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EFFICACY OF BULB EXTRACTS OF *ALLIUM CEPA* VARIETIES (RED, WHITE AND SMALL ONION): AN *IN VITRO* ANTIFUNGAL AND ANTIOXIDANT ACTIVITY

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ABSTRACT

The study was performed to evaluate the efficacy of different extracts of bulb of the *Allium cepa* varieties on antifungal and antioxidant activity. The extracts were prepared with aqueous, ethanol, chloroform and petroleum ether and subjected for preliminary phyto-chemical, antioxidant and antifungal activity. The total ash and water content, organic, inorganic were evaluated along with the different extracts of onion bulb. Total phenolic content were analyzed by HPLC method. The presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phyto-chemical analysis. DPPH free radical, nitric oxide, superoxide anion radical scavenging assays showed strong antioxidant activities with increase in concentration of bulb extracts of the aqueous, ethanol, chloroform and petroleum ether fractions of the three onion varieties. Antifungal activity was assessed by the paper disc agar diffusion method. The results of the antifungal activity showed that among the onion varieties analyzed, the small onion exhibited strong fungal growth inhibition in its aqueous, chloroform and petroleum ether extraction while white onion is effective in its ethanolic extract and red onion is effective in its chloroform extract. Screening of antioxidant and antibacterial activity performed on *A.cepa* bulb extracts shows that they are endowed with potentially exploitable free radical scavenging and antifungal activity. These results showed that onion bulb can be a source of compounds that can serve as templates for future fungicides against *Aspergillus niger, Aspergillus, fumigates, Candida albicans and Aspergillus flavus.* Hence, bulb extracts of *A.cepa* varieties could be used as an easy accessible source of natural antioxidants, and antifungal agent making it one of the potent therapeutic phytomedicines.

KEYWORDS: Anti fungal activity, bulb extracts, antioxidant activity, onion varieties

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INTRODUCTION

Since ancient times, onions (*Allium cepa, L.*) have been an important dietary resource and have also been of interest for medical purposes (Rose *et al*., 2005). Traditionally, onions and plants belonging to the *Allium* genus have been used as an herbal remedy for a wide range of ailments, due to their association with many pharmacological effects (Yin & Cheng, 1998; Rose *et al*., 2005). Biological effects attributed to onions have been commonly ascribed to the volatile sulfur-containing compounds, such as thiosulfinates, mainly responsible for the characteristic taste, aroma and lachrymatory effects (Lanzotti, 2006). These compounds are formed from cysteine sulfoxide precursors and the effect of the enzyme alliinase which is released from cell vacuoles when tissues are damaged (Krest & Keusgen, 2002). However, these volatile products are highly unstable and recently attention has been focused on the effects of phenolic compounds, such as flavonoids, which are more stable (Ioku *et al*., 2001). Onion is known for being a good natural source of flavonoids mainly represented by the flavonols - quercetin and kaempferol, which are present as their glycosides (Fossen *et al*., 1998). In recent years, many publications have reported evidence of beneficial health effects attributed to flavonoids including antiallergenic, antiinflammatory, cardioprotective, vasodilatory, anticarcenogenic and antioxidant properties (Shon *et al*., 2004). Several epidemiological studies have also associated the consumption of flavonoids with a reduction of the risk of chronic diseases including, cancer, diabetes and coronary heart problems (Hirvonen *et al*., 2001; Kosmider & Osiecka, 2004). The antibacterial and antifungal properties reported to be possessed by flavanoids (Rauha *et al*., 2000) has increased the interest of the food industry in these natural compounds as components to improve food stability against microbiological spoiling agents (Taguri *et al*., 2004; Sofia *et al*., 2007). Protection of food from pathogens and spoilage organisms has been traditionally achieved by chemical methods, but during recent years there has been an increase in consumer interest in developing foods which contain a low level or are free of chemical preservatives (Viuda-Martos *et al*., 2008). The emergence of pathogens which are resistant to classical preservatives has also created an urgent necessity to find alternative antimicrobial agents (Xu & Lee, 2001). In consequence, the food industry is interested in developing natural components for the total or partial replacement of synthetic antimicrobials (Grohs & Kunz, 2000).

Onions can be considered as a good source of natural additives to retard food deterioration (Navas *et al*., 2006). However, the application of thiosulfinates and volatile compounds for food preservation is limited due to their strong flavour and biochemical instability (Benkeblia, 2004). These properties focus attention on the more stable flavonoids as additives to enhance food shelf-life by inhibiting microbial spoiling and oxidative deterioration, due to their antimicrobial and antioxidant properties (Ramos *et al*., 2006; Naz *et al*., 2008). Therefore, this study intended to investigate the antifungal activity of bulb onion extract on selected fungi and to explore the preliminary phyto-chemical analysis, physico-chemical analysis and to find if the anti-bacterial properties of bulb extracts of *A.cepa* varieties are responsible for its pharmacological properties.

MATERIALS AND METHODS

1. Chemicals

All chemicals and reagents used in the present study were of molecular and analytical grade and they were purchased from Sigma Chemicals Company, St. Louis, MO, USA; and Sisco Research Laboratories (SRL), Mumbai, India. Quercetin standard was purchased from Sigma-Aldrich Company Ltd (Gillingham, UK) for the assessment of TLC and HPLC. The standard drug, Ketoconazole for fungi were procured from High media Company.

2. Micro organism

Antifungal activities of aqueous, ethanol, chloroform and petroleum ether extracts of various *A.cepa* varieties (red, white and small onion) were studied. Ketoconazole was used as standard drug. The microorganisms, maintained on Nutrient Agar (Merck, Darmstadt, Germany), were supplied by the microbiology laboratory of the University. Four species of fungi, *Aspergillus niger, Aspergillus fumigates, Candida albicans and Aspergillus flavus* were used in this study.

3. Collection and identification of plant materials

The plant materials, three types of onion (red, white and small onion) were selected for the investigation. Fresh onions were procured from the local market in Chennai, Tamil Nadu, India. The voucher specimens of the plants were authenticated by Taxonomist, Prof. A. Manoharan, Department of Plant Biology and Plant Biotechnology, Presidency College, Chennai, Tamil Nadu, India and were sorted for uniformity and absence of defects and stored at 4°C prior to analysis.

4. Extraction of plant materials

i) Aqueous extract

Fresh onions were peeled off their outer layer and 1 kg of onion was washed thoroughly with distilled water and then the bulb was cut into pieces and was made into a crude paste. This paste was soaked in 1 litre of sterile distilled water for 24 hours at 4°C and it was then filtered thrice using a sterile muslin cloth. The filtrate was poured into a beaker and concentrated on a water bath at 100°C to obtain semi-solid residue and aqueous extract was weighed and this was immediately subjected to preliminary phyto-chemical and antifungal analysis using standard method.

ii) Ethanolic extract

After cleaning 1kg of onion as described earlier they were made into a paste which was soaked in 500ml of ethanol for 15 days at room temperature then it was filtered using sterile muslin cloth and the filtrate was poured into a beaker and concentrated on a water bath at 70-80°C to obtain semi-solid residue. The weight of the yield was noted and this was subjected to preliminary phyto-chemical and antifungal analysis.

iii) Chloroform extract

After making a paste of 1kg onion of different varieties as described earlier, they were separately soaked in 300 ml of chloroform for a week at room temperature. It was then filtered using sterile muslin cloth and the filtrate was concentrated in a beaker at 60-62°C to obtain semi-solid residue. This was weighed and subjected to preliminary phyto-chemical and antifungal analysis*.*

iv) Petroleum ether extract

Following the earlier procedure, onion bulb of different varieties were prepared and soaked in 200 ml of petroleum ether for 15 days at room temperature. Then it was filtered and the filtrate was concentrated at 40-60°C. The extract was weighed and subjected to preliminary phyto-chemical and antifungal analysis**.**

