

## Evaluation of Free Radical Scavenging Activity of Sauce and Formulation of Tamarindus indica (L.) And Prunus domestica (L.) Fruits

Zulnoreen Sarfaraz

*Department of Botany, Government College Women University Sialkot, Pakistan, moodi977@gmail.com*

Mehmooda Munazir

*Department of Botany, Government College Women University, Sialkot, Pakistan, moodi977@gmail.com*

Mubashrah Munir

*Department of Biological Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan, mubashrah.munir@uvas.edu.pk*

Asma Ahmed

*Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan, asma.ahmed@imbb.uol.edu.pk*

Rehana Badar

*Department of Biological Sciences, Superior University Lahore, Pakistan, rehanabadar45@gmail.com*

*See next page for additional authors*

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## Evaluation of Free Radical Scavenging Activity of Sauce and Formulation of *Tamarindus indica* (L.) And *Prunus domestica* (L.) Fruits

### Authors

Zulnoreen Sarfaraz, Mehmooda Munazir, Mubashrah Munir, Asma Ahmed, Rehana Badar, and Ayesha Tahir

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## EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF SAUCE AND FORMULATION OF *TAMARINDUS INDICA* (L.) AND *PRUNUS DOMESTICA* (L.) FRUITS

ZULNOREEN SARFRAZ<sup>1</sup>, MEHMOODA MUNAZIR<sup>1\*</sup>, MUBASHRAH MUNIR<sup>2</sup>, ASMA AHMED<sup>3</sup>, REHANA BADAR<sup>3,4</sup>, AND AYESHA TAHIR<sup>1</sup>

<sup>1</sup>Department of Botany, Government College Women University Sialkot, Pakistan

<sup>2</sup>Department of Biological Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan

<sup>3</sup>Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan

<sup>4</sup>Department of Biological Sciences, Superior University Lahore, Pakistan

Corresponding authors email: moodi977@gmail.com

### ABSTRACT

The formulation and sauce of *Tamarindus indica* (L.) and *Prunus domestica* (L.) are very popular recipes across globe. But, not even a single study existed on free-radical scavenging activity (FRSA) of afore-mentioned recipes. So, the present study was designed to bridge this gap of information. The FRSA of individual samples were also determined and compared with that of combinations of both. The highest value for FRSA among all samples was that of *T. indica* ( $158.9732 \pm 12.658$  % at  $100 \mu\text{g ml}^{-1}$ ). It was followed by that of individual extract of *P. domestica* fruit ( $96.647 \pm 0.554$  %). The percent FRSA of both combinations was less than that of individual sample extracts. The highest SA recorded was  $93.886 \pm 0.471$  % and  $77.64 \pm 16.391$  % respectively. The fruit length was  $10.18 \pm 0.34$  cm and  $4.7 \pm 0.2$  cm for *T. indica* and *P. domestica* respectively. The width of fruit was  $1.6 \pm 0.3$  cm and  $4.55 \pm 0.12$  cm for *T. indica* and *P. domestica* respectively. The weight of fruit was  $7.2 \pm 0.6$  g and  $44.8 \pm 0.37$  g for *T. indica* and *P. domestica* respectively, Pulp weight was  $48 \pm 0.43$  % and  $94.58 \pm 0.352$  % for *T. indica* and *P. domestica* respectively. The stone weight was  $30 \pm 0.421$  % and  $5.42 \pm 0.224$  % for *T. indica* and *P. domestica* respectively. The values of squared R reflected that there was a strong correlation between the concentrations and percent SA for all the studied samples ( $p < 0.05$ ) with values of  $R^2$  0.896 and 0.946 in case of *T. indica* and *P. domestica* extracts respectively and 0.980 and 0.875 for sauce and formulation respectively. It was concluded that the mixture of both ingredients has considerable potential for scavenging free radicals.

