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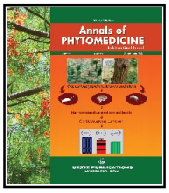

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Studies on nutrient and phytochemical composition and assessment of *in vitro* antioxidant and enzyme inhibitory properties of watermelon fruit by-products

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Abstract

Watermelon (*Citrullus lanatus* L.) by-products with high nutritional value have gained a lot of interest because these are highly valued, and have great potential to be added to the human diet. Peel and rind are usually discarded as the major by-products of watermelon fruit processing industries which could be a good source of many bioactive compounds. The present study was carried out to study the nutritional, anti-nutrient and phytochemical composition and bioactivities of the watermelon peel and rind extract. It was found that peel and rind are good sources of essential nutrients and also has negligible amounts of anti-nutrients. The extracts of peel and rind powder exhibited significant antioxidant activity, which could be attributed to the presence of phytochemicals like phenolic compounds, flavanols and flavonoids in the extract. Methanolic extracts of peel and rind showed efficient *in vitro* antidiabetic activity by inhibiting the carbohydrate hydrolyzing enzymes, hypolipidemic effect by inhibiting pancreatic lipase enzyme and anti-inflammatory activity by inhibiting hyaluronidase enzyme. This observation is supported by the presence of high levels of total phenolics, flavonoids and flavanols in the peel and rind which contributed to the *in vitro* enzyme inhibitory activity of these by-products.

1. Introduction

Fruit and vegetable processing industries generate significant amount of food wastes during processing. These large amounts of food waste arising from the processing of fruit and vegetable for the production of various food products are known as by-products which raise problems in disposal and also lead to loss of valuable biomass and nutrients. These by-products can be converted into value added products for the production of novel and functional foods from fruit and vegetable waste. Food waste utilization in product and drug designing could enhance food supply, health and the environment. The exploitation of fruit peels as a source of functional compounds for treatment of various metabolic disorders is the current focus area in the medical research (Saravana Kumari *et.al*., 2020). Research by Peschel *et al.* (2006) showed that by-products in general are composed of wide range of various biologically active compounds which are mostly discarded as wastes. Discarding without further utilization not only causes a loss in the valuable resources but also raises a problem of waste disposal. Utilization of these wastes could create alternative functional foods for human consumption. Such waste burden could be managed by increasing the dietary intake, product development and industrial utilization of the wastes, which leads to the basic investigative studies on the properties of processing food wastes. One such plant is watermelon (*C. lanatus*) and every part of this fruit including rind, peel and the seeds has got nutritional value. watermelon is a fruit commonly consumed in many countries. It is widely grown due to its large edible fruits that contain a hard-green rind and a watery reddish pulp inside.Watermelon is very rich in phytochemicals such as lycopene and carotenoids which are of great interest due to their antioxidant capacity. Watermelon is also a rich source of (non-essential) amino acid such as citrulline, it is an efficient hydroxyl radical scavenger and a strong antioxidant. It is an important product used in the production of various food products. The most useful part of the watermelon in the food industries is its pulp. The peel and rind are usually discarded as waste. In addition, rind is a good source of dietary fiber and phenolic compounds (Phisut Naknaen *et al*., 2016). But, more than 90% of the rind isusually discarded into the environment, thereby constituting environmental pollution. Watermelon peels account for roughly 30% of the total mass of the fruit. As a high-emission processing waste, watermelon peel has become a serious environmental concern, making it necessary to understand the sustainable value of the peel from both ecological and economic perspectives (Begum *et al*., 2017). A protection to the body may be due to the phytochemicals with their anti-inflammatory and immunomodulatory properties and there is abundance of naturally available medicinal plants with these properties that has some health benefits (Aditya Baruah *et.al*., 2021).These by-products of watermelon are not presently being utilized for any value addition due to limited research done on them which lead to the focus on conversion of this waste to value added products. Therefore, the aim of this research is to study the nutritional, anti-nutritional and phytochemical content of watermelon by-products. Peel and rind were assessed for its *in vitro* antioxidant and enzyme inhibitory properties.

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2. Materials and Methods

2.1 Procurement and preparation of raw materials

2.1.1 Procurement

Watermelon fruit peel and rind were procured from small scale juice processing units in Anantapur. Analytical grade chemicals were procured from SRL, SD Fine and Sigma companies.

