

# *Ribes nigrum* and *Juglans regia*, Potential Functional Foods as a Source of Protein Tyrosine Phosphatase Enzyme: Biochemical and Kinetics Studies

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Received: 20.11.2021; Accepted: 18.12.2021; Published: 2.02.2022

**Abstract:** Functional foods have gained substantial attention in the healthcare industry for their less explored bioactive ingredients with significant therapeutic potential. Extensive research in the fields of plants is required to identify the sources of PTPase, a key enzyme in regulating proteins and involved in various ailments. *Ribes nigrum* (Munakka; M), *Juglans regia* (Walnut; W); dry fruit and nut, have been purified through salt fractionation. In M, 0-45% fraction exhibited a 3.51-fold increase in purification with specific activity of 10.39 U.mg<sup>-1</sup> (M), whereas in W, 45-90% fraction exhibited values of 7.20-fold and 31.35 U.mg<sup>-1</sup> (W). PTPase from W and M exhibited thermostability up to 70°C and 60°C, respectively, with 75% activity retention. K<sub>m</sub> values were determined to be 4.67 mM (W) and 14.29 mM (M), respectively whereas, the activation energy (E<sub>a</sub>) values were 30.59 KJ.mol<sup>-1</sup> (W) and 51.84 KJ.mol<sup>-1</sup> (M). Fe<sup>2+</sup>, Mn<sup>2+</sup>, and Zn<sup>2+</sup> ions showed prominent inhibitory effects on PTPase; K<sub>i</sub> value showed Mn<sup>2+</sup> ions exhibit strong binding affinity differentially in PTPase from two sources of 0.075 mM(W), 0.103 mM(M) may have clinical importance in the future. The findings demonstrate that dry fruit and nuts are sources of PTPases as potential functional foods that may serve significant multifunctional eatery for holistic development.

**Keywords:** PTPase; specific activity; inhibition; functional food; holistic development; biochemical; kinetics.

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## 1. Introduction

The concept of functional foods comprises additional bioactive components that can benefit consumer health by minimizing disease-related risk factors [1-3]. Global consumption of functional foods and their market worth was 162 billion USD in 2018 and are predicted to reach 280 billion USD by 2025, growing at an annual rate of 8% [4]. Numerous studies focus on functional foods derived from plants as antioxidants, antibacterial, anticancer, antihypertensive, antihypercholesterolemic, anticoagulant, and anti-inflammatory agents [5-7]. Functional foods are affordable and cost-effective for pharmaceutical product development. However, only a few potential natural sources have been clinically documented as moderate evidence. The mechanism of action of many functional foods, such as nuts, berries, garlic, and dried fruits, is still unknown [8-11].

The food and pharmaceutical sectors' primary focus toward functional food is advancing their scientific knowledge of formulation and processing to meet customer expectations for health and sustainability claims [12,13]. *Ribes nigrum* (Munakka; M) and *Juglans regia* (Walnut; W) are a few examples of such potential functional foods. *J. regia*, more commonly referred to as Persian or English walnut, is a member of the genus *Juglans* and family *Juglandaceae* [14]. Many people eat raw nuts, and they have also been used to flavor various cuisines. They contain a rich source of flavonoids, vitamins, minerals, lipids, proteins, and polyunsaturated fatty acids [15,16]. In addition to this, *J. regia* has therapeutic significance in neurodegenerative diseases, antioxidant potential, anti-diabetic potential, antihyperlipidemic, hypoglycaemic, antimicrobial, and antihypertensive effects [17-20]. *Ribes nigrum* (Munaka; M) is a commonly consumed berry that contains a variety of medicinal components, including vitamins, phenolic compounds, flavonoids, anthocyanins, polysaccharides, and organic acids [21,22]. As an immunostimulatory, anticancer, antioxidant, antibacterial, and anti-inflammatory agent [23,24], it contributes to promoting a healthy lifestyle. All of this into account, *R. nigrum* (M) and *J. regia* (W) were considered probable sources of phosphatase activity, particularly at tyrosine residues.