**Keywords:** Antioxidant activity, sauce, formulation, *Tamarindus indica*, *Prunus domestica*

### INTRODUCTION

Plants are well known around the globe for having therapeutic properties (Anwar et al., 2009; Chandra et al., 2020). Many unique and well known medicinal plants are the great blessing in this world (Inoue et al., 2019; Krishnaiah et al., 2009). At present time, we are using many drugs attained from medicinal plants (Ajaiyeoba et al., 2006). Different parts of medicinal plants

are frequently used in medicinal system and their consumption level is different depending upon their therapeutic properties (Kitula 2007; Shinwari et al., 2017). More than 80,000 of medicinal plant species have been found in Asia and Pakistan is enriched with a lot of species of medicinal plants (Qureshi et al., 2007). A huge number of medicinal plants are used in pharmaceutical industries worldwide (Anand et al., 2019). There exists a huge demand for plants for

biopharmaceuticals and for the preparation of functional foods (Santana and Macedo, 2019).

In human body, numerous metabolic reactions take place. These metabolic reactions along with external environmental factors produce countless free radicals like reactive oxygen species (ROS). These reactive oxygen species comprise of some free radicals like hydroxyl ion, hydrogen peroxide and super oxides collectively called oxidants (Srivastava et al., 2017). A free radical can be any atom or molecule having unpaired electron (Milenković et al., 2017). These free radicals are mostly produced naturally in our body as a result of metabolic activities. The strongest free radical which is produced after intake of oxygen into the cell is (O<sup>-</sup>) superoxide anion radical (Losada-Barreiro and Bravo-Diaz, 2017). Reactive oxygen species may prove constructive for body when in lesser amount, however their higher concentration may lead to many toxic effects on RNA, lipids and proteins (Ahmad et al., 2017). Many diseases have been reported which are caused by oxidative stress (Preiser 2012; Sharma et al., 2018).

*Tamarindus indica* L. (Tamarind) is an important medicinal plant species of third largest dicotyledonous flowering plant of family Caesalpiniaceae. It comprises of total 727 genera and 19,327 species (Adedayo et al., 2016; Lewis et al., 2005). Its origin is from Africa but mostly grows in tropical and humid areas at an average temperature of 25 °C (Luzia and Jorge 2011). It also grows in sub-tropical areas like India, Spain, China, Indonesia, Thailand, Philippine and Pakistan (Komutarin et al., 2004). Its seed coat has wound healing property (Naik et al., 2017). Its seeds also contain immunomodulatory effect (Aravind et al., 2012). Its seed has some specific enzyme called snake venom enzyme which

provide relief against hypertension, inflammation and local tissue damage (Ushanandini et al., 2006), and it help to relief dry eye syndrome (Rolando and Valente, 2007). *T. indica* contains phenolic constituents, alkaloids, flavonoids and tannins at chemical level (Sheela et al., 2019). The fruit of *T. indica* is mostly used as spice. It is usually in form of an elongated pod having brown bark with 3-8 seeds covered with pulp which contain tartaric acid, citric acid, acetic acid and little amount of sugar contents (Tsuda et al., 1994). Due to its laxative and digestive ability, the pulp of *T. indica* is used in different juices, curries, sauces, beverages by folks and also used in medicines (Siddhuraju 2007).

*Prunus domestica* L. (Plum) is an important and well-known climacteric stone fruit. It was first time documented as genus *Prunus* comprising of 2000 species by Weinberger in 1975 The plants of *P. domestica* are medium sized flowering trees The height of this plant species ranges from 10-12m. The dried fruits of *P. domestica* are commonly called prunes (Birwal et al., 2017; Jabeen and Aslam, 2011). Its seed is enclosed in a hard covering. It contains proteins, lipids and is a source of food, pharmaceuticals and cosmetics products (Savic et al., 2020).

It has been observed that the medicinal effects of different plant species may vary if used in combinations. The medicinal impacts of different plant derived compounds may either have additive or synergistic effect or the resultant product may have reduced activity. Since many of the fruits and/or medicines are used in combinations, so their cumulative effect needs to be investigated. Antioxidants stop the progression of free radicals during infections in a living system (Suleman et al., 2019). Formulations and sauces with varying recipes are used across the globe as a part of human diet. A number of such

formulations are used by the local residents of every region. The free radical scavenging activity (FRSA) of a number of individual dietary foods have been documented but the unique combinations have been neglected so far. In Pakistan, formulation and sauce of *T. indica* and *P. domestica*, generally known as imli-alu-bukkharay-ka-sharbat and imli-alu-bukkharay-ki-chatni respectively, are quite famous across the country especially in Punjab province during Summer season. The stalls of formulation across roads during Summer season are quite common and well-loved by the public. In present study, the main focus was the first time assessment of in vitro free radical scavenging activity of traditional Pakistani *P. domestica* and *T. indica* formulations and sauce. Such studies can prove valuable not only in studying the cumulative effect of different components of our diet, but can make possible the discovery of some novel bioactive constituents in future and can prove helpful to validate the presence of antioxidants in such daily food components. The study protocol was in compliance with relevant institutional guidelines and regulations.