2.1.2 Preparation of raw materials

Watermelon peels and rind were washed thoroughly with deionized water. They were sliced further with a stainless-steel knife. Any adhering pulp was scooped out from the rind with the spoon.

2.1.3 Preparation of peel and rind powder

The fruit peel was grated and rind was cut into small pieces with the stainless-steel knife and they were kept for drying in the tray drier at 40ºC. After drying, they were powdered using the laboratory mixer. It was stored in an airtight container for nutrient composition and antinutrient content analysis.

2.1.4 Preparation of solvent extracts

The total phenolic content and antioxidant activity is considerably affected by the properties of extracting solvents in fruits and vegetables. Higher the polarity, the better the extraction of phenolic compounds (Archana *et al.*, 2015). Different solvents like methanol, ethanol, ethyl acetate and acetone were used for the extraction of phytochemicals. Each solvent had different extraction efficiency. Methanol showed higher extraction efficiency than other solvents.Watermelon peel and rind were extracted with methanol and the extracts were stored in amber vials at 4ºC for phytochemical content analysis and assessment for *in vitro* antioxidant and enzyme inhibitory properties.

2.2 Analysis of watermelon fruit peel and rind extracts

Watermelon fruit peel and rind were estimated for nutrient composition, antinutrient content and phytochemical content. Peel and rind methanolic extracts were assessed for its *in vitro* antioxidant and enzyme inhibitory properties by using standard procedures. Standard methods were adopted as follows: Moisture, ash, total carbohydrates, fat, fiber and protein (AOAC, 2000), neutral detergent fiber, acid detergent fiber and hemicellulose (Van Soset and Wines, 1967), iron (Wong,1928), calcium, phosphorus by fiske and Subba Rao method and oxalates (Raghuramulu *et al*., 2003), tannins and phytates (Davies and Reid, 1979), total phenolic content (Singleton and Rossi, 1965), flavanols (Kumaran and Karunakaran, 2006), flavonoids (Chang *et al.*, 2002), 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity (Sreejayan and Rao, 1996), N,N-dimethyl-p-phenylenediamine (DMPD) scavenging activity (Fogliano *et al*., 1999), hydroxyl radical scavenging activity (Klein *et al*., 1991), reducing power and iron chelating capacity (Yamaguchi *et al*., 2000), α-amylase inhibitory activity (Miller 1959), α-glucosidase inhibitory activity (Mc Cue *et al*., 2005), Pancreatic lipase inhibitory activity (Kim and Kang, 2005), Hyaluronidase inhibitory activity (Fujitani *et al*., 1999).

2.3 Statistical analysis

Analysis of samples was carried out in triplicates. Values were expressed as means of three independent samples analysed in triplicate ± standard error of mean (SEM). The data analysis was done using Microsoft Excel software 2019.

3. Results

3.1 Proximate composition of peel and rind powder

Proximate composition of peel and rind powder is given in Table1. Moisture content of peel and rind were 80.58% and 93.57%. Fat in peel and rind was estimated to be 3.92% and 1.5%. Protein was found to be 7.1% in peel and 6.12% in rind. Fiber was estimated to be 22% for peel and 16% for rind which is almost comparable to the watermelon peel 26.31% as reported by Feumba Dibanda Romelle *et al.* (2016). Peel and rind had 1.02%, 1.15% of ADF, NDF was 1.12%, 1.19% in peel and rind. Hemicellulose content was found to be 0.58%, 0.72% in peel rind. Total carbohydrate content in peel and rind was 43.97% and 54.8%. Results showed that ash content of peel was higher than the rind. The ash content of peel and rind was 9.13% and 8.18%. The calcium content of rind in the present study was higher (600 mg/kg) than peel (400 mg/kg) and phosphorous content was lesser in rind 63.61 mg/kg when compared with the peel 92.4 mg/kg.

