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Determination of Total Phenol, antioxidant and Antimicrobial Activity of Beetroot and Strawberry in Sulaimani City - Kurdistan Region, Iraq

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Abstract

Strawberry and beet root are both extensively utilized crops that contain a variety of bioactive chemicals. Using various solvents, in this study, the bioactive and antibacterial characteristics of beet root and strawberry was investigated. In strawberry and beet root, the methanol extract gave the greatest total phenol concentration (175.5 mg gallic acid/g and 150.6 mg/g, respectively). The reducing power (FRAP) assay, (DPPH) scavenging activity, and Trolox equivalent capacity (TEAC) assay were used to measure antioxidant activity. With increasing concentrations, the decreasing power of all beet root and strawberry extracts rose. The extracts' DPPH -free radical scavenging activity was measured as EC50 and varied from 34.9 µg /ml to 127.1 µg /m for beet root and 25.7 µg /m to 158.1 µg /m for strawberry. Total phenol concentration in methanol extract and antioxidant activity of strawberry and beet root were shown to be significantly correlated. By comparing three distinct methods, it was shown that antioxidant activity (AOA) generated a greater quantity in methanol extract, followed by H2O. In three separate methods, chloroform extract generated the least amount of AOA. All of the extracts (methanol, water, ethyl acetate, and chloroform) inhibited Gram -negative bacteria more effectively than Gram -positive bacteria in antibacterial tests. Strawberry and beet root methanol extracts had the strongest antibacterial and anticandidal properties. These findings may be useful when it comes to strawberry and beet root intake.

Key words: Beet Root; Strawberry; antioxidant; antimicrobial activity; phenolic compounds.

Introduction

Polyphenols are found in a variety of fruits and vegetables, and they may have health -promoting properties as antioxidants in addition to their anticancer and anti -carcinogenic properties. [1] Chronic illnesses are worldwide health issues that claim the lives of millions of people. The development of various chronic illnesses is caused by high levels of oxidants such as reactive oxygen and nitrogen compounds in the human body systems. Various studies reveal that the free radicals present in the human body result in oxidative damage to different biomolecules, like lipid, protein, and nucleic acid linked to the degenerative disease initiation process. Many distinct dietary phytonutrients are found in fruits and vegetables, and they help to prevent numerous chronic illnesses linked with aging, such as cancer, cardiovascular disease, cataracts, and brain and immunological dysfunction. [8 ,2,3] These fruits and vegetables contain several antioxidant components, particularly in their peels, which are high in phenolic compounds as well as a variety of many other biologically active constituents, which reduce the harmful effects of free radicals [4]. Beet Root - Beta Vulgaris L. root is a member of the Chenopodiaceae family and comes in a variety of colors, from yellow to red, in its bulbs. Beet roots with a deep red color are the most preferred for human consumption,either cooked or raw in salads and beverages. [5 -7] With an entire phenolic constituent of 50 –60 µmol/g weight (dry), beet root is one of the ten most effective vegetables in terms of antioxidant activity. [9, 10] Beet Root could be a possible source of useful water -soluble nitrogen

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pigments, called betalains, consisting of two fundamental classes, red betacyanins and yellow betaxanthins. Fruits of Strawberry Arbutus Uneda L. The Ericaceae family belongs to an evergreen bush, a native Mediterranean plant also grown in numerous locales of Eastern Europe. [11, 12] Strawberries (Fragaria ananassa), which belong to the Rosaceae family, are a good source of phenolic compounds, as well as antioxidant and antiproliferative properties. Because of their nutritional value and flavor, they are commonly consumed. [13, 14] Strawberry's antioxidant characteristics are attributed to their high level of total phenolic chemicals rather than vitamin C, according to research. [14] Strawberry quality is influenced by the total phenolic compounds present, which contribute to organoleptic and sensory qualities as well as health benefits [15] Strawberry production and consumption have increased globally as a result of these many health benefits in addition to its nutritional quality, making strawberries the first most important soft fruit species [16]. Strawberry fruits include important polyphenols such as hydrolysable (ellagitannins and gallotannins), flavonols, anthocyanins, and condensed tannins. [17, 18].

