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Study of Antioxidant Potential of *Solanum tuberosum* peel extracts

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ABSTRACT

The hydroxyl and nitric oxide, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities and total phenolic content of Solanum tuberosum peels extracts in ethyl acetate and methanol were investigated in vitro. Total Polyphenolic Contents were determined in terms of Gallic Acid Equivalent. Reducing power Assay was also determined by standard method. In the present study it has been demonstrated that, ethyl acetate extract contains higher amount of Polyphenolic Contents as compared to methanolic extract as well as DPPH Radical Scavenging is concentration dependent and Solanum tuberosum peel extract in ethyl acetate extract shows more DPPH Radical Scavenging than methanol extract. To conclude, Solanum tuberosum peels extracts showed a good antioxidant activity and might be an alternate to synthetic antioxidants available in the market.

Keywords: Antioxidant, methanol extract, Hydroxyl radical DPPH, Total Polyphenolic Contents

INTRODUCTION

Human population is exposed to a number of chemicals either accidentally, occupationally or through life style habits. Many of these foreign substances exhibit their toxic effects by generating reactive oxygen species and other free radicals which cause damages to cells and various cellular molecules. The reactive oxygen species are causative factors in the etiologies of many diseases such as cancers and hepatopathies[1].

Medicinal plants have always been considered a healthy source of life for all people. Therapeutically properties of medicinal plants are very useful in healing various diseases and the advantages of these medicinal plants is being 100% natural[2].

An antioxidant is capable of inhibiting oxidation of other molecules. Oxidation reaction can produce free radicals. In turn, radicals can start chain reactions that can damage cells and tissues in body. These are atoms, molecules, or ions with unpaired electrons on an open shell configuration. They may also be involved in Parkinson's diseases, drug -induced deafness, schizophrenia, and Alzheimer's disease. The classic free- radical syndrome, the iron-storage disease hemochromatosis, is typically associated with a constellation of free-radical Related symptoms including movement disorders, psychosis, skin pigment melanin abnormalities, deafness, arthritis, and diabetes mellitus . The free radical theory of aging proposes that free radicals underlie the aging process itself, whereas the process of mitohormesis suggests that repeated exposure to free radicals may extend life span[1] . An antioxidant may be added for this purpose to pharmaceutical products subjected to deterioration by oxidative process.

Antioxidants are among the most important variables in controlling or preventing the free radical reaction. An antioxidant if present in low concentration can prevent oxidation of substances like proteins lipids and DNA the major biological antioxidants are ascorbyl palmitate, tocopherol, betacarotene, plant phenolics and thiols containing compounds[3]. Because free radicals are necessary for life, body has a number of mechanisms to minimize free radical induced damage and to repair damage that occurs, such as the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. In addition, antioxidants play a key role in the defense mechanisms. These are often present in three vitamins vitamin A, vitamin C and vitamin E and polyphenol antioxidants. A large number of medicinal plants and their purified constituents[4] have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum, Piper cubeba Linn., Allium sativum Linn., Terminalia bellerica, Camellia sinensis Linn., Zingiber officinale Roscoe* and several Indian and Chinese plants.[5,6]

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The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins.[7] Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes,[8] Alzheimer's disease and cancer[9].The antioxidant and radical scavenging activities of black pepper (*Piper nigrum Linn.*) seeds have been well reported.[10] Both water extract and ethanol extract of black pepper exhibited strong antioxidant activity. Antimicrobial[11] larvicidal[12] and anti-cancer[13] activities of *Piper nigrum Linn.* have been reported. Antioxidant activity of potato have been determined and compared with that of broccoli, onion, carrot and bell peppers was higher than all except broccoli[14]. *Solanum tuberosum* (Potatoes) has become plant of interest in the scientific community in the last seven to ten years. It has free scavenging effects, decrease the risk of coronary heart disease, nourishes the spleen and benefits the stomach; stops relieves urgency, detoxifies and relieves swelling they are highly digestible and used to treat hard, greasy and wrinkled skins. A literature survey made on the plant *Solanum tuberosum* revealed the presence of antioxidant properties in root. The present study has been undertaken to access the preparation of ethyl acetate and methanolic extracts of *Solanum tuberosum* peels and to determine the antioxidant potential through various biochemical methods.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The fresh and healthy peels of ***Solanum tuberosum*** were collected from Punjab. The peels were processed for studying the antioxidant potential in Pharmaceutical Chemistry Laboratory of Chandigarh group of college Gharuan, Mohali. The average weights of fresh peels were 76.8 g. After drying average weight of dried peels were found to be 15 g. The moisture content for the peels was 80.47%.