Table 1: Proximate composition of watermelon fruit peel and rind

Parameters	Peel	Rind
Moisture (%)	80.58 ± 0.023	93.57 ± 0.011
Ash (%)	9.13 ± 0.01	8.18 ± 0.002
Total carbohydrates (%)	43.97 ± 0.02	54.8 ± 0.02
Protein (%)	7.1 ± 0.025	6.12 ± 0.032
Fat (%)	3.92 ± 0.001	1.5 ± 0.04
Crude fibre (%)	22.00 ± 0.01	16.00 ± 0.032
Acid detergent fibre (%)	1.02 ± 0.02	1.15 ± 0.01
Neutral detergent fibre (%)	1.12 ± 0.011	1.19 ± 0.02
Hemicellulose (%)	0.58 ± 0.01	0.72 ± 0.01

Values are mean ± SE (n=3).

Table 2: Mineral composition of watermelon fruit peel and rind

Parameters	Peel	Seed
Iron (mg/100 g)	1.46 ± 0.03	1.05 ± 0.01
Calcium (mg/100 g)	400 ± 0.16	600 ± 0.05
Phosphorous (mg/100 g)	92.4 ± 0.05	63.61 ± 0.14

Values are mean ± SE (n=3).

3.2 Determination of antinutrients in watermelon fruit peel and rind

Since fruits are important sources of nutrients, it is important to analyse the antinutrient content of fruits as they limit the bioavailability of nutrients. Peel and rind of some fruits have good amounts of vitamins, minerals, fibre and other essential nutrients than the other parts of the fruit. Therefore, it is important to analyse the antinutrients content in them. The antinutrient composition in peel and rind are shown in Table 3.

Table 3: Antinutrient composition of watermelon fruit peel and rind

Antinutrients	Peel	Rind
Oxalates (mg/100 g)	8.00 ± 0.002	6.00 ± 0.001
Tannins (mg/100 g)	10 ± 0.002	5 ± 0.002
Phytates (mg/100 g)	7 ± 0.001	4 ± 0.002

Values are mean ± SE (n=3).

3.3 Solvent extraction

The total phenolic content and antioxidant activity is considerably affected by the properties of extracting solvents in fruits and vegetables. Higher the polarity, the better the extraction of phenolic compounds. Watermelon peel and rind were extracted with the methanol which was further concentrated and stored in a glass vial at 4ºC.

3.4 Phytochemical analysis of watermelon fruit peel and rind extract

The total phenolic content of peel and rind extract is shown in Table 4. The TPC of peel and rind extracts was 2173.45 and 1012 mg GAE/ 100 g. Flavonoids possess a number of health benefits such as antioxidant, anti-inflammatory and antiviral properties. Flavonoids help regulate cellular activity and fight against free radicals in the body. Flavonoid content was 20.14 mg, 15.36 QE/100 g in peel and rind. Intake of flavanols is found to be associated with a wide range of health benefits which include antioxidant potential and risk of vascular disease. Flavanol content was 110.87 mg, 98.74 RE /100 g in peel and rind.

Table 4: Phytochemical composition of passion fruit by-products

Parameters	Peel	Rind
Total phenolic content (mg GAE/100 g)	2,173.45 ± 0.01	1,012.00 ± 0.02
Flavonoids (mg QE /100 gm)	20.14 ± 0.01	15.36 ± 0.02
Flavonols (mg RE/100 gm)	110.87 ± 0.02	98.74 ± 0.02

Values are mean ± SE (n=3).

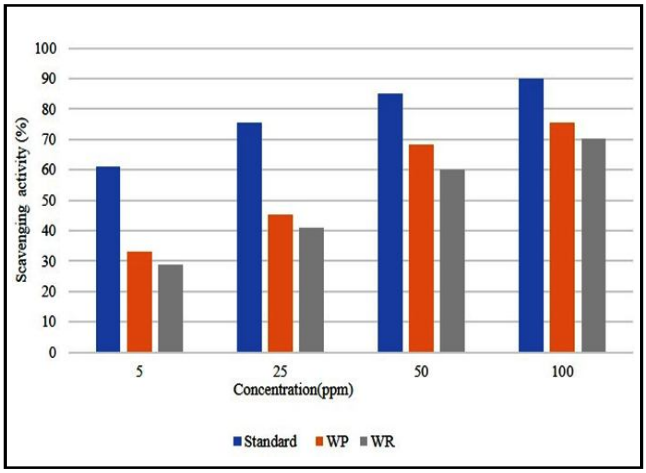

Figure 1: DPPH radical scavenging activity of watermelon fruit peel and rind.

