Analysis of antioxidant and anticancer potentials of
Urginea indica, an endangered medicinal plant

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Received on:12-06-2012; Revised on: 17-07-2012; Accepted on:26-08-2012

ABSTRACT

Cellular damage caused by reactive oxygen species (ROS) has been implicated in several diseases. Natural antioxidants have significant importance in human health. Urginea indica, an endangered medicinal plant, commonly known as sea onion and methanolic extract of its bulbs has been reported to possess hypoglycemic and anticancer properties. In the present study, antioxidant status of different parts of U. indica varieties including leaf, bulb, stem and roots were analyzed using most commonly accepted assays. In this study, different parts of red and white varieties of U. indica were used to study their reducing ability, antioxidant activity, radical scavenging ability, anti-proliferative activity against ER positive and negative breast cancer cell lines and cathepsin B and MMP-9 enzyme inhibition activity. Our study demonstrated that both red and white varieties of U. indica have potential antioxidant activity. This study also demonstrated that U.indica have significant inhibitory effect on tissue remodeling enzymes. In conclusion, the potential antioxidant and anti-cancer activities of U.indica may be due to DPPH, superoxide, hydroxyl radical and hydrogen peroxide scavenging, anti-proliferative activity and cathepsin B and MMP-9 inhibitory activities. Therefore, U.indica may serve as a novel therapeutic agent for the treatment of radical mediated diseases.

Keywords: Urginea indica, antioxidant, reactive oxygen species, MMP-9 and Cathepsin B.

INTRODUCTION

In recent times, focus on medicinal plant research has increased all over the world even though large body of evidences has been collected to show immense potential of medicinal plants (Dahanukar et al., 2000). The use of medicinal plant products as antioxidants is wide spread and still medicinal plants are huge source of antioxidants that might serve as novel drugs for the treatment of oxidative damage related diseases such as cancer (Linn and Huang, 2002).

Urginea indica is rare, endangered and threatened Indian medicinal plant belongs to Liliaceae family, commonly called as sea onion (Deb and Dasgupta, 1976). It is a perennial plant having fibrous roots from the bottom of a large, tunicated and globular bulb. There are two varieties of U. indica, which differ in the color of bulb scales, have been reported to possess medicinal properties (Kirtikar & Basu, 1999). Nearly all parts of the plants are reported to have therapeutic value; recently, bioactive compounds of bulbs have received much attention due to their anti-cancer properties (Harini et al., 2008).

Proteolytic enzymes such as cysteine proteases and matrix metalloproteinases are required for tissue remodeling in invasion of various cancers (Ramara et al., 2011). It is well known that radical-related damage of DNA and proteins causes the development of age dependent diseases such as neurodegenerative diseases, atherosclerosis, arthritis and cancer (Collins, 2005; Sies, 1993). In addition, reactive oxygen species also related to both growth arrest as well as induction of cell proliferation (Schulze et al., 1995). Recent studies have demonstrated that ROS activates matrix metalloproteinase-9 (MMP-9) and cathepsin B to mediate the breakdown of extra cellular matrix (Liu et al., 2005). Several studies have demonstrated that reactive species can be eliminated by a number of enzymatic and non-enzymatic antioxidants (Boullier et al., 2001).

In the present study, we have demonstrated that extracts of U. indica exhibited significant antioxidant and anti-radical activities compared to natural antioxidant like rutin and synthetic antioxidant, BHT. In addition, aqueous extracts also significantly inhibited the activity of tissue remodeling enzymes like cathepsin B and MMP-9. Therefore, inhibition of radicals, cathepsin B and MMP-9 activities may be contributed to the anticancer activity of U. indica.

MATERIALS AND METHODS:

Chemicals and reagents:
Thiobarbituric acid (TBA), 1,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-1-s-triazine (TPTZ), nitroblue tetrazolium (NBT), Brij, Bacterial Collagenase-I, were purchased from Sigma, USA. Rutin, Ascorbic acid, Sodium dodecyl sulphate (SDS), Sodium azide (NaN₃) were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents used were of analytical grade.

