

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY BASED COMPARISON STUDY OF KIWIFRUIT PULP AND PEEL

Noor ul Ain Mumtaz^(1*), Samahar Rana ⁽²⁾, Mauzzmah Shahid ⁽³⁾, Rimsha Raees ⁽⁴⁾ Huma Shahid ⁽⁵⁾,

¹: Department of Biochemistry, Riphah International University Faisalabad

²: Department of Biochemistry, Riphah International University Faisalabad

³: Department of Chemistry, University of Engineering and Technology, Lahore-54890

⁴: Department of Physics, Institute of Computing and Applied Sciences, (RICAS) Riphah International University, Lahore-54000

⁵: Department of Physics, Institute of Computing and Applied Sciences, (RICAS) Riphah International University, Lahore-54000

ABSTRACT

Kiwifruit, *Actinidia deliciosa*, is the edible berry of several *Actinidia* species'. "Hayward," is the green-fleshed Kiwifruit, most widely available and commercially important. The amounts of polyphenols, vitamins, dietary fiber, and functional ingredients as well as bioactive phytochemicals are found in the edible and also non-edible parts of kiwifruit in variations. The objective of this research was to compare the Kiwifruit peel and pulp regarding bioactive compounds, antioxidants and antimicrobial activity. Kiwifruit pulp shows higher TFC, expressed as (QE mg/100 g of dw), as compared to kiwifruit peel extract. The TPC, expressed as (GAE mg/100 g of dw), 269.5 in pulp as compared to 267.3 in peel. The antioxidant activity of Kiwifruit pulp shows higher antioxidant activity with DPPH and ABTS⁺ assay as compared to kiwi peel. Kiwifruit pulp shows higher antibacterial activity than kiwi peel. Anti-microbial activity of Kiwifruit is dose dependent. Kiwifruit pulp shows remarkable antioxidant and antimicrobial activity due to presence of phytochemicals as compared to peel. Kiwifruit pulp with higher phenolics and flavonoids exerts more potent antioxidant, antibacterial activity than pulp. This study provides evidence for the development of Kiwifruit based novel natural products with excellent bioactivity

Keywords: *Actinidia Deliciosa, Bioactive compounds, Hayward, Antioxidant, Antimicrobial, proximate composition*

^(3*)**Mauzzmah Shahid**

Department of Chemistry, University of Engineering and
Technology Lahore:

INTRODUCTION

Plants are a wonderful source of natural medicines, and people have used them since ancient times. There are many methods used to treat various ailments, including Homeopathy, allopathy, Unani, and Ayurvedic medical systems and these frequently rely on, one way or another, plant stuff. The use of natural medicines is approved worldwide due to its effectiveness, affordability, and lack of negative effects (Ansari et al., 2023). Fruits are a great source of several bioactive substances All of the roots, leaves, stems, bark fruit, and seeds contain these substances like phenolics, flavonoids, alkaloids, glycosides, saponins, essential oils, mucilages, and tannins. They are capable of treating a variety of illnesses in both people and animals (Walia et al., 2022). Kiwi fruit commonly known as Kiwi or Chinese goose berry is nutritive eatable berry of numerous woody vines of a specie *Actinidia*. Although popularly grown in New Zealand, Kiwis actually have their roots in eastern China. Kiwi fruit has egg like oval shape of about 4-9 cm in length and around 5-6 cm in diameter. It has fuzzy hairy edible skin of camel brown color and green (Hayward), yellow (golden) or red pulpy flesh with characteristics round rows of tiny black seeds which are also edible. Flesh is soft pulpy having distinctive sweet tart bold flavor (Hazarika et al., 2022). It has high amount of vitamin C, E and K and with negligible fats and proteins. Typically, 62% of the fatty acids like ALA can be found in Kiwifruit seed oil. Tetraterpenoids such as provitamin A, beta-carotene, lutein, and zeaxanthin can be present in Kiwifruit pulp (He et al., 2019).