3.5 Assessment of *in vitro* antioxidant activity of peel and rind extracts

3.5.1 DPPH radical scavenging activity

Dose dependent DPPH radical scavenging activity of methanolic extracts are presented in the Figure 1. Methanolic extract of peel and rind exhibited a significant scavenging activity at 500 ppm with IC_{50} values of 32.63 µg/ml and 45.26 µg/ml, respectively.

3.5.2 N, N-dimethyl-p-phenylenediamine (DMPD) scavenging activity

DMPD radical cation decolorization method has been developed for the measurement of the antioxidant activity in food and biological samples. The assay can equally be applied to the determination of antioxidant activity of both hydrophilic and lipophilic antioxidants. The experimental procedure is rapid and ensures sensitivity along with reproducibility in the measurement of antioxidant activity of both hydrophilic and lipophilic compounds, and thus has a promising aspect of use in screening fruit samples. Dose dependent DMPD radical scavenging activity of peel and rind methanolic extract is shown in Figure 2. It exhibited a significant scavenging activity at 500 ppm with IC_{50} values of 353.60 µg/ml and 384.78 µg/ml of peel and rind, respectively.

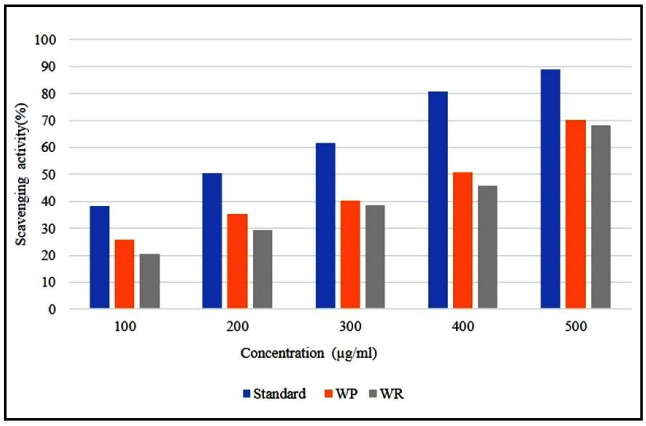

Figure 2: DMPD activity of watermelon fruit peel and rind.

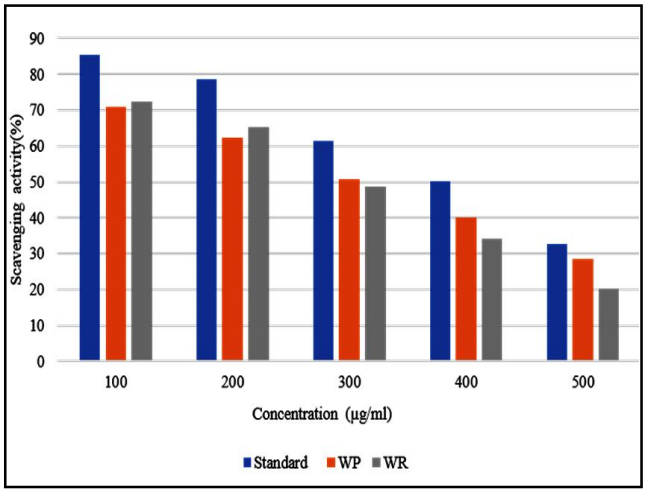

Figure 3: HRSA activity of watermelon fruit peel and rind.

3.5.3 Hydroxyl radical scavenging activity (HRSA)

Concentration dependent hydroxyl radical scavenging activity exhibited by methanolic extract of peel and rind is shown in Figure 3. The highest activity was shown at 500 ppm with IC_{50} values of 294.50 µg/ml and 314.80 µg/ml of peel and rind, respectively.

3.5.4 Reducing power

There was an increase in the absorbance level as the concentration increased. With increase in the amount of extract, in the range of 1-20 µg/ml, the reducing power also increased as shown in the Figure 4. The reducing power measures the ability of the extract to donate electrons to Fe (III). The results indicate that the peel can be used as a natural antioxidant as it exhibited significant radical scavenging activity and reducing power.

