Prunus Domestica L.: A Domestic Source of Natural Antioxidants

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Abstract

The aim of this study is to evaluate crude extract, ethyl acetate, chloroform and butanol fractions of *P. domestica* for their *in vitro* antioxidant activities using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging and reducing power assay on 1.25%, 2.5% and 5% concentrations. According to the results fraction of ethyl acetate showed maximum free radical scavenging up to 94% at the concentration of 5%, 93% at the concentration of 2.5% and 67% at the concentration of 1.25% followed by crude extract that showed 85, 54 and 41% activity at the concentrations of 5, 2.5 and 1.25% respectively. Chloroform fraction showed 70, 55 and 39% scavenging activity at 5, 2.5 and 1.25% concentrations respectively. While butanol fraction exhibited least activity i.e. 39, 36 and 9% on 5, 2.5 and 1.25% concentrations respectively. On the other hand, by reducing power assay method, ethyl acetate exhibited 90, 70 and 55% percent reducing power, followed by crude extract which exhibited 84, 62 and 41%, while chloroform extract exhibited 70, 42 and 28% and the least activity was shown by butanol extract i.e. 45, 22 and 12% at the concentrations of 5, 2.5 and 1.25% respectively. This study showed that ethyl acetate fraction exhibited best antioxidant potential and can be further isolated for biologically active constituents for further studies.

**Keywords:** *P. domestica* fractions, DPPH radical scavenging, reducing power assay.

INTRODUCTION

Free radical are compounds normally produce by body play a major role regarding human health, as they are capable to initiate many degenerative diseases like Alzheimer’s, Parkinson’s, Diabetes mellitus, atherosclerosis and many more [1]. Therefore to eliminate free radicals from body antioxidants are required. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these life threatening disorders [2, 3]. Although both synthetic and natural antioxidants are available but due to harmful effects of synthetic antioxidant there is now strong restrictions have been placed on their use [4, 5]. Natural antioxidants from fruits and vegetables play important role to reduce degenerative diseases. Currently there has been an increased interest to identify natural antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine that protect the cell constituents against destructive oxidative damage, inhibit hydrolytic and oxidative enzymes including lipid peroxidation [6]. Considering the growing demand and greater popularity of medicinal plants possessing the antioxidant capacity, we tried to identify the antioxidant effect in crude extract and different fractions of *P. domestica* dry fruit by using DPPH scavenging method and reducing power assay.

*Prunus domestica* (Rosaceae) or plum indigenous to Pakistan and other Asian countries with local name Alu-Bukhara or Alucha [7]. A number of pharmacological activities reported include measles, gastric problems, anti-cancer, anti-diabetic, anti-obesity, CVS problems, dyspepsia, nausea, vomiting, thirst, bilious fevers, liver diseases, gynaecological problems, analgesic, antioxidant, anxiolytic and respiratory tract diseases [8-10]. It is reported that *P. domestica* contains all necessary dietary constituents including carbohydrates, proteins, vitamins, minerals and dietary fibers. Other reported chemical constituents are benzaldehyde, linalool, ethyl nonanoate, methyl cinnamate, γ-decalactone, benzaldehyde, 2-furancarboxyvaldehyde, ethyl cinnamate, chlorogenic acid, neochlorogenic acid, caffeic acid, coumaric acid, rutin, proanthocyanidin and melanodins [11-13]. Keeping in view the reported pharmacology and...
phytochemistry of *P. domestica* and its medicinal importance, it was planned to screen and compare antioxidant activity in crude ethanol extract and different fractions of *P. domestica* by two different antioxidant methods.

**MATERIAL AND METHOD**

**Chemicals**

1,1-diphenyl-2-picryl-hydrazil (DPPH) and all solvents were purchased from Roche Diagnostics, Mannheim, Germany. DMSO, potassium hexacyanoferrate [K₃Fe(CN)₆], sodium carbonate (Na₂CO₃), butylated hydroxyanisole (BHA), Ferric chloride, trichloroacetic acid (TCA) were obtained from MP Biomedical (France).

