

## RESEARCH ARTICLE

### EVALUATION OF ANTIOXIDANT AND CYTOTOXICITY PROPERTIES OF AMYGDALIN EXTRACTED FROM *PRUNUS DULCIS*

Sushma, P<sup>1</sup>, Bindu Jacob<sup>1</sup> and R.T. Narendhirakannan<sup>2\*</sup>

<sup>1</sup>Department of Biotechnology, School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore-641114, Tamil Nadu, India.

<sup>2</sup>Department of Biochemistry, Kongunadu Arts and Science College, G.N. Mills (PO), Coimbatore-641029, Tamil Nadu, India.

#### ABSTRACT

Free-radical reactions have been implicated in the pathology of many human diseases like atherosclerosis, ischemic heart disease, aging process, inflammation, diabetes, immuno-suppression, neurodegenerative disease etc. Radicals and other reactive oxygen species are formed constantly in the human body and are removed by the enzymatic and non-enzymatic antioxidant defence. The disturbance in 'redox homeostasis' that occurs when antioxidant defences are inadequate can damage lipids, proteins, carbohydrates and DNA. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing tissue injury. Phytochemicals with antioxidant property are naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration. Amygdalin, also known as vitamin B17 is a cyanogenic glycoside found in several sources mainly in apples, pears, apricots, plums, peaches. Several reports claim amygdalin to be good chemopreventive agent, however these claims are not often backed by proper scientific evidence. Thus the present study is aimed to evaluate the therapeutic potential of amygdalin isolated from *Prunus dulcis* by studying its in vitro antioxidant and cytotoxic properties.

**Keywords:** Amygdalin, Antioxidant, Cytotoxicity, Extract, *Prunus dulcis*

#### 1. INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias. An increasing number of studies reveal that dietary antioxidants are capable of blocking neuronal death in vitro and many therapeutic properties in animal models of neurodegenerative diseases including Alzheimer's and Parkinson's diseases (1). The antioxidant activity of dietary polyphenols is considered to be much greater than that of the essential vitamins. Hence, studies on the evaluation and exploitation of phyto-nutrient compounds particularly phenolic acids, flavonoids and high molecular weight tannins of legumes as natural antioxidants have assumed great significance (2). Amygdalin also known as "Vitamin B17" is a product of the metabolism of phenylalanine in the bitter almond. The safety levels of amygdalin include the fact that according to researchers, healthy cells break vitamin B17 down into beneficial product and cancerous cells break it down into toxins. Studies regarding indigenous

groups which are principally free of cancer have revealed a common diet that is rich in vitamin B17. People of the Abkhazians, the Hunzas, the Eskimaux and the Karakorum obtain their vitamin B17 through variety of foods and may consume about 250-3000 mg of B17 nitriloside a day. When this food habits get changed due to their move into urban from tribe, there is increase risk of cancer. The average western diet tends to have less than 5 mg a day (3). It has been observed that not much research work has been carried out in elucidating the therapeutic efficacy. Several sources have reported the amygdalin content found in the apricot kernels to possess significant anti-cancer and anti-oxidant properties. However, these reports are not backed up by strong scientific evidence. Thus the present study is aimed to bridge this gap and provide a scientific evidence of the efficacy of amygdalin as a cytotoxic and antioxidant agent.

#### 2. MATERIALS AND METHODS

##### 2.1. Chemicals

Diethyl ether, Ascorbic acid, DPPH-2,2-diphenyl-1-picrylhydrazyl, Sulphuric acid, Ammonium molybdate, Sodium phosphate, Sodium

\*Correspondence: Narendhirakannan, R.T., Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: bionaren\_phd@yahoo.co.in

dihydrogen phosphate monohydrate, Disodium hydrogen phosphate anhydrous, Potassium ferricyanide, Trichloroacetic acid, Ferric chloride, Hydrogen peroxide, Rutin, PBS-Phosphate Buffered Saline, 10% FBS-Foetal Bovine Serum, DMEM-Dulbecco's Modified Eagle's Medium, DMSO-Dimethyl Sulfoxide, Trypsin were purchased from Hi Media Laboratories Ltd., All chemicals were of analytical grade and were used as such for assays.