The size, hairiness, and colour of the fruit's skin vary. The colour, juiciness, texture, and flavour of flesh of all Kiwifruits is distinctive. While some fruits taste significantly better than the bulk of commercial cultivars, others are unpleasant to eat (Follett et al., 2019). Commonly Kiwifruit is consumed raw or use as juice or fruit salads also used in bakery and also prepared with meat. Humans can eat the entire fruit, including the skin, however because of its texture, the fuzzy kinds' of skin is frequently rejected (Yuan et al., 2022). In China it was not consumed as fruit, but it was taken as medicine. Traditionally Chinese used to give Kiwifruit as medicine to children to enhance their growth and for recovery of women who gave birth. Actinidain, also known as Actinidin, is found in raw Kiwifruit is used commercially as a meat tenderizer and may potentially have digestive benefits (He et al., 2019). Kiwis have a wide range of nutrients and bioactive phytochemicals because of their genetic diversity. Kiwifruit has variety of organic

acids, dietary fiber (DFs), vitamins, and minerals (Gautam et al., 2022). Polyphenols and non-starch polysaccharides, among other bioactive phytochemicals, can be found in Kiwifruit (Commisso et al., 2019). Anti-oxidative, anti-proliferative, anti-inflammatory, anti-microbial, anti-hypertensive, neuro-protective, and digestive health are all helped by phytochemicals. The acceptance of Kiwifruit by consumers and its commercial success depends on its chemical and biological properties (Kai et al., 2020).

Materials and Method

The fresh Kiwifruits, green kiwi, were collected from local mart, Imtiaz Mart Faisalabad, Pakistan, and the specimens were identified from herbarium botany department, Ayyub Agricultural Research Institute (AARI), Faisalabad. The fruits were washed with distilled water. Peels were separated from fruit flesh with the help of spoon. Separated Peels and cut flesh was oven dried at 40C for 3 days and then ground in fine powder and stored at dry place (AOAC International, 2012).

Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH) (99%) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS⁺) was purchased from Sigma-Aldrich. Methanol and all other chemicals, reagents and solvents used in study were of analytical grade and purchased from local store in Faisalabad.

Extract preparation

Using a mixer grinder, the whole fruit pulp and separated fruit peel from *Actinidia Deliciosa* were ground into small particles. These particles were then steeped in, 70% methanol. All samples were soaked for 48 hours at room temperature with shaking, filtered through muslin cloth, and then extracted three times using Whatmann No. 1 filters paper. The resulting filtrate was concentrated in a rotary evaporator at a temperature of 40 C until dry. After that, the extracts were maintained in a refrigerator at 8°C to be analysed for additional factors (phytochemicals, antioxidant activity, and antimicrobial activity) (Dias et al., 2020).

For Total Phenolic content

Total phenolic contents of Kiwifruit pulp and peel of different extracts was determined spectrophotometrically using Folin-Ciocalteu reagent assay, Gallic acid is used as standard. 50 μ l samples and 50 μ l Folin-Coicalteu reagent were taken and added in the 750 μ l of distilled water and kept this mixture at room temperature. After 10 min 150 μ l of 20% sodium carbonate (Na_2CO_3) was added. This mixture was heated at 40°C in water bath for 20 min, and then it was cooled in ice bath. Then the absorbance was determined at 755nm using UV spectrophotometer (double beam spectrophotometer Uvikon XL, Bio-Tek instruments, Bad friedrichshall, Germany). The total phenolic content in the samples was expressed as mg gallic acid equivalents (GAE)/g dry weight sample. All samples were examined in triplicate (Mulye et al, 2020).