**Instruments**

Rotary evaporator (Buchi), ELISA plate reader (Spectramax plus 384 Molecular Device, USA), spectrophotometer (Spectro 2000, Germany)

**Extraction of Plant material**

The dried fruit *P. domestica* was purchased from local market of Karachi, Pakistan. The sample was properly identified by Plant taxonomist, of PCSIR Labs Complex, Karachi. Plant specimens were submitted in Herbarium bearing voucher no. PDK-090-2010. After washing and air drying at room temperature the dried fruit of *P. domestica* (200g) was soaked into 1.5 Liter of 95% methanol which was added and centrifuged for 10 min at 3000 rpm. The supernatant was poured into separating funnel and left for one day. Next day ethyl acetate layer was collected and evaporated to collect ethyl acetate fraction. The left over aqueous layer was mixed with equal volume of DMSO/ methanol.100 ml of 0.1 % Ferric chloride [Fe Cl₃] was added and the absorbance was measured at 700 nm by using spectrophotometer. Reduction capability was calculated in terms of percentage with respect to reference standard (BHA).

**RESULTS**

The ethyl acetate fraction exhibited maximum free radical scavenging activity up to 94% at 5% conc. 93% at 2.5% conc. and 67% at 1.25% conc. Crude extract showed 85, 54 and 41% at 5, 2.5 and 1.25% conc. respectively. While chloroform fraction showed 70, 55 and 39% scavenging activity at 5, 2.5 and 1.25% conc. Butanol fraction showed the least activity i.e. 39, 36 and 9% at 5, 2.5 and 1.25% conc. respectively (Fig. 1).

The crude extract and fractions of *P. domestica* were also examined for reducing power and found very effective results. The ethyl acetate fraction exhibited 90, 70 and 55% reducing power, followed by crude extract which exhibited 84, 62 and 41%, while chloroform extract showed 70, 42 and 28% and the least activity was shown by butanol extract i.e. 45, 22 and 12% at 5, 2.5 and 1.25% concentrations respectively (Fig. 2).

**DISCUSSION**

Free radicals compounds were generated normally by body’s own physiological and biochemical processes. Over production of these compound leads to many chronic diseases. Therefore to reduce or eliminate these diseases body requires antioxidants [15, 16]. Because of carcinogenic effects of synthetic antioxidants, globally attention is diverted towards natural antioxidants [17]. Natural herbs have numerous medicinal activities because of their certain bioactive chemical constituents [18]. Therefore medicinal herbs are catching attention to be commercial source of antioxidants to combat with these free radicals and can be a better replacement for the treatment of life threatening chronic diseases. The phytochemical screening of various fractions and crude extract of *P. domestica* for antioxidant abilities revealed that it
possesses biologically active constituents particularly ethyl acetate fraction that showed significant reducing power and free radical scavenging ability in dose dependent manner.

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Substances which have reduction potential react with potassium ferricyanide [Fe]$^{3+}$ to produce potassium ferrocyanide [Fe]$^{2+}$ which then reacts with ferric chloride [FeCl]$^{3+}$ to form ferric ferrous complex that has absorbance maxima at 700nm. This principle helps us to evaluate reducing potential of any material. These results clearly indicate that ethyl acetate fraction has great amount of responsible constituents which make it strongest representative of reducing power among all fractions and extract of *P. domestica*. While the crude extract and chloroform fraction also has capacity of reduction of ferric ions to ferrous state but butanol fraction was devoid of significant reducing potential. Many publications reported that plants possesses antioxidant activity due to their chemical compounds like tannins and flavnoids and our results also support these statements as *P. domestica* possesses these compounds [8].

CONCLUSION

From this study it is concluded that among the crude extract and various fractions of *P. domestica* including ethyl acetate, chloroform and butanol, the ethyl acetate fraction is found to have best antioxidant potential and can be further isolated for future studies. This study strongly supports the idea that plant constituents with antioxidant activity can be capable of exerting protective effects against oxidative stress in biological systems and further in vitro studies can be based to be performing in future.

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