PREPARATION OF EXTRACTS

Fresh peels were separated and washed thoroughly with distilled water and freeze dried. The dried samples were ground to powder and stored airtight. The powdered peels were extracted with solvents of varying polarity such as methanol and ethyl acetate according to their boiling points for 24 hrs with a mass to volume ratio by using soxhlet apparatus. Final extracts were distilled by using distillation apparatus and evaporated to dryness under vacuum on a rotator evaporator. Then solid mass was obtained. All the extracts were stored at 4° C until used.

SAMPLE PREPARATION

0.1g of sample was dissolved in 10 mL of solvent and final volume was made to 100 mL with solvent. Six test tubes were taken and labeled as 1, 2, 3, 4, 5, 6. In the test tube no. 1 blank was taken. 0.2, 0.4, 0.6, 0.8, 1.0 ml of sample solution was added to test tube 2, 3, 4, 5, 6 respectively. Solvent was added to each test tube to made final volume upto 1 mL. Thus samples were prepared for both methanol and ethyl acetate extracts of following concentrations 200, 400, 600, 800 and 1000 µg/mL. Sample S1 stands for ethyl acetate extract and S2 stands for methanol extract of *Solanum tuberosum*.

BIOCHEMICAL ASSAY

(A) TOTAL POLYPHENOLIC CONTENTS

The total Polyphenolic Contents in both extracts of *Solanum tuberosum* were determined by using Folin-Ciocalteu method[11]. The extracts were oxidized with Folin-Ciocalteu Reagent, and the reaction was neutralized with Sodium Corbonate. The absorbance of the resulting blue colour was measured at 725nm UV-VIS Spectrophotometer. Total Polyphenolic Contents are calculated in terms of Gallic Acid equivalents for both extracts. 1.0 mL sample of various concentrations mixed with 1.0 mL of solvent. 5.0 ml distilled water and 0.5 ml of 50% Folin-Ciocalteu Reagent was mixed with above solution. Reaction mixture was allowed to react for 5 minutes and 5% Sodium Carbonate solution was added. Reaction mixture was mixed thoroughly and placed in dark place for 1 hour. After that absorbance was measured at 725 nm from UV-Vis Spectrophotometer. Total Polyphenolic Contents were expressed as Gallic Acid Equivalents

STANDARD CALLIBRATION FOR GALLIC ACID

Dilution of Gallic Acid was made with both solvents (Ethyl Acetate and Methanol) separately and above procedure is used for the preparation of standard calibration curve of Gallic Acid.

(A) REDUCING POWER ASSAY

Fe^{3+} Reducing Power Assay of the extract was determined by Oyaizu method[12]. 0.75 ml of extract at various concentrations was mixed with 0 .75ml of Phosphate Buffer (0.2 M, pH 6.6) and Potassium Ferric Cyanide (1%w/v) and incubated at 50°C in water bath for 20 minutes. This reaction was stopped by adding 0.75 mL of Trichloroacetic Acid solution (10%) and centrifuged at 800 rpm for 10 minutes. Then 1.5 mL of supranatant was mixed with 1.5 mL of distilled water and 0.1 mL of Ferric Chloride (0.1%). After 10 minutes absorbance was measured at 700 nm with UV-Vis Spectrophotometer.

(B) HYDROXYL RADICAL SCAVENGING ACTIVITY

The scavenging activity for hydroxyl radical was measured with Fenton Reaction. 1.5 mL of extract was mixed at various concentrations with 60 µl of 1.0 mM Ferric chloride ($FeCl_3$) , 90 µl of mM 1,10 Phenanthroline, 2.4 mL of 0.2 M Phosphate Buffer (pH 7.8) and 150 µL of 0.17 M Hydrogen Peroxide (H_2O_2). Absorbance of reaction mixture was measured at 560 nm with UV-Spectrophotometer after incubation at room temperature for 5 minutes. Then Hydroxyl radical scavenging activity was calculated by using following equation.

