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RESEARCH ARTICLE

Nutritional, antioxidant and antiproliferative properties of persimmon (*Diospyros kaki*) -a minor fruit of J&K India

Amreen Nazir[1], *S. M. Wani[1], Adil Gani[1], F. A. Masoodi[1], E. Haq[2], S. A, Mir[1], Umaya Riyaz[1]

1. Food Science & Technology, University of Kashmir Srinagar, India-190006.

2. Department of Biotechnology, University of Kashmir Srinagar.

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Corresponding Email:
wanisajad82@gmail.com

Abstract

Extracts from persimmon using various solvents i.e. ethanol, methanol, ethanol + water (50:50 v / v), water & acidified methanol (99 ml methanol +1 ml HCl) were analyzed for DPPH radical scavenging activity, Reducing power, Total phenolics, Hydroxyl radical scavenging activity, FRAP & Lipid peroxidation. Antiproliferative activity was determined by MTT assay using brain cancer cell lines. Antioxidant activity in all the solvents increased in a dose dependent fashion with increase in volume of extracts used. Amongst different solvents used, ethanol in general showed highest antioxidant activity. Extracts showed a good antiproliferative property as evidenced by % increase in cell death on addition of fruit extracts. Persimmon has a promising potential to be used against many deadly diseases, which may be owed to its good antioxidant properties.

Introduction

Persimmon (*Diospyros kaki* L), belonging to family Ebeneceae, has been cultivated for thousands of years. Persimmon is believed to have originated in China (Luo & Wang, 2008), before spreading to Korea and Japan, where it is a traditional crop and then to other regions of the world where it is considered an exotic fruit. World production of persimmons reached 3,627,575 t in 2008, with an upward trend since 1965 (FAOSTAT, 2010); the main producers are China (2,533,899 t), Korea (430,521 t), Japan (244,800 t), Brazil (169,000 t), Azerbaijan (132,179 t), Spain (70,000 t), Italy (50,000 t), Israel (30,089 t) and Uzbekistan (31,000 t). The fruit has attracted attention of several researchers because of its health promoting properties. Persimmon is generally recognized as an outstanding source of biologically active compounds related to both nutritional and nutraceutical values.

Persimmons are noted for their high level of water soluble dietary fibre, minerals, trace elements and contain significant antioxidants (β-carotene, β-cryptoxanthina, lutein, zeaxanthin, and lycopene). Some are active with provitamin A (β-carotene and β-cryptoxanthine). Among phenolic acids, gallic, chlorogenic, vallinic and protocatechuic acids (benzoic acids derivatives), caffeic, ferulic and p-coumaric acids (cinnamic acid derivatives) have been determined in fresh persimmon by different authors (Chen et al., 2005; Jung et al., 2008; Suzuki et al., 2005). Persimmon is also a good source of biologically active compounds such as ascorbic acid and condensed tannins and these are related to various physiological functions including a protective role against oxidative stress-related diseases, antimutagenic and anticarcinogenic capacities (Suzuki et al., 2005). This fruit possesses hypolipidimic and antioxidant properties and can be used in anti-atherosclerosis diets. Persimmons are particularly rich in vitamin C, carotenoids and polyphenols, all of which are usually considered as powerful antioxidants that protect against free radicals, prevent risk of cardiovascular disease, diabetes and cancer (George & Redpath, 2008; Park et al., 2008; Piretti, 1991; Uchida,\ et al., 1990). Diets supplemented with this fruit improve plasma lipid metabolism and increase total antioxidant activity in rats. The lipid lowering effect of persimmon was more evident when whole persimmon or its parts were added to the diet of rats with nutrition-induced hypercholesterolemia (Gorinstein et al., 2000). Persimmons are suggested

to exert a chemoprotective effects against different cancerous cells, such as oral carcinoma cells, human lymphoid leukaemia cells (Takayuki, 2005) and precancerous colon polyps in women (Takayuki, 2005). Catechins and lycopene, both present in quite high amounts in persimmons, have been found to be active in both in vitro and in vivo tests against different kinds of cancers (Dorgan et al., 1998).