5. METHODS

a. Determination of water content

Water content of *A. cepa* varieties (Red, white and small onion) was determined using the method of Nwinuka *et al.* (2005). Thermal drying method was used in the determination of water content of the samples. 10g bulbs of different *A. cepa* varieties were weighed in triplicate and placed in washed, dried and weighed crucible. This was placed in an oven and dried at 105°C (Hot air oven, AUSCO Company, Chennai) for three hours. The samples were allowed to cool in a desiccator and then reweighed. The percentage of water content was calculated by expressing the loss in weight on drying as a fraction of the initial weight of sample used and multiplied by 100.

$$WC\ (\%) = Wo/\ W_i \times 100$$

Where, Wo = loss in weight (g) on drying and Wi = initial weight of sample (g).

b. Determination of total ash

A known weight of *A.cepa* varieties dry bulb which ignited to ash and the weight of the ash thereby obtained was expressed in terms of percentage. In a clean clay crucible, three varieties of *A.cepa* dry bulb were taken and weighed. Weighed dish was placed over a tripod stand carefully. The crucible were opened partially and directed to the tip of the flame for gradual heating at 500°C. The onion samples were heated gently to avoid catching fire. When the smoke subsides the burner was placed underneath the dish. It was gradually ashed continuously, till it becomes a white ash. Then the dish was cooled to room temperature and weighed with its contents. Again the sample was heated to effect for any possible ashing and weighed. The process was repeated till three consecutive weighing and complete combustion was taken. The total ash was then determined and recorded. The percentage of ash content was calculated using the formula:

$$\text{Ash (\%)} = Ma/Ms \times 100$$

Where, M_a = Mass of ash (g) and M_s = Mass of sample used (g).

c. Determination of pH

pH of different *A.cepa* varieties were determined as per the method of Park and Chin (2010) and the pH values of *A.cepa* were measured using a digital pH-meter (Elico, LII20, digital pH-meter, Taiwan). 10g bulbs of different *A. cepa* varieties were homogenized with 90 ml of sterile double distilled water, after which the pH values were measured five times for its concordancy and expressed in average values.

d. Preliminary phytochemical screening

Qualitative phytochemical screening of different extracts (aqueous. ethanol, chloroform, petroleum ether, successive chloroform and successive ethanol) of *A.cepa* varieties [Big (Red, White) and Small] were analyzed in the present investigation by the methods of Harbone and Baxter (1993).

e. *Determination of total phenolic content* (*TPC*)

Total phenolic contents in the ethanolic extract of *A.cepa* were analysed by HPLC according to the method of Hertog *et al.* (1992). The aquepus, ethanol, chloroform and petroleum ether extracts of *A.cepa* varieties were analysed in HPLC system from Agilent 1000 series HPLC system, UV- absorbance Diode Array Detector (DAD). The Merk column was C_{18} (4.6 mm X 250 mm, 5 µm at 40°C) and mobile phase constituted of solvent A (0.1% formic acid) and solvent B acetonitrile) with gradient elution, (i.e., solvent B was increased from 7 to 45% within 30 minutes and then dramatically decreased to 7% within 1 minute. The flow rate was 1.4 ml/minutes and detection was made at 275 nm. Identification of total phenolic contents were analysed by HPLC by comparing the retention time of standards. The results are expressed in percentage of mean±SD.

7. *In vitro* antioxidant studies

a. DPPH free radical scavenging activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the method of Hatano *et al*, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of different extracts of *A.cepa* varieties in methanol at different concentrations (100, 200, 300,400 and 500µg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%).

b. Nitric oxide radical scavenging activity

Scavenging of nitric oxide radical was assayed by the method of Garrat *et al*. (1964). In the total volume of 3ml reaction mixture, 2ml of sodium nitroprusside, 500µl of phosphate buffered saline (PBS) were mixed with 500µl of different concentrations (100, 200, 300,400 and 500µg/ml) of extracts of A.cepa varieties and incubated for 1 hour 30 minutes at 25°C. Then, 500µl of reaction mixture containing nitrite was mixed with 1 ml of sulfanilic acid and allowed to

stand for 5 minutes for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 minutes at 25°C. A pink colored chromophore is formed in diffused light. Ascorbic acid at the same concentrations was used as standard. The activity was measured at 550 nm and the results were expressed in percentage (%).

c. Super oxide anion scavenging activity

Scavenging of superoxide anion activity was assessed by the method of Liu *et al*. (1997). Superoxide anions were chemically generated in a mixture of phenazine methosulphate (PMS) and NADH. The reaction was quantified by coupling superoxide generation to the reduction of nitroblue tetrozolium (NBT). In this experiment, the superoxide radicals were generated in 3ml of Tris-Hcl buffer (16mM, pH 8.0) containing 1ml of NBT (50 µM), 1ml of NADH (78 mM) and 1ml of various concentrations (100, 200, 300, 400, 500 µg/ml) of *A.cepa* varieties extracts. Ascorbic acid at the concentrations was used as standard. The reaction mixture was incubated at 25°C for 5 minutes and the activity was measured at 560nm. Results were expressed in percentage (%).

d. Protective effect of A.cepa varieties (red, white and small onion) on free radical-mediated DNA Sugar Damage

Free radical-mediated DNA sugar damage was assessed by the method of Haliwell and Guteridge, (2000). The reaction mixture in a total volume of 1.24ml contained 0.5ml of calf thymus DNA, 0.5ml of phosphate buffer, 0.2ml of ascorbic acid (1mM) and 0.0 4ml of ferric chloride. To this reaction mixture various concentrations of *A.cepa* varieties extracts (100, 200, 300, 400, 500 µg/ml) were added. The reaction mixture was incubated for 1 hour at 37°C. After the incubation, 1ml of 0.67% TBA was added to the reaction mixture and then it was kept in boiling water bath for 15 minutes. The scavenging activity was measured at 535nm and results were expressed in percentage (%).

8. Determination of antifungal activity

The disc diffusion method was used (Gillespia. 2002) to evaluate the antifungal activity against *Aspergillus niger, Aspergillus, fumigates, Candida albicans and Aspergillus flavus.* The sterilized (autoclaved at 120 C for 30 minutes) medium (40°C-50°C) was inoculated with the suspension of various microorganisms and poured into petridishes to give a depth of 5mm, various concentration of *A.cepa* aqueous (100, 200, 300µg/ml), ethanol (25, 50, 100µg/ml), chloroform (50,100,150µg/ml) and 50,100,150µg of petroleum ether extracts of the various *A.cepa* (Red, White and Small) were prepared separately. Sterile disc (made from Whatman filter paper-41 was sterilized in UV lamp) dipped in specified concentration of the extracts and standard (ketoconazol, 50µg/ml). The impregnated discs are allowed to dry and dried discs were placed on the surface of agar plates. A disc dipped in solution of different concentration of *A.cepa* extracts, standard and blank were placed on the surface of agar plates. The plates were left for 1hour at room temperature and incubated at 37°C for 24 hour. The diameter of zone of inhibition of extracts and standards were measured. All tests were performed in duplicate and antifungal activity was expressed as the mean diameter of inhibition zones (mm) produced by the formulated bulb extracts of *A.cepa varieties*.

9. Statistical analysis

All the samples and readings were prepared and measured in triplicate. The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between the groups and results were expressed as mean ± SD using a computer-based software (SPSS 7.5 for windows student version; SPSS, Chicago, IL). In the Tukey's test, the significance was considered at the level of $p<0.05$.

9. RESULTS AND DISCUSSION

In the present investigation, preliminary physical and phytochemical analyses were done in bulb

extract of onion varieties. Water, organic, inorganic, ash contents and pH of all the three types of bulb extracts of onion are listed in the table 1a. Water content of *A.cepa* varieties was found to be 80.5, 80.6 and 90% in red, white and small onion, respectively. Organic content was found to be 16.4, 15.38 and 7.48% in red, white and small onion, respectively. Total ash content was 3.26, 4.02 and 2.60%, respectively and the pH was found to be 5.80, 5.78 and 5.87 (Table 1b).

Table 1a

Determination of water, organic and ash content in onion varieties (red, white and small onion)

S.No	Name of the onion	Water content (%)	Organic content (%)	Ash content (%)
1	Red	80.73±0.25	16.50±0.41	5.25±0.31
2	White	82.06±1.5	15.20±0.20	4.12±0.176[a]
3	Small	91.0±1.2[a,b]	7.75±0.30[a,b]	2.75±0.128[a,b]

Each value represents mean±S.D of 3 observations. Significance at p<0.05.
a- compared with red onion; b-compared with white onion.