Strawberries help to prevent cancer, obesity, cardiovascular disease, inflammation -related illnesses, and Alzheimer's disease because of their high antioxidant content and favorable effects on human health [19 –21]. Because of their strong antibacterial properties, phenolic compounds have been substituting artificial antioxidants and antimicrobial agents in food production, according to research. These compounds might be used in the production of food products and medicines for health -promoting effects. [22]

Fig (1) Beta vulgaris (Red Beet root)

Fig.(2) Strawberry (Arbutus Unedo)

Experimental procedure
Materials and methods:
1-Sample collection and drying:

Strawberry and Beet root plants were collected in Sulaimanyah city, to maintain a strategic distance from the corruption of colors and poly phenolic compounds, the aerial part of such plants air-drying, in temperature of 23-25 °C within a dim place, and in order to uniform molecule size the plants were powdered.

2-Preparation of the Extracts:

Methanol was extracted to the powdered plants for about 48 hours. Then, the extracted methanol was liquefied in water, broken via solvents made from ethyl acetate and chloroform. Next, the extricates were sifted and after that intensed under suction at 40°C employing a rotating evaporator(RE-2000E). The buildups remained stored in a refrigerator until more advanced tests could be performed

3- Determining total levels of phenols:

The overall phenolic compounds of such plant extricates were decided concurring to the Folin Ciocalteu strategy Folin-Denis reagent [23]. The results were communicated as mg gallic acid counterpart strategies.

Methods :

4- Free radical-scavenger activity assessment (DPPH° assay):

Extreme potential for scavenging was calculated according to the recorded technique [24]. At different concentrations, 1.5 ml of 0.25 mM DPPH mixture and 1.5 ml of extract were diluted in ethanol. For a constant state, this combination was vigorously shaken at ambient temperature. After thirty minutes

DPPH decolarization was measured by taking a spectrophotometer(Agilent technologies, Cary 60 UV-Vis) to measure the absorbance at 517 nm. Then, the following equation was used to calculate how scavenging DPPH radicals works.

Scavenging activity equation $= [\frac{A0-A1}{A0} \times 100]$

A0 denoted the control absorbance (unqualified, with no extraction)

A1 Denoted the absorbance when the excerpt or regular example is present.

5- Ferric Reducing Antioxidant Power Measurement (FRAP assay):

The reduction power was calculated with a few adjustments using the FRAP test [25]. In short, The test composites (0.2 ml) is combined with the newly prepared FRAP mixture (1.8 ml), consisting of TPTZ mixture (2.5 ml, 10 mm) in HCl (40 mM) and with FeCl3 (2.5 ml, 20 mM) and acetate buffer (25 ml, 0.3 M) (pH 3.6). Then the retention of such blend was determined at 593 nm. To get the calibration curve, Ethanolic solutions with several concentrations with Fe (II) utilized used. The FRAP results were computed by correlating the absorbance alter in tests at 593 nm to those that contain ferrous ions with recognized awareness.

6- Trolox Equivalent Antioxidant Capacity Measurement (TEAC assay)

TEAC measure is grounded upon $ABTS^{\circ+}$ scavenging [26]. 7 mM of ABTSo+ solution was arranged in PBS (100 mM) (pH 7.4) and then oxidized for 10 hrs utilizing potassium per sulfate (2.45mM) in dim. The solution ABTSo+ was weakened with PBS to 0.7 ± 0.05 absorbance at 734 nm. 10 µL of the test was blended via 990 µL of ABTSo+ mixture for measuring antioxidant ability. After 5 min absorbance of the blend was measured at 734 nm. Absorption decline was utilized to measure TEAC standards. Next, the absorbance decrease of the ABTSo+ solution at various Trolox concentrations was calculated to create a standard bend.

Blank estimates were done correctly, and the results were recorded. Trolox counterparts of plant extricates shown in the TEAC values.

7-Antimicrobial Activity:

The extracts were tested separately toward of 11 microorganisms, including 8 bacteria such as: *Bacillus pumlls (B. pumlls), Escherichia coli (E.coli), Bacillus cereus (B.Cereus), Staphylococcus aureus (S.aureus), Klebsiella Pneumoniae (K. Pneumoniae) , Bacillus subtilis (B.Subtilis) , , Pseudomonas aeruginosa (P. aeruginosa) (Phosphate Solublizing Bacteria) Staphylococcus epidermidis (S. epidermidis), Enterococcus(E. faecalis) and 2 fungi, Saccharomyces cerevisiae (S ac.cerevisiae) and Candida albicans(C. albicans).*

A- Assay for Disk Diffusion

Agar-well diffusion approach was used to assess the activity of antimicrobials of crucial oil and extracts [27]. In short, 0.1 ml of a research microorganism suspension (108 cells ml^{-1}) dispersed on plates with Mueller – Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for the fungi.