## 2.2. Processing of *Prunus dulcis*

Powdered almonds were subjected to reflux conditions using ethanol solvent at 78.5°C. The mixture was boiled under reflux for 40, 80, 120 and 180 min. After reflux, the suspension was completely filtered with the help of Whatman filter paper No 1 and the solvent was evaporated using of rotary evaporator. To the dried sample, 10 ml of diethyl ether was added and it was vortexed for 1 min at room temperature (22°C) to get the precipitate. The diethyl ether was made to evaporate overnight in a fume hood. The sample obtained was stored in airtight container and was used for further analysis.

## 2.3. In vitro antioxidant studies

### 2.3.1. DPPH scavenging activity

Different concentrations of sample and standard solutions were prepared and made up to 100 µl with methanol. To the samples, 5 ml of DPPH was added and was incubated for 20 mins in dark. The absorbance was recorded at 517 nm. Methanol alone served as blank. A mixture of methanol and DPPH was used as control.

The percentage of inhibition is calculated by the following formula:

$\% \text{Inhibition} = \frac{[(\text{control OD} - \text{Sample OD}) / \text{Control OD}] * 100}{100}$ . A graph is plotted with % of inhibition on Y-axis and concentration on X-axis.

### 2.3.2. Reducing Power ability

Different aliquots of standard and sample solutions were prepared in a series of test tubes. The volume in the test tubes was made up to 1ml in all the test tubes. A test tube with 1 ml of methanol act as a blank. In all the test tube, 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide was added including the blank. The solution mixture was incubated at 50°C for 20 min followed by addition of 2.5 ml trichloroacetic acid. The solutions were centrifuged at 1000 rpm for 10 mins at room temperature. After centrifugation, the upper layer of solution was mixed with distilled

water and chloride was added. The absorbance of the green colour is read at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. A graph is plotted with absorbance in Y-axis and concentration on X-axis.

### 2.3.3. Hydroxyl radical scavenging activity

Different concentrations of both sample and standard were prepared in a series of test tubes and each tube was made up to 100 µl with the phosphate buffer. Then 1 ml of EDTA, 0.5 of EDTA, 1ml of DMSO and 0.5 ml of ascorbic acid was added to all test tubes followed by uncubation for 15mins at 80°-90° C. After the incubation, 1ml of trichloro acetic acid, 3ml of Nash reagent was added and was incubated for 15 mins. The test tube containing Phosphate buffer along with all reagents serves as positive control. The phosphate buffer served as a blank. The absorbance was read at 412 nm. The percentage of hydroxyl radical scavenging activity is calculated by the following formula:

$\text{Scavenging activity (\%)} = \frac{[(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] * 100}{100}$ . A graph was plotted with percentage of scavenging activity in Y-axis against concentration in X-axis.

## 2.4. In vitro cytotoxicity studies

The Cell toxicity assays were done by seeding HeLa cells in a 96 well plate and incubating it at 37°C with 5% CO<sub>2</sub> in CO<sub>2</sub> incubator. A series of dilution of the samples were added to the cells containing medium in 96 well plates. Wells containing only media and cells were taken as control wells. After the incubation of 24 h, MTT reagent was added to wells and then the 96 well plates were kept for few hours for the reaction to take place. When purple crystals are clearly visible; DMSO is added and kept in dark for few hours. Optical density of the formazan product was read at 495 nm using scanning multi well spectrophotometer. The results were given as mean of three independent experiments.

## 3. RESULTS AND DISCUSSION

The presence of cyanide peak in a FTIR chromatogram confirms the presence of amygdalinas the major component of amygdalin is the nitrile moiety (4). In recent years, researchers had stated that the anti-oxidants can delay or restrain the oxidation of molecules. The process is mainly done by inhibiting the initiation of the free-radical induced chain reactions (5). Depending upon the assay performed, the results of antioxidant studies are usually scattered and that is to evaluate

the percentage of inhibition. The DPPH assay was performed for the extract, commercial amygdalin and standard.