Persimon is a minor fruit crop of J &K, India and is not very frequently consumed despite enormous health benefits. It is grown mainly without any use of chemicals which makes the fruit and its products free from harmful residues. Keeping in view the above mentioned health benefits of persimmon; present study was carried out to study the effect of extraction solvents on its antioxidant activity which will pave way for its popularization of its consumption.

Material and Methods

2.1 Proximate composition

2.1.1 Moisture Content

Moisture content was determined by digital moisture analyzer (Sartorius-MA 100) at 100 ^{0}C.

2.1.2 Ash Content

Ash content of the persimmon pulp was determined according to the procedure of AOAC (1995). 3 g of sample were placed in pre-weighed crucible, charred over a flame and kept in a muffle furnace at a temperature of 600 ^{0}C for about 2 hrs. The crucible was cooled in a dessicator and weighed accurately.

2.1.3 TSS

Soluble solids were determined using refractometer (Digital Abbe Refractometer, Kruss Optronic) (AOAC, 1980), expressed as ^{0}Brix. A drop of the solution was squinted on the prism of refractometer. The percentage of TSS was obtained from direct reading of the instrument.

2.1.4 pH

The pH of the persimmon pulp was determined by using digital pH meter. The pH meter was first calibrated using buffer of pH 4.0 & pH 7.0 at room temperature. The sample was taken in 100 ml beaker, stirred & electrode of pH meter put in it and direct reading from pH meter was taken when the reading stabilized.

2.1.5 Titratable acidity (TA)

Acid content in persimmon was determined by titration. For the titratable acidity (TA), approximately 10 mL of sample was diluted with 100 mL of distilled water and 3-4 drops of phenolphthalein indicator were added. Acids in the persimmon were titrated with 0.1N NaOH. The percentage of malic acid was calculated according to the following expression:

% Acidity = (titre vol. × normality of NaOH × vol. made up × eq.wt. of acid / **(**aliquot of sample × vol. of sample × 1000) × 100.

2.1.6 Ascorbic acid (Vitamin C)

Ascorbic acid content of persimmon was estimated by titration method (Ranganna, 1986) using 2, 6-dichlorophenol indophenol dye solution. The method of estimation involves the reduction of 2, 6-dichlorophenol indophenol dye to a colorless form by ascorbic acid in an alkaline solution. The reaction is quantitative and particularly specific for ascorbic acid in solution in the pH range of 1-3.5. In the procedure followed, the dye solution was first standardized against standard ascorbic acid in order to determine the dye factor. The sample was diluted with 3% metaphosphoric acid and then the phosphoric acid extract of the sample was titrated against the dye solution until a pink color was obtained which persists for 15seconds. Dye factor was determined by the following equation:

Dye factor=0.5/ Titrate vol.

Ascorbic acid was estimated as mg of ascorbic acid / ml, and was determined by the following equation:

mg of ascorbic acid / ml = titrate vol. (ml of dye used) × dye factor × vol. made up × 100 / aliquot of sample taken for estimation × vol. of sample.

2.1.7 Reducing Sugars

The quantification of reducing sugars in persimmon pulp was carried out using Lane & Eynon method (Ranganna, 1986). 5 g of sample were placed in a measuring cylinder, added with 100 ml distilled water and stirred thoroughly. The sample was neutralized with 1 N NaOH using phenolphthalein as indicator. It was followed by addition of 5 ml of 45% lead acetate, and then 5ml of 22% potassium oxalate was added after 10 minutes. Final volume was made upto 250 ml (using distilled water). The above formed solution was filtered and marked as solution A. The Fehling's solutions were titrated against solution A on hot plate till brick red colour developed. After adding 5-7 drops of methylene blue, the same was again titrated till permanent brick red colour was obtained. Percent reducing sugar was determined using the following equation:

% reducing sugar =0.5 ×vol. made / titrate value × weight of sample × 100

2.2 Antioxidant Activity:

2.2.1 Sample preparation:

The extraction of persimmon pulp was carried out using different solvents like ethanol, methanol, ethanol + water (50:50 v / v), water & acidified

methanol (99ml methanol +1 ml HCl). 2 g of pulp were mixed with 8 mL of each of the above mentioned solvents followed by centrifugation at 10000×g for 10 minutes. The supernatant was collected and was used for analysis of antioxidant activity.

