C for further evaporating. After few days, the extract became concentrated and removed from the water

bath.(Krakowska-Sieprawska et al., 2022) The China dish was covered with the aluminum foil with few holes on the surface. The extract was kept in the refrigerator to protect the extract from contamination.(Abubakar & Haque, 2020; Gazola et al., 2022)

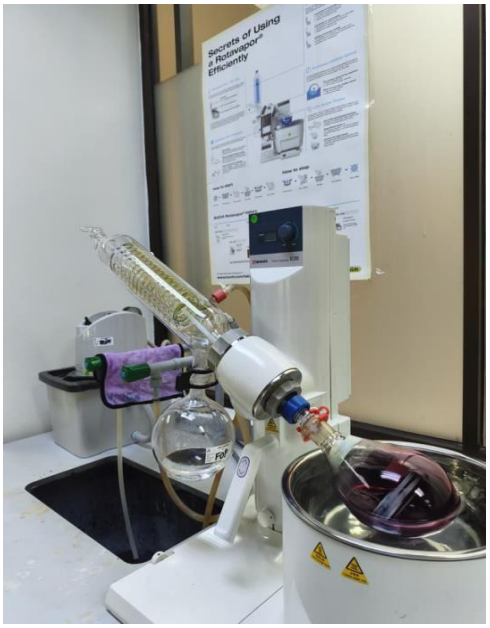


Figure 9: Evaporation of solvent using rotatory evaporator.

Phytochemical screening

The following phytochemical test were carried out after the extraction according to (Dubale et al., 2023; Evans & Trease, 1989; Shaikh & Patil, 2020):

- **Test for alkaloids**

Dragendoff's test: 1.0 ml of the blueberry extract was warmed with 2.0 ml of 2.0% sulphuric acid for 2 minutes. Few drops of Dragendoff's reagent was added. The orange red precipitate observed shows the presence of alkaloids.

- **Test for reducing sugars**

Benedict's test: 1.0 ml of blueberry extract was added with few drops of Benedict's reagent. The solution was boiled for 2 minutes. Presence of green color indicate the reducing sugars.

- **Test for saponins**

Foam test: 1 ml of the blueberry extract was shaken together with 5ml of sodium bicarbonate solution vigorously. Persistent froth or foam indicate the presence of saponins.

- **Test for terpenoids**

Salkowski's test: 1.0 ml of blueberry extract was mixed with 2.0 ml chloroform ($CHCl_3$) and 3.0 ml concentrated sulfuric acid (H_2SO_4) was carefully added. A layer formed and reddish brown coloration present at the interface indicate the presence of terpenoids.

- **Test for anthraquinones**

Borntrager's test: 1.0 ml of blueberry extract was boiled with 2.0 ml of 10.0% hydrochloric acid (HCl) solution for few minutes in a water bath. The test tube allowed it to cool. Equal volume of chloroform ($CHCl_3$) was poured into the test tube. A few drops of 10.0% ammonia, were added to mixture and heated. A rose pink color observed showed the presence of anthraquinones.

- **Test for glycosides**

10% NaOH test: 1.0 ml of blueberry extract was hydrolyzed by 1.0 ml of 10.0% hydrochloric acid (HCl) solution and neutralized with 1.0 ml of 0.2 M sodium hydroxide (NaOH) solution. After that, few drops in Fehling's solution A and B was added. The present of red precipitate shows the glycosides.

- **Test for phenolic compounds and tannins**

Iodine test: 1.0 ml of blueberry extract was added few drops of dilute iodine solution. A transient red solution shows the presence of phenolic compounds and tannins.

- **Test for flavonoids**

Alkaline reagent test: 1.0 ml of blueberry extract was dissolved in 1.0 ml of diluted 0.2 M sodium hydroxide (NaOH) and 1.0 ml of 10% hydrochloride acid (HCl) solution were added. Yellow solution observed indicated the presence of flavonoids.

- **Test for protein and amino acids**

Millon's test: 2ml of blueberry extract was added with few drops of Millon's reagent. Formation of a white precipitate indicates the presence of proteins.

- **Test for carbohydrates**

Molisch's test: 0.2 ml of Molisch's reagent was added to 1.0 ml of blueberry extract in a small test tube. After mixing, the test tube was titled. 0.5 ml of concentrated sulfuric acid (H_2SO_2) was carefully added without stirring by pouring down the side of the test tube. A red violet ring observed at the interface between the bottom and upper laver showed the presence of carbohydrates.

- **Test for anthocyanins**

HCl test: 2ml of blueberry extract was mixed with 2ml 2N HCl. Pink red solution which turned blue violet after addition of few ml of ammonia indicate the presence of anthocyanins.

Antioxidant activity determination

DPPH Radical Scavenging Assay

Antioxidant activity determination was carried out using DPPH radical scavenging assay which was introduced by Marsden Blois in 1958. This method is known as the most simple, rapid and inexpensive method to determine antioxidant capacity as no sophisticated equipment is required. (Gulcin & Alwasel, 2023a; Kedare & Singh, 2011; Munteanu & Apetrei, 2021)

Preparation of Sample

1. 1g of extract was dissolved in 100ml 95% ethanol to prepare 10mg/ml stock solution.
2. Six different concentrations of extracts were prepared at 100, 200, 400, 600, 800 and 1000 µg/ml using serial dilution method.
3. From the stock solution, 1, 0.8, 0.6, 0.4, 0.2, 0.1 ml were pipetted into 6 different 10ml volumetric flasks labelled accordingly. The volume was then made up to 10 ml using 95% ethanol.
4. To prepare 1000 µg/ml concentration, 1 ml was taken directly from the stock solution and diluted with 9 ml of ethanol.
5. To prepare 800 µg/ml concentration, 0.8 ml was taken from the stock solution and diluted with 9.2 ml of 95% ethanol.
6. To prepare 600 µg/ml concentration, 0.6 ml was taken from the stock solution and diluted with 9.4 ml of 95% ethanol.
7. To prepare 400 µg/ml concentration, 0.4 ml was taken from the stock solution and diluted with 9.6 ml of 95% ethanol.
8. To prepare 200 µg/ml concentration, 0.2 ml was taken from the stock solution and diluted with 9.8 ml of 95% ethanol.
9. To prepare 100 µg/ml concentration, 0.1 ml was taken from the stock solution and diluted with 9.9 ml of 95% ethanol.
10. The final volume of each dilution was 10ml.

Preparation of Standard

The procedures of preparation of standard solutions was same as the preparation of sample. However, the extract was changed to the ascorbic acid. The stock solution should be made at 100 µg/ml by dissolving 10mg of ascorbic acid at 100ml ethanol. Six different concentrations of extracts were prepared at 1, 2, 4, 6, 8 and 10 µg/ml using serial dilution method. From the stock solution, 1, 0.8, 0.6, 0.4, 0.2, 0.1 ml were pipetted into 6 different 10ml volumetric flasks labelled accordingly. The volume was then made up to 10 ml using 95% ethanol.

DPPH assay procedure

1. 11.83mg DPPH was weighed and dissolved in 100ml 95% ethanol to produce 0.3mM DPPH solution.
2. 6 different test tubes were labelled for the six concentrations of blueberry extract.
3. 2.5ml of each concentration was added into respective test tube.
4. 1ml of 0.3mM DPPH solution was added into each test tube.
5. The test tubes were covered with aluminum foil and incubated in dark cupboard for 30 minutes.
6. Same procedure was carried out for ascorbic acid concentrations.
7. Control was prepared by mixing 2ml 95% ethanol and 1ml of 0.3mM DPPH solution.
8. Blank was prepared by using 3ml 95% ethanol.
9. Approximate amount of each mixture was then transferred into a cuvette, and the absorbance was measured at 517 nm using a spectrophotometer.
10. The absorbance for both blueberry extract and ascorbic acid solutions were tabulated, and calibration curves were constructed.

