Polyphenol Composition and in Vitro Antioxidant Activity of Prunus Amygdalus as Affected by Sprouting

Neeti Sharma[1], Ajinkya Ashokrao Shitole [2] and Ashwin Vivek Wadaskar [2]

[1] Symbiosis School of Biomedical Sciences
Symbiosis International University
Gram- Lavale; Taluka- Mulshi
Pune, India
Corresponding author email: neetimohan27 {at} gmail.com

[2] Symbiosis School of Biomedical Sciences
Symbiosis International University
Gram- Lavale; Taluka- Mulshi
Pune, India

[2] Symbiosis School of Biomedical Sciences
Symbiosis International University
Gram- Lavale; Taluka- Mulshi
Pune, India

ABSTRACT— ***The presence of natural antioxidant in plants is well known. Plant phenolics constitute one of the major groups of components that act as primary antioxidant free radical terminators. This paper reports for the first time the change in the antioxidative activity of methanolic extract of Prunus amygdalus as a result of sprouting. The amounts of total phenolics in the solvent extracts (methanol extract) were determined spectrometrically. From the analyses, methanolic extract of the peel/skin only had the highest total phenolic content (193 µg GA/1000 µg extract) and antioxidant activity (37%) using DPPH method. Increasing the concentration of the extracts resulted in increased antioxidant power for the extracts. Finally, a relationship was observed between the antioxidant potential and total phenolic levels of the extract of Prunus amygdalus.***

Keywords— Prunus amygdalus, Free radicals, Antioxidant activity, DPPH

1. INTRODUCTION

Nuts are traditionally considered as a nutritious component of almost all diet types. Almonds are the most popularly consumed tree nut, seed of the fruit of the almond tree. Almonds are classified into two categories: sweet (Prunus amygdalu var. dulcis) and bitter (Prunus amygdalu var. amara). Sweet almonds are the type that is eaten. They are oval in shape, usually malleable in texture and wonderfully buttery in taste. Almonds belong to Rosaceae family and are an important product due to high commercial value of its fruits [1]. Almond fruit consists of 3 or, more accurately, 4 portions: the kernel or meat, the middle shell, the outer green shell cover or almond hull, and a thin leathery layer known as the brown skin of the meat or the seed coat [2]. The nutritional importance of the almond fruit is related to its kernel. The hull splits open when the fruit reaches maturity and is then separated from the shelled almond (the whole natural almond).

Various studies on almonds indicate their contribution in improving serum lipid profiles, cholesterol status [3,4,5] and changes associated with reduced risk of cardiovascular diseases. Almonds are rich source of Vitamin E, protein, magnesium, calcium, phosphorus, niacin, omega-3, omega-6 fatty acids sterols, and flavonoids which has been associated with an anti-obesity effect in women and reduced risk of stroke, cardiovascular disease, and some forms of cancer [6,7,8,9,10].

Almonds are consumed, most often, after sprouting however there are no studies reporting the effect of sprouting on the antioxidant activity hence the objective of the present study was to investigate the effect of sprouting on the antioxidant activity of almonds as compared to the antioxidant activity of the dried almonds.

2. MATERIALS AND METHODS

2.1 Preparation of crude extract

The fruits of these almonds were collected, dried at room temperature, and exposed to the sun. For extraction of the antioxidant compounds, a fine dried powder of the sample (0.5 g) was extracted using 50 ml of methanol by sonication at room temperature for 20 min. The extracts were filtered through whatman filter paper No. 1 paper.

2.2 Antioxidant activity (Radical scavenging activity)

DPPH was used to evaluate the free radical scavenging activity of plant extracts as described by Hatano and others 1989[11]. Briefly methanolic extract of almonds were diluted to get final concentration 1mg/ml. From 1mg/ml stock serial dilutions were performed to get final concentrations 1000, 500, 250, 125, 62.5,31.25, 15.62 and 7.81µg/ml. Diluted samples (1ml each) were mixed with 1ml of methanolic solution of DPPH (1mg/ml). DPPH was filtered through Whatman filter paper no.1 after preparation. After 30 min incubation in darkness at room temperature (25^0C), the absorbance was recorded at 517nm.Control sample contained all the reagents except the plant extract. Percentage inhibition was calculated using equation given below:

$$\text{Inhibition (\%)} = \frac{\text{Abs 517 (control) - Abs 517 (extract)}}{\text{Abs 517 (control)}} * 100$$

The IC50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Ascorbic acid was used as the reference. All tests were performed in triplicate.