2.2.2 DPPH (1,1-dihpenyl-2-picrylhydrazyl) radical scavenging activity

The assay was carried out according to the method of Shimada et al. (1992). For DPPH, three different concentrations of persimmon extracts (0.1, 0.2, 0.3 µL) were added to 1.0 mL of 0.01% methanolic solution of DPPH. Absorbance at 517 nm was measured after 30 min. The % inhibition was calculated against a control using formula as:

% inhibition = A control 517- A sample 517/A control 517 × 100.

2.2.3 Reducing power

The reducing power of persimmon extracts was determined according to the method of Oyaizu (1986). 2.5 mL of fruit extract were mixed with phosphate buffer (2.5 mL, 2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50 ^{0}C for 20 min. 2.5 mL of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 1500×g for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.2.4 Total Phenolics (TP)

TP concentrations were assayed using Folin-Ciocalteu method as described by Jayaprakasha et al. (2001). Briefly, in a 10 mL Eppendorf tube, 7.9 mL of distilled water, 0.1 mL of extract, and 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) were mixed. After 1 min, 1.5 mL of sodium carbonate (20 g per 100 mL) was added and mixed well. The reaction solution was then incubated at room temperature for 2 h in the dark before absorbance was read at 765 nm. The TP concentration was calculated from a calibration curve using gallic acid as standard.

2.2.5 Hydroxyl radical scavenging activity

Hydroxyl radical-scavenging activity was assayed using the 2-deoxyribose oxidation method given by Chung et al. (1997) with minor modifications. The reaction mixture contained 0.45 mL of 0.2 M sodium phosphate buffer (pH 7.4), 0.15 mL of 10 mM 2-deoxyribose, 0.15 ml of 10 mM $FeSO_4$–EDTA, 0.15 mL of 10 mM hydrogen peroxide, 0.525 mL of distilled water, and varying concentration of persimmon extract in a tube. The reaction was started by the addition of hydrogen peroxide. After incubation at 37^{0}C for 1 h, the reaction was stopped by adding 0.75 mL of 2.8% trichloroacetic acid and 0.75 mL of 1.0% thiobarbituric acid. The mixture was boiled for 10 min and then absorbance was measured at 520 nm. Hydroxyl radical-scavenging ability was evaluated as the inhibition rate of 2-deoxyribose oxidation by hydroxyl radicals. The results were calculated as the percentage inhibition according to the following formula:

% inhibition = $A_{control}$ 532- A_{sample} 532 /$A_{control}$ 532 × 100

Where $A_{control}$ 532 was the malondialdehyde produced by Fenton reaction treated alone, and

A_{sample} 532 was the malondialdehyde produced in presence of extract.

2.2.6 FRAP

FRAP of each persimmon extract was measured according to the modified protocol developed by Benzie & Strain (1996) with minor modifications. Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants contained in the samples to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm . The assay was carried out with 100µL, 200µL, 300µL & 400µL of extracts. To prepare FRAP solution a mixture of 0.1M acetate buffer (3.6), 10mM TPTZ (2,4,6-tripyridyl-s-triazinesolution in 40 mM HCl) & 20 mM ferric chloride (10:1:1,V/V/V) was made. To 1.9 ml of reagent 0.1 ml of extract was added. Absorption maximum was taken using a UV-visible spectrophotometer. The absorbance of reaction mixture was measured after 4 minutes against reagent blank. Value was expressed in µM FRAP/g fresh weight material. Ascorbic acid was taken as standard.

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute / Change in absorbance of standard from 0 to 4 minute) X FRAP value of standard.