The antioxidant activity of the ascorbic acid and blueberry extract was determined using this formula.(Nesrine Benkhaira, 2021)

% DPPH radical scavenging activity = (A0 - A1) / A0 x 100

Where A0 = absorbance of the blank solution and A1 = absorbance of the standard/sample

Graph of percentage of % scavenging activity against concentration (µg/ml) were plotted for both ascorbic acid and blueberry extract to determine IC_{50}. A lower IC_{50} value means stronger antioxidant activity.

Antibacterial activities determination

The broth microdilution method was applied to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the blueberry extracts. The sterile distilled water was inoculated with the bacteria culture until the turbidity was standardized to the 0.5 McFarland standards. The concentration that inhibits visible bacterial growth is assessed as MIC. The lowest concentration at which 99.9% of the inoculum is killed is assessed as MBC. The experiment is carried out in triplicate for all the sample (Balouiri et al., 2016). This is laid down in Figures 10,11.

Figure 10: Subculture mother plate of *Bacillus subtilis.*

Figure 11: Subculture mother plate of *Escherichia coli.*

Minimum Inhibitory Concentration (MIC)

1. 0.5 g of the dried blueberry extract was dissolved in 5 ml of distilled water to obtain a concentration of 100 mg/ml.
2. Six different concentrations of extract were prepared at 100, 80, 60, 40, 20 and 10 mg/ml using serial dilution.
3. To obtain 100 mg/ml concentration, 1 ml of the extract solution was transferred into a clean autoclaved test tube containing 1 ml of MHB and added with 0.1 ml of the standard McFarland culture (*Bacillus subtilis*).
4. To obtain the 80 mg/ml concentration, 0.8 ml of the extract solution was transferred into a clean autoclaved test tube containing 1 ml of MHB and diluted with 0.2 ml of distilled water. 0.1 ml of the standard McFarland culture (*Bacillus subtilis*) was then added.
5. To obtain 60 mg/ml concentration, 0.6 ml of the extract solution was transferred into a clean autoclaved test tube containing 1 ml of MHB and diluted with 0.4 ml of distilled water. 0.1 ml of the standard McFarland culture (*Bacillus subtilis*) was then added.
6. To obtain the 40 mg/ml concentration, 0.4 ml of the extract solution was transferred into a clean autoclaved test tube containing 1 ml of MHB and diluted with 0.6 ml of distilled water. 0.1 ml of the standard McFarland culture (*Bacillus subtilis*) was then added.
7. To obtain the 20 mg/ml concentration, 0.2 ml of the extract solution was transferred into a clean autoclaved test tube containing 1 ml of MHB and diluted with 0.8 ml of distilled water. 0.1 ml of the standard McFarland culture (*Bacillus subtilis*) was then added.
8. To obtain the 10 mg/ml concentration, 0.1 ml of the extract solution was transferred into a clean autoclaved test tube containing 1 ml of MHB and diluted with 0.9 ml of distilled water. 0.1 ml of the standard McFarland culture (*Bacillus subtilis*) was then added.
9. The entire process was repeated for *Escherichia coli*, however higher concentration stock solution with 1g/ml was made by dissolving 5g of dried blueberry extract into 5ml of distilled water. Six different concentrations of extract were prepared at 1000, 800, 600, 400, 200 and 100 mg/ml using the serial dilution.
10. Positive tube was prepared by adding 1ml of McFarland culture and 0.1ml of Ciprofloxacin into 1ml MHB.
11. Negative tube was prepared by adding 1ml of McFarland culture into 1ml of MHB.
12. The tubes were then incubated at 37°C for 24 hours.
13. The MIC activity was evaluated by observing the turbidity of the tubes after incubation.
14. The tube that does not show any visible turbidity indicates the MIC.

Minimum Bactericidal Concentration (MBC)

1. The tubes which did not show any visible turbidity from the MIC test were taken and proceeded with the MBC determination.
2. One loop was taken from the tube, and streaking was performed onto MHA plate.
3. The plates were then incubated at 37°C for 24 hours.
4. The MBC activity was evaluated by observing the presence of bacterial growth on the agar after incubation. The plate that does not have presence of bacterial growth indicates the MBC.

Fourier Transform Infrared (FTIR) spectroscopy analysis

Fourier Transform Infrared (FTIR) spectroscopy was conducted to identify the functional groups present in the blueberry extract and provide qualitative evidence of its phytochemical compounds. Blueberry extract was analyzed by Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR). To ensure measurement accuracy, the crystal surface was thoroughly cleaned with isopropyl alcohol (IPA), followed by 70% ethanol using lint-free wipes before starting sample analysis. Then one drop of the extract was directly deposited onto the ATR crystal using a spatula.

Spectral data was acquired across the range of 4000-400 cm^{-1} with a spectral resolution of 4 cm^{-1}. The recorded spectra was analyzed by comparing the peaks to reference spectra to identify specific phytochemicals. (Schuttlefield & Grassian, 2008) as shown in Figure 12.

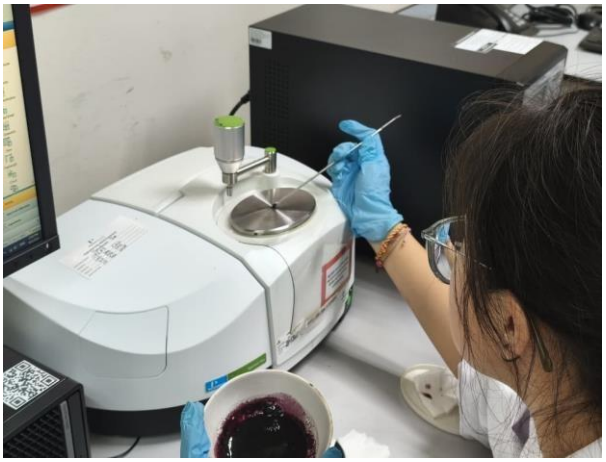


Figure 12: Placing a drop of extract on ATR crystal.

RESULTS

Phytochemical screening

Table 5: Results of phytochemical present in fresh blueberry extract prepared by maceration.

S. No	Test	Name of test	Observation	Result
1.	Test for alkaloids	Dragendoff's test	Absence of orange red precipitate	-
2.	Test for reducing sugars	Benedict's test	Presence of green color	+
3.	Test for saponins	Foam test	Absence of persistent froth or foam	-
4.	Test for terpenoids	Salkowski's test	Presence of reddish- brown coloration at the interface	+
5.	Test for anthraquinones	Borntrager's test	Presence of rose pink color	+
6.	Test for glycosides	10% NaOH test	Presence of red precipitate	+
7.	Test for phenolic compounds and tannins	Iodine test	Presence of transient red solution	+
8.	Test for flavonoids	Alkaline reagent test	Presence of yellow solution	+
9.	Test for protein and amino acids	Millon's test	Absence of white precipitate	-
10.	Test for carbohydrates	Molisch's test	Presence of red violet ring observed at the interface between the bottom and upper layer	+
11.	Test for anthocyanins	HCl test	Pink, red solution which turned blue-violet after addition of ammonia	+

+ : Present - : Absent

Results of phytochemical present in fresh blueberry extract prepared by maceration are shown in Table 5.