Table 1b

Determination of pH in Allium cepa varieties (red, white and small onion)

S.No	Name of onion	pH
1.	Red	5.89
2.	White	5.75
3.	Small	5.86

Each value represents mean of 3 observations.

The results of phytochemical screening of qualitative organic analysis of aqueous, ethanolic, chloroform and petroleum ether extracts of *A. cepa* varieties were studied (Table 2a). The result revealed the presence of phenols, flavonoids, triterpenoids, tannins, alkaloids, saponins, acid, carbohydrates, glycosides, proteins and amino acids. However, steroids were found to be absent in aqueous extract of all the *A.cepa* varieties while alkaloids were found to be absent in the ethanolic, chloroform and petroleum ether extracts of all the varieties. Among different extracts of *A.cepa*, the ethanolic extract of small onion was found to have more constituents compared to other extracts. The result of the inorganic analysis of aqueous extract of *A.cepa* varieties revealed the presence of acid radicals, such as sulphate, phosphate, carbonate, nitrate and nitrite and basic radicals such as aluminum, iron, zinc, magnesium, calcium, sodium and potassium. However, sulphide, fluoride, oxalate, lead and mercury are found to be absent in the aqueous extract of small onion bulb (Table 2b). In accordance with the present study, phytochemical analysis of the crude extracts of various plants indicated the presence of major phytocompounds including phenols, alkaloids, glycosides, flavonoids, tannins, etc. The therapeutic value of plant is attributed their active constituents, which are being investigated to serve as pharmacological tools to provide health wellness (Liu, 2005). In addition to this, Panduranga Murthy *et al.* (2011) have reported the presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds and saponins in wild onion. Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities and also as a reducer of cardiovascular complications (Yen *et al.,* 1993). The scavenging ability of the phenolics is mainly due to the presence of hydroxyl group. To know the concentration of total phenolic compounds,

HPLC was performed. Figures 1, 2 and 3 represent the total phenolic content in the bulb extracts of onion varieties. In the present study, bulb extracts of *A.cepa varieties* showed presence of total phenolic rich constituents such as quercetin, kaempferol, ferrulic acid and protocatechuic acid. However, quercetin was found to be the highest followed by kaempferol. Among these five total phenolic constituents, the quercetin concentration

Table 2a

Qualitative organic analysis in the extracts of Allium cepa varieties

Test	Aqueous extract			Ethanolic extract			Chloroform extract			Petroleum ether extract		
	R	W	S	R	W	S	R	W	S	R	W	S
Steroids	–	–	–	++	+	++	++	+	++	++	+	++
Triterpenoids	++	+	++	++	+	++	++	+	++	++	+	++
Flavonoids	++	+	+++	++	+	+++	+	+	++	+	+	++
Phenols	++	++	+++	++	++	+++	++	++	+++	+	+	++
Tannins	+	+	+	+	++	++	+	+	++	++	+	++
Alkaloids	++	+	+++	–	–	–	–	–	–	–	–	–
Saponins	++	+	+++	+	+	++	+	+	++	+	+	++
Acid	++	++	+++	++	++	+++	++	++	+++	++	++	+++
Carbohydrates	+	+	++	+	+	+	+	+	+	+	+	+
Glycosides	++	+	++	+	+	+	+	+	+	+	+	+
proteins	++	++	++	+	+	+	+	+	+	+	+	+

Results are based on colour reaction. R-red onion; W-white onion; S-small onion.

Table 2b

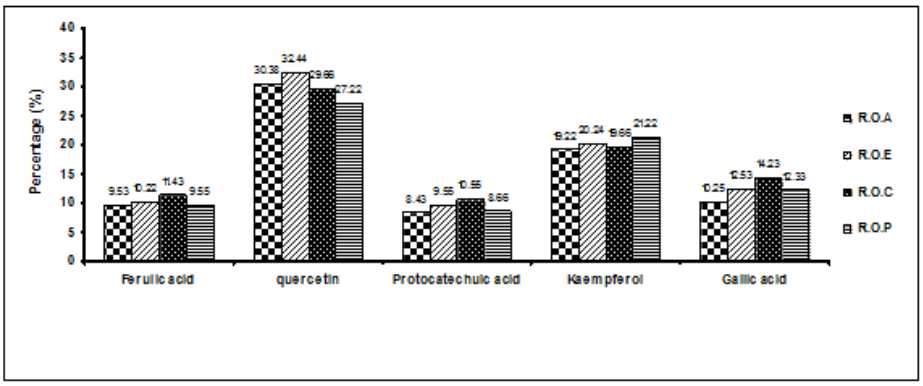
Qualitative inorganic analysis in the extracts of Allium cepa varieties

S.No	Test	Aqueous ash		
	Acid radicals	**Red onion**	**White onion**	**Small onion**
1	Sulphate	+	+	+
2	Sulphide	-	-	-
3	Chloride	-	-	-
4	Phosphate	+	+	+
5	Carbonate	+	+	+
6	Nitrate	+	+	+
7	Nitrite	+	+	+
8	Fluoride & Oxalate	-	-	-
	BASIC RADICALS			
9	Lead	-	-	-
10	Alluminium	+	+	+
11	Iron	+	+	+
12	Zinc,magnesium,	+	+	+
13	Mercury	-	-	-
14	Calcium	+	+	+
15	Sodium	+	+	+
16	Potassium	+	+	+

+ = Present, - = Absent.

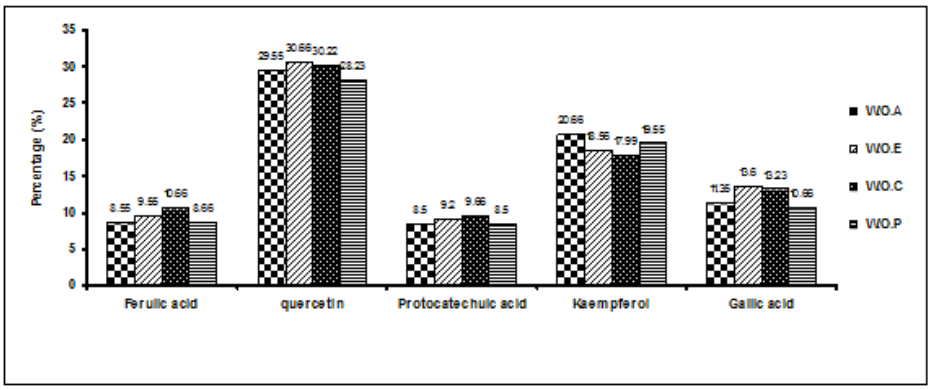
was found to be the highest in the *A.cepa* ethanolic extract. The percentage of quercetin was found to be higher than the standard.These results agree well with recent reports where flavonoids were mainly present in the ethyl acetate sub fraction but represented only a small percentage of total phenols present in the aqueous subfractions (Singh *et al.,* 2009). This is consistent with the low solubility of flavonols in water with quercetin and kaempferol being the most common flavonoids present in onion extracts (Nuutila *et al.,* 2002; Lanzottii, 2006). In addition to this, it has been reported that the edible part of the yellow onion variety had higher total phenol and flavonoid content (Sellappan and Akoh 2002; Yang *et al.,* 2004). In the present study also, the edible part of the red, white and small onion extracts showed the highest phenolic rich constituents making them potential antioxidants. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari and Chatterjee, 2008). DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It is reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration-dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups (Nanjo *et al.,* 1996).