Protein Tyrosine Phosphorylation is an enzyme that contributes to the regulation of cellular signal transduction by catalytically removing the phosphate group from phosphorylated tyrosine residues on proteins via protein tyrosine kinases (PTKases) and protein tyrosine phosphatases (PTPases) [25,26]. There are 107 protein tyrosine phosphatases, all of which share the conserved active site domain C(X)<sub>5</sub>R, which causes drug discovery difficult. They are critical in the regulation of cells in both plants and animals. Attempts have been made to identify potential compounds capable of selectively inhibiting oncogenic Tyr phosphatases using various moieties. Still, no strong preclinical candidate has been identified, reinforcing the notion that Tyr phosphatases are not a druggable protein class [27-29]. Genomic screening, biochemical characterization, and inhibition of phosphatases all contribute to understanding protein phosphatase's functional relevance, notably in plants [30-33]. However, our understanding of the molecular mechanisms behind PTPases in many signaling cascades remains limited. Considering all of this, the PTPase enzyme is a great pharmacological target and plays a critical role in inhibition studies. The sources cited previously have a limited amount of data on genetic relevance. As a result, the current study evaluated dry fruits and nuts as prospective sources of PTPase and conducted biochemical and kinetic analyses on enzymes.

## 2. Materials and Methods

### 2.1. Biological material and chemical.

Green earth products Pvt. Ltd. supplied biological material (dry fruits and nuts). SIGMA chemicals (St. Louis, Missouri, USA) provided the O-phospho-L-tyrosine substrate. All other chemicals were of analytical grade purchased from standard commercial firms.

### 2.2. Partial purification of protein tyrosine phosphatases.

All operations were carried out between 0-4 degrees Celsius (unless stated). Homogenization buffer (50 mM Tris-HCl, pH-7.6, 50 mM NaCl, 0.04 % -mercaptoethanol, 5 mM EDTA, 1 mM PMSF) was used to crush plant tissue in a Waring blender. The homogenate

was centrifuged and filtered through four layers of pre-sterilized cheesecloth. The supernatant obtained in this manner is referred to as crude extract.

### 2.3. Enzyme assay and estimation of total protein.

#### 2.3.1. Assay for the activity of Protein Tyrosine Phosphatase (PTPase).

The activity of the PTPase was determined using O-phospho-L-tyrosine (SIGMA) as the substrate. The reaction mixture was assayed for inorganic phosphate ( $P_i$ ) release using the Malachite green assay described by Lanzetta *et al.* (1979) [34], and the amount of  $P_i$  released was quantified by referring to  $Na_2HPO_4$  as a  $P_i$  standard at 660 nm.

A unit of enzyme activity is defined as the amount of protein required to release 1 nM of  $P_i$  per minute per mL under standard test conditions.

#### 2.3.2. Protein quantification.

The method of Lowry *et al.* (1951) [35] was used for the estimation of proteins using BSA as standard.

#### 2.3.4. Specific activity determination and purification fold.

The samples' specific activity (PTPase) was calculated by the ratio of the total number of enzyme units to the total amount of protein (in mg). Purification-fold was defined as the ratio of the sample's specific activity to that of a crude enzyme.

### 2.4. Biochemical characterization of partially purified PTPase from dry fruits and nuts.

The following parameters were determined for partially purified protein tyrosine phosphatases (PTPases) for *R. nigrum* and *J. regia*.

#### 2.4.1. Optimal pH values and pH stability.

Appropriate buffers with a pH range of 3.0-9.0 were added to the reaction mixture under standard assay conditions, and the relative PTPase activity was calculated and represented as a percentage. To test pH stability, PTPases were incubated alone in buffers ranging from 3.0 to 9.0 under normal conditions, and the relative PTPase activity was calculated and expressed as a percentage.

#### 2.4.2. Temperature stability and optimum temperature values.

PTPase activity was evaluated at temperatures ranging from 10<sup>0</sup>C to 90<sup>0</sup>C using a standard protocol. The activity was quantified in terms of relative values (%). Thermostability was determined by incubating the PTPases alone for 24 hours at temperatures ranging from 10<sup>0</sup> to 90<sup>0</sup> Celsius and assaying the enzyme activity under standard conditions; enzyme activity was represented as relative PTPase activity (in percentage).