Antioxidant activity determination DPPH radical scavenging assay

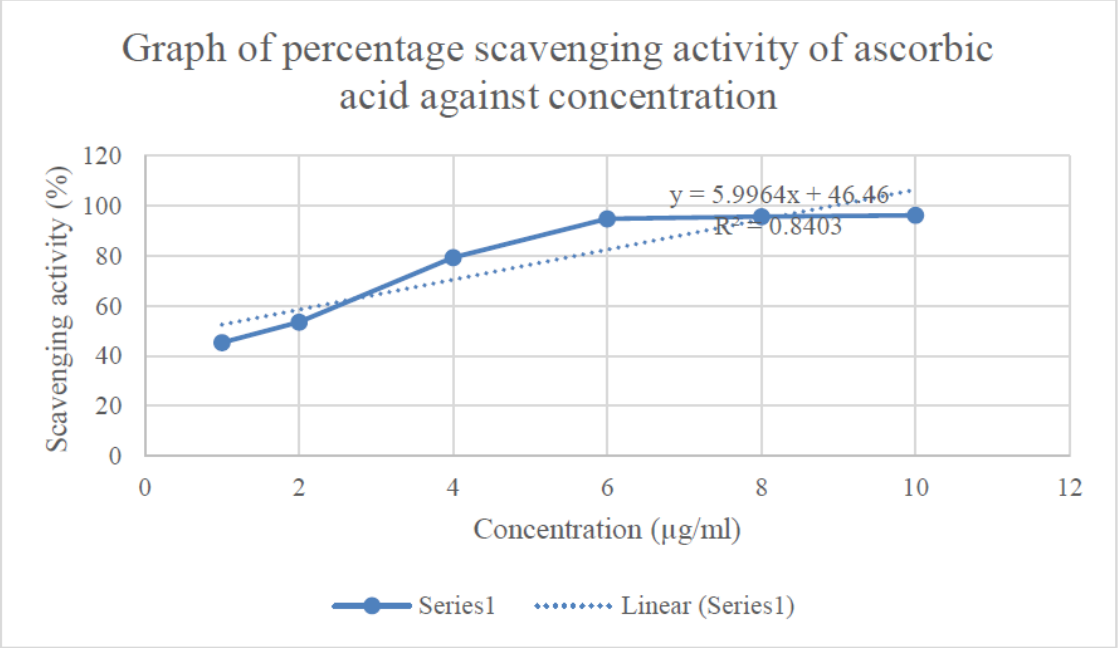
The following results show the absorbance and corresponding percentage scavenging activity of ascorbic acid standard solution and ethanolic maceration extract of fresh blueberries. The results are shown in Tables 6,7 and in Graphs 1 and 2.

Table 6: Absorbance and corresponding scavenging activity (%) of ascorbic acid.

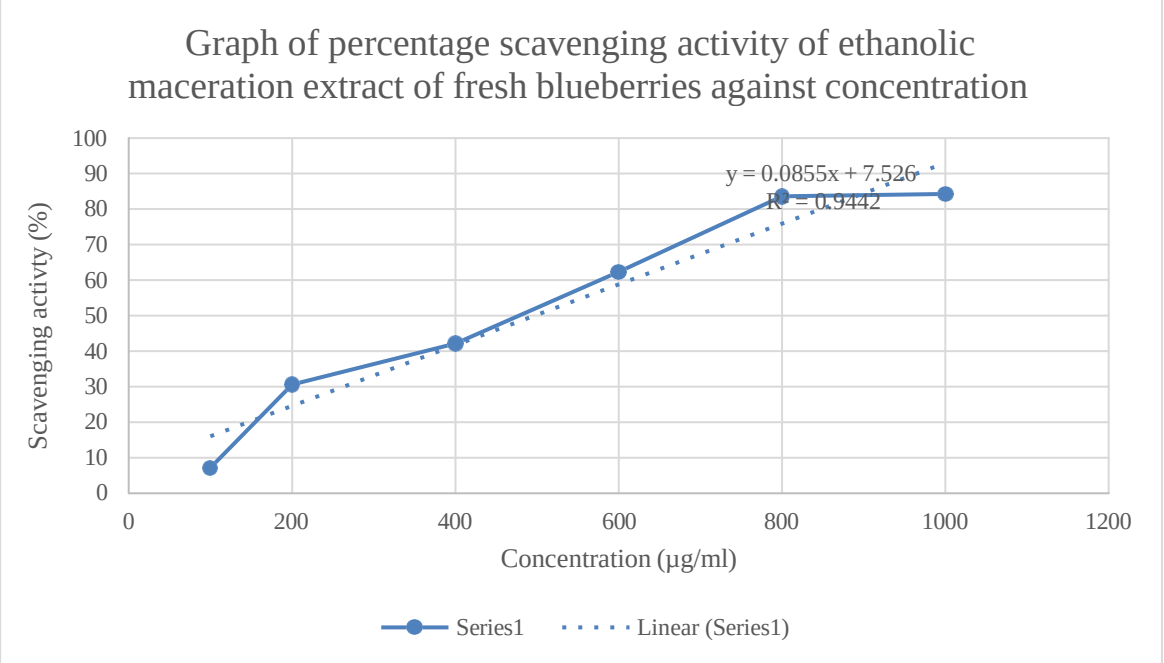
Concentration (µg/ml) of ascorbic acid standard solution	UV Absorbance	Scavenging activity (%) (A0 - A1) / A0 x 100
Control (A0)	0.526	-
1	0.288	45.25
2	0.245	53.42
4	0.109	79.28
6	0.027	94.87
8	0.023	95.63
10	0.020	96.20

Table 7: Absorbance and corresponding scavenging activity (%) of fresh blueberries.

Concentration (µg/ml) of ethanolic maceration extract of fresh blueberries	UV Absorbance	Scavenging activity (%) (A0 - A1) / A0 x 100
Control	0.624	
100	0.579	7.21
200	0.433	30.61
400	0.361	42.15
600	0.235	62.34
800	0.102	83.65
1000	0.098	84.29



Graph 1: Graph of percentage scavenging activity of ascorbic acid against concentration.



Graph 2: Graph of percentage scavenging activity of ethanolic maceration extract of fresh blueberries against concentration.

Calculation of concentration needed to reduce absorbance by 50% (IC50)

Ascorbic acid IC_{50} = 0.59 µg/ml Y = mx + c
50 = 5.9964x + 46.46
5.9964x = 50-46.46
5.9964x =3.54 x= 3.54/5.9964
x=0.59 µg/ml

Blueberry extract IC_{50} = 496.77 µg/ml

Y = mx + c
50 = 0.0855x + 7.526
0.0855x = 50-7.526
0.0855x =42.474 x= 42.474/0.0855
x=496.77 µg/ml

Antibacterial activity determination Minimum inhibitory concentration (MIC)

MIC of *Bacillus subtilis* was observed to be 80 mg/ml due to less turbid compared to the previous test tubes.

MIC of *Escherichia coli* was observed to be 200 mg/ml due to less turbid compared to the previous test tubes.

Minimum bactericidal concentration (MBC)

After MIC incubation, tubes with extract concentration of 60mg/ml, 80mg/ml and 100mg/ml concentration for *Bacillus subtilis* were further tested for its minimum bactericidal concentration (MBC). MBC of *Bacillus subtilis* approximately happened at 100mg/ml.

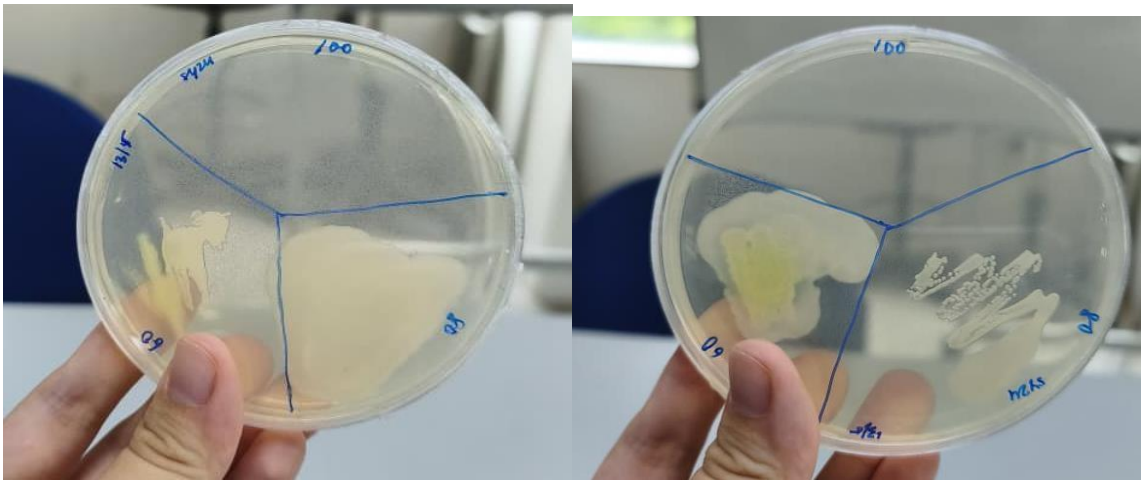


Figure 14: Result of MBC test for *Bacillus subtilis.*

After MIC incubation, tubes with extract concentration of 200mg/ml, 400mg/ml, 600mg/ml, 800mg/ml and 1000mg/ml concentration for *Escherichia coli* were further tested for its minimum bactericidal concentration (MBC). MBC of *Escherichia coli* approximately happened at 400mg/ml.