To test the flavonoid contents,

Total flavonoid content of Kiwifruit pulp and peel in different extracts was determined spectrophotometrically using quercetin as standard. 0.5ml of each pulp and peel extract was taken and mixed them with 0.5ml of ALCL_3 2% methanol solution. This mixture was incubated for 10 minutes at room temperature. After 10 min, absorbance was determined at 368nm in UV spectrophotometer. Total flavonoids in the sample were expressed as mg quercetin equivalents (QE)/ g dry weight. All samples were analyzed in triplicate (Mulye et al, 2020).

Antioxidant Activity

DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for measuring the antioxidant activity. The antioxidant capacity of all extracts (EtoH, MeoH, Ace. Aque.) was determined using DPPH assay previously described by (Siddique et al., 2021). 0.9 mL of the DPPH solution was added to each well, so that the final concentration of DPPH is 0.1 mM then mixed the contents of each well thoroughly and incubated the plate at room temperature for 30 minutes. Absorbance of each well was measured at 517 nm using a spectrophotometer. Then

calculated the antioxidant activity for each kiwi peel and pulp extract by comparing the absorbance of the mixture to a standard curve of BHT using the following eq.

$$\% \text{ DPPH radical scavenging activity} = \frac{(\text{Ablank} - \text{Asample}) \times 100}{\text{Ablank}}$$

Where Ablank is the absorbance of the DPPH solution alone and Asample is the absorbance of the Kiwi fruit extract + DPPH solution.

ABTS⁺ Assay

The ABTS⁺ assay is a laboratory method used to measure the antioxidant activity of a substance. It works by measuring the ability of the substance to scavenge the ABTS⁺ radical, which is a stable, blue-green colored radical that is formed from the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and potassium persulfate (Deng et al., 2018). The assay is typically performed in aqueous solutions but can also be performed in other solvents such as ethanol, methanol, and acetone.

ABTS⁺ radical solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate in water and allowing the mixture to stand in the dark for at least 12 hours and then extract was centrifuged in the solvent of choice to separate the solid and liquid phases. Antioxidant extract was diluted to the desired concentration and mixed it with the ABTS⁺ radical solution. Then absorbance of the mixture was measured at 734 nm using a spectrophotometer. Antioxidant activity of the kiwi fruit extract was calculated by comparing the absorbance of the mixture to a standard curve of BHT using the following eq.

$$\text{Antioxidant activity (AA)} = \frac{(\text{A sample} - \text{A blank})}{(\text{A control} - \text{A blank})}$$

Anti-Microbial Activity

Antimicrobial activity of fruit samples was determined against bacterial and fungal strains. For antibacterial activity, Gram positive (*Bacillus subtilis* NRRL B-94, *Staphylococcus aureus* NRRL B-313) bacterial strains and gram negative (*Escherichia coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL B-32) bacterial strains were used. Agar diffusion method was used to evaluate antibacterial activity with some changes (Siddique *et al.* (2021).

Bacterial species were grown on nutrient broth media and incubated for 24 hrs., The remaining spore containing solution was used for inoculation. To prepare the plate, 50 ml of agar media at 50°C was combined with simple inversion with 1 ml of Tween 20 and 500 µl of inoculating media. After some time, using sterile tubes, 6mm diameter wells were made in hardened or solid agar. Add the extract samples and positive control separately in each dish and wait for 30 minutes for diffusion of sample in agar. Then incubate the bacterial sample for 48hrs at 30°C each. After incubation periods microbial growth inhibition and clear microbial free inhibition zone was measured and activity was evaluated. Diffusion of the sample is (200, 400 and 600µg/l).

Results

Total Flavonoids

Table 1 Total flavonoids contents of Kiwi pulp and peel

Sr. no.	Peel	pulp
1	108.9	131.7
2	110.11	130.2
3	107.6	132
Mean	108.87	131.3
SD	1.255	0.964

Table1 and figure 1 represents the total flavonoids content found in peel and pulp of Kiwi when extract was prepared by using different solvents (water, methanol, ethanol, and acetone). Results depicted that Kiwi pulp contains a significant amount of total flavonoids as compared to

kiwi peel extract. Statistical analysis revealed that extracts of pulp showed a significant amount of total flavonoids as compared to peel extract in methanol with p values $<0.005^{**}$ and $<0.0001^{****}$ respectively. Kiwifruits biological activities are due to presence of phytochemicals like flavonoids. Kiwi fruits have significant amount of flavonoid due to which it shows anticancer, antifungal, and antioxidant activities. Kiwi fruit demonstrates more flavonoid content (Alim et al., 2020).