#### 2.4.3. Determination of $K_m$ (Michaelis-Menten constant) and $V_{max}$ (Maximum velocity).

The concentration of the substrate (O-phospho-L-tyrosine) was changed under alternative optimum assay conditions, and the Michaelis-Menten constant ( $K_m$ ) and maximum

velocity ( $V_{max}$ ) values were calculated using a Lineweaver-Burk reciprocal plot of  $1/V_0$  vs  $1/[S_0]$ .

#### 2.4.4. Metal ions have an effect.

The effect of metal ions on PTPases was investigated by adding 30 mM concentrations of  $Cu^{2+}$ ,  $Mg^{2+}$ ,  $Zn^{2+}$ ,  $Mn^{2+}$ ,  $Ca^{2+}$ ,  $Fe^{2+}$ , and  $Ni^{2+}$  in the form of respective salts to the reaction mixture and incubating for an hour at  $30^{\circ}C$ . The activity was determined upon incubation. The control sample was assumed to have 100% activity, and the residual activity (%) of treated samples was measured in relation to the control sample's activity.

#### 2.5. Inhibition kinetics.

By altering the substrate concentration, PTPase activity was detected in the absence and presence of inhibitors at various concentrations. The nature of inhibition for each inhibitor was evaluated using the Line weaver-Burk reciprocal plot of  $1/V_0$  against  $1/[S_0]$ .

##### 2.5.1. $V_{max}$ and $K_m$ .

The  $V_{max}$  and  $K_m$  values for the PTPases from *R. nigrum* (M) and *J. Regia* (W) were calculated by plotting  $1/V_0$  versus  $1/[S_0]$  (Double reciprocal plot) using different substrate concentrations (2.5 mM-25 mM) described in standard protocols to measure the rate of catalysis  $V_0$  (reaction velocity) for various substrate concentration  $[S_0]$  by plotting  $1/V_0$  versus  $1/[S_0]$  (Double reciprocal plot) using Lineweaver–Burk method (1934).

##### 2.5.2. Energy of activation.

The activation energy and reaction rate correlation were calculated by the Arrhenius equation:

$$k=A.e(-Ea/RT)$$

where  $k$  is the rate of reaction,  $A$  denotes the preexponential factor,  $Ea$  denotes the activation energy,  $R$  denotes the gas constant, and  $T$  denotes the temperature (in Kelvin),

The activation energy of the PTPases was determined by calculating enzyme activity at  $40^{\circ}C$  and  $50^{\circ}C$ .

##### 2.5.3. Coefficient of temperature.

The temperature coefficient of PTPases from *R. nigrum* (M) and *J. regia* (W) was determined by measuring the rate of reaction increments for each 10-degree ascend in temperature.

The temperature coefficient ( $Q_{10}$ ) is calculated by the following formula:

$$Q_{10}=(R_2/R_1) (10/T_2-T_1)$$

where  $R_1$  denotes the initial rate,  $R_2$  denotes the final rate,  $T_1$  denotes the initial temperature in ( $^{\circ}C$ ),  $T_2$  denotes the final temperature in ( $^{\circ}C$ )

#### 2.6. Inhibition kinetics of PTPases using Divalent cations as inhibitors.

The type of inhibition was determined by adding metal ions acting as PTPase inhibitors in varying concentrations (ranging from 2.5 mM to 25 mM) and determining the type of inhibition by plotting  $1/V_0$  versus  $1/[S_0]$  (Lineweaver Burk plot) and comparing to the reaction

performed in parallel without any inhibitor; the pattern of plots indicating competitive, uncompetitive, or mixed inhibition.

2.6.1. Calculation of the Inhibition constant ( $K_i$ ).

Due to the fact that metal ions,  $Fe^{2+}$ ,  $Zn^{2+}$ , and  $Mn^{2+}$ , in their respective salts, significantly inhibited PTPase activity at various concentrations ranging from 0.5 mM to 5.0 mM were added and the experiment performed. The inhibition constant ( $K_i$ ) values were established by graphing  $1/V_0$  versus  $I_0$ .

2.7. Statistical analysis.

The differences in control and samples (triplicate runs) were determined by one-way ANOVA (SPSS). The values were considered to be statistically significant at  $p < 0.05$ .

3. Results and Discussion

Five different nuts and dried fruits were studied for PTPase enzyme. After extraction, the crude extract was evaluated for PTPase specific activity using the enzyme assay and protein quantification.