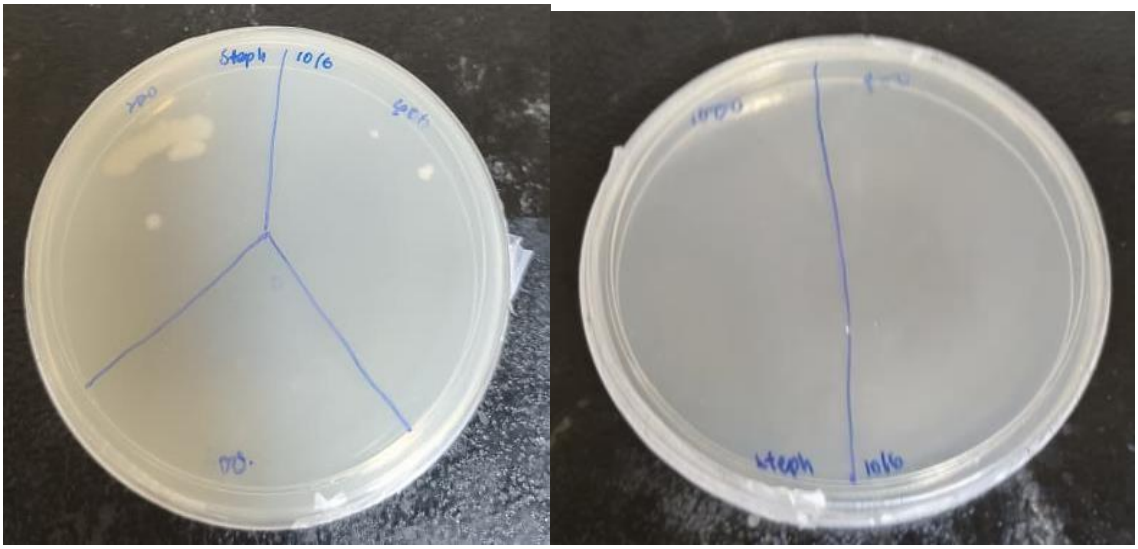


Figure 15: Result of MBC test for *Escherichia coli.*

Fourier Transform Infrared (FTIR) spectroscopy analysis

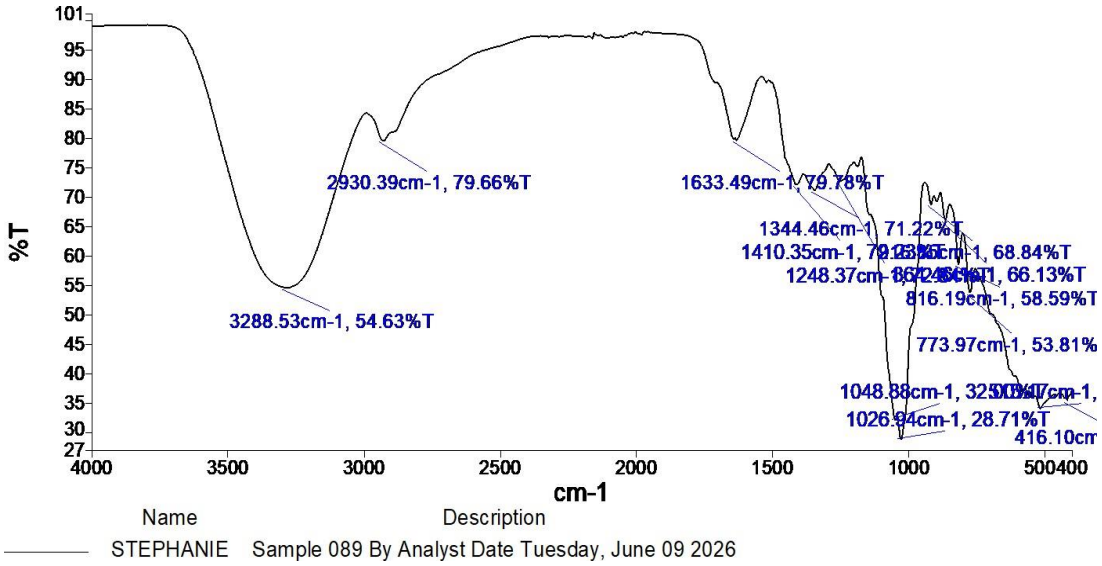


Figure 16: FTIR spectrum of fresh blueberries ethanolic extract.

Table 8: FTIR spectral peak values and functional groups of fresh blueberries extract.

IR spectra (cm^{-1})	Possible Functional Group	Compound Class	Phytochemicals
3288.53	O-H stretching	Hydroxyl group	Presence of phenols, flavonoids, anthocyanins or water molecules involved in H-bonding
2930.39	Methylene C-H stretch	Alkyl group, alkanes	Presence of aliphatic hydrocarbons likely from carbohydrates, fatty acids or long chain organic molecules
1633.49	C=O stretching	Carbonyl group	Organic acids (citric acid, malic acid), esters, flavonoids, anthocyanins, pectin
1410.35	C-C stretching	Aromatic or alkene	Aromatic compounds, or unsaturated hydrocarbons, likely phenolic or flavonoids derivatives
1344.46	O-H bending	Phenolic groups	Indicates presence of phenolic groups
1248.37	C-O stretching	Ether, esters or carboxylic acids	Suggests carbohydrate, glycosides or ester compounds
1048.88	C-O stretching	Ether, esters or carboxylic acids	Suggests carbohydrate, glycosides or ester compounds
1026.94	C-O stretching	Ether, esters or carboxylic acids	Suggests carbohydrate, glycosides or ester compounds
816.19	C-H bending (aromatic)	Alkenes	Presence of aromatic compounds like flavonoids or phenols
773.97	C-H bending (aromatic)	Alkenes	Aromatic compounds, confirms aromatic ring system

DISCUSSION

The objective of this research is to determine the phytochemical constituents, antioxidant potential, antibacterial activity potential and determine the functional group using Fourier Transform Infrared (FTIR) spectroscopy.

Qualitative phytochemical screening, quantitative DPPH antioxidative assay, MIC and MBC using broth dilution method and FTIR analysis were the four main approaches employed in this study. Maceration method was used for the extraction process by using 95% ethanol as solvent.

In this qualitative phytochemical screening, fresh blueberry extract was tested to have the phytochemicals of reducing sugars, terpenoids, anthraquinones, glycosides, phenolic compounds and tannins, flavonoids, carbohydrates and anthocyanins. While in test for alkaloids, saponins, protein and amino acids, blueberry extract show the negative results.

The Dragendorff's test showed the absence of orange red precipitate; thus, alkaloids were not detected in the blueberry extract. Alkaloids are nitrogen containing compounds which normally biosynthesized by certain botanical families of plant instead of blueberry(Dey et

al., 2020). Alkaloids normally is derived from amino acid such as tryptophan and tyrosine yet in phytochemical screening amino acid was not detected in blueberry extract. Thus, potassium iodide and bismuth nitrate in Dragendorff's reagent are unable to react with nitrogen due to absence of nitrogen containing alkaloid compound in the blueberry.