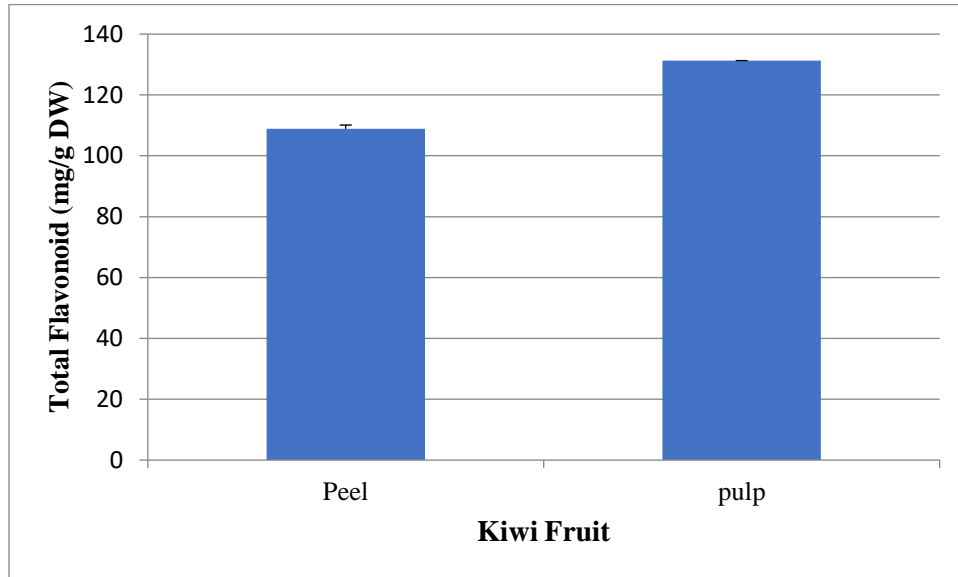


Figure 1 Graphical representation of total flavonoids contents in different extracts of Kiwi peel and pulp

Total Phenol

Table 2 Total phenol contents in different extracts of Kiwi peel and pulp.

Sr. no.	Peel	Pulp
1	267.3	269.5
2	266.12	271.57
3	268.48	267.43
Mean	267.3	269.5
SD	1.18	2.07

Table 2 and figure 2 represents the total phenol content found in peel and pulp of Kiwi when extract was prepared by using methanol. Results depicted that Kiwi pulp contains a significant amount of total phenol. It is also concluded that both extract pulp and peel represent a significant amount of total phenol but pulp extract showed a greater amount of total phenol. Statistical analysis revealed that pulp extract showed a significant amount of total phenol as compared to peel with p values $<0.005^*$ and $<0.0001^{****}$ respectively. Kiwifruit has many biological activities like antioxidant activity anti-inflammatory activity, anticancer and antimicrobial activity also promote gut health and metabolic health due to presence of fiber and phenols, which is in accordance with the result (Alim et al., 2019).