Figure 1
Total phenolic contents in the red onion extracts



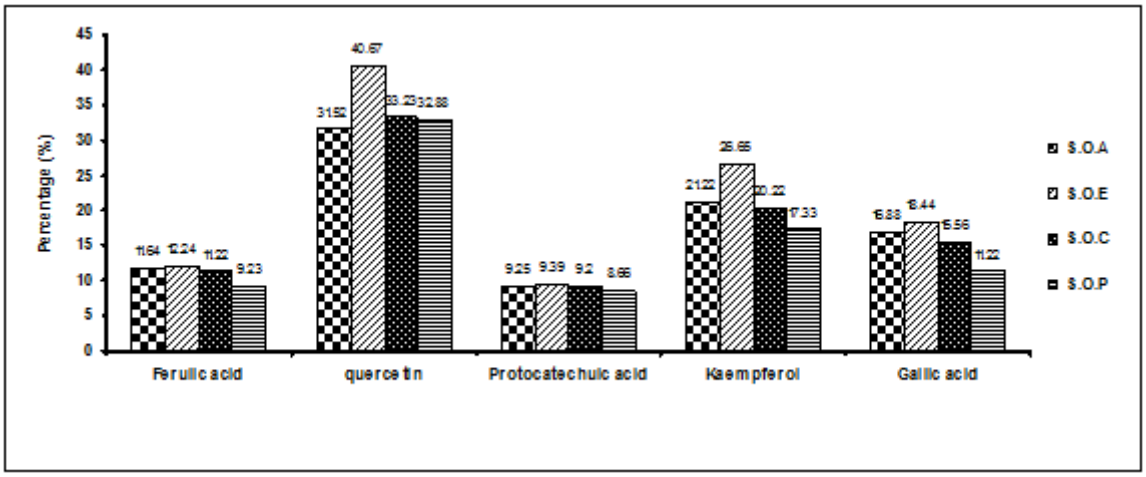
Each bar represents mean values of 3 observations.

Figure 2
Total phenolic contents in the white onion extracts



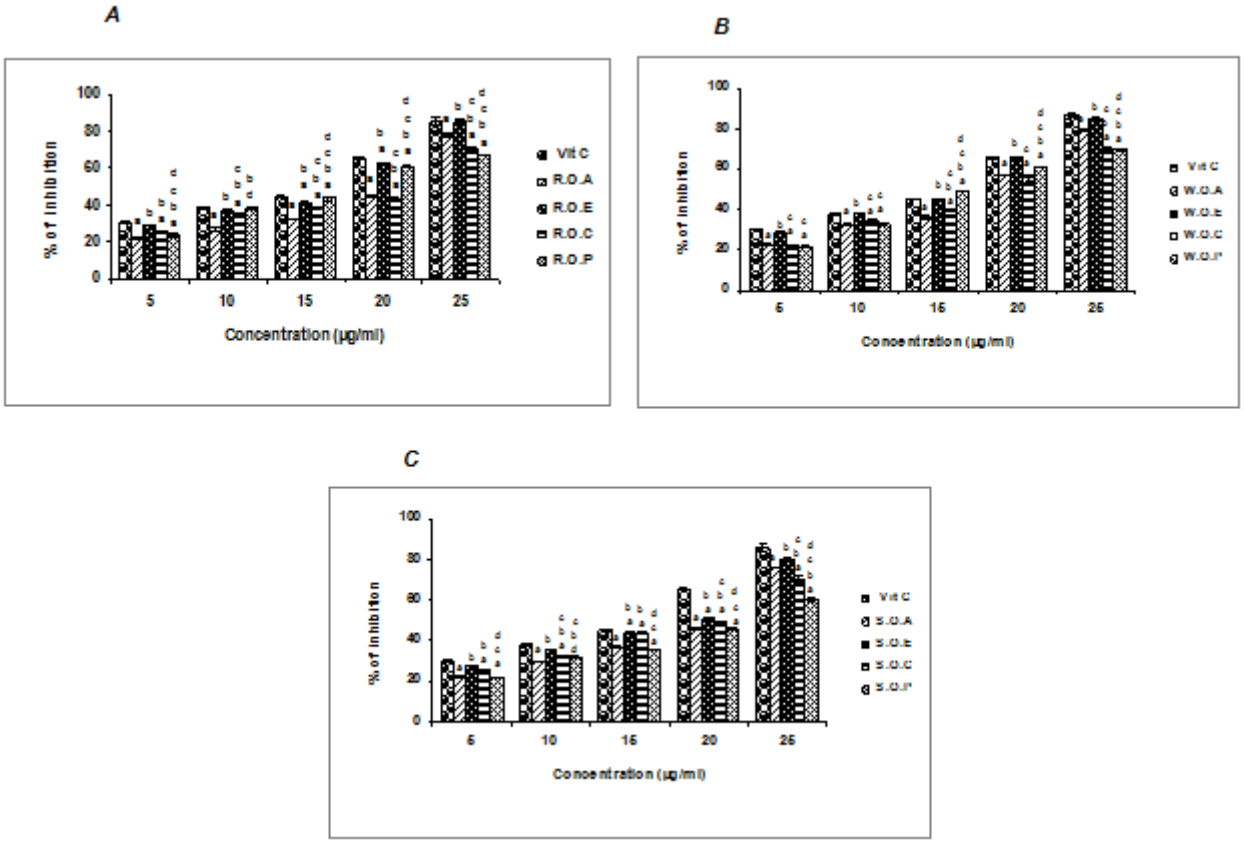
Each bar represents mean values of 3 observations.

Figure 3
Total phenolic contents in the small onion extracts



Each bar represents mean values of 3 observations.

Figure 4
DPPH radical scavenging activity of different extracts (aqueous, ethanol, chloroform and petroleum ether) of onion varieties



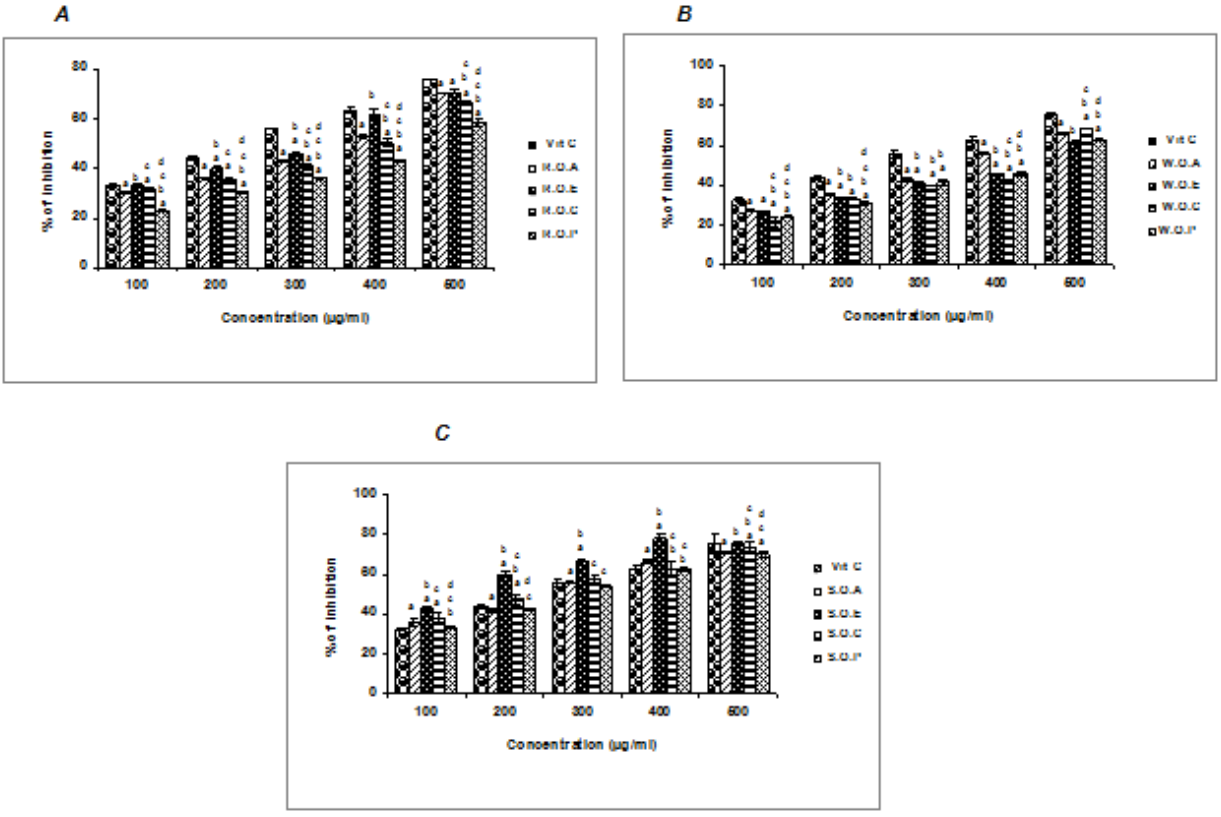
Each bar represents mean ± SD of three observations. Significance at p<0.05.