## MATERIALS AND METHODS

### Collection of Samples

The fruits of *T. indica* L. and *P. domestica* L. were collected from the local market of District Sialkot, Punjab, Pakistan. The collected samples were cleaned and washed with running tap water to remove dust. Then the clean fruits were stored in the refrigerator at 4 °C for further use. For estimation of physical properties, three fruits (for each of *T. indica* and *P. domestica*) were randomly chosen and analyzed for their size and weight.

### Determination of Total Moisture Contents

To check the total moisture contents of *P. domestica* and *T. indica*, the fresh weight of both fruits was recorded then these fruits were dried at 40-60 °C. Total weight was measured and mean value of each of three replicates was calculated by using the following equation;

$$\text{Total weight} = \text{Moisture Content} = \frac{W_f - W_d}{W_f} \times 100$$

Here, Mc stands for Moisture Content in percentage

Wf was the fresh weight of samples.

Wd was the weight of dried samples.

### Preparation of Extracts

First of all, the individual extracts of both fruits were prepared. For this purpose, fifty grams of each of the fruit was soaked in water (1:50 ratio) for 24 hours while constantly being shaken at room temperature. Then the samples were filtered. The residue was washed well with distilled water and the filtrate obtained so was kept in hot air oven for evaporation of water. Different concentrations of dried extracts were prepared ranging from 10 to 100 µg ml<sup>-1</sup> to 100 µg ml<sup>-1</sup> in methanol. Then, Fifty grams of each plant fruit pulp was soaked overnight and then the formulation and sauce was prepared by using traditional methods of Pakistani culture. For formulation, sugar (100 grams) was mixed well with the soaked pulp and the volume was made up to 500 ml. While for the sauce, 20 grams sugar and 2 grams salt was mixed well and the volume was made up to 100ml. The extracts prepared by afore-mentioned method were heated using hot plate in temperature range of 40-60 °C until the extracts got dried completely. Total moisture was evaporated and the

remaining sticky material from the container was used to prepare different concentrations ranging from 10 to 100µg ml-1. This was done by first weighing the dried extracts and then their methanolic extracts were prepared at different concentrations ranging from 10 µg ml-1 to 100 µg ml-1 for reaction with DPPH to assess the free radical scavenging capacity.

### Free Radical Scavenging Assay

The determination of free radicals from the formulation and sauce of *T. indica* and *P. domestica* and their individual extracts was carried out by using DPPH free radical scavenging assay by the method described by Munazir et al. (2015) and Shen et al. (2010) with some modifications. Briefly, 0.3 mM DPPH solution was prepared in methanol and 1ml of this solution was added to 2.5 ml solution of all extracts at different concentrations (10, 20, 40, 60, 80 and 100 µg ml-1). These mixtures were shaken well and were kept for 30 minutes at room temperature in the dark. Then the absorbance was measured at 517 nm with the help of UV visible mass spectrophotometer. Ascorbic acid was used as the standard. The DPPH

radical scavenging capacity was calculated with the help of following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = (A_0 - A_1)/A_0 \times 100$$

Here, A<sub>0</sub> is the value of absorbance of the control reaction, and A<sub>1</sub> is the absorbance value of experimental mixture

### Data Analysis

All experiments were replicated thrice and data was presented as the mean ± standard deviation (SD). Analysis of Variance and Regression Analysis was performed through SPSS version 24 for windows to analyze the results.

## RESULTS

The physical properties (including length, width, weight, total moisture content) of fresh fruits were studied. The results of size parameters are given in Table 1. The total moisture content of both *T. indica* and *P. domestica* were recorded which showed moisture contents of *T. indica* and *P. domestica* were 86 % and 91 % respectively. And their weight reduction was 14 % and 9 %, respectively.