3.1. Specific activity of PTPases from nuts and dried fruits.

The specific activity of PTPase isolated from various plant sources is summarised in Table 1. In addition, the specific activity of PTPases was determined from *R. nigrum* (M), and *J. regia* (W) was found to be higher and, therefore, characterized further.

**Table 1.** The specific activity of protein tyrosine phosphatases (PTPases) obtained from various dried fruits and nuts.

S.No.	Plant Source	Specific Activity of PTPase (U.mg <sup>-1</sup> )
1.	Walnut ( <i>Juglans regia</i> )	4.364
2.	Munakka ( <i>Ribes nigrum</i> )	2.964
3.	Cashew ( <i>Anacardium occidentale</i> )	1.602
4.	Almonds ( <i>Prunus dulcis</i> )	2.641
5.	Raisins ( <i>Vitis vinifera</i> )	0.86

\* Results are mean of triplicates

3.2. Partial purification of PTPases by ammonium sulfate fractionation.

The crude aqueous extracts of *R. nigrum* (M) and *J. regia* (W) were separated into two fractions using ammonium sulfate fractionation as 0–45% and 45–90%.

**Table 2.** Purification profile of PTPase from potential dried fruits and nuts using ammonium sulfate fractionation.

Fractions	Specific Activity (Umg <sup>-1</sup> )		Purification-fold	
	<i>Juglans regia</i> (W)	<i>Ribes nigrum</i> (M)	<i>Juglans regia</i> (W)	<i>Ribes nigrum</i> (M)
Crude	4.36	2.96	1.00	1.00
0-45%	9.49	10.39	2.18	3.51
45-90%	31.35	7.67	7.19	2.59

\* Results are mean of triplicates

The fractions were tested for determining their specific activity, as given in Table 2. In the case of *R. nigrum* (M) specific activity in 45-90% fraction was found to be 10.39 U.mg<sup>-1</sup> whereas in *J. regia* (W) and values being 31.35 U.mg<sup>-1</sup> (0-45%). The purification level was 7.19-fold *J. regia* (W), and 3.51-fold *R. nigrum* (M) fold increase, respectively.

### 3.3. Biochemical characterization of PTPase from nuts and dried fruits.

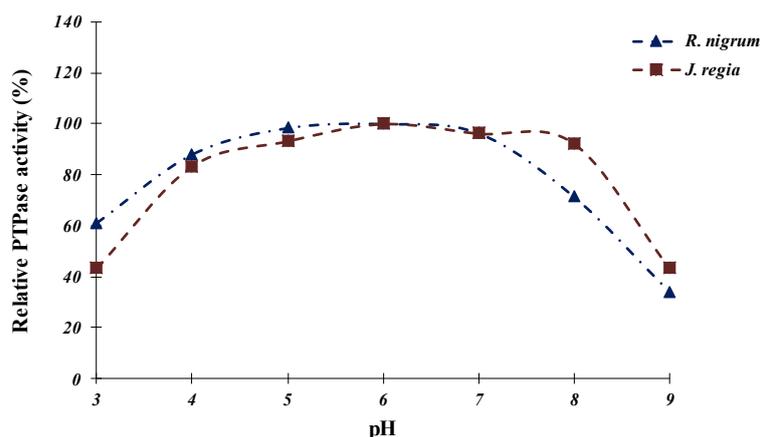
**Table 3.** Kinetic parameters of PTPase isolated from dry fruit and nuts.

S. No.	Parameter	Values	
		<i>Juglans regia</i> (Walnut; W)	<i>Ribes nigrum</i> (Munakka; M)
1.	pH optima	7.0	6.0
2.	Temperature optima	40°C	30°C
3.	pH stability	4.0-7.0	5.0-7.0
4.	Temperature stability	50°C	60°C
5.	Temperature coefficient (Q <sub>10</sub> )	1.130	1.852
6.	Energy of activation (E <sub>a</sub> )	10.86 KJ.mol <sup>-1</sup>	51.84KJ.mol <sup>-1</sup>
7.	K <sub>m</sub>	8 mM	14.29 mM
8.	V <sub>max</sub>	31.25 nM. min <sup>-1</sup>	25 nM. min <sup>-1</sup>