Benedict's test was carried out to test for reducing sugars. The result was positive due to glucose and fructose that found in blueberries(Padmanabhan et al., 2016). Thus, Molisch's test to test for the presence of carbohydrates also show a positive result. Foam test to determine the saponin content was tested to be negative due to absence of constituent which able to reduce the surface tension of water and form the stable foam during agitation(Chen et al., 2010).

Salkowski's test to verify the presence of terpenoids was found to be positive due to it is an important constituent found in blueberries to provide fruity and floral aroma of the blueberries(M. Zhang et al., 2025).

In alkaline reagent test, blueberries extract was tested positive for the flavonoid constituents (Godlewska et al., 2023).

Blueberries are one of the richest sources of flavonoids mainly their high anthocyanin glycosides content thus in test for glycosides and anthocyanin, blueberries show positive result. Besides that, iodine test was conducted to detect the presence of phenolic compounds and tannins.

Blueberries extract show the positive result in iodine test with the formation of transient red solution. These compounds play an important role in showing the antioxidant properties of blueberries.

Next, the antioxidant potential of fresh blueberries extract was assessed using DPPH radical scavenging assay and compared with the standard ascorbic acid. The ethanolic extract of fresh blueberries exhibited a concentration-dependent increase in antioxidant activity.

When the extract concentration increase from 100 to 1000 µg/ml, the absorbance gradually decreased from 0.579 to 0.098, while the DPPH scavenging activity increased from 7.21% to 84.29%. DPPH is a stable free radical which gives a strong deep color and absorb light at the wavelength of 517nm.

When the antioxidant was added into DPPH, it will donate the electron to DPPH free radical. DPPH free radical is reduced and cause the solution turning pale yellow color, this will result less light is being absorbed at 517nm(Gulcin & Alwasel, 2023b). Thus, absorbance reading will be reduced, and this indicate higher antioxidant properties.

Initially concentration of ascorbic acid standard solution from 100 to 1000 µg/ml was used to facilitate the comparison of antioxidant potential between ethanolic extract of blueberry and standard solution. However, UV spectrophotometer in our lab cannot detect absorbance which is too low. Thus, concentration of 1,2,4,8 and 10 µg/ml were used instead, the absorbance decreased from 0.288 to 0.020 while percentage of scavenging activity increased from 45.25% to 96.20%.

Percentage of scavenging activity was calculated using the formula of (A0 - A1) / A0 x 100 where A0 represent absorbance of control and A1 represent absorbance of sample.

Thus, concentration that needed to reduce 50% of free radical (IC_{50}) was used as a parameter to compare the antioxidant properties of ethanolic extract of blueberry.

IC_{50} was calculated by plotting absorbance value of blueberry extract and ascorbic acid on the graph respectively and best fit line was drawn to determine the linear equation. IC_{50} of ascorbic acid standard solution is 0.59 µg/ml derived from $y = 5.9964x + 46.46$ while for blueberry extract is 496.77 µg/ml derived from $y = 0.0855x + 7.526$. The higher the amount of IC_{50}, the weaker the antioxidant properties. Thus, the blueberry extract exhibited a lower antioxidant activity compared to ascorbic acid. The difference is expected due to ascorbic acid is a purified antioxidant compound.

However, according to (Gonçalves et al., 2023), blueberry extract only 5 times less effective than ascorbic acid, but my research showing 840 times less effective compared to ascorbic acid. This may be due to the incomplete ethanolic extraction of anthocyanins from fresh blueberries. The antioxidant properties of blueberry was further proving the phytochemical screening is correct where the presence of anthocyanins, flavonoids and phenolic compound is positive.

Next, antibacterial activity of blueberry was determined using the broth dilution method to determine MIC and MBC of each bacteria strain which is gram positive bacteria, *Bacillus subtilis* and gram -negative bacteria, *Escherichia coli.* For *Bacillus subtilis,* concentration of 10, 20, 40, 60, 80 and 100 mg/ml were used. MIC was determined after 24 hours of incubation. MIC of *Bacillus subtilis* was observed to be 80 mg/ml due to the test tube appearing to be less turbid compared to the test tube that contain smaller concentration of blueberry extract. Thus, 60, 80 and 100mg/ml containing test tubes were chosen and proceeded with the MBC by streaking on the MHA plate. After incubation of 24 hours, section that streaked with 100 mg/ml showing a clear region which indicate no *Bacillus subtilis* growth compared to other concentration. Thus, MBC of *Bacillus subtilis* approximately happened at 100mg/ml.

Initially, same concentration of blueberry extract that was tested in *Bacillus subtilis* was used to determine MBC of *Escherichia coli*. However, it found that even the highest concentration of blueberry extract cannot stop and kill the bacteria growth due to *Escherichia coli* is a gram negative bacteria which has a stronger bacterial wall harder for the extract to penetrate which consists of lipopolysaccharides(Breijyeh et al., 2020). Thus for *Escherichia coli*, concentration of 100, 200, 400, 600, 800 and 1000 mg/ml were used. MIC was determined after 24 hours of incubation.

MIC of *Escherichia coli* was observed to be 200 mg/ml due to the test tube appearing to be less turbid compared to the test tube that contain smaller concentration of blueberry extract. Thus, 200, 400, 600, 800 and 1000mg/ml containing test tubes were chosen and proceeded with the MBC by streaking on the MHA plate.

After incubation of 24 hours, section that streaked with 600, 800 and 1000 mg/ml showing a clear region which indicate no *Escherichia coli* growth compared to other concentration. Thus, MBC of *Escherichia coli* approximately happened at 600 mg/ml.

FTIR spectrum of blueberry extract were shown. From each peak form in the FTIR spectrum we can actually identify the constituents contain in the blueberry extract by determining their functional group. Due to lack of FTIR spectrum of pure standard of anthocyanins, direct comparison between the FTIR spectrum of blueberry extract and anthocyanin cannot be made. However, we can determine the functional group from each peak using the wavelength range of each functional group. IR spectra of 3288.53 cm^{-1} indicate O-H stretching which may represent the presence of phenols, flavonoids, anthocyanins or water molecules involved in H-bonding.

IR spectra of 2930.39cm^{-1} indicate Methylene C-H stretching which may represent the presence of aliphatic hydrocarbons likely from carbohydrates, fatty acids or long chain organic molecules. IR spectra of 1633.49cm^{-1} indicate C=O stretching which may represent the presence of organic acids (citric acid, malic acid), esters, flavonoids, anthocyanins or pectin. IR spectra of 1410.35^{-1} indicate C-C stretching which may represent the presence of aromatic compounds, or unsaturated hydrocarbons, likely phenolic or flavonoids derivatives.

IR spectra of 1344.46cm^{-1} indicate O-H bending which may represent the presence of phenolic groups. IR spectra of 1248.37cm^{-1}, 1048.88cm^{-1} and 1026.94cm^{-1} indicate C-O stretching which may represent the presence of carbohydrate, glycosides or ester compounds. IR spectra of 816.19cm^{-1} indicate C-H bending which may represent the presence of aromatic compounds like flavonoids or phenols. IR spectra of 773.97cm^{-1} indicate C-H bending which confirm the presence of aromatic ring system.

These further verified the result of phytochemical screening and antioxidant activity where functional group of phenolic compound, flavonoids, carbohydrate, aromatic ring and anthocyanins were identified in FTIR spectrum.

CONCLUSION

In conclusion, hypothesis is accepted. Freshly extracted blueberries contain significant amount of phytochemical which can identified through the phytochemical screening. Blueberries are expected to exhibit antioxidant and antibacterial activity by carrying out the antioxidant and antibacterial assay. Structure and functional group of the blueberries extract can be elucidated by FTIR.