2.2.7 Lipid Peroxidation

The antioxidant activity of persimmon extracts was determined spectrophotometrically according to the method described by Wallin et al. (1993) with minor modifications. Different concentrations (100µL, 200µL, 300µL & 400µL) of the extract were mixed with 1 mL of linoleic acid (0.1g in 100 ml of pure ethanol), 0.2 mL of H_2O_2 (30mM), 0.2 mL of ascorbic acid (100mM) and 0.2ml of ferric nitrate (20mM). This was followed by incubation at 37^{0}C in water bath for 1h. The reaction was stopped by the addition of 1.0 mL TCA (Trichloroacetic acid, 10% w/v); followed by addition of 1.0 ml of TBA

(Thiobarbituric acid, 1% w/v) and all the tubes were placed in a boiling water bath for 20 minutes. The tubes were then centrifuged at 5000 rpm for 10 min. The amount of malondialdehyde formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm against a reagent blank.

2.3 Antiproliferative Activity:

Antiproliferative activity was investigated according to the method of Mosmann et al. (1983) with minor modifications. Human brain cancer cell line was used for the MTT assay. Cells were plated in 96 well plate at 5000-7000 cell density per well. Cell were grown overnight in 100 µL of 10% FBS. After 24 hours cells were replenished with fresh media and persimmon extracts were added to the cells. Different concentrations, 1µL, 5µL, 10µL, 15µL & 20µL of the methanolic extracts of pulp samples were added to wells in triplicates. Cells were incubated with the extract for 24 hours. After 24 hours 20 µL of MTT dye (5mg/mL) were added to each well and further incubated for 3 hours. Before read-out, precipitates formed were dissolved in 150 µL of DMSO using shaker for 15 minutes. All the steps performed after MTT additions were performed in dark. Absorbance was measured at 590 nm.

Result and Discussion

3.1 Proximate composition

Chemical composition of persimmon fruit is presented in Table 1. Total soluble solids(%), titratable acidity(%), moisture content(%) & total sugars(%) recorded values of 10±0.42, 0.210±0.01, 68.9±1.27 & 7.40±0.31 respectively. pH, vitamin C(mg/100g), Ash(%) & reducing sugar(%) content of persimmon fruit were found to be 5.96±0.21, 6.9±0.24, 0.32±0.02 & 2.87±0.10 respectively.

Table: 1. Proximate composition of persimmon

Parameter	Results
Moisture content (%)	68.9 ±1.27
Ash content (%)	0.32 ±0.02
Reducing sugars (%)	2.87 ±0.10
TSS (%)	10 ±0.42
pH	5.96 ±0.21
Titratable acidity (%)	0.21 ±0.01
Total sugars (%)	7.40 ±0.31
Vitamin C (mg/100g)	6.9 ±0.24

3.2 Antioxidant properties

Antioxidants are the substances that reduce oxidation and so counteract the reactive species. Reactive oxygen species (ROS) are major free radicals generated in many redox processes, which may induce oxidative damage to biomolecules including carbohydrates, proteins, lipids, and DNA. DNA base alterations, strand breakage & mutations are problems that are usually associated with free radical attack on DNA. Reactive oxygen species affect living cells, which mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, chronic inflammatory diseases, cancers, and other degenerative diseases (Dermott, 2000). Consumption of antioxidants has been associated with reduced levels of oxidative damage to lymphocytic DNA (Mushtaq & Wani, 2013). Antioxidants interfere with the production and the activation of free radicals, resulting in protecting the human body from free radicals, that may cause some chronic diseases including cancer and cardiovascular diseases (Kinsella et al., 1993). They are also used to preserve food by retarding rancidity, discoloration or deterioration due to auto-oxidation (Huang et al., 2003). Although synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate are very effective & are used for industrial processing but they possess potential health risks & toxic properties to human health and should be replaced with natural antioxidants (Mayakrishnan et al., 2012). Fruits can be rich sources of various vitamins, minerals and fibers required by human body for optimal health. Epidemiological studies have shown that high fruit intake can be associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer one possible mechanism is attributed to the antioxidant activity (Lampe 1999; Amarowicz et al., 2004).