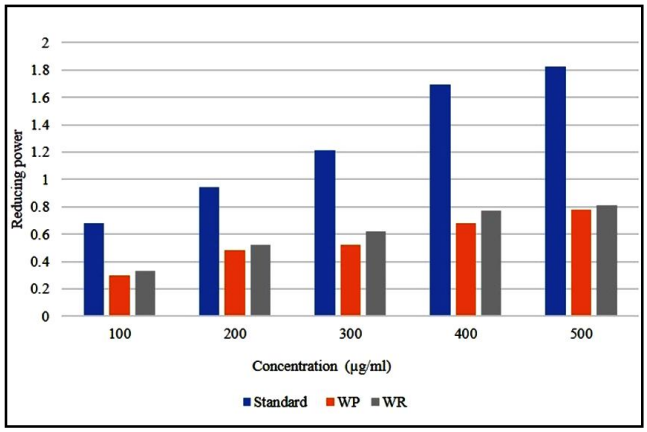

Figure 4:Reducing power of watermelon fruit peel and rind.

3.5.5 Iron chelating activity

Iron chelating effect exhibited by methanolic extract of peel and rind is displayed in Figure 5. In the present analysis, it exhibited potential activity at 500 ppm with IC_{50} values 170.53 µg/ml and 140.67 µg/ml, respectively.

3.6 Assessment of *in vitro* enzyme inhibitory analysis of peel and rind extracts

3.6.1 Enzyme inhibitory analysis

3.6.1.1 Antidiabetic activity

The antidiabetic activity of methanolic extracts of peel and rind was assessed in terms of inhibition of carbohydrate hydrolysing enzymes, *i.e.*, α-amylase and α-glucosidase.

3.6.1.2 α-amylase inhibition activity

The concentration dependent α-amylase inhibition activity exhibited by the methanolic extracts of peel and rind is shown in Figure 6. Methanolic extracts exhibited a significant inhibition activity on α-amylase with IC_{50} values of 287.43 µg/ml in peel and 334.21 µg/ml in rind, respectively. As the concentration increases the samples showed increased, activity on α-amylase inhibition. There exists a significant difference (p<0.05) between peel and rind inhibiting activity.

3.6.1.3. α-glucosidase inhibition activity

Concentration dependent α-glucosidase inhibition activity exhibited by the methanolic extracts of watermelon fruit peel and rind is represented in Figure 7. The highest activity was shown at the concentration 500 µl with IC_{50} values 343.43 µg/ml in peel and 385.85 µg/ml in rind, respectively. There exists a significant difference (p<0.05) between peel and rind inhibitory activity.

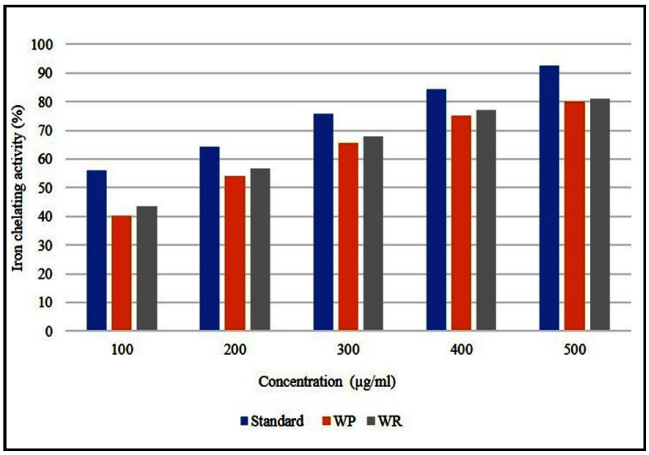

Figure 5: Iron chelating capacity of watermelon fruit peel and rind.

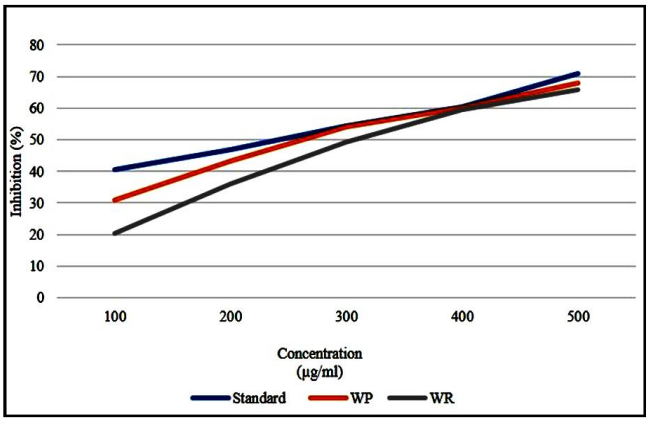

Figure 6: α-amylase inhibitory activity of methanolic extracts of watermelon fruit peel and rind.