**Table 1: Physical properties of fresh fruits of *T.indica* L. and *P. domestica* L.**

Size parameter	<i>T.indica</i>	<i>P. domestica</i>
fruit length (cm)	10.18±0.34	4.7±0.2
width of fruit (cm)	1.6±0.3	4.55±0.12
weight of fruit (g)	7.2±0.6	44.8±0.37
Pulp weight (%)	48±0.43	94.58±0.352
Stone weight (%)	30±0.421	5.42±0.224

It was observed that antioxidant activity of both these ingredients increased in a concentration dependent manner i.e. with the increase in concentrations, the percent scavenging activity also increased (Figure 1 and 2). The analysis of data reflected that the value of R Square is 0.896 and 0.946 in case of *T. indica* and *P. domestica* extracts

respectively (Table 2 and 3). It reflected that 89.6 and 94.6 percent of variation in dependent variable is explained by the independent variable for *T. indica* and *P. domestica* extracts respectively. Here dependent variable is scavenging activity expressed as percentage and independent variable is different concentrations of aqueous extract of *Tamarindus indica* and

*P. domestica* fruit. R-squared is a statistical measure of how close the data are to the fitted regression line. The p value is less than 0.05 ( $p < 0.05$ ) in case of both individual extracts which indicated

that, overall, the regression model predicts the outcome variable in statistically significant manner (Table 4 and 5).

**Table 2: Model summary showing the R Square value (0.896) and standard error of estimate for percent scavenging activity of *T. indica* fruit extracts at varying concentrations ranging from 10-100µg ml-1**

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.953 <sup>a</sup>	.908	.896	9.748

a. Predictors: (Constant), activity

**Table 3: Model summary showing the R Square value (0.946) and standard error of estimate for percent scavenging activity of *P. domestica* fruit extracts at concentrations ranging from 10-100µg ml-1**

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.976 <sup>a</sup>	.952	.946	7.005

a. Predictors: (Constant), activity

**Table 4: ANOVA table showing the significance of results ( $p < 0.01$ ) of percent scavenging activity of *T. indica* fruit extracts at concentrations ranging from 10-100µg ml-1**

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	7489.863	1	7489.863	78.826	.000 <sup>b</sup>
	Residual	760.137	8	95.017		
	Total	8250.000	9			

a. Dependent Variable: concentration

b. Predictors: (Constant), activity

**Table 5: ANOVA table showing the significance of results ( $p < 0.01$ ) of percent scavenging activity of *P. domestica* fruit extracts at concentrations ranging from 10-100µg ml-1**

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	7857.449	1	7857.449	160.131	.000 <sup>b</sup>
	Residual	392.551	8	49.069		
	Total	8250.000	9			

a. Dependent Variable: concentration

b. Predictors: (Constant), activity

**Table 6: Model summary showing the R Square value (0.980) and standard error of estimate for percent scavenging activity of sauce of fruits of *T. indica* and *P. domestica* at varying concentrations ranging from 10-100µg ml-1**

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.991 <sup>a</sup>	.982	.980	4.285

a. Predictors: (Constant), activity

After investigation of individual extracts, the combinations of extracts of both fruits were studied for their DPPH-radical scavenging capacity. It was observed that the percent DPPH-scavenging activity increased in a concentration-dependent manner (Figure 3 and 4). The value of squared R was 0.980 and 0.875 for sauce and formulation respectively (Table 6 and 7). It reflected

that 98 and 87.5 percent of variation in dependent variable is explained by the independent variable for sauce and formulation respectively. The p value is less than 0.05 ( $p < 0.05$ ) in case of sauce and formulation which indicated that, overall, the regression model predicts the outcome variable in statistically significant manner (Table 8 and 9).