Phytochemicals are bioactive compounds found in fruits, vegetables and other plant foods that are known to reduce the risk of major chronic diseases. Phenolic compounds are one of the main groups of dietary phytochemicals, known for their antioxidant activity which has been linked to slowing down the ageing process and lowered risks of many prevalent diseases such as cancer and coronary heart disease. Persimmon fruit contains a large number of components such as condensed tannins, dietary fiber, carotenoids, gallic acid, catechins and flavonoids etc. Phenolic compounds in plant foods include a wide range of compounds and a broad spectrum of functional activities. Traditionally, these compounds have been considered important in plant foods because of their impact on flavor and colour, but there is substantial current interest in them because of

their health promoting properties. They are increasingly recognized as potential health promoters on account of their antioxidant & anticancerous properties.

In the present study, the persimmon pulp extracted with different solvents was assayed for antioxidant activity. The use of different solvents, during the study presents the advantage of assaying their effects on antioxidant activity of persimmon. In our study, DPPH, Reducing power, Total Phenolics, Lipid Peroxidation & FRAP assays were used for the evaluation of antioxidant activity.

3.2.1 DPPH radical scavenging activity:

The effects of different solvent extracts of persimmon on DPPH radical scavenging activity are presented in Fig 1. The ethanolic extracts of persimmon pulp showed highest % inhibition (67%), among all the solvents used for extraction. For other solvents like methanol, ethanol + water, acidified methanol & water the values obtained were 45%, 50%, 25% & 12% respectively. Thus the order of %inhibition obtained for different solvents was as: ethanol > ethanol+water > methanol > acidified methanol > water. Furthermore during our study, dose dependant responses were obtained i.e. the % inhibition increased with increasing concentration of extracts from 0.1 to 0.3µL for each solvent used in the study. The DPPH radical scavenging assay is widely used to evaluate the antioxidant capacity of plant extracts. Unlike other free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Yildirim et al., 2000). The essence of DPPH assay is that the antioxidants react with the stable free radical 1, 1-Diphenyl-2-picrylhydrazyl (deep violet color) and converts it to 1, 1-Diphenyl-2-picrylhydrazine with a yellow color. The degree of discoloration indicates the scavenging potential of the sample antioxidant resulting in a decrease in absorbance at 517nm. Radical scavenging activity might possibly be ascribed to the phytochemicals present in the extract which could act as hydrogen donors.

3.2.2 Reducing Power:

Reducing power may serve as a significant indicator of potential antioxidant activity. In this study, reducing activity was determined based on the ability of persimmon extracts to reduce Fe^{3+}/ferricyanide complex to form Fe^{3+} ferrous complex. The amount of Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm. The dose– response curve for the reducing activity of persimmon extracts with different solvents is shown in Fig 2. In the present study, the ethanolic extracts showed a significantly higher reducing power. The reducing power of all extracts increased with increasing concentration and these varied in the order: ethanol > ethanol+water > methanol > acidified methanol > water. Reducing power assay measures the electron-donating capacity of an antioxidant. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+} / ferricyanide complex used in this method to the ferrous form may serve as a significant indicator of its antioxidant capacity. The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom (Singh & Rajini, 2004). The reduction of the Fe^{3+} / ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution. Absorbance of Fe^{2+} can be measured at 700 nm. The reducing power of the compound is related to its electron donating ability and may thereforeserve as significant indicator of its antioxidant activity (Ajila et al., 2007).

3.2.3 TOTAL PHENOLS:

The Total Phenolic content in persimmon fruit extract obtained through different solvents is presented in Fig 3. The TPC attained through different solvents from the same fruit were significantly different to each other. The observed trend was as: ethanol + water > ethanol > methanol > acidified methanol > water. The highest values were obtained with ethanol + water (80.84 mg GAE/100g) & lowest with water (11.28 mg GAE/100g). Many studies have shown that the physiological functions of natural ingredients linked usually to the antioxidant activity of phenolic compounds. During the study, the effect of extraction solvent was evident by the difference in the amount of phenolics obtained with each solvent. The highest phenolic content obtained with ethanol + water, might be attributed to the ability of aqueous ethanol to solubilise more phenolics present in the studied fruit. The combination of aqueous & organic solvents has improved effects on TPC as compared with water or organic solvent alone (Musa et al., 2011).