2.3 Determination of Total Phenolic Content

Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteu reagent and Gallic acid as standard [12]. About 1.0 ml of plant extract (5µg/ml) was taken in a test tube. Then 5 ml of Folin-ciocalteu (diluted 10 fold) reagent solution and 4 ml of sodium carbonate solution (7.5%) was added into the test tube. The test tube was incubated for 30 minutes at 20˚C to complete the reaction. Then the absorbance of the solution was measured at 765 nm using spectrophotometer against blank. The total content of phenolic compounds in plant ethanol extracts in Gallic acid equivalents (GAE) was calculated.

2.4 Statistical analysis

All experiments were performed with at least 3 replicates. One-way ANOVA was applied to determine the significance of results between different treatments. All the statistical analyses were done using SPSS v.11.5 for Windows.

3. RESULTS AND DISCUSSION

Phytochemicals especially polyphenols constitute a major group of compounds that act as most active antioxidants [13].They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because of they are stable radical intermediates [14]. Generally, the outer layers of plants such as the peel, shell, and hull contain large amount of polyphenolic compounds to protect the inner materials. A number of phenolic acids are linked to various cell wall components such as arabinoxylans and proteins [15].

Relative oxygen species and associated free radicals have been implicated in the etiology of various human diseases including inflammation, metabolic disorders, cellular aging and atherosclerosis, heart disease, diabetes mellitus, cancer, malaria, rheumatoid arthiritis and HIV/AIDS [16, 17,18].

Plants are known to possess diverse substances possessing antioxidant properties having ability to protect the human body against cellular oxidation. Anti-oxidants possess the ability to protect the body from damage caused by free radicals inducing oxidative stress [19,20]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [21].

In the present investigation the antioxidant properties and phenolic content of dried and soaked sprouted almonds was studied. As far as we are aware this is the first study to investigate the antioxidant properties and phenolic content of dried and sprouted almonds. Both dried and sprouted (soaked) almonds are considered beneficial for health as it is a rich source of Vitamin E, protein, magnesium, calcium, phosphorus and niacin, omega-3 as well as omega-6 fatty acids etc. Besides this soaked almonds are also beneficial as a cosmetic product. It has been observed that worldwide after soaking the almond peel or skin is discarded. The reducing power of almond hull and shell in different species increases significantly with phenol content.

In our study it was noted that the overall the free radical scavenging activity was increased on sprouting of almonds as compared to dried almonds. It was also observed that the free radical scavenging activity of skin of soaked almonds alone was found to be even higher than the soaked seeds alone (Fig. 1).

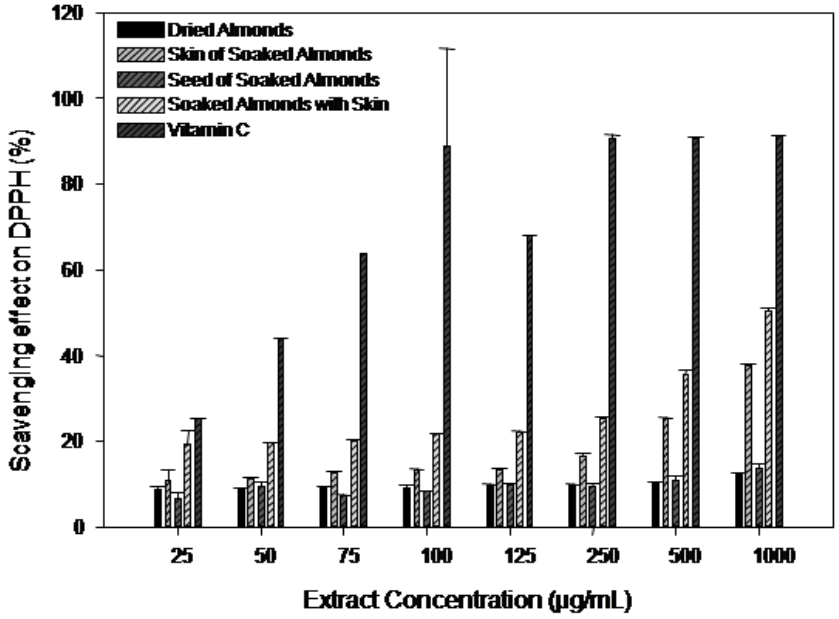

Figure 1: Free radical-scavenging activity of Prunus amygdalus at different concentrations.

The fold increase in scavenging activity of skin was three times alone as compared to four times increase in the activity of soaked almond with skin as observed (Fig. 2).