The microbial lawns were mounted on sterilized disks (6 mm) with 10 ml of samples each. The bacteria plates were brooded for 24 hours at 37 degrees Celsius, while the fungal plates were brooded for 48 hours at 30 degrees Celsius. The inhibitory zone was measured in mm around the halo. In all of the experiments triplicate examinations were carried out.

B- Minimum Inhibitory Concentration Determination (MIC):

Micro dilution astestsay was used to assess the MIC values [26]. In 96-well micro titer plates, serial duplicate reductions of the tests were performed in Mueller – Hinton Broth with 0.5 percent Tween 80 regarding the bacteria and 0.5 percent Sabouraud Dextrose Broth for the fungus.

To give a final concentration of 5×10^5 organism's ml^{-1} fresh microbial suspensions arranged within the same media were included from overnight crops. Microorganisms were utilized as medium controls, or the measurements were used individually. Microplates were produced at 37 degrees Celsius for 24 hours for bacteria and 30 degrees Celsius for 48 hours for fungus. The initial dilute was reported as the MIC, with no microbial growth.

Results and discussion

Organic solvents such as methanol, ethanol, ethyl acetate, chloroform, are widely used in the extraction of bioactive substances such as phenolic compounds or other compounds having antioxidant potential.

1-Total polyphenolic content (TPC)

Folin—Ciocalten reagent (FCR) was used to determine the polyphenolic amount of the extract, and this experiment was carried out on the whole BR bulb.The gallic acid equivalent per 100 gram of BRE was used to assess the results, which were compared to the calibration series. The influence of extraction solvents (methanol, water, ethyl acetate, chloroform) on the overall polyphenol amount of Beet root and Strawberry is shown in Tables 1 and 2, and figure 3, 4. The results suggest that the methanol extract was more effective, with the greatest (TPC) in both Beet root and Strawberry (150.6 mg, 175.5 mg) GAE/gm, followed by water extract (131.6 mg, 155.3 mg) GAE/gm with both Beet root and Strawberry. This was crucial for recovering the phenolic compound. However, in chloroform extract (39.2mg, 24.6 mg) GAE/gm, the Beet root and Strawberry exhibited the lowest (TPC).

As a result, the strategy of performing two consecutive extracts on the two samples (Beet root and Strawberry) appears to be sufficient for improving phenolic recovery. Furthermore, most extractions are done in batches, requiring many stages to separate the recovered components from the solvents utilized so that the solvents may be recovered. Nonetheless, this technique will ultimately create extracts containing residual solvent, which may restrict their application in some cases. Furthermore, these solvents can produce large amounts of waste, which can be damaging to the environment in most cases.

As a result, for industrial applications, utilize clean solvents such as water is very advantageous, since it is less expensive and easier to get, and it is more ecologically friendly. (28, 29).

Table 1: Shows the overall phenolic content and antioxidant capacity of strawberry extracts obtained using various techniques (DPPH, FRAP and ABTS).

Extracts	DPPH	FRAP	ABTS	Total phenol
	IC_{50} µg/mL	µmol Fe^{2+}/g extract	µmol Torolox /g extract	mg galic acid/g extract
Methanol	25.7±0.1	2600.4±3.0	836.3±2.5	175.5±2.8
water	31.6±0.2	2224.2±3.1	764.3±2.1	155.3±2.2
Ethyl acetate	54.8±0.3	1746.3±2.8	508.2±2.2	101.3±0.8
Cloroform	158.1±0.5	1504.4±1.7	500.2±1.6	24.6±0.7
BHT	23.0±0.2	-	-	-

Table 2: Illustrates the total phenol and antioxidant activities of Beet root extracts that three different techniques (DPPH, FRAP, and ABTS) were used.