3.2.4 Hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity of persimmon in different solvents is presented in Fig 4. The effect of the extracts is evaluated by monitoring the degraded DNA fragments through the formation of MDA (malondialdehyde). % inhibition observed with different solvents can be shown as: ethanol (30 to 72%) > ethanol+water (22 to 54.2%) > methanol (18 to 50%) > acidified methanol (12 to 40%) > water

(10 to 30%). The highest inhibitory effects were observed with ethanol & lowest with water as solvent. It was observed that the persimmon plant extracts exhibited dose dependant inhibitory responses. Active oxygen species or free radicals, such as singlet oxygen (O_2), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), & hydroxyl radical (OH^-) are considered to cause oxidative damage to DNA, which bring about a variety of diseases as well as ageing. Persimmon contains many bioactive compounds such as polyphenols, flavonoids, & carotenoids. Carotenoids, the most potent biological quenchers of singlet O_2, act as chain breaking antioxidants (Liebler, 1993), and flavonoids inhibit the enzymes responsible for O_2 production (Hanasaki et al., 1994). 2-Deoxyribose is oxidized by hydroxyl radicals formed by the Fenton reaction and degrades to malondialdehyde (Gutteridge, 1984). The effect of the extracts on OH radical generated by Fe^{3+} ions was measured by determining the degree of deoxyribose degradation. The antioxidant(s) in the plant extracts competed with deoxyribose against the OH radical generated from the Fe^{3+} dependent system. The antioxidant(s) in these plant extracts could be acting as chelators of the Fe^{3+} ions in the system, thereby preventing them from complexing with the deoxyribose, or simply donating hydrogen atoms and accelerating the conversion of H_2O_2 to H_2O (Wang et al., 2007).

3.2.5 Ferric reducing antioxidant power (FRAP):

The FRAP values for different solvent extracts of persimmon are presented in Fig 5. Of all solvent extracts, the highest FRAP value expressed in µM FRAP/g fresh weight basis was found in ethanolic extract (11.00) and the lowest value in aqueous extracts of persimmon pulp (1.28) respectively. The order of FRAP activity for different solvent extracts was as: ethanol > ethanol+water > methanol > acidified methanol > water. During our work, a concentration- dependant response was observed *i.e.* FRAP value of extracts increased with increasing concentration. FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduced oxidant system. The principle underlying the assay is that at low pH, reduction of ferric tripyridyl triazine (Fe^{3+} TPTZ) complex to ferrous form Fe^{2+} (which has an intense blue colour) can be monitored by measuring the change in absorption at 593nm. The assay measures the reducing capability by increased sample absorbance based on the formed ferrous ions. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture (Amin et al., 2013). Generally the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. Furthermore the reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process, so that they can act as primary and secondary antioxidants (Yen & Chen, 1995).

3.2.6 Lipid Peroxidation:

Inhibition of lipid peroxidation was assessed by the amount of malondialdehyde (MDA) produced. The % inhibition with varying concentrations is presented in Fig 6. All extracts showed a dose- related inhibition among which methanolic extracts showed highest inhibitory effects with values ranging from 50.2% to 80.1%. Lipid peroxidation involves the formation and propagation of lipid radicals with numerous deleterious effects, including destruction of membrane lipids, metabolic disorders and inflammation, and production of malondialdehyde (MDA) is a hallmark of this process. The assay involves the oxidation of linoleic acid in presence of Fe^{2+} & ascorbic acid. Malondialdehyde (MDA), a secondary end-product of the oxidation of polyunsaturated fatty acids, reacts with TBA, yielding a pinkish red chromogen with an absorption maximum at 532 nm. During our study, the extracts exhibited significant inhibitory effects on lipid peroxidation which may account for cytoprotective effects of persimmon against oxidative damage.