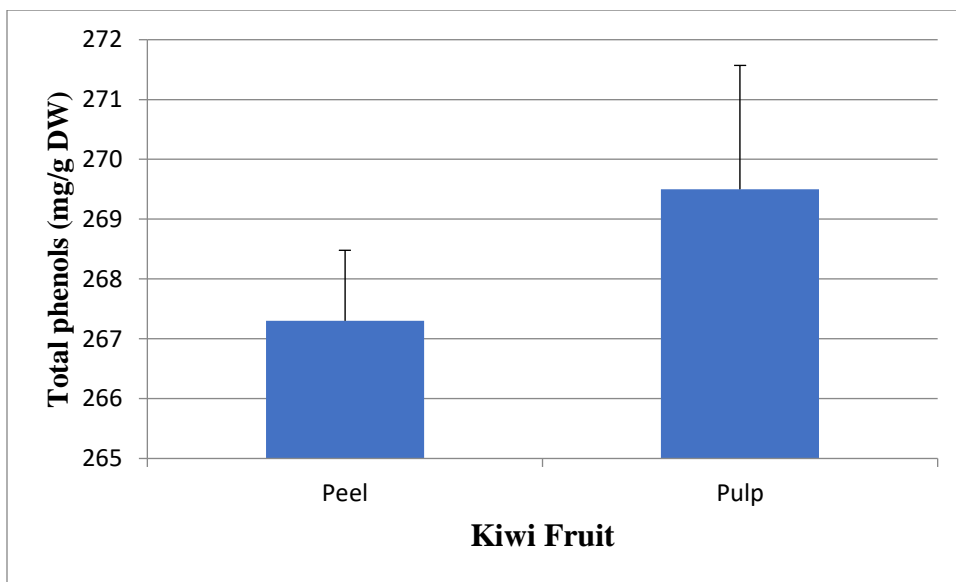


Figure 2 Graphical representation of total phenols contents in different extracts of Kiwi peel and pulp

Anti-Oxidative Properties

DPPH Assay

Table 3 DPPH radical scavenging activity of Kiwi peel in different extracts

Sr. no.	Control (BHT)	Peel	Pulp
1	4.73	89.42	107
2	4.02	89.78	108.01
3	4.67	90.45	107.03
Mean	4.473333	89.88333	107.3467
SD	0.393743	0.522717	0.574659

Table 3 and figure 3 represents the DPPH radical scavenging activities of peel and pulp of Kiwi when extract was prepared by using methanol. Results depicted that Kiwi pulp contains a significant amount of antioxidants than extract of kiwi peel. It can be due to presence of vitamin C in pulp. Statistical analysis revealed that the extracts of peel and pulp showed a highly significant radical scavenging property as compared to control with p value $< 0.0001^{****}$. Research shows that due to presence of Vitamin C, phenols, flavonoids, and other phytochemicals such as anthocyanin etc, fruit has high antioxidant activities. Research study shows that extract shows high DPPH value which indicates that fruit has favorable antioxidant activity (Mulye et al., 2020).

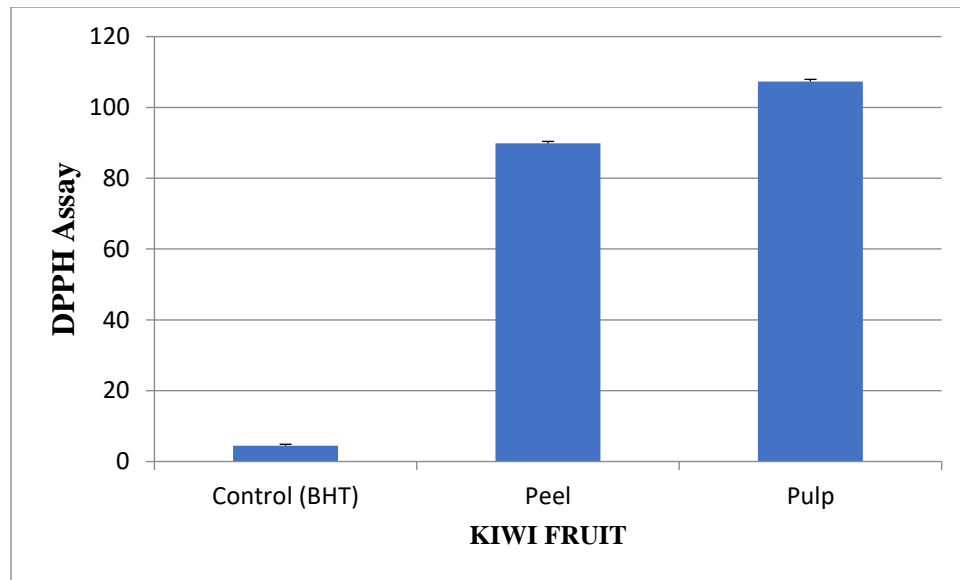


Figure 3 Graphical representation of DPPH radical scavenging activity of Kiwi peel and pulp in different extracts