Extricates	DPPH	FRAP	ABTS	Total phenol
	IC_{50} µg/mL	µmol Fe^{2+}/g extract	µmol Torolox /g extract	mg galic acid/g extract
Methanol	34.9±0.2	2040.4±3.0	700.0±2.4	150.6±2.9
water	37.3±0.3	1899.9±2.1	624.2±2.0	131.6±1.7
Ethyl acetate	88.6±0.4	1443.3±3.2	500.6±2.2	53.4±0.6
Cloroform	127.1±0.5	903.2±3.1	418.4±1.8	39.2±0.2
BHT	23.0±0.2	-	-	-

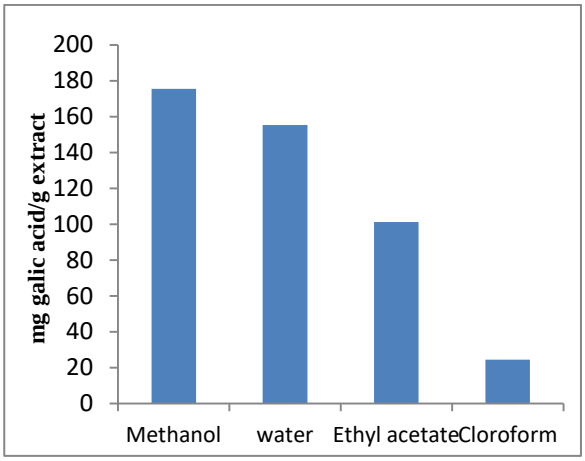


Figure 3: Total phenolics content of Strawberry (T2) extracts expressed as gallic acid equivalent (GAE).

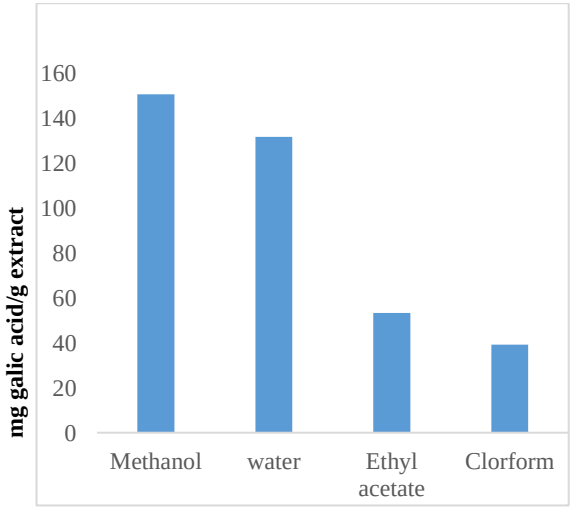


Figure 4: Total phenolics content of Beetroot (T1) extract expressed as gallic acid equivalent (GAE)

Measurement's antioxidant activity (AOA)

The obtained results with three approaches when looking at antioxidant activity were highly variable, which originates from the chemical components of the compounds and the reaction engaged. Because antioxidant molecules run through various processes, many techniques of measuring antioxidants have mostly been widely employed. In the reaction matrix, each has a distinct target. Because varied chemical reactivity can lead to varying degrees of antioxidant potential in various chemical tests, phenolic compounds have diverse locations with free radical scavenging capabilities.

1-Measurement of free-radical scavenging activity (DPPH)

The DPPH test is a simple and effective method for determining antioxidant activity (AOA). The antioxidant impact, or DPPH, is a stable free radical that accepts an electron or hydrogen radical to form a stable diamagnetic molecule. Antioxidants caused a drop in DPPH radical absorbance at 517nm, which was used to measure their reduction capabilities. The dosage response curve for the radical scavenging ability of the (methanol, water, ethyl acetate, and chloroform) extract is shown in Figures (5) and (6). With rising extract concentration, the scavenging of DPPH radicals increased. The EC_{50} value is a metric generally used to assess the free radical scavenging activity. It is defined as the concentration of the extract necessary for 50 percent scavenging of radicals under the experimental condition used. (UVELIER et al .1992).

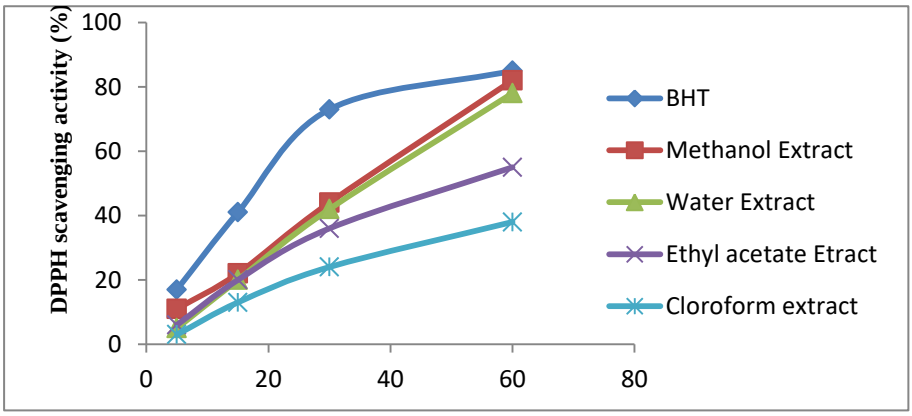


Figure 5: Shows the percentage of Beet root extracts' DPPH radical scavenging activity