**Table 7: Model summary showing the R Square value (0.875) and standard error of estimate for percent scavenging activity of formulation of fruits of *T. indica* and *P. domestica* at varying concentrations ranging from 10-100 µg ml-1**

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.943 <sup>a</sup>	.889	.875	10.693

a. Predictors: (Constant), activity

**Table 8: ANOVA table showing the significance of results (p<0.01) of percent scavenging activity of sauce of *T. indica* and *P. domestica* fruit extracts at concentrations ranging from 10-100 µg ml-1**

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	8103.107	1	8103.107	441.307	.000 <sup>b</sup>
	Residual	146.893	8	18.362		
	Total	8250.000	9			

a. Dependent Variable: concentration  
 b. Predictors: (Constant), activity

**Table 9: ANOVA table showing the significance of results (p<0.01) of percent scavenging activity of formulation of *T. indica* and *P. domestica* fruit extracts at concentrations ranging from 10-100 µg ml-1**

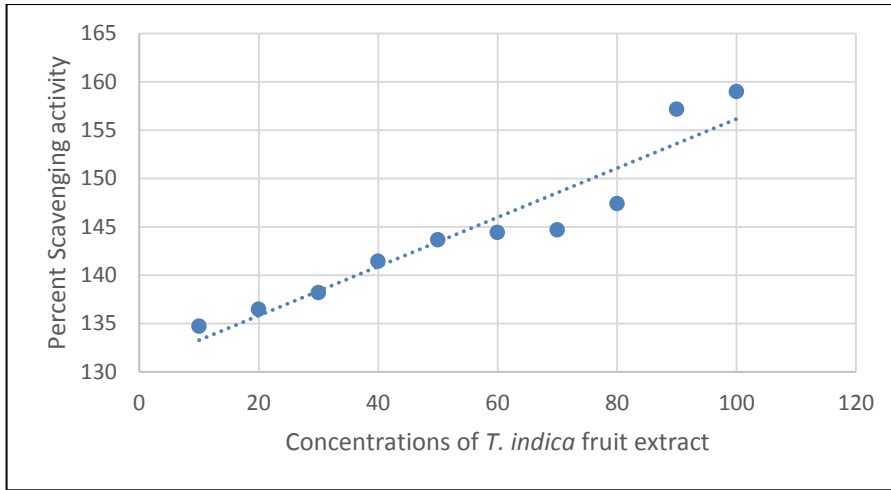
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7335.272	1	7335.272	64.153	.000 <sup>b</sup>
	Residual	914.728	8	114.341		
	Total	8250.000	9			

a. Dependent Variable: concentration  
 b. Predictors: (Constant), activity

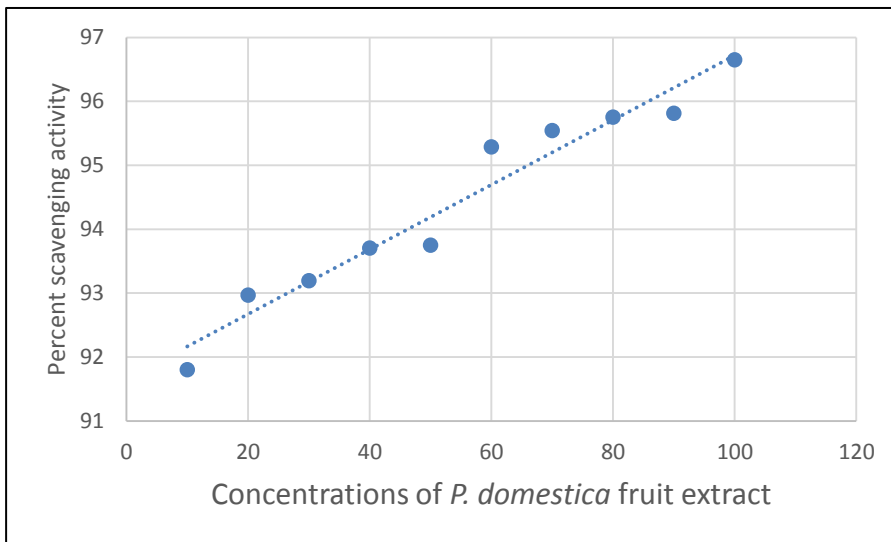
The highest value for free-radical scavenging activity among all samples was that of *T. indica* with  $158.9732 \pm 12.658$  % SA at a concentration of  $100 \mu\text{g ml}^{-1}$ . It was followed by that of individual extract of *P. domestica* fruit ( $96.647 \pm 0.554$  %). Whereas, the percent SA of both

combinations i.e. sauce and formulation was less than that of individual sample extracts. The highest SA recorded in case of sauce and formulation was  $93.886 \pm 0.471$  % and  $77.64 \pm 16.391$  % respectively.