3.2.7 Antiproliferative activity:

The Antiproliferative effects of methanolic extracts of persimmon on cancer cells is present in Figc7. During the experiment, blank (without extract) was taken as reference for which no significant cytotoxic effects were observed. The % cell death rates for concentration 1µL, 5µL, 10µL, 15µL & 20µL were found as: 3.45%, 21.23%, 35.05%, 48.89% and 54.02% respectively. The methanolic extracts showed cytotoxicity on cancer cell lines in a dose dependant manner i.e. highest cell death rate was witnessed at 20µL (54.02%). The methanolic extracts exhibited potent antiproliferative effects on human brain cancer cell lines. The Antiproliferative effects of methanolic extracts may be due to polyphenolics present in persimmon.

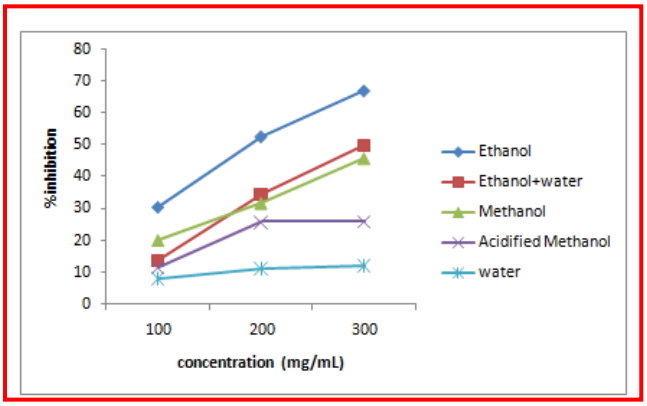

Fig. 1. DPPH radical scaven ging activity of persimmon in different solvents.

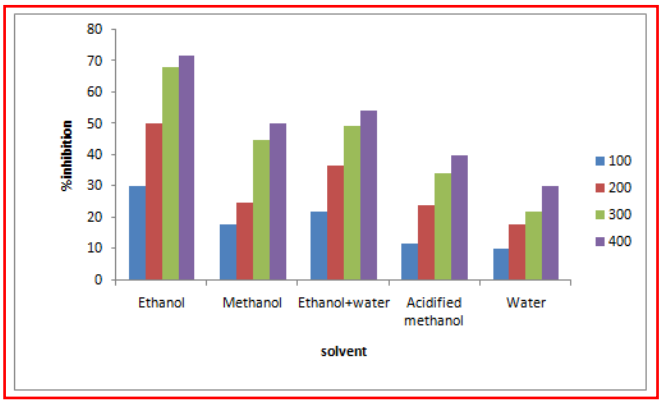

Fig 4. Hydroxyl radical scavenging activity of persimmon extracts in different solvents

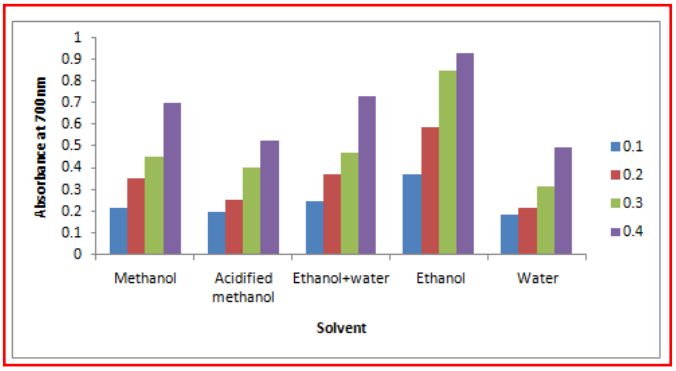

Fig 2. Reducing power exhibited by persimmon fruit extracts with different solvents.