Objectives of this research were achieved. Blueberry extract was prepared by using maceration process with 95% ethanol for 7 days. Besides that, phytochemical screening to determine phytochemicals in the blueberry extract was performed. Blueberry extract was proved to have reduced sugars, terpenoids, anthraquinones, glycosides, phenolic compounds and tannins, flavonoids, carbohydrates and anthocyanins. These phytochemicals played an important role in showing the antioxidant properties and antibacterial properties. Besides that, these phytochemicals also provide the fruit aroma to the blueberry. Next, antioxidant assay was performed to determine the antioxidant ability of blueberry compared to ascorbic acid by using DPPH radical scavenging assay.

It proved that blueberry extract exhibited concentration dependent increase in antioxidant activity which having 84.29% of scavenging activity at 1000 µg/ml which less effective than pure ascorbic acid.

Besides, antibacterial assay was performed to determine antibacterial activity of blueberry extract using broth dilution method. Blueberry extract shows inhibitory effect at 80 mg/ml and bactericidal effect at 100 mg/ml for gram positive bacteria. While for gram negative bacteria, blueberry extract showing inhibitory effect at 200 mg/ml and bactericidal effect at 600 mg/ml.

Presence of hydroxyl groups within polyphenols, phenolic and alcohol compounds interact with the bacterial cell membrane and affect enterotoxin production and enzyme activities(Suriyaprom et al., 2022).

Functional group of blueberry extract was determined via FTIR. It further verify the result of phytochemical screening. Phenolic compound, flavonoids, carbohydrate, aromatic ring and anthocyanins were identified in FTIR spectrum.

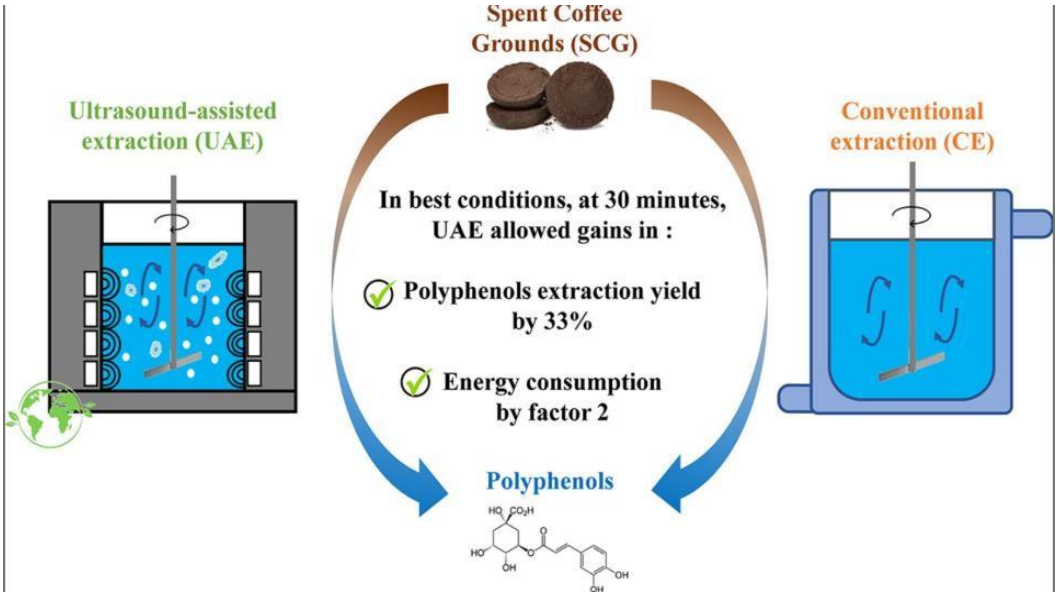


Figure 17: Comparison between ultrasound-assisted extraction and conventional extraction (Beaudor et al., 2023).

Secondly, the antioxidant activity of blueberry extract can be carried out via multiple assay for example ABTS and FRAP to provide a more comprehensive evaluation (Floegel et al., 2011). Besides that, procedure to determine total phenolic content can be carried out to identify the value of AAE/g of the blueberry extract when compared to ascorbic acid. For antibacterial activity, we can investigate a wide range of gram- negative bacteria and gram- positive bacteria to verify the result and increase the reliability of the research. Besides that, methods like well diffusion or disc diffusion method should be employed to identify the extent of the blueberry antibacterial effect. For FTIR analysis, due to the lack of the spectrum of the reference standard, we couldn't confirm the constituents in blueberry. However, we can apply HPLC coupled with MS to confirm the identity of anthocyanin(Z. Zhang et al., 2004).

REFERENCES

1. A. Satashia, S., & Pandya, N. K., Comparative Analysis of Antioxidant Capacities and Antimicrobial Activities in Selected Berry Fruits. *International Journal of Current Microbiology and Applied Sciences*, 2025; *14*(3): 10–19. https://doi.org/10.20546/ijcmas.2025.1403.003
2. Abubakar, A. R., & Haque, M., Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy & Bioallied Sciences*, 2020; *12*(1): 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
3. Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G., Antimicrobial resistance: Impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health*, 2024; *2*: 100081. https://doi.org/10.1016/j.glmedi.2024.100081
4. Alias, N., Wan-Fei, K., Mang, C. Y., Nasaruddin, N. H., & Omar, M. A. (n.d.). *AGE-ADJUSTED DEATH RATES FOR LEADING CAUSES OF DEATHS IN MALAYSIA, 2019*.
5. Al-Kelani, M., & Buthelezi, N., Advancements in medical research: Exploring Fourier Transform Infrared (FTIR) spectroscopy for tissue, cell, and hair sample analysis. *Skin Research and Technology*, 2024; *30*(6): e13733. https://doi.org/10.1111/srt.13733
6. American Physiological Society. (n.d.). *Blueberry proanthocyanidins and anthocyanins improve metabolic health through a gut microbiota-dependent mechanism in diet-induced obese mice | American Journal of Physiology-Endocrinology and Metabolism | American Physiological Society*. Retrieved January 1, 2026, from https://journals.physiology.org/doi/full/10.1152/ajpendo.00560.2019
7. Amin Salehi, M., Chehregani Rad, A., & Afshar, S., Anticancer and Antibacterial Effects of Blueberry Fruit (Vaccinium corymbosum L.) in Three Developmental Stages. *Iranian Journal of Medical Microbiology*, 2023; *17*(5): 613–619. https://doi.org/10.30699/ijmm.17.5.613
8. Ashique, S., Mukherjee, T., Mohanty, S., Garg, A., Mishra, N., Kaushik, M., Bhowmick, M., Chattaraj, B., Mohanto, S., Srivastava, S., & Taghizadeh-Hesary, F., Blueberries in focus: Exploring the phytochemical potentials and therapeutic applications. *Journal of Agriculture and Food Research*, 2024; *18*: 101300. https://doi.org/10.1016/j.jafr.2024.101300
9. Aziz, S. S., Qureshi, M. H., Baba, H., Begum, N., & Najmuddin, M., Phytochemical profiling and FTIR-based analysis of aqueous extracts of the Unani blueberry formulation. *Journal of Pharmacognosy and Phytochemistry*, 2025; *14*(2): 232–238. https://doi.org/10.22271/phyto.2025.v14.i2c.15294
10. Bader Ul Ain, H., Tufail, T., Javed, M., Tufail, T.,

Arshad, M. U., Hussain, M., Gull Khan, S., Bashir, S., Al Jbawi, E., & Abdulaali Saewan, S., Phytochemical profile and pro-healthy properties of berries. *International Journal of Food Properties*, 2022; *25*(1): 1714–1735. https://doi.org/10.1080/10942912.2022.2096062