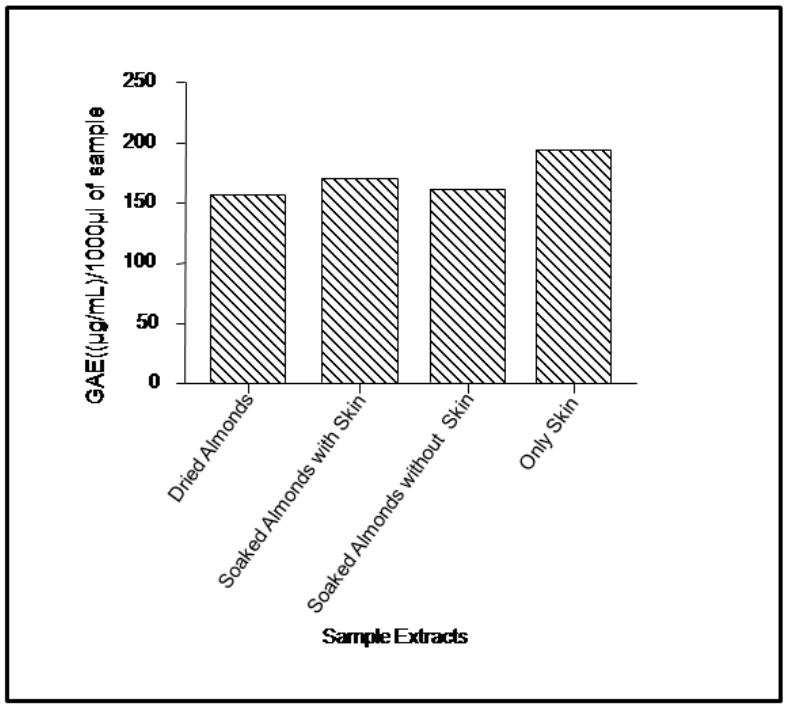

Figure 2: Total phenol content of *Prunus amygdalus* solvent extracts.

We also observed that the amount of total phenolic content was in accordance to the observed anti oxidant activity i.e. the total phenol content increased on sprouting (Fig. 3).

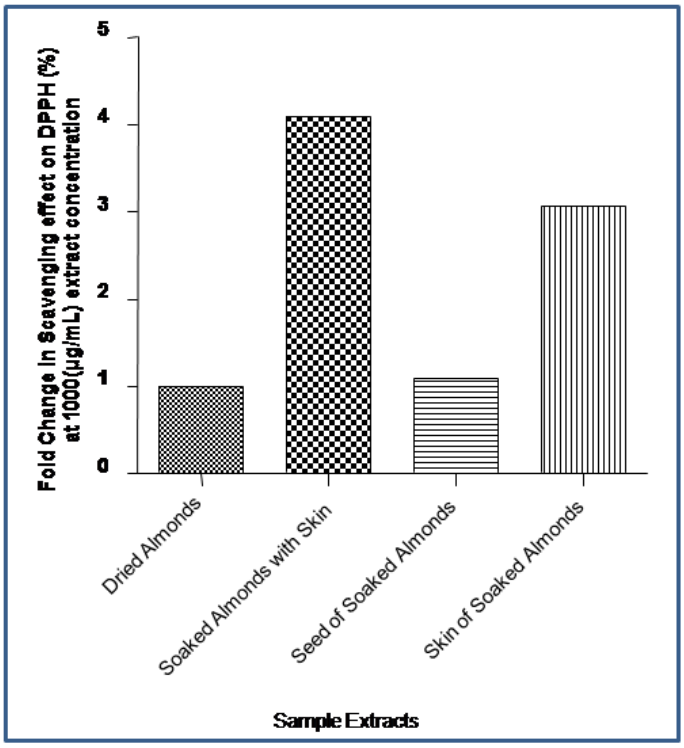

Figure 3: Fold increase in the free radical scavenging activity of Prunus amygdalus as a result of sprouting.

Sprouted (soaked) almonds had higher phenol content as compared to dried almonds. The phenol content was highest in the skin of the sprouted (soaked) almonds as compared to the seed of the sprouted (soaked) almonds.

Thus our findings indicate that sprouting increases the total phenol content and subsequently the antioxidant capacity is also increased (Fig. 1, Fig. 2, Fig. 3). This increase in total phenol content and the subsequent increase in the antioxidant capacity directly indicate the significance of consuming sprouted almonds.

4. CONCLUSION

This study affirms an increase in the in vitro antioxidant potential of solvent extracts of Prunus amygdalus skin and seed, with results comparable to those of the standard compounds such as Ascorbic acid. Sprouted Prunus amygdalus along with skin had higher phenol content and free radical scavenging activity as compared to dried Prunus amygdalus and can therefore be proposed as new potential sources of natural additives for the food and/or pharmaceutical industries. However, the components responsible for the antioxidant activities of the extracts were not identified and further work should be conducted to isolate and identify these bioactive compounds.

5. REFERENCES

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