\* Results are mean of triplicates

#### 3.3.1. pH optima and pH stability of PTPases.

The stability of an enzyme's conformation and its catalytic activity is proportional to its pH [36]. The effect of pH on PTPase assessed by varying pH (3.0 to 9.0) with values are mentioned in table 3. The maximum PTPase activity in *R. nigrum* (M) was found at pH 6.0, whereas, in the case of *J. regia* (W), the value was pH 7.0. Good agreement was found when comparing results from Jiang *et al.* (2015) [37] on osteoclastic protein tyrosine phosphatase shows maximum activity at pH 7.0. Remarkably, PTPase activity was stable after incubation of the enzyme for 24 h at pH range 4.0-7.0 in the case of *R. nigrum*, whereas in *J. regia* the PTPase retained activity up to pH range 5.0-8.0, as illustrated in Figure 1.



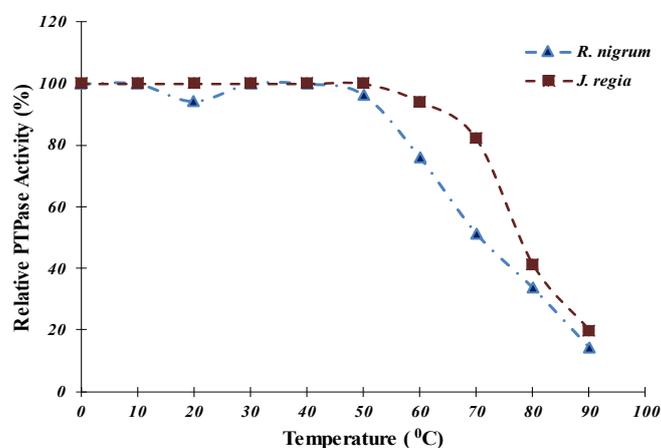
**Figure 1.** pH stability curve of PTPase enzyme from *Juglans regia* (Walnut; W) and *Ribes nigrum* (Munakka; M) by incubating enzyme alone for 2 h with buffers of pH values ranging from 3.0 to 9.0 and further assayed using optimum conditions. The results are expressed as relative PTPase activity (taken in percentage). Mean error bars in the figure represent the mean ± standard error from the triplicate samples tested (p< 0.0005).

The above results suggested that the obtained PTPases retain 70% activity till pH 8.0. This pattern of observation has been shown in previous works on microbial species such as *Lactobacillus Rhizopus* microspores, *Metarhizium anisopliae* Strain CQMa102, *Aspergillus*

*caespitosus*, *Geobacillus thermodenitrificans*, with optimum pH 5.0-8.0 and stable up to pH 3.0-9.5 [38-42].

### 3.3.2. Temperature optima and stability of PTPases.

The temperature optima for partially purified PTPase obtained from *J. regia* (W) and *R. nigrum* was found to be 50<sup>0</sup>C, and 30<sup>0</sup>C, respectively. These results were also supported by studies on *Fenneropenaeus merguensis*, *Alcaligenes faecalis* [43-44] having optimum up to 50<sup>0</sup>C whereas *Bacillus flexus* and *Rhizobium sp.* being stable till 35<sup>0</sup>C [45-46]. PTPase is stable up to 50- 60<sup>0</sup> C, as shown in Figure 2. Around 75 % of enzyme activity was maintained in *J. regia* (W) till 70<sup>0</sup> C, whereas *R. nigrum* (M) was stable up to 60<sup>0</sup>C. These results match well with existing studies on *O.megacantha* Salm-Dyck, *Macrotylomaui florum* seeds, *Rhizopus* microspores var. *rhizopodiformis*, *G. thermodenitrificans* which are stable up to 65<sup>0</sup>C [47-48,40,38]. Similarly, *Trachyspermum ammi*, *Cinnamomum verum*, *Metarhizium anisopliae* strain CQMa102 PTPase are stable up to 70<sup>0</sup>C [49, 39].