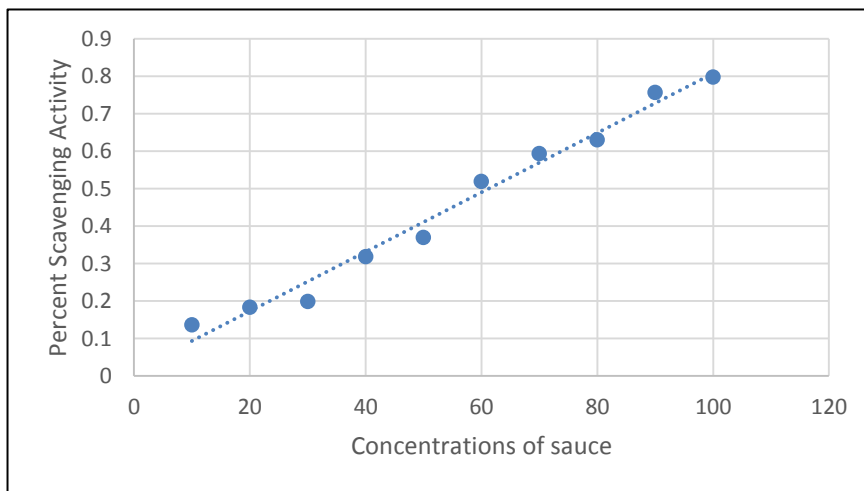




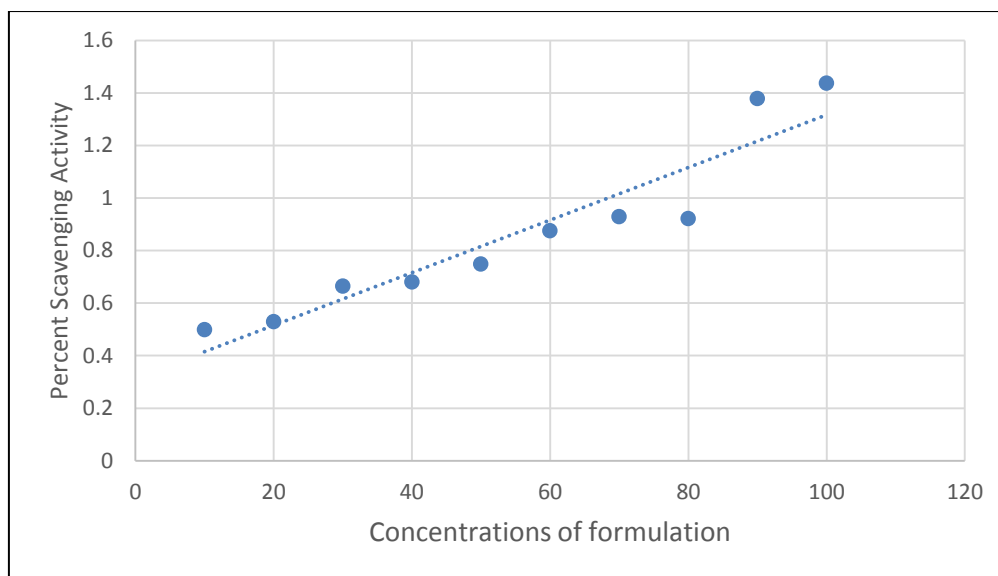
**Figure 1: Percent scavenging activity at varying concentrations of *T. indica* fruit extract**



**Figure 2: Percent scavenging activity at varying concentrations of *P. domestica* fruit extract**



**Figure 3: Percent scavenging activity at varying concentrations of sauce of *T. indica* and *P. domestica* fruit extract**



**Figure 4: Percent scavenging activity at varying concentrations of formulation of *T. indica* and *P. domestica* fruit extract**