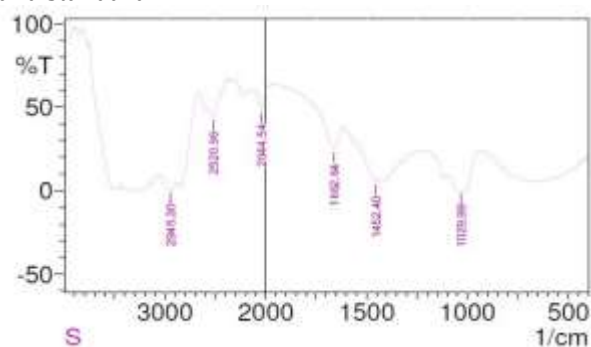


Fig. 1. FTIR for commercial amygdalin

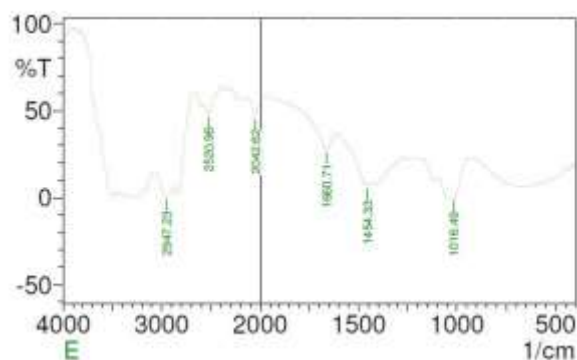


Fig. 2. FTIR for extract (Reflux)

Table 1: FTIR peak values for Std amygdalin

S. No.	Peak values	Groups present
1	2945.3	C-H
2	2520.96	O-H
3	1662.64	$\alpha, \beta$ Unsaturated
4	1452.4	C=C
5	1029.99	C-N

Table 2: FTIR peak values for extract

S. No.	Peak values	Groups present
1	2947.23	C-H
2	2520.96	O-H
3	1662.71	$\alpha, \beta$ Unsaturated
4	1454.33	C=C
5	1069.49	C-N

Due to the DPPH radical hydrogen donating ability, the scavenging effects of DPPH was radical was observed in all the samples (6). The scavenging activity of hydroxyl radical is an important index for measuring the antioxidant capacity (7). From the

graph, it was concluded that the percentage of inhibition for

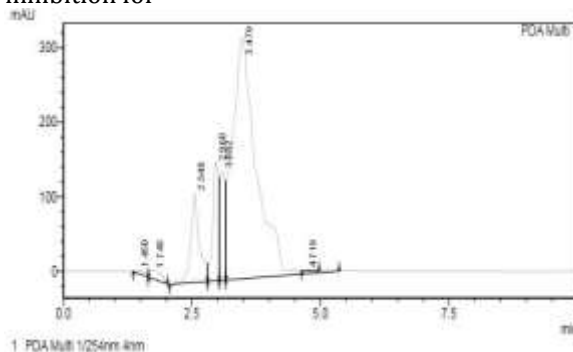


Fig. 3. HPLC for Standard

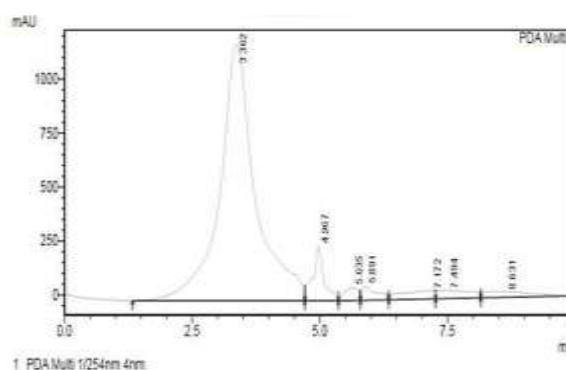


Fig. 4. HPLC for extract (Reflux)