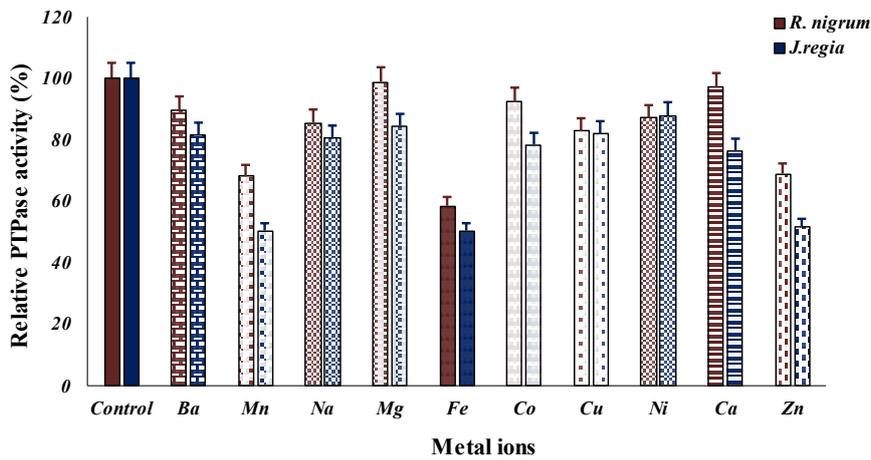


**Figure 2.** The temperature stability curve of PTPase from *Juglans regia* (Walnut; W) and *Ribes nigrum* (Munakka; M) was determined by incubating the enzyme (alone) at temperatures from 10<sup>0</sup>C to 90<sup>0</sup>C for 2 h and further assayed under standard conditions. The results are expressed as relative PTPase activity (taken in percentage). Mean error bars in the figure represent the mean ± standard error from the triplicate samples tested (p< 0.0005).

Wang *et al.* (2009) also reported similar observations against *Thermus thermophilus* HB27[50]. The stability of protein depends on the presence of amino acid residues such as proline, isoleucine, and valine, as well as polar charged amino acid residues that offer stability to the protein. The presence of disulfide bridges is to enhance the thermostability through strong interactions and can stabilize the protein by decreasing the entropy of the unfolding state [51-53]. The present work results show PTPase from the *J. regia* (W) shows substantial thermostability.

### 3.3.3. Effect of metal ions on PTPases.

Metal ions can activate cofactors in a catalytic mechanism to accelerate or inhibit reactions. They can also interact with the protein to alter its structural conformations. Studies on divalent metal ions such as Cu<sup>2+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>, Ca<sup>2+</sup>, Fe<sup>2+</sup>, Ni<sup>2+</sup> used at 10 mM concentration, and the results are illustrated in Figure 3. Fe<sup>2+</sup>, Zn<sup>2+</sup>, and Mn<sup>2+</sup> showed a noticeable inhibitory effect on PTPase from all three sources.



**Figure 3.** Effect of various metal ions on PTPase activity of *Juglans regia* (Walnut; W) and *Ribes nigrum* (Munakka; M) by adding metal ions to the reaction mixture for one-hour incubation at 30°C. Untreated metal ions can be taken as control. The results are expressed as relative PTPase activity (taken in percentage). Mean error bars in the figure represent the mean ± standard error from the triplicate samples tested ( $p < 0.0005$ ).

The disulfide bond alterations on PTPase helps to target cysteine residues to regulate its activity via the change in molecular architecture [54]. This might be achieved with the help of zinc and other metal ions. Similar results reported by Kim *et al.* (2000) showed that  $Fe^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ , and  $Cd^{2+}$  inactivates human dual-specificity protein phosphatase VHR *in vitro*[55]. These findings also go well with *Nelumbo nucifera*, *B. licheniformis* MTCC 1483, lactic acid bacteria, *Metarhizium anisopliae* strain CQMa102, *Geobacillus thermodenitrificans*, *Pinctada fucata* based phosphatases were inhibited by  $Mn^{2+}$  and  $Zn^{2+}$  [39,38,56-59]. Likewise,  $Fe^{2+}$  can also inhibit phosphatases from *Vigna radiata* seeds (Saeed *et al.*, 2014)[60].

