
**ISSN Print:** 2394-7500
**ISSN Online:** 2394-5869
**Impact Factor:** 8.4
IJAR 2020; 6(12): 90-92
www.allresearchjournal.com
Received: 18-10-2020
Accepted: 20-11-2020

**Shavita Bali**
M.Phil. Researcher Scholar, Department of Botany, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

**Dr. Suchi Modi**
Assistant Professor, Department of Botany, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

**Corresponding Author:**
**Shavita Bali**
M.Phil. Researcher Scholar, Department of Botany, Rabindranath Tagore University, Bhopal, Madhya Pradesh, India

# An *in vitro* study of some apple varieties of Kashmir

**Shavita Bali and Dr. Suchi Modi**

**Abstract**
Apples are sub-acid foods that have a rich pectin content and have proven rather beneficial to diabetes patients. Pectin, one of the most potent components in apples, acts as a detoxifier of the body by supplying an inordinate amount of glucuronic acid. India is considered the original home of apples (*Pyrus malus* L). Phenolic compounds are believed to impart resistance to diseases in plants and Polyphenol oxidase (Catecholase and Cresolase) enzyme has been reported to be responsible for *in vivo* synthesis and accumulation of these compounds. In present study Polyphenol oxidase activity in different varieties of apple fruit samples (found in Kashmir) has been correlated with polyphenolic compounds and antioxidant activity. It was found that increase in Polyphenol oxidase (PPO) activity and total phenolic content in apple fruit samples of *Ambri* and *Red Delicious* varieties were maximum in accordance with antioxidant activity to that of *Kessi* and *A. Trel* which have reduced Polyphenol oxidase activity, total phenolic content and antioxidant activity. In the present investigation a relation between Polyphenol oxidase activity, Total Phenolic Content (TPC) and antioxidant activity has been established. Thus on the basis of PPO activity, polyphenolic content and antioxidant activity, the different varieties of the fruits can be differentiated. From the results of present study it can be concluded that apples having maximum antioxidant activity should be consumed more since these fruits will be much beneficial for health.

**Keywords:** *Pyrus malus* L., PPO, catecholase, cresolase, total phenolic content (TPC), antioxidant activity

## Introduction
In recent years more interest has been paid to protect foods and human beings against oxidative damage caused by free radicals like hydroxyl, peroxyl and Superoxide radicals. It is well known that many polyphenolic compounds such as phenolic acids, flavonoids, anthocyanidins and tannins possess remarkable antioxidant and anticancer activities, are rich in plant materials. Apple (*Pyrus malus* L.) is believed to be the good source of antioxidants. Thus it is said to have an apple a day prevents doctor away. India can be considered as the home of apple (*Pyrus malus* L.), maximum of the varieties are also grown commercially around the world. Kashmir is endowed to have the richest pilgrim of different varieties of apples. Many of the varieties are found at the same season. Apples are exported from Kashmir not only to India but also to the different parts of the world. Phenolic compounds are believed to impart resistance to diseases in plants and polyphenol oxidase (Catecholase and Cresolase) enzyme has been reported to be responsible for *in vivo* synthesis and accumulation of these compounds [1, 2]. In the present investigation the different varieties of apple belonging to the same season were compared on the basis of Polyphenol oxidase activity, Total phenolic content and *in vitro* antioxidant activity. The objective of the present investigation was to categorize different varieties of apple on the basis of *in vitro* antioxidant activity.

**Statement of the research problem:** The statement of the research problem is reported as under:

**Objectives of the study:** The objectives of the study is reported as under.

**1) To explore the in vitro study of some apple varieties of Kashmir**
**Materials and Methods:** Various samples of different varieties of apple fruits, growing in a same period i.e. August-September were selected from local gardens of Kashmir.

The samples were washed, crushed and homogenized. Polyphenol oxidase (both catecholase and cresolase) activity, Total Phenolic Content (TPC) and *in vitro* antioxidant activity were determined. Correlation between PPO activity, Total Phenolic Content (TPC) and antioxidant activity were calculated.

**Extraction**

The pulp of crushed apple plantlets was extracted separately in a rotary shaker with 200 ml of 50% ethanol at 30 °C for 24 h. The extract was then filtered, concentrated using a rotary evaporator to obtain the crude ethanol fraction (CF) [3]. The freeze dried extract was dissolved in 50% ethanol for further determination of Total Phenolic Content (TPC) and antioxidant activity.

**Estimation of total phenolic content (TPC)**

Total Phenolic content of the extract was determined [4] and was then expressed as µg/g Gallic acid equivalents. In brief, a 100µl aliquot of the samples was added to 2 ml of 0.2% w/v $Na_2CO_3$ solution. After two minutes of incubation, 100 µl of 500 ml/l Folin Ciocalteu reagent was added and the mixture was then allowed to stand for 30 minutes at 25 °C. The absorbance was measured at 750 nm using systronics UV-VIS spectrophotometer. The blank consisted of all reagents and solvents but without the sample. The Total Phenolic Content (TPC) in µg/g was determined using the standard Gallic acid calibration curve.