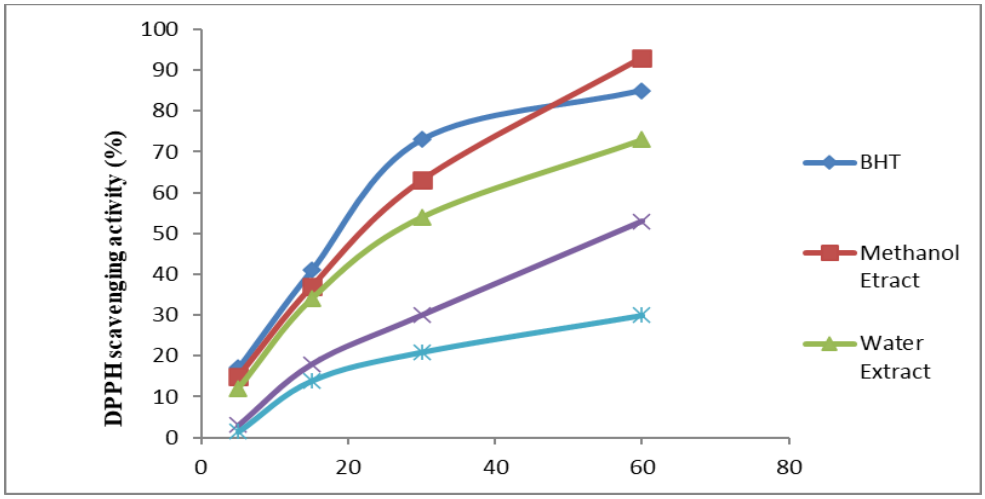


Figure 6: demonstrates the percentage of Scavenging activity of Strawberry extracts on DPPH radicals

A greater antioxidant activity is associated with a lower $EC_{50\ DPPH}$ value. Tables 1, 2, and figure 7, 8 show the $EC_{50\ DPPH}$ value of Beet root and Strawberry extracts based on radical scavenging activities (SADPPH) of different solvent extracts. Methanol and water extract both had a high amount of DPPH radical scavenging activity, however chloroform extract performed poorly in the DPPH test for Beet root and Strawberry. The EC50 DPPH =0.0280.001 mg/ml when BHA was used as the pure reference component.

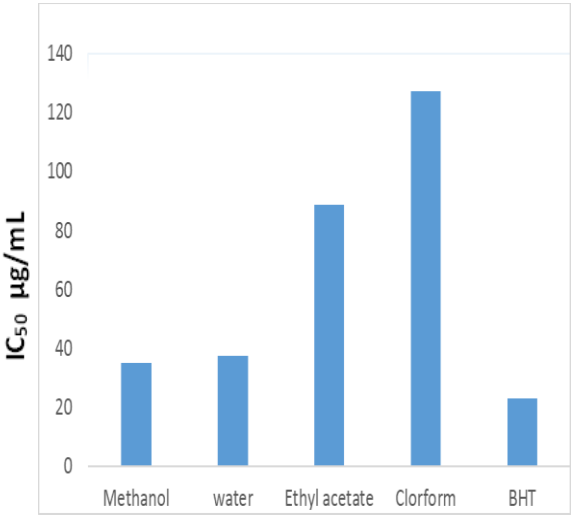


Figure 7: the DPPH radical scavenging activities of Beet root (T1) extracts

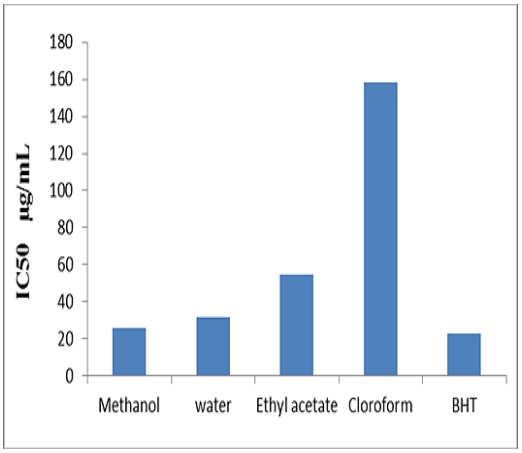


Figure 8: Demonstrates the Strawberry extracts' DPPH radical scavenging ability

2- Anti-oxidant ferric reduction (FRAP assay)