ABTS⁺ Assay

Table 4 ABTS⁺ radical scavenging activity of Kiwi peel and pulp in different extracts

Sr. no.	Control (BHT)	Peel	Pulp
1	15.43	195.01	257.67
2	15.67	197.03	258.9
3	15.02	195.78	260.01
Mean	15.37333	195.94	258.86
SD	0.328684	1.019461	1.170513

Table 4 and figure 4 represents the ABTS⁺ radical scavenging activities of peel and pulp of Kiwi when extract was prepared by using methanol. Results depicted that Kiwi peel showed a significant radical scavenging property but kiwi pulp has showed greater antioxidant activity. It is also concluded that extract represent a significant amount of antioxidants but pulp extract in methanol showed a greater ABTS⁺ radical scavenging property. Statistical analysis revealed that the extracts of peel and pulp showed a highly significant radical scavenging property as compared to control with p value < 0.0001****. Wang et al., (2018) studied the different varieties

and genotypes of kiwifruit and determined their antioxidant activity using ABTS⁺ assay according to result the Hayward, kiwi has high antioxidant activity than other which are gold kiwi and red kiwi.

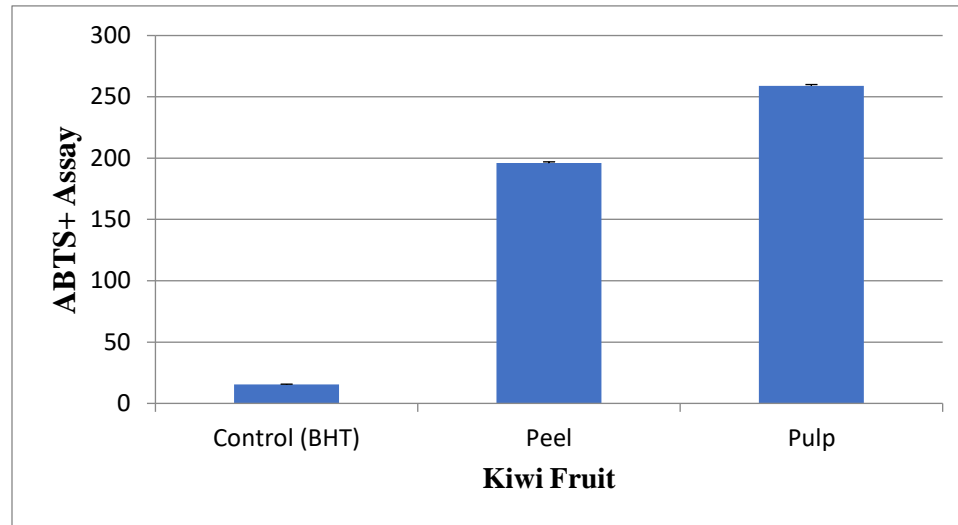


Figure 4 Graphical representation of ABTS radical scavenging activity of Kiwi peel and pulp in different extracts

Antibacterial Activities

Table 5 Antimicrobial activities of different extracts of Kiwi peel and pulp against bacterial strains

Bacterial strain	Peel	Pulp	Peel	Pulp	Peel	Pulp
	200 (µg/ mL)		400 (µg/ mL)		600 (µg/ mL)	
<i>B. subtilis</i>	0 ± 0.00	0 ± 0.00	13.2 ± 3.45	14.16 ± 2.34	18.16 ± 3.67	19.82 ± 1.99
<i>S. aureus</i>	0 ± 0.00	0 ± 0.00	11.3 ± 2.98	11.73 ± 0.67	17.65 ± 3.56	15.5 ± 0.01