**Preparation of enzyme extract**

The crude enzyme extract was prepared at 4 °C [5]. Apple samples from different varieties about 1g were chopped and ground with 5ml of 100mM phosphate buffer (pH 7.3) containing 10mM Sodium ascorbate and was then further homogenized in a homogenizer for 2 minutes, filtered and was then centrifuged at 3000 rpm for 30 minutes. The precipitate was re-extracted for 15 minutes with 5ml of 1.5% Triton-X prepared in 100mM phosphate buffer (pH, 7.3). The final volume of extract was made up to 25ml with phosphate buffer (pH, 7.3) and was then further centrifuged at 10,000 rpm for 10 minutes. The supernatant was taken as an enzyme source.

**Enzyme assay**

Both catecholase and cresolase activities were measured spectrophotometrically at 400nm. Catecholase activity was measured (CMC) as substrate made up in 10mM sodium acetate buffer (pH, 4.5). Three ml 100mM phosphate buffer (pH, 7.3) was added to 1ml substrate at zero time. The change in absorbance at 400 nm was recorded in Systronics UV-VIS spectrophotometer. Cresolase activity was measured in the same way, except that 4-methyl phenol (p-cresol) was used as substrate, made up in 10mM phosphate buffer (pH 7.0). Enzyme activity was represented as a change in absorbance at 400nm/g of tissue weight per minute.

**Determination of *in vitro* antioxidant activity**

*In vitro* antioxidant activity was determined by Superoxide Anion Radical Scavenging Activity method [6]. The fraction was mixed with 3ml of reaction buffer solution (pH, 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction solution was illuminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance was measured at 560 nm using a spectrophotometer.

Ascorbic acid was used as positive control and the reaction mixture without any sample was used as negative control.

The Superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{Ao - As}{Ao} \times 100$$

Where

Ao = Absorbance of positive control

As = Absorbance of sample

**Results and discussion**

Catecholase and Cresolase activity and Phenolic content varied widely among various apple varieties. The results are illustrated in Table 1. Catecholase and Cresolase activities were found highest in Ambri and Red Delicious i.e. 1.85; 1.62 and 1.50; 1.35 $g^{-1}min^{-1}$ respectively while Catecholase and Cresolase activities were found lowest in Kessi and *A. Trel* (American Trel) i.e. 0.86; 0.52 and 0.46; 0.25 $g^{-1}min^{-1}$ respectively. Phenolic content followed a same pattern as that of Catecholase and Cresolase activity. Ambri variety of fruit samples contained the maximum phenolic content (28.22 µg/g fresh tissue weight) followed by Red Delicious (26.16 µg/g). Kessi and *A. Trel* contained the least phenol content i.e. 15.12 and 12.10 µg/g respectively. The antioxidant activities were thus found prominent in Ambri followed by Red Delicious i.e. 86 and 80% respectively. Antioxidant activities were found lowest in Kessi followed by *A. Trel* i.e. 65 and 60% respectively. These studies distinguished the different varieties of apples from a specific region. A close relation was found between Total Phenolic Content (TPC), PPO activity and Antioxidant activity. These results were in accordance with previous work done [7] on mango plantlets for screening germplasm against malformation. These results indicate that PPO activity can be used to screen quality of apple varieties on the basis of PPO activity and Total phenolic Content (TPC). It has been determined that antioxidant activity is directly correlated with the Total Phenolic Content (TPC). This study can further be used as a character for selecting parental stock. A correlation between PPO activity and phenolic content in various varieties of apple can be used as criteria to estimate the antioxidant levels in different varieties of apple from Kashmir, Shimla etc. in India and various other regions of the world. Thus various apple varieties and other fruits, vegetables having medicinal importance can be classified and categorized according to their antioxidant levels. This is the first time work done ever in India to screen apple varieties in Kashmir.

**Table 1:** Determination of PPO (Catecholase and Cresolase) Activity, Total Phenolic Content (TPC) and Antioxidant Activity

| S. No. | Apple variety | Catecholase activity ($g^{-1}$ $min^{-1}$) | Cresolase Activity ($g^{-1}$ $min^{-1}$) | Total Phenolic content (µg/g) | Superoxide Anion Radical Scavenging Activity (%) |
|---|---|---|---|---|---|
| 1. | Ambri (Amri) | 1.85 | 1.62 | 28.22 | 86 |
| 2. | Kessi (Cox orange Pippin) | 0.86 | 0.52 | 15.12 | 65 |
| 3. | Red Delicious | 1.50 | 1.35 | 26.16 | 80 |
| 4 | *A. Trel* | 0.46 | 0.25 | 12.10 | 60 |

## References


1. Mayer AM, Harel E. Polyphenol oxidase in higher plants: A Review. Phytochemistry 1979;18:193-215.
2. Vaughan KC, Duke SO. Function of polyphenol oxidase in higher plants. Plant Physiol 1984;60:106-112.
3. Sharma RR, Sharma HC, Goswami AM. Polyphenol oxidase activity and phenolic content pattern during shoot development of grape (*Vitis vinifera* L.) in different growing seasons. J Plant Biochem 1994;3(2):145-147.
4. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents. American Journal of Ecology and Viticulture 1965;16:144-158.
5. Valero E, Sanchez-Ferrer A, Varon V, Garcia Carmona F. Evaluation of grape polyphenol oxidase activity and phenolic content pattern during maturation and vinification. Vitis 1989;28:85-95.
6. Duan X, Wu G, Jiang Y. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules 2007;12(4):759-771.
7. Sharma RR, Singh CN, Chhonkar OP, Goswami AM, Singh SK. Polyphenol oxidase activity as an index for screening mango (*Mangifera indica* L.) germplasm against malformation. PGR News Letter 2006;124:41-43.