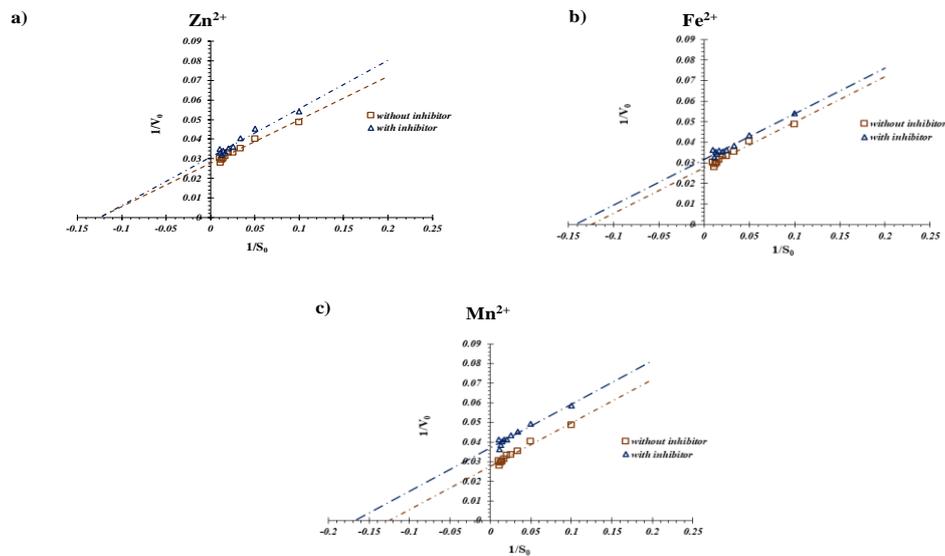
### 3.3.4. Kinetic parameters.

The Michaelis constant ( $K_m$ ) expresses the enzyme's affinity for a substrate, with smaller values indicating greater affinity. The kinetic parameters such as  $K_m$  and  $V_{max}$  were calculated by increasing substrate concentration and plotting a double-reciprocal (Lineweaver–Burk) plot of PTPase obtained from *J. regia* (W); the corresponding values were 4.67 mM and 20.83 nM.min<sup>-1</sup>, respectively. In *R. nigrum* (M),  $K_m$  and  $V_{max}$  values were determined to be 14.29 mM and 25 nM.min<sup>-1</sup>. The temperature coefficient and energy of activation values for the enzyme were calculated to be 1.410 and 30.59 kJ.mol<sup>-1</sup>, respectively, in *J. regia* (W) and for *R. nigrum* (M) values are 1.852 and 51.85 kJ.mol<sup>-1</sup>, as presented in Table 3; The lower activation energy signifies faster enzymatic reaction and lesser  $K_m$  values signify higher affinity of the enzyme toward the substrate. The energy of activation results reaches closer values comparable with *O. megacantha* (28.90 kJ.mol<sup>-1</sup>)[47], *Phaseolus vulgaris* embryonic axes (32.70 kJ.mol<sup>-1</sup>)[61], garlic seedling (41.84 kJ.mol<sup>-1</sup>) [62].

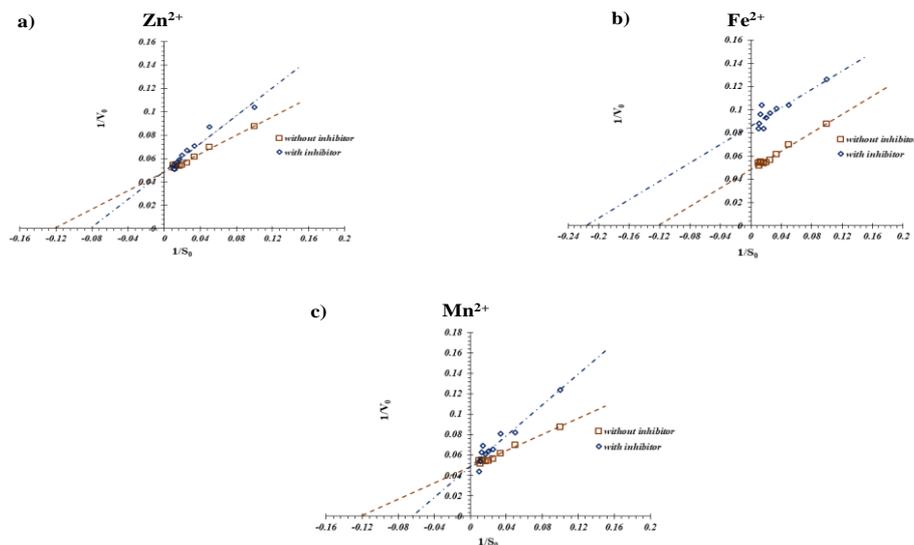
### 3.4. Inhibition kinetics.