<i>E.coli</i>	0 ± 0.00	0 ± 0.00	10.23 ± 3.02	13.5 ± 1.09	18.15 ± 2.98	19.52 ± 2.78
<i>P. aeruginosa</i>	0 ± 0.00	0 ± 0.00	10.23 ± 3.90	12.56 ± 2.78	18.15 ± 2.67	19.5 ± 3.67

Table 5 and figure 5 represents the antimicrobial activities of Kiwi peel and pulp when extract was prepared by using different concentrations (200, 400 and 600 µg/mL). Results depicted that 400 and 600 µg/mL concentrations of Kiwi pulp extract showed a significant zone of inhibition in bacterial strains. Results also demonstrated that 600 µg/mL extract of Kiwi showed maximum zone of inhibition which concluded that the antimicrobial activity of Kiwi pulp is higher than kiwi peel and dose dependent i.e., higher the amount of Kiwi extract high will be the zone of inhibition. Elkichaoi et al., (2015) has studied the antimicrobial activity in kiwifruit peel and pulp and came to result that kiwi fruit also has good antibacterial activity that is in harmony of the result which also shows good antibacterial activity that can be due to presence of phenols and flavonoids.

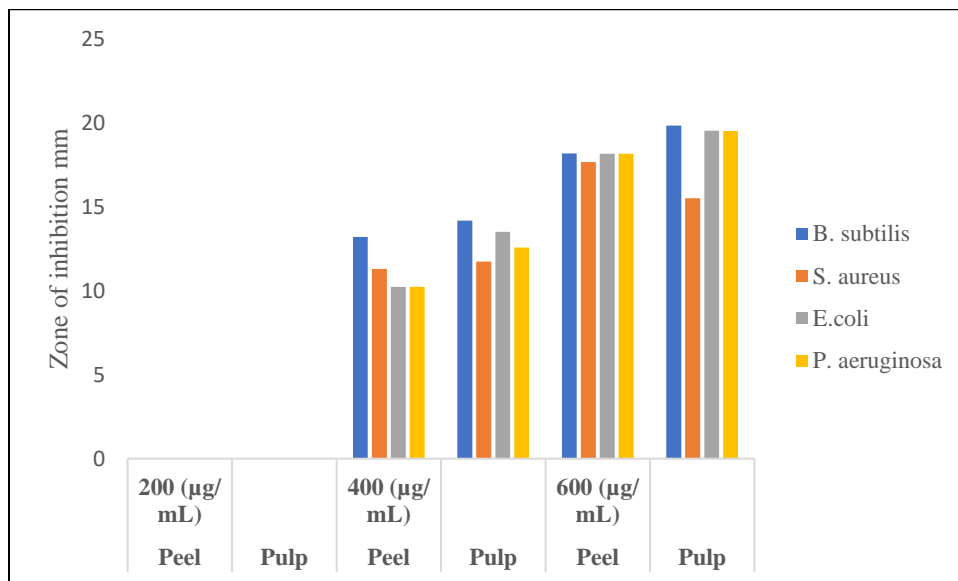


Figure 5 Graphical representation of antimicrobial activities of different extracts of Kiwi peel and pulp against bacterial strains

DISCUSSION

The kiwi fruit (*Actinidia Deliciosa*) is renowned for its health-promoting qualities, including low caloric value, low glycaemic index (GI), more vitamins (especially vitamin C), high potassium content, high dietary fibre content, presence of a variety of pigments (including chlorophylls, carotenoids, lutein, and anthocyanin), presence of a few key enzymes (such as proteases), low allergic response (AR) (Satpal et al., 2021). Its little seeds are dispersed throughout the entire fruit pulp and are edible, having their own health benefits. (Follett et al., 2019).