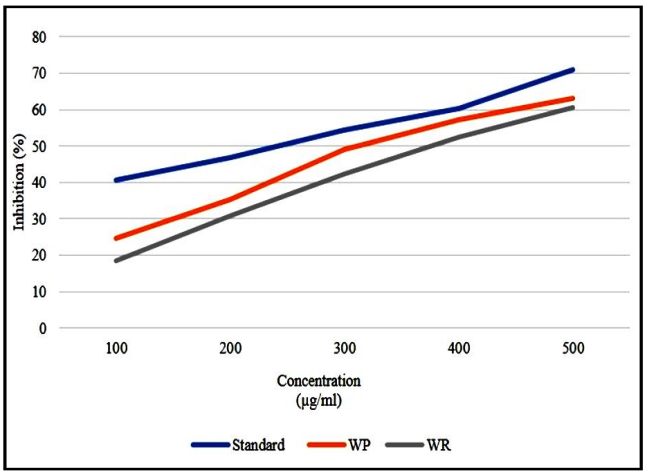

Figure 7: α-glucosidase inhibitory activity of methanolic extracts of watermelon fruit peel and rind.

3.6.2 Hypolipidemic effect

3.6.2.1 Pancreatic lipase inhibitory activity

The results are represented in Figure 8 shows the concentration dependent pancreatic lipase inhibition activity exhibited by the methanolic extracts of peel and rind. Methanolic extracts of peel and rind exhibited a significant inhibition activity on pancreatic lipase with IC_{50} values 332.34 µg/ml and 373.82 µg/ml, respectively. As the concentration increased, the samples showed increased activity on pancreatic lipase inhibition. There exists a significant difference (p<0.05) between peel and rind inhibition activity.

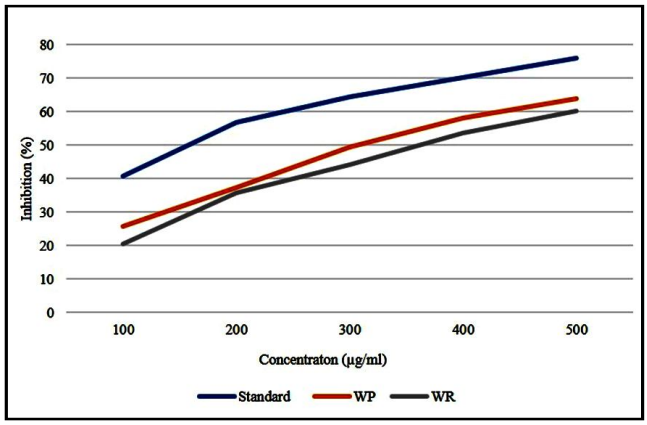

Figure 8: Pancreatic lipase inhibitory activity of methanolic extracts of watermelon fruit peel and rind.

3.6.3 Anti-inflammatory activity

3.6.3.1 Hyaluronidase inhibitory activity

The results represented in Figure 9. shows the concentration dependent hyaluronidase inhibition activity exhibited by the methanolic extracts of peel and rind. Methanolic extracts of peel and rind exhibited a significant inhibition activity on hyaluronidase with IC_{50} values 341.01 µg/ml and 408.58 µg/ml, respectively. As the concentration increased, the samples showed increased activity on hyaluronidase inhibition. There exists a significant difference (p<0.05) between peel and rind inhibition activity.

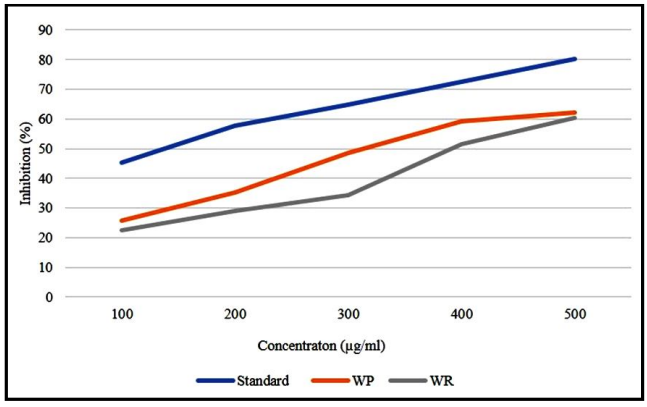

Figure 9: Hyaluronidase inhibitory activity of methanolic extracts of watermelon fruit peel and rind.