11. Balouiri, M., Sadiki, M., & Ibnsouda, S. K., Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 2016; *6*(2): 71–79. https://doi.org/10.1016/j.jpha.2015.11.005
12. Beaudor, M., Vauchel, P., Pradal, D., Aljawish, A., & Phalip, V., Comparing the efficiency of extracting antioxidant polyphenols from spent coffee grounds using an innovative ultrasound-assisted extraction equipment versus conventional method. *Chemical Engineering and Processing - Process Intensification*, 2023; *188*: 109358. https://doi.org/10.1016/j.cep.2023.109358
13. Borozan, A.-B., Popescu, S., Dobrei, A., Moigradean, D., Poiana, M.-A., Dobrei, A., Popa, M.-V., Raba, D., & Bordean, M., Bioactive compound content and biological activity of blueberry leaves and fruits. A review. *JOURNAL of Horticulture*, 2025; *29*.
14. Breijyeh, Z., Jubeh, B., & Karaman, R., Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules*, 2020; *25*(6): 1340. https://doi.org/10.3390/molecules25061340
15. Britannica Editors. (2025, November 28). *Blueberry | Description, Types, Nutrition, Cultivation, & Facts | Britannica*. https://www.britannica.com/plant/blueberry-plant
16. Chen, Y.-F., Yang, C.-H., Chang, M.-S., Ciou, Y.-P., & Huang, Y.-C., Foam Properties and Detergent Abilities of the Saponins from Camellia oleifera. *International Journal of Molecular Sciences*, 2010; *11*(11): 4417–4425. https://doi.org/10.3390/ijms11114417
17. Dembińska, K., Shinde, A. H., Pejchalová, M., Richert, A., & Swiontek Brzezinska, M., The Application of Natural Phenolic Substances as Antimicrobial Agents in Agriculture and Food Industry. *Foods*, 2025; *14*(11): 1893. https://doi.org/10.3390/foods14111893
18. Dey, P., Kundu, A., Kumar, A., Gupta, M., Lee, B. M., Bhakta, T., Dash, S., & Kim, H. S., Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). *Recent Advances in Natural Products Analysis*, 2020; 505–567. https://doi.org/10.1016/B978-0-12-816455-6.00015-9
19. Donlan, R. M., Biofilms: Microbial Life on Surfaces. *Emerging Infectious Diseases*, 2002; *8*(9): 881–890. https://doi.org/10.3201/eid0809.020063
20. Dróżdż, P., Šėžienė, V., & Pyrzynska, K., Phytochemical Properties and Antioxidant Activities of Extracts from Wild Blueberries and Lingonberries. *Plant Foods for Human Nutrition*, 2017; *72*(4): 360–364. https://doi.org/10.1007/s11130-017-0640-3
21. Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S., Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of Experimental Pharmacology*, 2023; *15*: 51–62. https://doi.org/10.2147/JEP.S379805
22. Evans, W. C., & Trease, G. E., *Trease and Evans' pharmacognosy* (13. ed), 1989.
23. Fais, A., & Era, B., Phytochemical Composition and Biological Activity. *Plants*, 2024; *13*(3): 331. https://doi.org/10.3390/plants13030331
24. Farneti, B., Khomenko, I., Grisenti, M., Ajelli, M., Betta, E., Algarra, A. A., Cappellin, L., Aprea, E., Gasperi, F., Biasioli, F., & Giongo, L., Exploring Blueberry Aroma Complexity by Chromatographic and Direct-Injection Spectrometric Techniques. *Frontiers in Plant Science*, 2017; *8*. https://doi.org/10.3389/fpls.2017.00617
25. Felgus-Lavefve, L., Howard, L., Adams, S. H., & Baum, J. I., The Effects of Blueberry Phytochemicals on Cell Models of Inflammation and Oxidative Stress. *Advances in Nutrition*, 2021; *13*(4): 1279–1309. https://doi.org/10.1093/advances/nmab137
26. Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I., & Chun, O. K., Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 2011; *24*(7): 1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008
27. Fuentes, L., Figueroa, C. R., Valdenegro, M., & Vinet, R., Patagonian Berries: Healthy Potential and the Path to Becoming Functional Foods. *Foods*, 2019; *8*(8): 289. https://doi.org/10.3390/foods8080289
28. Gazola, M. B., Oliveira, C. T. D., Pesenti, M. C., Valdez, R. H., Pereira, E. A., & Teixeira, S. D., Phytochemical screening, total phenolics and antimicrobian action from the brazilian cherry, black mulberry and blueberry fruits' pulps. *Research, Society and Development*, 2022; *11*(8): e15911830495. https://doi.org/10.33448/rsd-v11i8.30495
29. Godlewska, K., Pacyga, P., Najda, A., & Michalak, I., Investigation of Chemical Constituents and Antioxidant Activity of Biologically Active Plant-Derived Natural Products. *Molecules*, 2023; *28*(14): 5572. https://doi.org/10.3390/molecules28145572
30. Golenia, A., & Olejnik, P., The Role of Oxidative Stress in Ischaemic Stroke and the Influence of Gut Microbiota. *Antioxidants*, 2025; *14*(5): 542. https://doi.org/10.3390/antiox14050542
31. Gonçalves, A. C., Nunes, A. R., Meirinho, S., Ayuso-Calles, M., Roca-Couso, R., Rivas, R., Falcão, A., Alves, G., Silva, L. R., & Flores-Félix, J. D., Exploring the Antioxidant, Antidiabetic, and Antimicrobial Capacity of Phenolics from Blueberries and Sweet Cherries. *Applied Sciences*,