The DPPH radical scavenging activity of different extracts (aqueous, ethanlol, chloroform and petroleum ether) of *A.cepa* varieties are shown in (Figure 4 A, B and C). In this study all the

onion extracts showed a significant ($p<0.05$) increase in DPPH radical scavenging activity in a dose-dependent manner (5, 10, 15, 20 and 25µg/ml) however, the small onion showed the maximum DPPH scavenging activity in all the extracts (aqueous, ethanol, chloroform and petroleum ether) compared with that of the standard. It is indicated from the above results that the ethanolic extract of small onion had strong DPPH radical scavenging activity. In the present study, the mechanism of the radical scavenging activity was observed based on the reduction in purple colour of DPPH solution. All the extracts of *A.cepa* varieties (aqueous, ethanolic, chloroform and petroleum ether) showed significantly higher inhibition percentage (hydrogen-donating ability) in a dose-dependent manner. From the results, it may be postulated that all the extracts of *A.cepa* varieties reduces the radical corresponding hydrazine when it reacts with hydrogen donors in antioxidant principles. However, the ethanolic extract of small onion showed the highest radical scavenging activity compared to that of other varieties of *A.cepa* and it can be positively correlated with total phenolic contents and presence of phytochemical constituents observed in the present investigation. In accordance with the present investigation, it is worth to recall the report of Nuutila *et al.* (2003) who reported that methanol extract of the edible portion of the yellow onion showed 50% DPPH radical scavenging activity. Moreover, it has been reported that wild onion significantly increased the DPPH radical scavenging activity in a dose-dependent manner (Panduranga Murthy *et al.,* 2011).

Figure 5

Nitric oxide radical scavenging activity of different extracts (aqueous, ethanol, chloroform and petroleum ether) of onion varieties



Each bar represents mean ± SD of three observations. Significance at p<0.05.

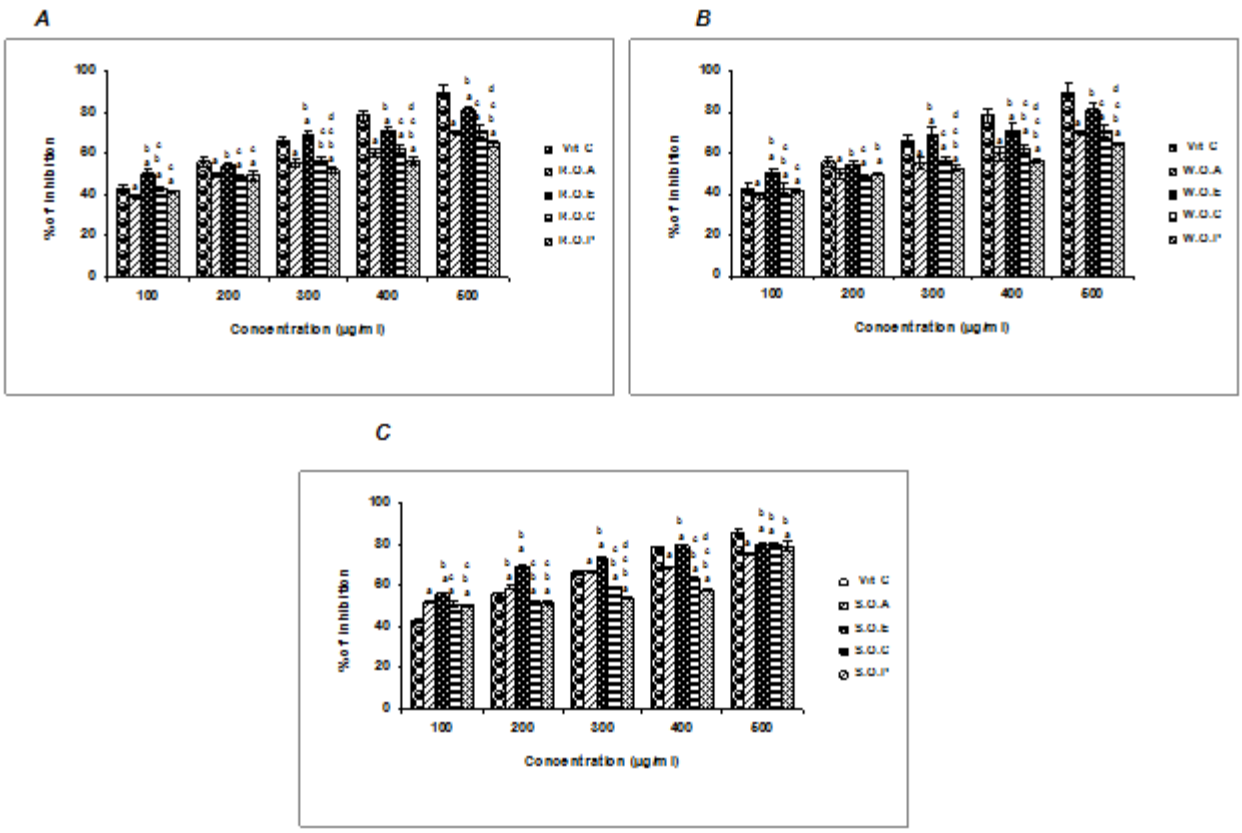
Nitric oxide is a free radical, produced in mammalian cells involved in the regulation of various physiological processes. However, excess production of nitric oxide has been implicated in various inflammatory and degenerative diseases (Guo X *et al.,* 1999; Pacher *et al.,* 2007). It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities (Miller *et al.,* 1993). Moreover, in pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules such as peroxynitrite (Guo X *et al.,* 1999). *In vitro* inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent (Marcocci *et al.,* 1994). Figures 5A, B and C represent the nitric oxide radical scavenging activity of different extracts of *A.cepa* varieties. The nitric oxide radical scavenging activity was found to be significantly ($p<0.05$) increased in all the concentrations used in the present study (100 to 500µg/ml). It is indicated from the results that among all the extracts of *A.cepa* varieties, aqueous and ethanolic extract of small onion ranked first, red onion ranked second and white onion ranked last for its nitric oxide radical scavenging activity. Whereas, in the chloroform and petroleum ether extracts of *A.cepa* varieties, the small onion ranked first, white onion ranked second and the red onion ranked the last for its nitric oxide radical scavenging activity. Small onion showed strong nitric oxide radical scavenging activity in all the extracts. All the extracts of *A.cepa* varieties decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. However, the small onion ethanolic extract showed highest scavenging effect in a dose-dependent manner when compared with the other onion extracts and standard. Our finding suggests that different extract of *A.cepa* varieties substantially inhibited nitric oxide production. Finally, the result indicates that the extract might contain compounds which may be able to inhibit nitric oxide and offers scientific evidence for the use of the extracts in indigenous system for various diseases condition.