4. Discussion

Ash content is generally due to the presence of minerals like calcium, iron and phosphorus. As both peel and rind showed significant mineral content, they can be used as an alternate natural source of minerals. It represents a novel source for new food products and can be used in fortified products.The antinutrient composition in peel and rind was found to be in trace amounts which would not interfere with the absorption of nutrients. Oxalates, phytates and tannin content of watermelon rind is slightly lesser than the watermelon peel.

Despite its great potential, the existing knowledge about phenolic composition in watermelon by-products is limited, especially in peel. These by-products found to be a good source of phytochemicals which could be used as natural ingredients in the food industries. Peel and rind extracts exhibited significant *in vitro* antioxidant activities. This observation is supported by the presence of high levels of dietary fiber, total phenolics, flavonoids and flavanols.

In the present study, α-amylase inhibition activity of methanolic extracts can be due to the presence of certain compounds like phenols, flavonoids and flavanols which had inhibiting capacity of enzymes. Carbohydrate hydrolyzing enzymes, *i.e.*, α-amylase and α-glucosidase convert complex carbohydrates and starch to simple sugars, facilitating the release of glucose into the blood. The inhibition of these enzymes delays the digestion of starch and impairs the digestion of disaccharides, thus being an effective tool for the management of diabetes (Nupur Mehrotra *et.al.*, 2019). Hence, watermelon fruit peel and rind proved to be a good therapeutic agent that could be used in diabetes therapy.

Disaccharides are broken down by α-glucosidase enzyme to simple sugars, which are readily available for intestinal absorption. In the digestive tract of human, inhibition of their activity is considered to be effective tool to control diabetes (Hara and Honda, 1990). Thus, in the present investigation, the presence of optimal content of phenolics, flavonoids and flavanols could be responsible for inhibition of α-glucosidase enzyme. Hence, the watermelon fruit peel and rind can be used in the treatment of diabetes since they possess inhibitors of α-amylase and α-glucosidase enzymes.

One of the strategies used in the discovery of antiobesity drug is through inhibition of activity of pancreatic lipase. Few classes of natural products were evaluated for their pancreatic lipase inhibitory activity which is essential for preventing obesity problem. The inhibitory capacity of extracts could be attributed to the total phenolics as polyphenols have been reported to have antiobesity effects by inhibiting the activity of pancreatic lipase (Lei *et al.*, 2007). Therefore, the watermelon fruit peel and rind can be used in the treatment of obesity and eating disorders.

Recent reports suggest that hyaluronidase, an enzyme known to be involved in tissue inflammation, participates in a type I allergic reaction. The IC_{50} value, equivalent to the concentration required for 50% inhibition of hyaluronidase inhibitory activity in *in vitro*, is used to compare the inhibitory activity. It has been used to denote a group of enzymes from different sources that catalyse the depolymerisation of certain acidic glycoaminoglycans involved in tissue inflammation (Tamakoshi, 1997). Many synthetic drugs have potent anti-inflammatory action, but due to significant side effects many drugs are less used for long term treatment (Punit Bhatt *et.al.*, 2019). Fruits contain many nutrients like omega-3-fatty acids,

vitamins. minerals and many phytochemicals which possess anti-inflammatory properties that are directly or indirectly connected to the immune system (Johra Khan, 2021). In the present study, the inhibition capacity could be due to the presence of phenolics and flavonoids.

5. Conclusion

Watermelon fruit by-products were good source of essential nutrients. Peel and rind had good amounts of phytochemicals such as total phenols, flavonoids and flavanols. Peel and rind extracts exhibited significant *in vitro* antioxidant activity which indicated the presence of more potent compounds in it. Methanolic extracts of peel and rind showed efficient *in vitro* antidiabetic activity, hypolipidemic activity and anti-inflammatory activity by inhibiting the enzymes and it could be attributed due to the presence of significant phytochemicals in it. Since results showed that peel and rind were good source of nutrients and bioactive components, they could be used in developing functional foods and for therapeutic purposes.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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