The FRAP test evaluates antioxidant activity by decreasing the complex of 2, 4,6-tripyridyl-S-triazine [Fe (III)- / g TpTz2]2 + in an intensely blue ferrous complex [Fe (II)-TpTz2]2 + in an acidic medium.

FRAP values are calculated using a 593 nm absorbance increase and then evaluated to a ferrous ion norm. Table 1, 2 and figure 9, 10 indicate the reducing power of beet root and strawberry as extracted by various solvents. When compared to those extracted with water, ethyl acetate, or chloroform, the reducing power of Beet root and strawberry extracted with methanol was much higher. Beet root extracts in methanol, water, ethyl acetate, and chloroform have absorbances of (2040.4, 1899.9,

1443.3, and 903.2µmol Fe^{+2}/gm) at 593 nm, respectively.

Strawberry also showed a similar pattern. The following order was used to determine the reducing power of beet root and strawberry extrats: chloroform < ethyl acetate <water <Methanol. For all extracts, the strawberry had a higher reducing power than the beet root. TPC and ferric reducing antioxidant power (FRAP) were shown to have a strong relationship in another investigation.

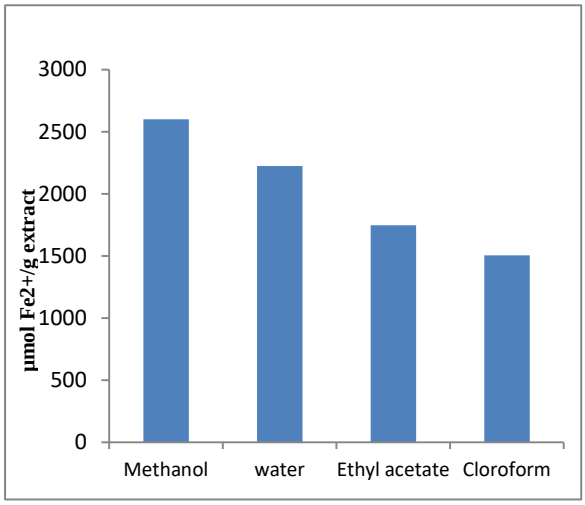


Figure9 : Shows Antioxidant capacity of Strawberry (T2) extracts using the FRAP assay

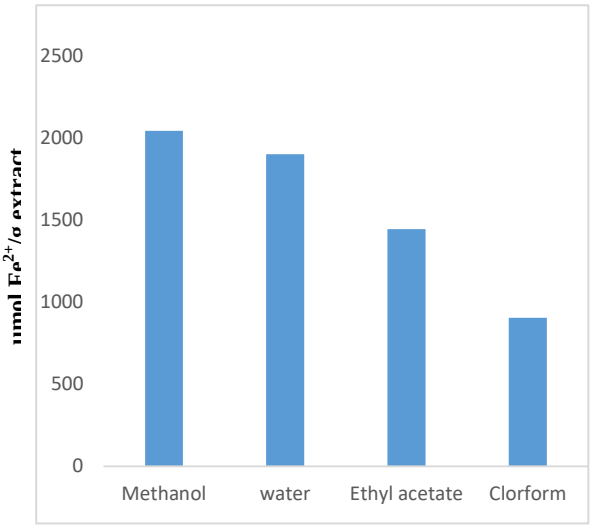
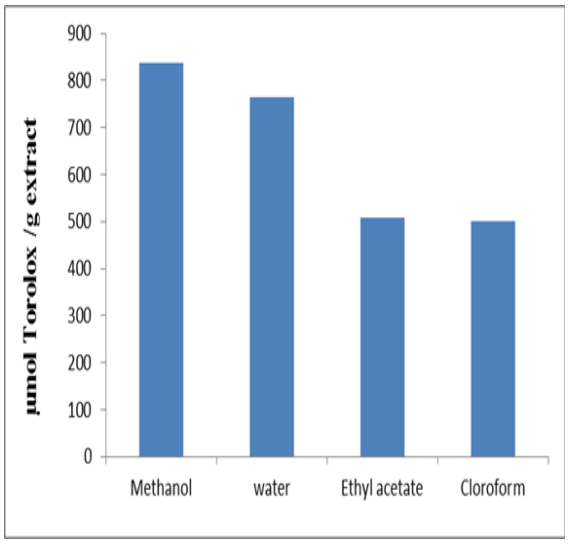