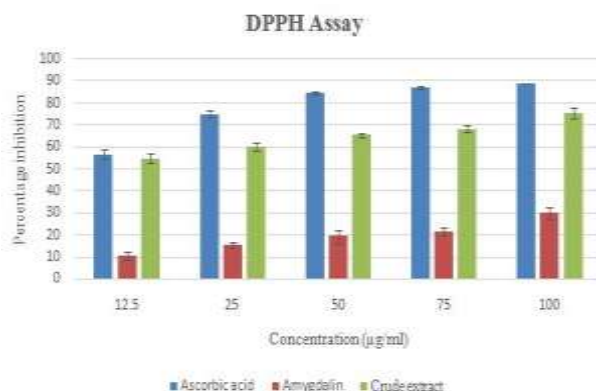
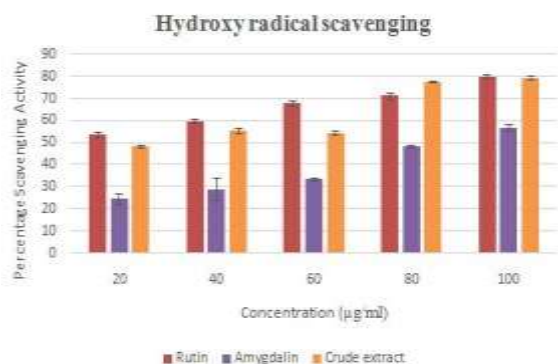
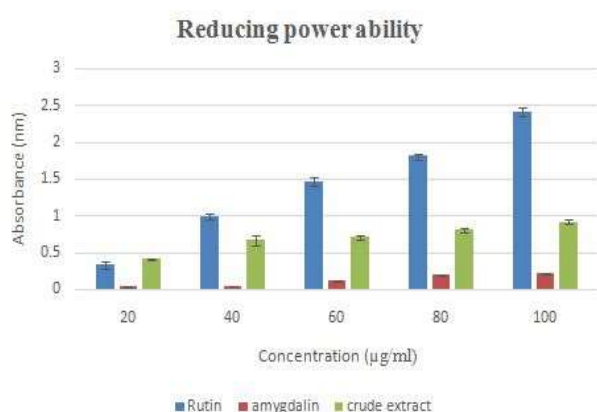


Fig. 5. Graph showing percentage of inhibition by DPPH assay

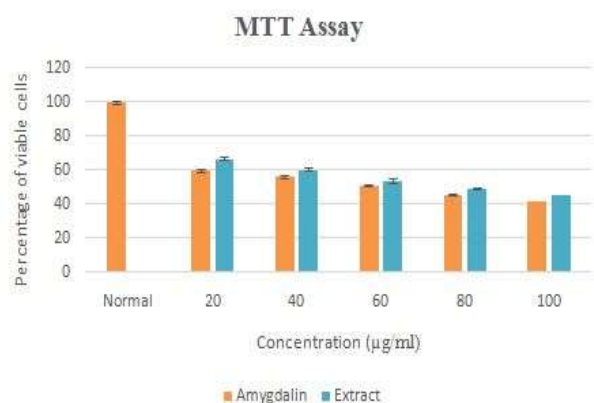
the extract is high when compared to the commercial amygdalin. In the reducing power assay, with the reduction of the  $Fe^{3+}$  cyanide complex to ferrous form, the green/blue is developed from the pale yellow colour. If the absorbance is higher, the reducing power is stronger. The intensity mainly depends upon the reducing power of the antioxidants. So the ferrous cation should be monitored by the absorbance at 700 nm (8).



**Fig. 6. Graph showing inhibition of free radicals by hydroxyl radical scavenging assay**



**Fig. 7. Graph showing percentage of inhibition by reducing power ability**



**Fig. 8. Effect of commercial amygdalin and extract on percentage of cell viability**

The HPLC has been done for the extract and standard and the curve was obtained. The HPLC was the method which was necessary to establish amygdalin in the sample. The ratio of methanol and water should be proper. In case if it is small, the analytical time will be long. If the methanol is large,

the amygdalin would not be separated well. Because of this reason only the gradient elution method should be followed (9). The MTT assay was also performed for the extract in different concentrations and also for the commercial standard also. It is mainly done to check the cell viability. In a study it was stated that amygdalin induces apoptosis in cancer cell lines (10). MTT assay can accurately determine the count of live cells and it is to find the cytotoxicity for cancer drugs (11).

#### 4. CONCLUSION

In the present study extraction of amygdalin from *P. dulcis* demonstrated by the reflux method was easy, fast, cost-effective, eco-friendly and non-toxic. The spectroscopic and chromatographic analysis such as FTIR and HPLC supported the synthesis of amygdalin. The amygdalin extract from *P. dulcis* exhibited a good antioxidant potential which was confirmed by various antioxidant assays. Amygdalin was found to have a significant cytotoxic effect against HeLa cancer cells. Further studies can be carried out to elucidate the biological properties of amygdalin.

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