2023; *13*(10): 6348. https://doi.org/10.3390/app13106348
32. Gulcin, İ., & Alwasel, S. H., DPPH Radical Scavenging Assay. *Processes*, 2023a; *11*(8): 2248. https://doi.org/10.3390/pr11082248
33. Gulcin, İ., & Alwasel, S. H., DPPH Radical Scavenging Assay. *Processes*, 2023b; *11*(8): 2248. https://doi.org/10.3390/pr11082248
34. Huang, W., Zhang, H., Liu, W., & Li, C., Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *Journal of Zhejiang University. Science. B*, 2012; *13*(2): 94–102. https://doi.org/10.1631/jzus.B1100137
35. Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R., Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Advances in Nutrition*, 2020; *11*(2): 224–236. https://doi.org/10.1093/advances/nmz065
36. Kauffmann, A. C., & Castro, V. S., Phenolic Compounds in Bacterial Inactivation: A Perspective from Brazil. *Antibiotics*, 2023; *12*(4): 645. https://doi.org/10.3390/antibiotics12040645
37. Kedare, S. B., & Singh, R. P., Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 2011; *48*(4): 412–422. https://doi.org/10.1007/s13197-011-0251-1
38. Krakowska-Sieprawska, A., Kiełbasa, A., Rafińska, K., Ligor, M., & Buszewski, B., Modern Methods of Pre-Treatment of Plant Material for the Extraction of Bioactive Compounds. *Molecules*, 2022; *27*(3): 730. https://doi.org/10.3390/molecules27030730
39. Leyane, T. S., Jere, S. W., & Houreld, N. N., Oxidative Stress in Ageing and Chronic Degenerative Pathologies: Molecular Mechanisms Involved in Counteracting Oxidative Stress and Chronic Inflammation. *International Journal of Molecular Sciences*, 2022; *23*(13): 7273. https://doi.org/10.3390/ijms23137273
40. Li, W., & Yang, S., Targeting oxidative stress for the treatment of ischemic stroke: Upstream and downstream therapeutic strategies. *Brain Circulation*, 2016; *2*(4): 153–163. https://doi.org/10.4103/2394-8108.195279
41. Liu, H., Wu, H., Wang, Y., Wang, F., Liu, X., & Zhou, J., Enhancement on antioxidant and antibacterial activities of Brightwell blueberry by extraction and purification. *Applied Biological Chemistry*, 2021; *64*(1): 78. https://doi.org/10.1186/s13765-021-00649-8
42. Looi, D., Moorthy, M., Chaiyakunapruk, N., & Devi Palanisamy, U., Impact of ellagitannin-rich fruit consumption on blood pressure: A systematic review and meta-analysis of randomized controlled trials. *Journal of Functional Foods*, 2022; *99*: 105320. https://doi.org/10.1016/j.jff.2022.105320
43. Mendoza, N., Silva, E. M. E., Mendoza, N., & Silva, E. M. E., Introduction to Phytochemicals: Secondary Metabolites from Plants with Active Principles for Pharmacological Importance. In *Phytochemicals—Source of Antioxidants and Role in Disease Prevention*. IntechOpen, 2018. https://doi.org/10.5772/intechopen.78226
44. Munteanu, I. G., & Apetrei, C., Analytical Methods Used in Determining Antioxidant Activity: A Review. *International Journal of Molecular Sciences*, 2021; *22*(7): 3380. https://doi.org/10.3390/ijms22073380
45. Nesrine Benkhaira., In vitro Methods to Study Antioxidant and Some Biological Activities of Essential Oils: A Review. *Biointerface Research in Applied Chemistry*, 2021; *12*(3): 3332–3347. https://doi.org/10.33263/BRIAC123.33323347
46. Nuszkiewicz, J., Wróblewska, J., Wróblewski, M., & Woźniak, A., Anthocyanin-Rich Purple Plant Foods: Bioavailability, Antioxidant Mechanisms, and Functional Roles in Redox Regulation and Exercise Recovery. *Nutrients*, 2025; *17*(15): 2453. https://doi.org/10.3390/nu17152453
47. Okan, O. T., Deniz, İ., Yayli, N., Şat, İ. G., Öz, M., & Serdar, G. H., Antioxidant Activity, Sugar Content and Phenolic Profiling of Blueberries Cultivars: A Comprehensive Comparison. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 2018; *46*(2): 639–652. https://doi.org/10.15835/nbha46211120
48. Padmanabhan, P., Correa-Betanzo, J., & Paliyath, G., Berries and Related Fruits. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of Food and Health*, 2016; (pp. 364–371). Academic Press. https://doi.org/10.1016/B978-0-12-384947-2.00060-X
49. Pasieczna-Patkowska, S., Cichy, M., & Flieger, J., Application of Fourier Transform Infrared (FTIR) Spectroscopy in Characterization of Green Synthesized Nanoparticles. *Molecules*, 2025; *30*(3): 684. https://doi.org/10.3390/molecules30030684
50. Pellegrino, D., Antioxidants and Cardiovascular Risk Factors. *Diseases*, 2016; *4*(1): 11. https://doi.org/10.3390/diseases4010011
51. PubChem. (n.d.). *Vaccinium*. Retrieved January 3, 2026, from https://pubchem.ncbi.nlm.nih.gov/taxonomy/Vaccinium
52. Rethemiotaki, I., Global prevalence of cardiovascular diseases by gender and age during 2010–2019. *Archives of Medical Sciences. Atherosclerotic Diseases*, 2023; *8*: e196. https://doi.org/10.5114/amsad/176654
53. Rodrigues, E., Poerner, N., Rockenbach, I. I., Gonzaga, L. V., Mendes, C. R., & Fett, R., Phenolic compounds and antioxidant activity of blueberry cultivars grown in Brazil. *Food Science and Technology (Campinas)*, 2011; *31*(4): 911–917. https://doi.org/10.1590/S0101-20612011000400013
54. Sandhu, H. K., Sinha, P., Emanuel, N., Kumar, N., Sami, R., Khojah, E., & Al-Mushhin, A. A. M., Effect of Ultrasound-Assisted Pretreatment on

Extraction Efficiency of Essential Oil and Bioactive Compounds from Citrus Waste By-Products. *Separations*, 2021; *8*(12): 244. https://doi.org/10.3390/separations8120244

55. Schuttlefield, J. D., & Grassian, V. H., ATR–FTIR Spectroscopy in the Undergraduate Chemistry Laboratory. Part I: Fundamentals and Examples. *Journal of Chemical Education*, 2008; *85*(2): 279. https://doi.org/10.1021/ed085p279
56. Shaikh, J. R., & Patil, M., Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 2020; *8*(2): 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834
57. Siddiq, M., Dolan, K. D., Perkins-Veazie, P., & Collins, J. K., Effect of pectinolytic and cellulytic enzymes on the physical, chemical, and antioxidant properties of blueberry (*Vaccinium corymbosum* L.) juice. *LWT*, 2018; *92*: 127–132. https://doi.org/10.1016/j.lwt.2018.02.008
58. Silva, S., Costa, E. M., Machado, M., Morais, R. M., Calhau, C., & Pintado, M., Selective Activity of an Anthocyanin-Rich, Purified Blueberry Extract upon Pathogenic and Probiotic Bacteria. *Foods*, 2023; *12*(4): 734. https://doi.org/10.3390/foods12040734
59. Silva, S., Costa, E. m., Mendes, M., Morais, R. m., Calhau, C., & Pintado, M. m., Antimicrobial, antiadhesive and antibiofilm activity of an ethanolic, anthocyanin-rich blueberry extract purified by solid phase extraction. *Journal of Applied Microbiology*, 2016; *121*(3): 693–703. https://doi.org/10.1111/jam.13215
60. Stevenson, D., & Scalzo, J., Anthocyanin composition and content of blueberries from around the world. *Journal of Berry Research*, 2012; *2*(4): 179–189. https://doi.org/10.3233/JBR-2012-038
61. Suriyaprom, S., Mosoni, P., Leroy, S., Kaewkod, T., Desvaux, M., & Tragoolpua, Y., Antioxidants of Fruit Extracts as Antimicrobial Agents against Pathogenic Bacteria. *Antioxidants*, 2022; *11*(3): 602. https://doi.org/10.3390/antiox11030602
62. Tobar-Bolaños, G., Casas-Forero, N., Orellana-Palma, P., & Petzold, G., Blueberry juice: Bioactive compounds, health impact, and concentration technologies—A review. *Journal of Food Science*, 2021; *86*(12): 5062–5077. https://doi.org/10.1111/1750-3841.15944
63. Wang, Y., Fong, S. K., Singh, A. P., Vorsa, N., & Johnson-Cicalese, J., *Variation of Anthocyanins, Proanthocyanidins, Flavonols, and Organic Acids in Cultivated and Wild Diploid Blueberry Species*, 2019. https://doi.org/10.21273/HORTSCI13491-18
64. Xu, X., Liu, A., Hu, S., Ares, I., Martínez-Larrañaga, M.-R., Wang, X., Martínez, M., Anadón, A., & Martínez, M.-A., Synthetic phenolic antioxidants: Metabolism, hazards and mechanism of action. *Food Chemistry*, 2021; *353*: 129488. https://doi.org/10.1016/j.foodchem.2021.129488
65. Zhang, M., Shen, Y., Fang, L., Yan, J., Ren, Y., Yang, F., Zong, Y., & Wang, X., Metabolomics and machine learning integrated analysis of flavor quality in 'Legacy' and a mutant blueberry. *Current Research in Food Science*, 2025; *12*: 101280. https://doi.org/10.1016/j.crfs.2025.101280
66. Zhang, Z., Kou, X., Fugal, K., & McLaughlin, J., Comparison of HPLC Methods for Determination of Anthocyanins and Anthocyanidins in Bilberry Extracts. *Journal of Agricultural and Food Chemistry*, 2004; *52*(4): 688–691. https://doi.org/10.1021/jf034596w
67. Zhou, L., Xie, M., Yang, F., & Liu, J., Antioxidant activity of high purity blueberry anthocyanins and the effects on human intestinal microbiota. *LWT*, 2020; *117*: 108621. https://doi.org/10.1016/j.lwt.2019.108621
68. Zimmer, K. R., Blum-Silva, C. H., Souza, A. L. K., WulffSchuch, M., Reginatto, F. H., Pereira, C. M. P., Macedo, A. J., & Lencina, C. L., The Antibiofilm Effect of Blueberry Fruit Cultivars Against Staphylococcus epidermidis and Pseudomonas aeruginosa. *Journal of Medicinal Food*, 2014; *17*(3): 324–331. https://doi.org/10.1089/jmf.2013.0037