Figure 10 : Antioxidant capacity of Beet root extract using FRAP assay.

3- ABTS

Table 1, 2 and figures 11 and 12 illustrate the findings of the ABTS technique for determining (AOA) in beet root and strawberry.

The ABTS radical test is one of the most widely used methods for determining antioxidant capability. In terms of ABTs, AOA has the greatest value (700, 836.3 µmole /gm Torolox) when using methanol extract for both beet root and strawberry respectively, and the lowest value (418.4, 500.2 µmole /gm Torolox) when using chloroform with both beet root and strawberry. The scavenging rate of beet root on ABTs (700-to 418.4 µmole /gm Torolox) was lower than strawberry extract (836.3 to 500.2 µmole /gm Torolox) in the ABTs assessment. Because the antioxidant activity (AOA) is dependent on the existence of polyphenol, which has a greater concentration in methanol extract, by assessing three methods (DPPH, FRAP AND ABTs) of AOA, this study found that beet root and strawberry had higher antioxidant activity (AOA) in methanol extract followed by water compared to other solvents. These findings were similar to those of Wootton-Beard et al. (30), who found that among 23 commercially available vegetable juices, the Beet root and Strawberry had the best radical scavenging ability in both the DPPH and ABTs tests, as well as the highest reduction capacity as determined by FRAP.



Figuger 11: The $ABTS^{+}$ scavenging activity of Strawberry extracts

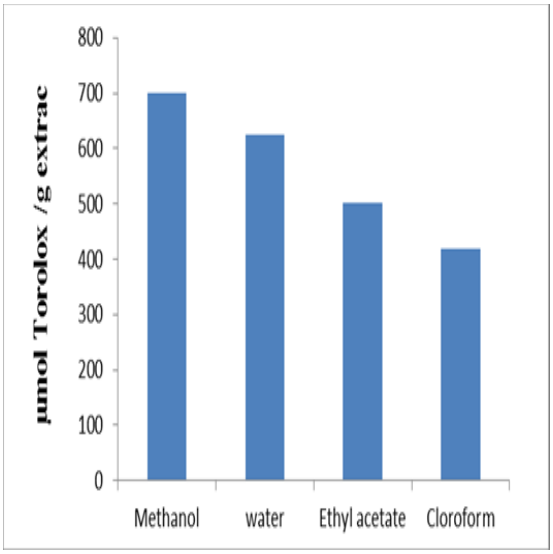


Figure 12: The ABTS^{+} scavenging activity of Beet root extracts**.**

4- Antimicrobial Activity study

The current research examined at the antibacterial and antifungal properties of beet root and strawberry extracts. Using chosen Gram-positive and Gram-negative bacteria as well as selected fungi, the antibacterial activity of methanol, water, ethyl acetate, and chloroform extracts of beet root and strawberry was tested using two methods: disc diffusion and microdilution. In table 3, 4, the diameter of the inhibition zone (ZI) and the lowest inhibitory concentration (MIC) are presented. As indicated, all of the extracts showed potential antibacterial efficacy against the test pathogens.The maximum antibacterial activity was found in methanol extracts of both beet root and strawberry.

Table 3: Strawberry Antimicrobial Activity (T2) extracts.