#### 3.4.1. Type of inhibition.

The result of metal ions (salts) was studied and found that  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$  acted as strong inhibitors; the types of inhibition, mainly determined to be competitive, uncompetitive, and non-competitive, as depicted in Figures 4 and 5 for *R. nigrum* and *J. regia*. In the case of *J. regia* exhibit non-competitive inhibition against  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$  and uncompetitive inhibition exhibited in the case of  $\text{Zn}^{2+}$ . Likewise, *R. nigrum* exhibited competitive inhibition against  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$  and non-competitive inhibition in  $\text{Fe}^{2+}$ . It indicates the presence of heterogeneity of peptides. It has been demonstrated previously that peptides with different inhibition mechanisms can coexist in a particular source [63]. As a consequence of heterogeneity, some peptides with alternative inhibitory mechanisms were retained.



**Figure 4.** Lineweaver-Burk plot showing the non-competitive type of inhibition exhibited by metal ions ( $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$  ion) in the form of their respective salts, whereas  $\text{Zn}^{2+}$  showing un-competitive type of inhibition on PTPase (source-*Juglans regia* (Walnut; W)) catalyzed reaction at a different concentration ranging from 10 mM to 60 mM. The results are the mean of triplicates.



**Figure 5.** Lineweaver-Burk plot showing the competitive type of inhibition exhibited by metal ions ( $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$  ion) in the form of their respective salts, whereas  $\text{Fe}^{2+}$  showing the non-competitive type of inhibition on PTPase (source-*Ribes nigrum* (Munakka; M)) catalyzed reaction at a different concentration ranging from 10 mM to 60 mM. The results are the mean of triplicates.

3.4.2. Inhibition constants ( $K_i$ ) of metal ions.

**Table 4.** Inhibition constants ( $K_i$ ) of Iron, Manganese, and Zinc ions for PTPase enzyme.

S.No.	Metal Ions	Inhibition constants ( $K_i$ ) (mM)	
		<i>Juglans regia</i> (Walnut; W)	<i>Ribes nigrum</i> (Munakka; M)
1.	Fe <sup>2+</sup>	0.136	0.140
2.	Mn <sup>2+</sup>	0.075	0.103
3.	Zn <sup>2+</sup>	0.154	0.118

Inhibition constants ( $K_i$ ) values of metal ions (inhibitors) (Table 4) and type of inhibition were identified using double reciprocal (Lineweaver-Burk) plots.  $K_i$  value provides information that assists in understanding the ability of the inhibitor to effectively block or reduce enzyme activity and the quantity of inhibitor needed either for the reaction to proceed or for the enzyme activities to be inhibited [64-66].

#### 4. Conclusions

Even though many functional foods include possible bioactive components, there is still considerable misunderstanding due to a lack of scientific evidence. A scientific approach to functional foods, such as their interaction with other components, safety, and efficacy, could help clarify the uncertainty. Extensive research on functional foods based on technological advances is necessary to promote global health by lowering healthcare expenses and increasing consumer life expectancy. There are no such things as good or bad foods in nature, as long as positive health behaviors such as healthy dietary patterns aid to enhance health and minimize illness risk are followed. In conclusion, our work established that *R. nigrum* and *J. regia* have major prospects for being industry-friendly due to their high specific activity and moderate stability of PTPases. This study aims to acquire a better knowledge of functional foods as a source of PTPase. As previously noted, PTPases are a highly anticipated therapeutic target for a variety of human diseases, including cancer. Additional research should be conducted to elucidate the structural and functional features of PTPases using an omics approach to establish their therapeutic relevance for the pharmaceutical sector. The observation of PTPase enzyme function and control by diverse moieties using inhibition kinetics will have clinical value in the future. These findings imply that the plant system may contain a highly active PTPase, which may aid in developing orally accessible PTPase inhibitors. To be a therapeutically significant source of PTPases, *R. nigrum* and *J. regia* offer many potential applications as functional foods.

#### Funding

This research received no external funding.

#### Acknowledgments

We would like to acknowledge the Director, Amity Institute of Biotechnology, Amity University Uttar Pradesh, with the support and infrastructure.

#### Conflicts of Interest

The authors declare that there exists no conflict of interest.

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