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 X\ 100]$$

Where A_0=Absorbance of the control in the absence of the sample (blank) A_1= Absorbance of sample (extract)

NITRIC OXIDE SCAVENGING ACTIVITY

Nitric Oxide Scavenging Activity was measured by reported method[13]. 2 mL of 10 mM Sodium Nitropruside and 0.5 mL of Phosphate Buffer saline (pH 7.4) were mixed with 0.5 mL of extract at various concentrations. Reaction mixture was incubated at 25°C for 150 minutes. Then 0.5 mL was taken from incubated reaction mixture and added into 1.0 mL of Sulphanillic Acid Reagent (33% in 20% Glacial Acetic Acid) and incubated at room temperature for 5 minutes . Finally 1.0 mL Naphthylenediamine dihydrochloride was mixed into the above mixture and incubated at room temperature for 30 minutes. Then absorbance was taken at 540 nm with UV- Vis. Spectrophotometer.

Nitric Oxide Scavenging Activity was calculated by following equation.

% Inhibition = $[(A_0 - A_1) / A_0 \text{ X } 100]$

Where A_0=Absorbance of the control in the absence of the sample (blank) A_1= Absorbance of sample (extract)

(E) DIPHENYL PICRYL HYDRAZYL (DPPH) SCAVENGING ACTIVITY

Diphenyl Picryl Hydrazyl Scavenging Activity was determined by the standard method[14].Various concentrations of extracts were mixed with 0.8 mL Tris HCl Buffer (pH 7.4) and 1.0 mL DPPH was added in this reaction mixture. Reaction mixture was shaken vigorously and left for 30 minutes and absorbance was measured at 517 nm with UV-Vis Spectrophotometer. Scavenging Activity was calculated by following equation.

$$\% \text{ Inhibition} = [A_0 - A_1 / A_0 \text{ X } 100]$$

Where A_0=Absorbance of the control in the absence of the sample (blank) A_1= Absorbance of sample (extract)

RESULTS AND DISCUSSION

TOTAL POLYPHENOLIC CONTENTS (TPC)

Total Polyphenolic Contents in ethyl acetate and methanol extract was determined from Calibration Curve

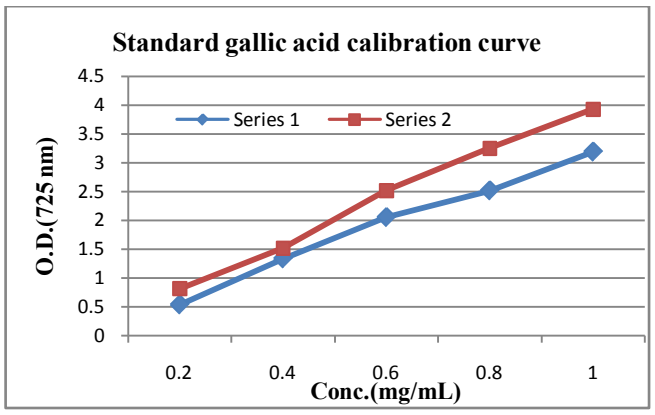


Figure 1. Standard Gallic Acid Calibration Curve

and expressed in Gallic Acid equivalents (GAE).

Total Polyphenolic Contents in terms of Gallic Acid Equivalent (GAE) is higher in ethyl acetate extract (S1) than methanol extract (S2) at each concentration and varies in dose dependent manner (highest for 1.0 mg/ml)

