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Original Research Article

Prunus Domestica L.: A Domestic Source of Natural Antioxidants

Hina Imran[1], Mehreen Latif[1], Zahra Yaqeen[1], Tehmina Sohail[1], Syed Rafay Yaqeen[2*], Syed Shafay Yaqeen[1], Wasif Iqbal[3]

[1]Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi Pakistan
[2]Baqai Medical University, Karachi Pakistan
[3]Dow Universities, Karachi Pakistan

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***Corresponding author:** Syed Rafay Yaqeen

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-furancarboxyaldehyde, 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 [K_3Fe $(CN)_6$], sodium carbonate (Na_2CO_3), butylated hydroxyAnisol (BHA), Ferric chloride, trichloroacetic acid (TCA) were obtained from MP Biomedicals (France).

Instruments

Rotary evaporator (Buchi), ELISA plate reader (Spectramax plus 384 Molecular Device, USA), spectrophotometer (Specord 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. domstica* (200g) was soaked into 1.5 Liter ethanol (70%) and kept at room temperature. After every 24 hours mixture was stirred by using a sterilized glass rod. After 7 days, soaked material was filtered and concentrated on rotary evaporator at 45ºC under reduced pressure. Recovered solvent was again used for percolation for another seven days. The process was repeated thrice and combined together to obtain the extract. A part of extract was utilized for further fractionation. 100ml ethyl acetate and 50ml water was added to the crude extract which was shifted to a 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 then treated with 100ml chloroform and with 100ml butanol and the above mentioned process was repeated to get these fractions. The fractions and the extract were stored in refrigerator for antioxidant studies.

DPPH Radical Scavenging Assay

The free radical scavenging activity was measured by using 1,1-diphenyl-2-picryl-hydrazil (DPPH) as free radical [14]. The solution of DPPH of 0.3 µM was prepared in ethanol. Each sample (5 µL) of different concentration (1.25-5%) was mixed with 95 µL of DPPH solution in ethanol. The mixture was dispersed in 96 well plates and incubated at 37 ºC for 30 min. The absorbance at 515 nm was measured by ELISA plate reader and percent radical scavenging activity was determined in comparison with the methanol treated control. BHA is used as standard.

DPPH scavenging effect (%) = Ac – As/Ac × 100
Where, Ac = Absorbance of control (DMSO treated);
As = Absorbance of sample.

Reduction Capability

Total reduction capability was estimated according to the method [14] with some modification.100 µl solution of each test (extract and fraction) in methanol with various concentrations was mixed with 250 µl of 0.2 M phosphate buffer (pH 6.6) then 250 µl solution of 1% potassium ferricyanide [K_3 Fe $(CN)_6$] was added. The mixture was incubated at 50°C for 20 min then, 250 µl trichloroacetic acid (10%) was added and centrifuged for 10 min at 3000 rpm, 250 µl from upper layer was separated and mixed with equal volume of DMSO/ methanol.50 µl of 0.1 % Ferric chloride [Fe Cl_3] 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 exhibited 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].

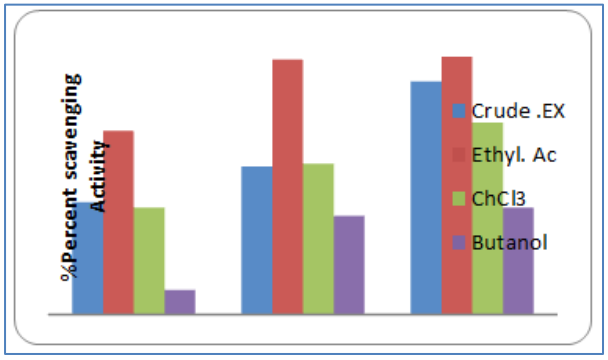

Fig-1: DPPH scavenging model by extract and fractions of *P. domestica*

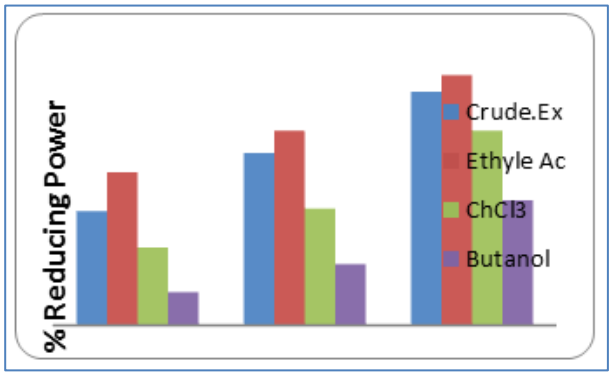

Fig-2: Reducing Power assay by extract and fractions of *P. domestica*

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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