Super oxide is a well known free radical of all oxygen derived species (McCord *et al.,* 1969). Therefore, it is the first intermediate in the sequential univalent reduction of oxygen that leads to formation of hydrogen peroxide (Harris, 1992). Superoxide radical is unique in that it can lead to the formation of many other reactive species, including hydroxyl free radical, hydrogen peroxide and perhydroxyl radicals (Pryor, 1986). It is involved in many pathological conditions. It mediates inflammatory tissue injuries in ischaemia-reperfusion, arthritis, gout and gastric ulceration. Superoxide radical has a low reactivity and a low capacity to penetrate the lipidic membrane layer, but it can generate hydrogen peroxide and highly reactive hydroxyl radical, via Haber-Weiss reaction (Rathee *et al.,* 2006). It induces oxidative damage in lipids, proteins and DNA (Pietta, 2000). Several phytochemical compounds were able to efficiently scavenge superoxide radicals (Valentao *et al.,* 2002). These compounds may react with the super oxide radical via one-electron transfer mechanism or by hydrogen abstraction mechanism to form the corresponding semiquinone (Wang *et al.,* 1996). In the present study, figure 6A, B and C show the superoxide anion scavenging activity of various extracts of *A.cepa* varieties. The scavenging activity was found to be significantly ($p<0.05$) increased as the concentrations of extracts as well as standard increased from 100 to 500 µg/ml at 100µg interval. At 500µg concentration, the aqueous extract of small onion showed the maximum scavenging activity (85.21%) which is near to that of the standard level (89.66%). While the red onion showed 70.66% and the white onion showed 69.66% of superoxide anion scavenging activity. In the ethanolic extract, small onion showed maximum scavenging activity (88.66%) which was also near to that of standard level (89.66%). Both red

and white onion varieties showed little lesser range of scavenging activity (86.11% and 80%, respectively) than the small onion.

Figure 6

Super oxide radical scavenging activity of different extracts (aqueous, ethanol, chloroform and petroleum ether) of onion varieties



Each bar represents mean ± SD of three observations. Significance at p<0.05.

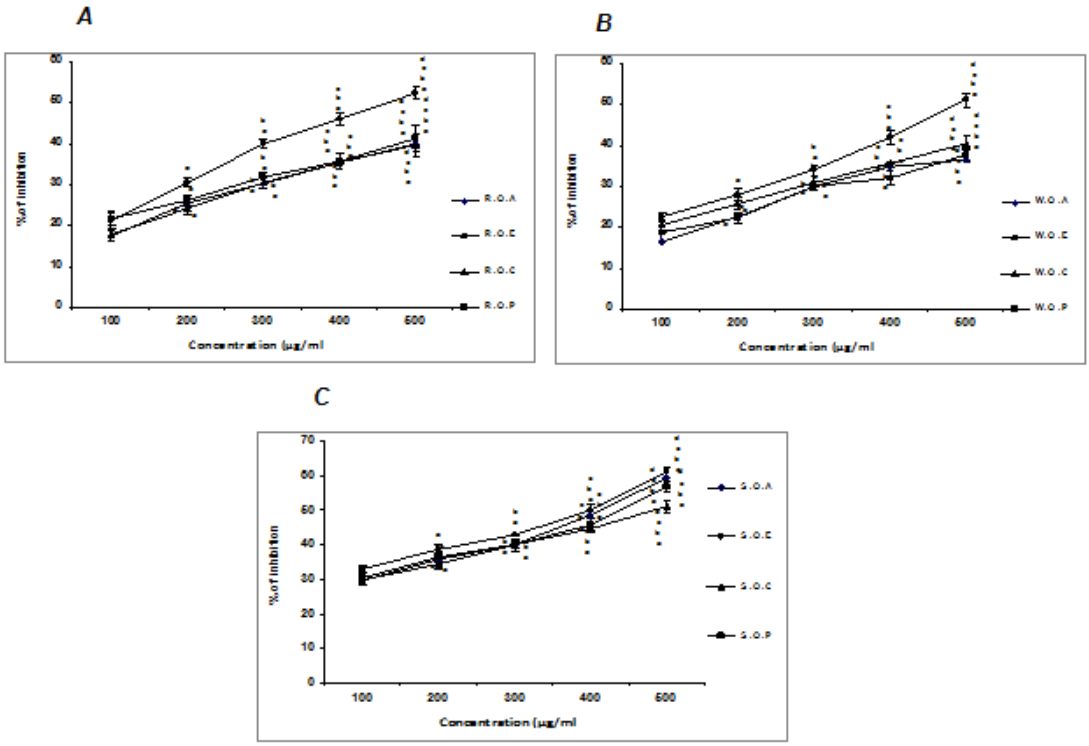
In the chloroform extract, the small onion showed maximum scavenging activity (80.22%), while the red and white onion varieties showed 72.66% and 70.36% which was the minimum activity. In the petroleum ether extract also, the small onion showed maximum superoxide anion scavenging activity (79.15%), while the red onion showed 70.66% and white onion showed 64.99% scavenging activity which was the least than the other *A.cepa* varieties. From the above results, it was revealed that among all the varieties of *A.cepa*, the small onion showed the maximum superoxide anion scavenging activity. Among all the extracts of *A.cepa* varieties, the ethanolic extract had strong superoxide anion scavenging activity. Whereas, the petroleum ether extract showed the least scavenging activity. These results revealed that the small onion extract had strong super oxide anion scavenging activity than the other two varieties of *A.cepa*. Compared with that of standard, the ethanolic extract of small onion showed superoxide anion scavenging activity. In the present study, all the *A.cepa* extracts eliminated superoxide radical generated by phenazine methosulphate/β-nicotinamide adenine dinucleotide system in a concentration-dependent manner. However, the small onion's ethanolic extract showed strong superoxide radical scavenging activity and this scavenging activity of extracts can be explained by its polyphenolic content recorded in the present investigation. Polyphenols in onion extracts may have directly scavenged the superoxide radical

by the hydrogen-donating capacity of their phenolic groups. In support of these findings, it has been reported that quercetin had been found to reduce the level of peroxynitrate, an extremely powerful oxidant in the brain, by scavenging superoxide anions (Shutenko *et al.,* 1999). The concentration dependent ferrous ion chelating capacity of red and violet varieties of onion increased from outer to inner layers. The most probable reason for the variation of free radical scavenging activity might be due to variation in the quantities of quercetin in the various layers of different varieties (Dhan Prakash *et al.*, 2007). These results were further supported by HPLC results of the specific phenolic composition which showed high amounts of quercetin in the outer layers of the red variety. The mechanisms of action of quercetin include free radical scavenging, chelation of transition metal ions and inhibition of oxidases (Lean *et al.,* 1999).

The plant prevents the DNA damage in a concentration dependent manner. Oxidative DNA damage has been implicated to be involved in various degenerative diseases (Jenner, 2003). Hydroxyl radicals generated by the Fenton reaction are known to cause oxidatively-induced breaks in DNA strands to yield its open circular or relaxed forms. It has been observed that proteins, lipids and DNA are the major targets of oxidative injury (Klaunig *et al.,* 2010). Of these, DNA damage may be of particular importance as its role has been recognized in a large number of genetic disorders including cancers (Cooke *et al.,* 2003). In this study, figure 7A, B and C illustrate protective effect of different extracts of *A.cepa* varieties on free radical mediated DNA sugar damage. All the extracts of *A.cepa* varieties significantly ($p<0.05$) increased the protective effect in a dose dependent manner (100,200,300,400 and 500 µg/ml). At 500 µg concentration the aqueous extract of small onion showed maximum protective effect (59.33%) than the red (39.43%) and white onion (36.25%). In the ethanolic extract, small onion exhibited the highest protective effect (60.65%) when compared to the other two varieties of *A.cepa*. The red onion showed 52.12% and the white onion showed 50.54% of protective effect on free radical mediated DNA sugar damage.

Figure 7

Protective effect of different extracts (aqueous, methanol, chloroform and petroleum ether) of onion varieties on free radical mediated DNA sugar damage



Each bar represents mean ± SD of three observations. Significance at p<0.05.