The kiwi fruit and peel had a large amount of fat, ash, soluble sugars, reducing sugars, proteins, fibre, moisture content, flavonoids, phenols, and tannins, according to a proximate study. According to Richardson et al. (2018), Kiwifruit also include flavonoids, phenols, and tannins in addition to many distinctive proteins, including the cysteine protease actinidin, which is the most prevalent protein in Kiwifruit. Nearly majority of the dietary fibre in kiwifruit comes from the plant cell walls, notably the polysaccharides that make up the bulk of these walls' structural elements. About 2-3% of the fresh weight of kiwifruit's cell walls are made up of non-starch polysaccharides, which are a valuable source of both soluble and insoluble fibre (Pinto et al., 2020). Nutritional fibre studies on green and gold have suggested that kiwifruit have roughly one-third soluble and two-thirds insoluble fibres, while Kiwi gold fruit have significantly less total fibre than green kiwifruit. The majority of the insoluble fibre is made up of cellulose and hemicelluloses, while the soluble fibre fraction is virtually entirely composed of pectic polysaccharides (Cremon et al., 2018). This study also reveals that Kiwi contains a significant amount of fibers in its fruit and peel.

The chemical component concentrations in the tissue of kiwifruit alter as they grow and ripen. The most significant alteration in the fruit's physiology occurs during ripening, which causes a quick drop in starch concentration and an increase in fructose and glucose as a result. While the kiwifruit is growing on the vine, the tissue is very hard, but as the fruit matures, the firmness of the flesh softens (Passafiume et al., 2020). Conversely, kiwis that are physically ready but have just begun to ripen can be picked and will successfully ripen off the vine. Immediately following harvest, cool storage slows the rate of ripening. These unique qualities of the kiwifruit enable producers like New Zealand to keep immature fruit and send it over a long

period of time to far-off markets. To make sure the fruit reaches the proper stage of growth before harvest, relevant markers of fruit maturity are applied. In order to accurately estimate the final eating quality of Kiwifruit, a "maturity value" that takes into consideration three changes—declining flesh hardness, starch conversion to sugar, and soluble solids concentration (to quantify sugar concentration—is utilised)(Richardson et al., 2018). This study also depicts that Kiwi contains a significant amount of soluble and reducing sugars in its fruit and peel.

The kiwifruit also contains a complex network of minor chemicals that may be linked to other advantageous physiological functions in addition to the major nutrients previously mentioned, for which there are dietary intake recommendations and well-described physiological functions. The antioxidant chemical profiles of many Actinidia species have undergone extensive analysis (Alim et al., 2019). As well as vitamins C and E, the other antioxidants include the carotenoids lutein, zeaxanthin and β -carotene, chlorophylls, quinic acid, caffeic acid glucosyl derivatives, β -sitosterol, chlorogenic acid, phenolics, including flavones and flavanones' to name but a few (Pérez-Burillo et al., 2018). Different in vitro chemical tests that track the quenching, scavenging, or delaying of free radical formation have been used to measure the antioxidant capacity of kiwifruit components (Dias et al., 2020). For instance, kiwifruit was shown to have a higher overall antioxidant capacity than apple, grapefruit, and pear, but less than raspberry, strawberry, orange, and plum (Razola-Díaz et al., 2022). The results of this study are also in accordance with previously reported studies that Kiwi peel has a significant amount of antioxidant as it showed remarkable properties of DPPH and ABTS⁺ radical scavenging properties.

Kiwi peel showed a greater antimicrobial activity against bacterial strains (Siddique *et al.* (2021) also reported that Kiwi fruit has shown antimicrobial activity apart from the antioxidant activity against many pathogenic bacteria as well as fungi and yeast also. With the presence of antibacterial, antifungal, and antioxidant activities in Kiwi fruit, it may be used as a potential medicinal fruit.

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