## DISCUSSION

Pakistani traditional food usually contain a complete combination of all types of nutrients, vitamins, phytochemicals and all essential contents which are necessary for normal body functioning and growth. These food combinations or formulations have been consumed since ancient times by the aboriginal people. They used different recipes and combinations to make food much more appealing and to provide good eminence to food. These traditional recipes were transmitted from generation to generation and with the passage of time it provoked the interest of researchers to study the importance of these food stuffs. Formulation and sauce of fruits of *Tamarindus indica* and *Prunus domestica* are well-known traditional recipes being used in various parts of the country. The fruits of these two species are used individually too in various forms and are well-known across globe for their astringent properties. But their combinations had not been tested for their potential antioxidant and health boosting properties so the aim of the present study was to assess the antioxidant potential of combined extracts (formulation and sauce)

of *Tamarindus indica* and *Prunus domestica*. But before doing that, the DPPH-free radical scavenging capacity of individual extracts of *T. indica* and *P. domestica* was assessed. For this purpose, varying concentrations of extracts of fruits of these two species were prepared (10-100  $\mu\text{g ml}^{-1}$ ) and reacted with DPPH at human body temperature i.e. 37  $^{\circ}\text{C}$ . The readings were taken by using spectrophotometer at a wavelength of 517 nm.

The ability of extract to donate electrons leads to color change of DPPH in methanolic solution. And absorbance peaks of the sample were calculated at 517nm spectrophotometrically (Christova-Bagdassarian et al., 2013). In DPPH assay, more decrease in absorbance reflects more scavenging of free radicals (Alves et al., 2010; Saeed et al., 2012). The substance that scavenges these free radicals is called antioxidant (Ebrahimzadeh et al., 2010). The whole method is carried out at 37 $^{\circ}\text{C}$  temperature (which is quite a reasonably normal temperature for plant extracts and DPPH) and in the presence of dark. Therefore, there is no risk of degradation of any type of compound due to heat in the sample mixture present for experimentation (Bondet et al., 1997).

DPPH-free radical scavenging assay was performed for all samples prepared by the methods described in the section on methodology.

The Percent SA of all tested samples increased in a concentration dependent manner and the highest SA was observed at the higher concentrations. This finding is supported by Chung et al. (2009), Ebrahimzadeh et al. (2010), Lalminghlui and Jagetia (2018), Munazir et al. (2015), Munir et al. (2020), Rahman et al. (2015).

Morabbi-Najafabad and Jamei (2014) investigated alcoholic samples of *P. domestica* for their antioxidant activity and they reported quite less value of SA for them i.e.  $49.10 \pm 1.24$  in case of fresh samples and  $79.78 \pm 1.34$  % for dried samples. The difference in our results may be attributed to the fact that they investigated the alcoholic extracts while we studied the aqueous extracts. The values of highest SA could be related to the tastes of all these tested samples. The order of sour taste is like *T. indica*>*P.domestica*>sauce>formulation and same is the order for highest SA for all these. The other reason for lower SA in case of sauce and formulation could be due to the fact that sauce and formulation contain less quantity of both ingredients as compared with the pure ones and secondly, some of the antioxidant chemical entities within both samples could react with each other upon mixing thus leading to a slight reduction in overall SA. It is noteworthy to mention that few fruits with astringent properties and sour taste are usually diluted in water and/or mixed with other fruits to reduce the sour taste for consumption. Since the more sour a fruit, the less amount can be consumed by a consumer. If diluted and mixed with other fruits, the taste may become better and tolerable for consumption and concentration of antioxidants may also be balanced or reduced. This could make it easy for human body cells to absorb the nutrients and antioxidants efficiently.

## CONCLUSIONS

The human body needs a balance between free radicals and scavengers of free radicals. The free radical scavenging activity of individual fruit samples was higher while that of combined extracts of fruits of *T. indica* and *P. domestica* was lower than the individual samples which reflects that the antioxidant potential is lower in case of combination of these two ingredients. Since these two fruits are usually sour and have astringent properties so they cannot be consumed by humans in higher quantities. But their recipes like formulation and sauce, being less sour and astringent are quite easily consumed. The free radicals present in these two might have reacted and got stabilized upon mixing, thus giving a balanced free radical scavenging activity to the human body. The formulation as well as sauce of these two may contain one or more novel antioxidant compounds if investigated in-depth. Such compounds can be further used at commercial and pharmaceutical level for further research and preparation of different useful products.

## AUTHORS CONTRIBUTION

Zulnoreen Sarfraz performed the experimental work, Mehmooda Munazir perceived the research idea, supervised practical work, provided guidance at all steps and prepared the manuscript, Mubashrah Munir and Asma Ahmed reviewed and edited the manuscript, Rehana Badar analyzed and interpreted the results, Ayesha Tahir helped in practical work and in preparing first draft of manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest

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