In the chloroform extract also the small onion showed the maximum protective effect (50.68%), while the white onion showed 39.67% and the red onion showed 39.38% protective effect. In the petroleum ether extract, the small onion showed high protective effect (56.87%) than the red (40.52%) and white onion (37.48%) varieties on free radical mediated DNA sugar damage. From the above results, it was revealed that among all the extracts of *A.cepa* varieties, small onion ranked first, red onion ranked second and white onion ranked the last for its protective effect on free radical mediated DNA sugar damage. However, the ethanolic extract proved to possess the highest protective effect on free radical mediated DNA sugar damage. Whereas, the aqueous, chloroform and petroleum ether extracts *of A.cepa* varieties had weak activity. Hence, among the three varieties of *A.cepa* extracts, the ethanolic extract of small onion proved to possess the high protective effect against the DNA sugar damage. Therefore, in the present study, the effects of differential crude extracts of *A.cepa* varieties assessed on the inhibition of free radical-mediated DNA damage. In the present study, the concentration dependent (100–500 μg/ml) free radical scavenging effect of different extracts of *A.cepa* varieties prevented DNA damage. However, the small onion's ethanolic extract exhibited the maximum effects when compared with that of the other varieties of *A.cepa* varieties. Dose-dependent decrease in the Fenton's reaction-mediated degradation of DNA by the presence of the bulb extracts of *A.cepa* varieties, suggest that these extracts have compounds which may combat against free radical-mediated degradation to deoxyribose sugar moiety of DNA and the extracts with high phenolic content showed better protection when compared to the others, indicating that protection was directly proportional to the concentration of phytochemicals as well as total phenolic constituents. Quercetin effectively protected DNA strand scission from tertbutylhydroperoxide

(Sestili *et al.,* 1998). Therefore, in the small onion variety, the presence of high quantities of quercetin may be responsible for better protection of DNA. Polyphenols are potential agents which protects DNA against the lethal effects of oxidative stress and redox-active transition metal ions. In line with the present study, it has been reported that outer layer of red onion's methanolic extract containing phenolic content showed better protection compared to the others, indicating that protection was directly proportional to the concentration of total phenolic content. However, in the present study, edible part (bulb) of the aqueous and ethanolic extracts of *A.cepa* varieties showed high phenolic constituents. It is inferred from the present findings that edible portion of red, white and small onion varieties, particularly in the small onion's extract was a rich source of phenols with promising antioxidant and free radical scavenging activities and ability to provide protection against DNA damage caused by reactive oxygen species. The present study together with previous work suggests the triple synergistic action of phenols in scavenging ROS, repairing DNA radicals and metal chelation (Zhao *et al.,* 2005). In recent years, a remarkable increase has been reported in the incidence of different mycoses due to aggressive cancer chemotherapy, widespread use of broad spectrum antibiotics, increasing number of immunosuppressive diseases and highly effective immunosupressants for organ transplantation (Anaissie *et al.,* 2003). Because of huge similarities between fungal and mammalian cells, there is a limited selective target for designing new antifungal formulations (Barrett, 2002). There is thus an urgent need for a new antifungal agent with new modes of action, broad fungicidal spectrum and fewer dose-limited side effects (Graybill, 1996). It has been reported that onion exerts a marked antifungal activity against both yeasts and mycelia fungi including dermatophytes (Barrett, 2002). To investigate, how different extracts of *A.cepa* varieties act toward the important fungi, *Aspergillus niger, Aspergillus fumigates, Candida albicans and Aspergillus flavus* were selected for the present study. Antifungal activity of aqueous (Table 3 and Plate1) ethanol (Table 4 and Plate 2), chloroform (Table 5, and Plate 3) and petroleum ether (Table 6, Plate 4) extracts were shown. The results reveal that the bulb of the onion varieties has potential antifungal properties. The aqueous extract of red and small onion showed highest zone of inhibition with *Aspergillus niger* and *Aspergillus flavus* while white onion exerted least inhibition on *Candida albicans.* In the ethanolic extract of white onion, *Candida albicans* was most sensitive. However, red onion had least growth inhibition on the same fungi.

In the chloroform extract of white onion, *Aspergillus fumigates* was highly inhibited, whereas the red onion chloroform extract showed least inhibition on *Candida albicans.* When the petroleum ether extract of small onion was found to have widest zone of inhibition on *Candida albicans,* the petroleum ether extract of red onion showed least inhibition against *Aspergillus niger.* Finally, among the onion varieties analyzed in the present investigation, the small onion exhibited strong fungal growth inhibition in its aqueous, chloroform and petroleum ether extraction while white onion is effective in its ethanolic extract and red onion is effective in its chloroform extract.

Table 3
Antifungal activity (inhibition in %) of aqueous extract of onion varieties against selected fungi

S.no	Name of the fungi	STD			100µg/ml			200µg			300µg		
		R	W	S	R	W	S	R	W	S	R	W	S
1	*Aspergillus niger*	38	39	37	20[51]	20[51]	20[51]	26[68]	23[58]	27[69]	32[84]	26[66]	33[84]
2	*Aspergillus fumigates*	38	38	37	21[55]	18[47]	23[58]	24[63]	24[63]	28[71]	29[76]	28[73]	31[79]
3	*Candida albicans*	38	38	38	20[51]	17[44]	18[47]	24[63]	19[50]	20[52]	28[73]	23[60]	24[63]
4	*Aspergillus flavus*	38	39	39	25[65]	20[51]	23[58]	29[76]	23[58]	28[71]	33[86]	28[71]	31[79]

R-red onion; W-white onion; S-small onion. The values are diameter of zone of inhibition at 100, 200 and 300µg/ml concentration.

Table 4
Antifungal activity (inhibition in %) of ethanolic extract of onion varieties against selected fungi

S.no	Name of the fungi	STD			25µg/ml			50µg/ml			100µg/ml		
		R	W	S	R	W	S	R	W	S	R	W	S
1	*Aspergillus niger*	38	38	38	19[50]	26[68]	20[52]	24[63]	32[84]	24[63]	28[73]	35[92]	30[78]
2	*Aspergillus fumigates*	38	38	37	16[42]	28[73]	19[51]	21[55]	33[86]	21[56]	25[65]	36[94]	27[72]
3	*Candida albicans*	38	38	38	16[42]	26[68]	18[47]	20[52]	34[89]	22[57]	23[60]	37[97]	28[73]
4	*Aspergillus flavus*	38	38	38	22[57]	27[71]	20[52]	26[68]	32[84]	27[71]	31[81]	35[92]	31[81]

R-red onion; W-white onion; S-small onion. The values are diameter of zone of inhibition at 25, 50 and 100µg/ml concentration.

Table 5
Antifungal activity (inhibition in %) of chloroform extract of onion varieties against selected fungi

S.no	Name of the fungi	STD			25µg/ml			50µg/ml			100µg/ml		
	Fungi	R	W	S	R	W	S	R	W	S	R	W	S
1	*Aspergillus niger*	38	38	38	16(42]	20[52]	20[52]	22[57]	24[63]	29[76]	29[76]	29[76]	32[84]
2	*Aspergillus fumigates*	38	38	38	21[55]	19[50]	19[50]	24[63]	25[65]	27[71]	32[84]	31[81]	31[81]
3	*Candida albicans*	38	38	37	23[58]	18[47]	19[51]	27[71]	26[63]	24[64]	33[86]	32[84]	30[81]
4	*Aspergillus flavus*	38	38	38	17[44]	21[55]	19[50]	20[52]	26[68]	23[58]	25[65]	31[81]	29[76]

R-red onion; W-white onion; S-small onion. The values are diameter of zone of inhibition at 25, 50 and 100µg/ml concentration.

Table 6
Antifungal activity (inhibition in %) of petroleum ether extract of onion varieties against selected fungi

S.no	Name of the fungi	STD			50µg/ml			100µg/ml			150µg/ml		
	Fungi	R	W	S	R	W	S	R	W	S	R	W	S
1	*Aspergillus niger*	38	38	38	9[24]	10[26]	13[34]	11[29]	12[32]	15[39]	13[34]	15[39]	19[50]
2	*Aspergillus fumigates*	38	39	38	10[26]	16[41]	16[42]	13[34]	19[49]	18[47]	16[42]	24[62]	22[58]
3	*Candida albicans*	39	38	38	10[26]	14[37]	17[45]	12[31]	18[47]	20[53]	15[38]	22[58]	25[66]
4	*Aspergillus flavus*	38	39	38	11[29]	17[44]	15[39]	14[37]	20[51]	19[50]	18[47]	23[59]	23[61]

R-red onion; W-white onion; S-small onion. The values are diameter of zone of inhibition at 50, 100 and 100µg/ml concentration.