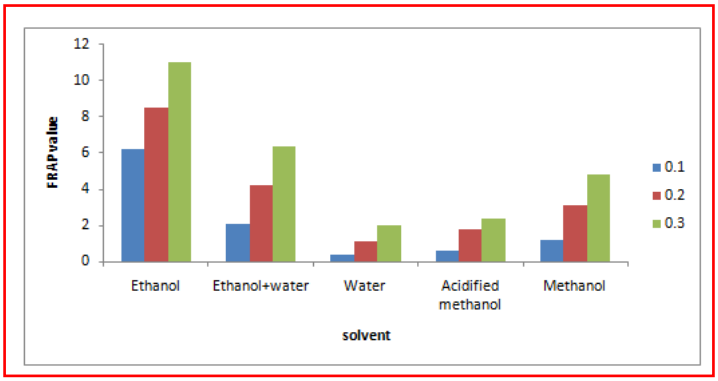

Fig 5. FRAP of persimmon extracts in different solvents with varying concentration

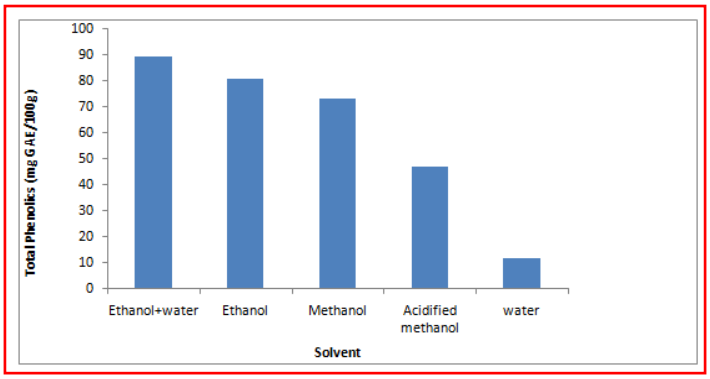

Fig 3. Total phenolics obtained for different extraction solvents.

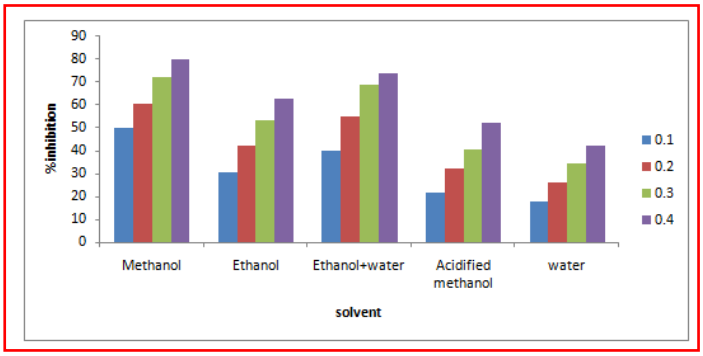

Fig 6. Lipid peroxidation exhibited by persimmon extracts in different solvents.

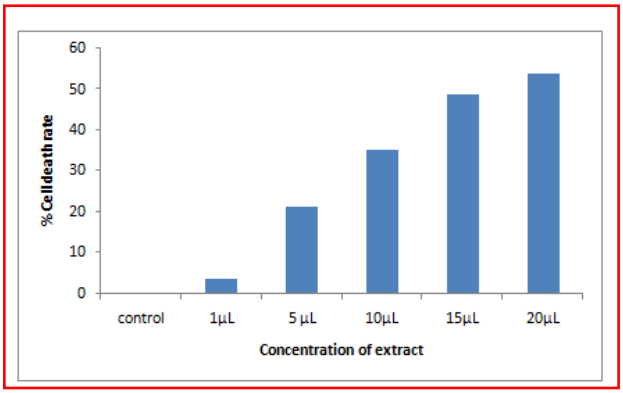

Fig 7. Antiproliferative activity of persimmon extract with varying concentrations.

Conclusion

Persimmon contains abundant amount of phytochemicals mainly polyphenols, tannins, carotenoids, vitamin C etc. From our study it can be concluded that persimmon possess good antioxidant activities, which were assessed through DPPH, Reducing power, Total phenols, Hydroxyl radical scavenging activity, Lipid peroxidation & FRAP. Furthermore, preliminary studies show that it possesses anticancerous properties as well, which became evident from antiproliferative effects of persimmon fruit extracts on human brain cancer cell lines.

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