	Microorganisms										
Extracts	B.PU	E.coli	S.au	B.Ce	KLb	E.NT	B.sub	St.Ep	PS	Can	Sac
Methanol	18[a] (7.5)[b]	20 (7.5)	18 (8)	18 (8)	14 (15)	20 (7.5)	18 (8)	14 (15)	18 (8)	12 (15)	11 (>10)
Water	14 (15)	14 (15)	14 (15)	12 (15)	18 (8)	14 (15)	11 (15)	14 (15)	12 (10)	12 (10)	11 (>10)
Ethyl acetate	20 (7.5)	12 (15)	14 (15)	20 (7.5)	12 (15)	12 (10)	14 (15)	11 (>10)	11 (>10)	10 (10)	10 (10)
Cloroform	14 (15)	-	12 (15)	11 (15)	12 (15)	-	12 (15)	12 (10)	11 (>10)	11 (10)	0

a Inhibition Zone Diameter (mm) with sterilized disk thickness (6 mm).
b Initial Concentration of Inhibitory, as mg per ml values.
c - , inactive, (7–14) medium active and (>14) strongly active

Table 4: Illustrates Beet root extracts Antimicrobial function.

Extracts	B.PU	E.coli	S.au	B.Ce	KLb	E.NT	B.sub	St.Ep	PS	Can	Sac
Methanol	14[a] (15)[b]	12(15)	12 (16)	12(16)	18 (8)	14 (15)	12 (15)	12 (15)	5 (32)	10 (10)	11 (10)
Water	14 (15)	11(15)	12 (15)	11 (15)	10 (10)	12 (15)	11 (10)	12 (15)	-	0	11 (10)
Ethyl acetate	11(15)	12 (15)	14 (15)	0	11 (15)	10 (10)	11 (10)	12 (15)	-	-	0
Cloroform	0	11(15)	-		-	-	10 (10)	10 (10)	-	-	-

a inhibition zones diameter (mm) with sterile disk diameter (6 mm).
b Initial Concentration of Inhibitory, values as mg / ml.
c -, not active; (7–14) medium active; (>14) strongly active

Bacillus pumilus, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae,Enterococcus faecalis, Bacilus Subtilis, Staphylococcus epidermidis, Pseudomonas aeruginosa, Candida albicans, and Saccharomyces cerevisiae were inhibited by a 14,12,12,18,14,12,12,5,10,11mm zone of beet root (methanolic) extract respectively. Similarly, against *Bacillus pumilus, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, Enterococcus faecalis, Bacillus Subtilis, Staphylococcus epidermidis, pseudomonas aeruginosa, Candida albicans, Saccharomyces cerevisiae*, strawberry (methanol) extract showed inhibitory zones of 18,20,18,18,14,20,18,14,18,14,18,12,11mm respectively. When compared to aqueous extracts, methanol extracts had rather greater antibacterial activity. All of the extracts were shown to have greater inhibitory zones against Gram-negative bacteria than against Gram-positive bacteria. This might be explained by the structural differences between Gram-negative and Gram-positive bacteria's cell walls. Gram-positive bacteria have a thick coating of peptidoglycan in their cell wall with covalently bonded teichuronic and teichoic acid, leaving them less vulnerable to the test agent's activity (31,32). In addition, as shown in table 3 and 4, antibacterial activity was assessed in terms of the MIC.

The lowest MIC value of 8mg/ml was found against *Klebsiella pneumoniae*, and the highest MIC value of 32mg/ml was observed against *pseudomonas aeruginosa*. Water and Ethyl acetate extract had equivalent antibacterial and antifungal efficacy against all species of bacteria and fungi. Likewise, against (*B.PU, Ecoli, and E.NT*) bacteria, strawberry (methanol) extract had the lowest MIC value of 7.5 mg/ml. All other extracts (water, ethylacetate, chloroform) demonstrated antibacterial action against all kinds of bacteria and fungus, with the maximum MIC value of 15 mg/ml against *Klb, St. Ep, and Can*. The MIC values of the extracts tested did not differ significantly.

Conclusion

The effects of different extraction solvents on the bioactive and antibacterial properties of beet root and strawberry were investigated. The results of this study demonstrate that methanol extract has the greatest bioactive property among the solvents. When compared to beet root, strawberry has more bioactive characteristics. The antiradical activities of beet root and strawberry were positively correlated with the total phenolic content, according to the correlation coefficients. The extracts had antimicrobial action against all of the pathogens tested, with the methanol extract having the maximum activity for both strawberry and beet root. Gram-negative bacteria showed greater inhibitory zones than Gram-positive bacteria. MIC values for the bacterial and fungal pathogens ranged from 5 to 20 mg/ml in extracts. Lastly, because the uses of the extracted phenolic compounds are targeted at integration into food products to boost their health-promoting features, such as antioxidant activity, it is preferable to reduce the usage of methanol in the health food business.

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