Plate 1
Aqueous extract of A.cepa varieties

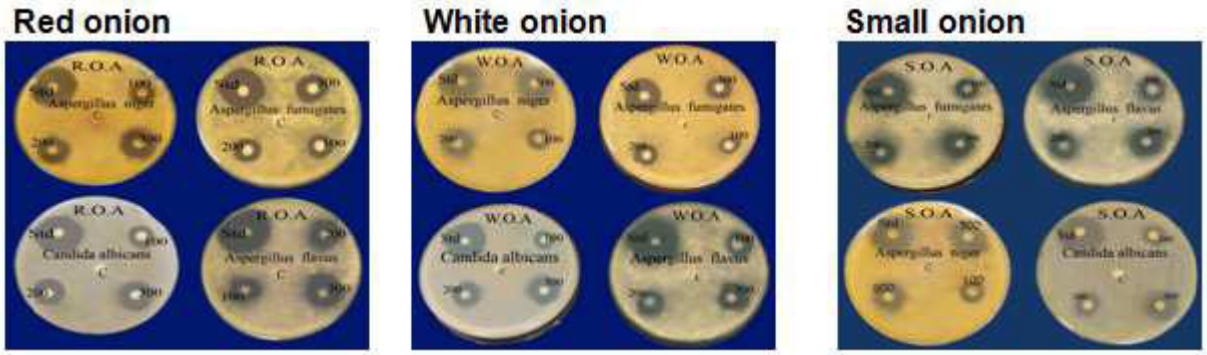


Plate 2
Ethanol extract of A.cepa varieties

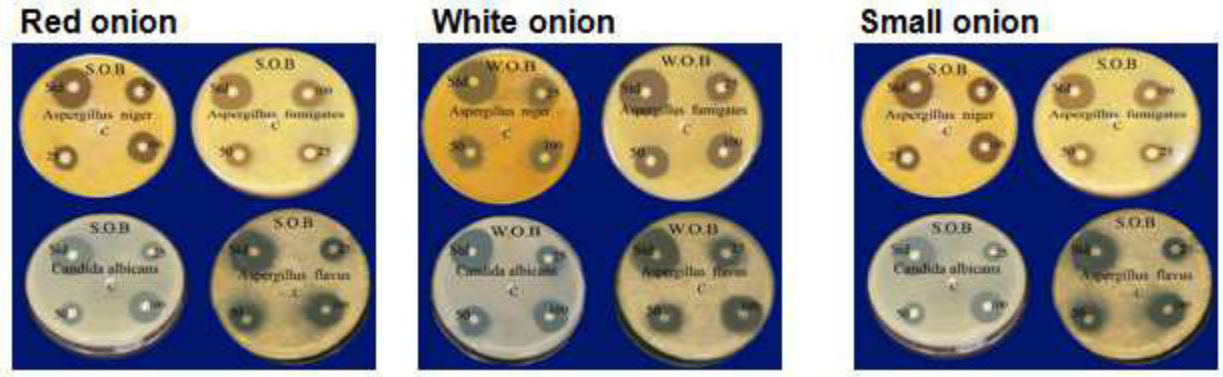


Plate 3
Chloroform ether extract of A.cepa varieties

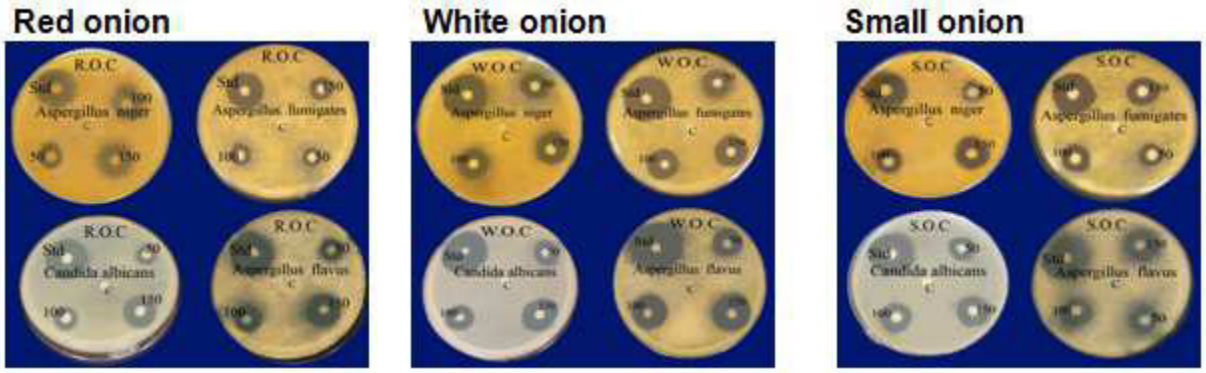
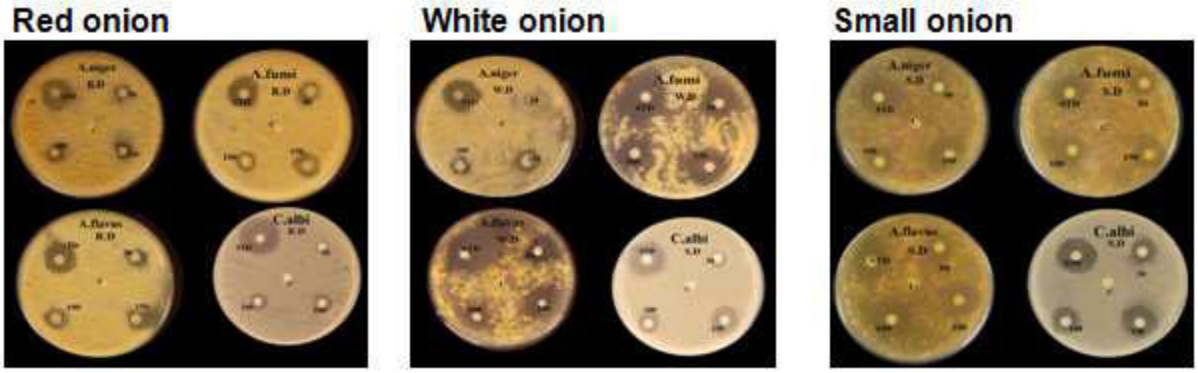


Plate 4
Petroleum ether extract of A.cepa varieties



Regardless of antifungal activities of onion varieties, it has been shown that crude extracts of onion may have potent antifungal and antibacterial properties (Elnima *et al.,* 1983). Phenolic compounds such as quercetin and kaempferol present in onion may contribute to this activity (Rauha *et al.,* 2000). In the present study also onion-induced dose-dependent fungal growth inhibition may be attributed to the presence of phenols and secondary metabolites. In accordance to these findings, reports by Skerget *et al.* (2009) can be quoted which recorded that the ethanol and acetone extracts of red onion's skin and edible part possess antifungal activity against *A. niger, T. viride* and *P. cyclopyum.* Moreover, it has been already reported that alliicin, thiosulfonates and other compounds of onion exhibit fungistatic activities against *A. niger, Rhodotorula nigricans, Penicillium italicum, Penicillium cyclopium, A. flavus, Cladosporium macrocarpum, A. fumigatus, A. alutaceus, A. terreus* and *Penicillium chryogenum* (Harris *et al.,* 2001). Finally, the onion-induced differential antifungal activities of *A. cepa* varieties may be due to the presence of phenolic constituents recorded in the present study and it may also be due to the presence of other compounds such as allicin, thiosulfonates, etc (Harris *et al.,* 2001).

CONCLUSION

In conclusion, the screening of antioxidant and antifungal activity performed on *A.cepa* bulb extracts, which is traditionally used as herbs, shows that they are endowed with potentially exploitable free radical scavenging and antifungal activity. Phytochemical constituents and total phenolic contents (quercetin and kaempferol) present in the onion varieties could

have contributed for the efficient inhibition of fungal growth. Hence, bulb extracts of *A.cepa* varieties could be used as an easy accessible source of natural antioxidants, and antifungal agent and therefore, onion bulb can be used as one of the effective therapeutic phytomedicines. However, additional purifications of the active compounds and *in vivo* evaluation of antioxidant and antifungal along with toxicity studies of the extracts from *A.cepa* are suggested for further studies.

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