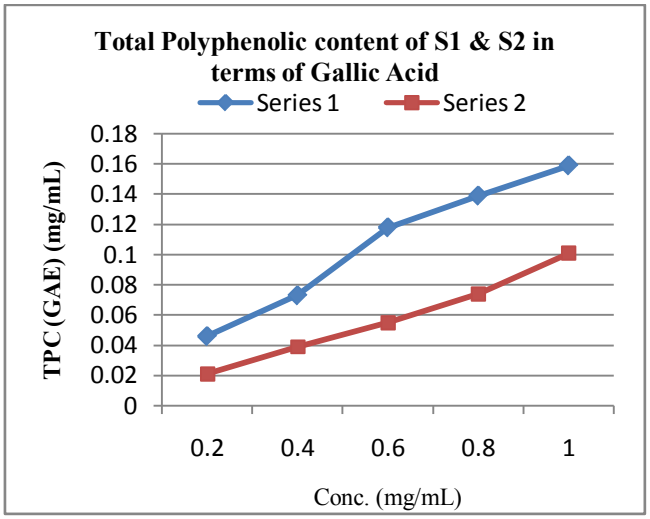


Figure 2. Graphical representation of Total Polyphenolic Contents

REDUCING POWER ASSAY

Fe^{+3} Reducing Power Assay is convenient and easy method. The increase in Absorbance gives the increase in reducing power extent.

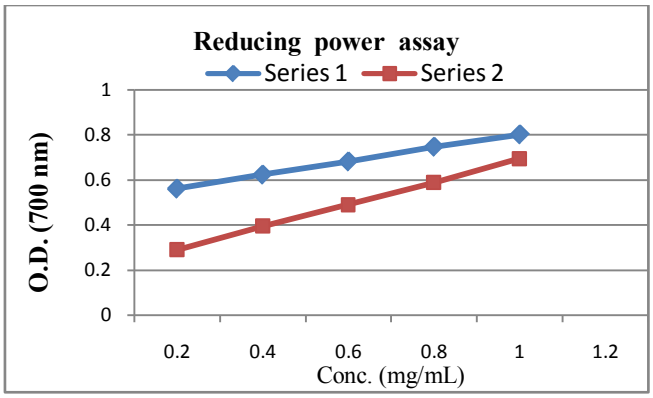


Figure 3. Reducing Power Assay of *Solanum tuberosum* peelextract in ethyl acetate (S1) & methanol (S2).

From the result it has been shown that *Solanum tuberosum* peel extract is showing higher Reducing Power in ethyl acetate (S1) than in methanol (S2) at each concentration and Reducing Power increases with increased in concentration of extract.

HYDROXYL RADICAL SCAVENGING ACTIVITY

Hydroxyl Radical is very reactive and can be generated in biological cells Through Fenton reaction. It has been observed that *Solanum tuberosum* peel extract in ethyl acetate (S1) and in methanol (S2) exhibits concentration

dependent scavenging activities against hydroxyl radicals generated in Fenton system.

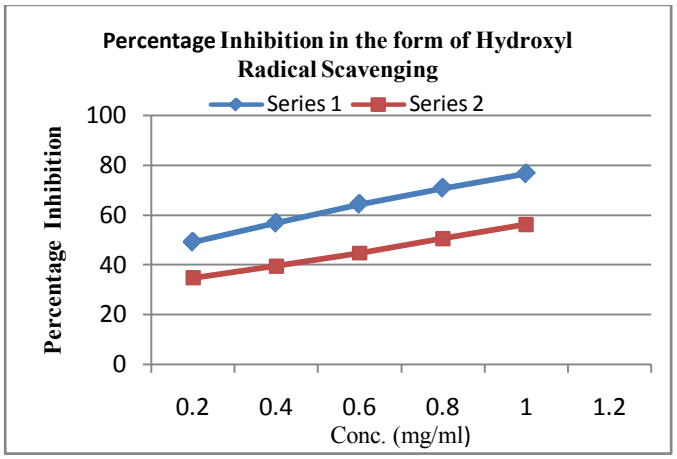
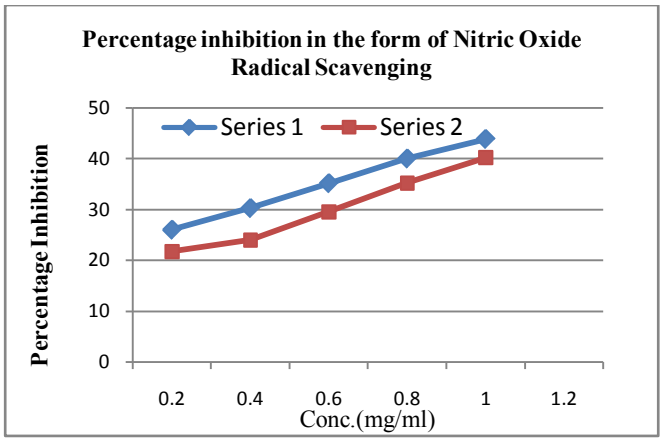


Figure 4. Percentage Inhibition in the form of Hydroxyl Radical Scavenging in *Solanum tuberosum* peel extract of ethyl acetate (S1) & methanol (S2). Blank O.D for S1 0.2760 and for S2 0.3790, O.D. at 560 nm

Solanum tuberosum peel extract demonstrates that Hydroxyl Radical Scavenging is concentration dependent and ethyl acetate (S1) show more Hydroxyl Radical Scavenging than methanol extract (S2).

NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

Nitric Oxide (NO) is potent Pleiotropic Mediator of physiological processes such as Smooth muscle relaxation, neuronal signaling of inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effecter molecule.



Blank O.D. for S1 0.7220 and for S2 0.8820 (O.D. at 517 nm)

Figure 5 Percentage Inhibition in the form of Nitric Oxide Radical Scavenging in *Solanum tuberosum* peel extracts of Ethyl Acetate (S1) Methanol (S2).

Solanum tuberosum peel extract demonstrates that Nitric Oxide Radical Scavenging is concentration dependent and ethyl acetate extract (S1) shows more Nitric Oxide Radical Scavenging than methanol extract (S2).

DIPHENYL PICRYL HYDRAZYL (DPPH) SCAVENGING ACTIVITY

This method is used for screening of antioxidant activity of many plant drugs. This method is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger.

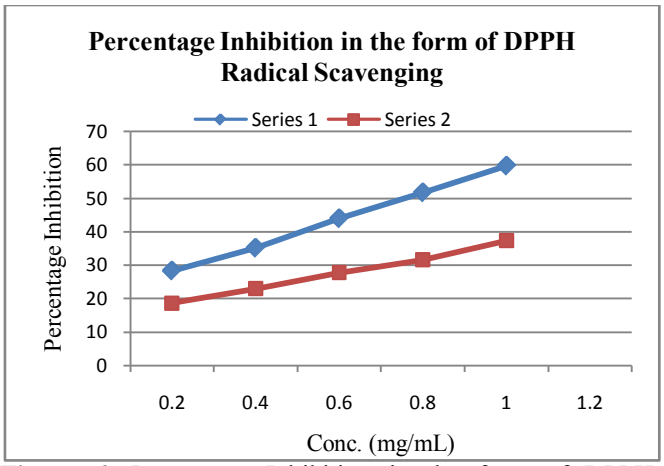


Figure 6. Percentage Inhibition in the form of DPPH Radical Scavenging in *Solanum tuberosum* peel extract of ethyl acetate (S1) & Methanol (S2).

Solanum tuberosum peel extract demonstrates that DPPH Radical Scavenging Is concentration dependent and ethyl acetate extract (S1) shows more DPPH Radical Scavenging than methanol extract (S2).

CONCLUSION

Free radicals are causative factors in the aetiologies of many diseases.[15-20] They are easily generated through normal metabolic pathways in the body. Antioxidants, on the other hand are able to quench free radicals thereby preventing diseases. Natural antioxidants of plant sources have been associated with reduction of chronic diseases through their capacity to terminate free radical propagation in biological systems.[21]

In the present statues, there is a strong need for an effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases like cancer, cardiovascular diseases, Age related macular degeneration and atherosclerosis. In the present study, we can conclude that *Solanum tuberosum* ethyl acetate extract (S1) contains higher amount of Polyphenolic Contents as compared to methanol extract (S2). Polyphenolic Contents (GAE) linearly increases with increase in concentrations of extract S1 and S2 respectively. Antioxidant Potential and reducing Power varies as function of concentration and total Polyphenolic Contents for both extracts, S1 shows higher values at every concentration as compared to S2. The results of the study helps us to conclude that *Solanum tuberosum* peel extract has higher amount of Antioxidant Potential in ethyl acetate (S1) in comparison to methanol (S2) because Hydroxyl radical

scavenging as well as Nitric Oxide Radical Scavenging is more in case of S1 rather than S2 . So *Solanum tuberosum* peels may play a role in preventing human diseases (e.g. Heart disease) in which free radicals are involved.

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