Collection of plant parts:
White and red varieties of U. indica were collected from Eastern Ghats of Vizianagaram district and authenticated by the faculty in the department of Botany, Andhra University, Visakhapatnam.

Preparation of plant extracts:
The fresh plant parts i.e. bulb, root, stem and leaves were collected and washed with distilled water and cut into small pieces, separately mashed in 10 ml of ice cold 0.1M phosphate buffer, pH 7.6 containing 0.1m M EDTA to get different concentrations using pre-cooled motor and pestle. Extracts

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Determination of total antioxidant activity:
The total antioxidant status of different parts of U. indica varieties were assayed by using FRAP method as described Wong et al. (2006) with some modifications. Briefly, 0.2ml of different concentrations (25, 50 and 100 mg/ml) of aqueous phosphate buffer extracts were added to 3.0 ml of FRAP reagent [mixture of 300 mM sodium acetate buffer (pH 3.6), 10 mM, TPTZ solution and 20 mM FeCl$_3$ in a ratio of 10:1:1]. The reaction mixture was incubated in a water bath at 37°C for 30 min. The increase in the absorbance measured using spectrophotometer at 593 nm. The total antioxidant capacity was assessed based on the ability to reduce ferric ions by the extracts. The percent of total antioxidant activity was calculated using a formula,

\[
\text{Percent of antioxidant activity} = \left( \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}} \right) \times 100
\]

Determination of ferric iron reducing ability:
The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu (1986). 1.0 ml of plant extract was mixed with 2.5 ml of 0.2M phosphate buffer (pH6.6) and 2.5 ml of 1% aqueous K$_3$Fe(CN)$_6$ solution. After 30 min of incubation at 50°C, 2.5ml of 10% trichloro acetic acid (TCA) was added and the mixture was centrifuged for 10 min at 3000rpm. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of water and 0.5 ml of 0.1% aqueous FeCl$_3$ and the absorbance was recorded at 700 nm. The results were expressed as ascorbic acid equivalents per gram of extract. Ascorbic acid was used as positive control.

Determination of DPPH Radical scavenging activity:
DPPH free radical scavenging assay was carried out by using Wong et al. (2006) method. The initial absorbance of DPPH in methanol was measured using spectrophotometer at 515 nm until the absorbance remains constant. A total of 40 µl of extract was added to 3 ml of 0.1 mM methanolic DPPH solution. The mixture was incubated at room temperature for 30 min and the change in the absorbance at 515 nm was measured. Rutin was used as positive control. The radical scavenging activity was expressed in terms of % control using the following equation.

\[
\% \text{ of DPPH scavenging activity} = \left[ \frac{(O.D \text{ of control} - O.D \text{ of sample})}{O.D \text{ of control}} \right] \times 100
\]

Determination of hydroxyl radical scavenging activity:
Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radical’s generated by fenton reaction, a method originally described by Gulhan et al., (2003). 0.1ml of plant extract was added to the reaction mixture containing 0.1ml of 3.0 mM deoxyribose, 0.5 ml of 0.1 m M FeCl$_3$, 0.5 ml of 1mM H$_2$O$_2$ and 0.8 ml of 20 mM phosphate buffer, pH 7.4 in a final volume of 3.0ml and incubated at 37°C for 1hr. The thiobarbituric acid reactive substances (TBARS) formed were measured by treating with 1.0 ml of TBA (1.0%) and 1.0 ml of TCA(2.8%) at 100°C for 20min. After the mixtures were cooled, absorbance was measured at 532 nm against control, which is devoid of plant extract. Percentage of inhibition was calculated as (I) = [(Absorbance of control- Absorbance of test / Absorbance of control)] x 100.

Determination of Superoxide radical scavenging activity:
The superoxide radical scavenging ability of U.indica extracts were determined by the method of Beauchamp and Fridovich (1976). To 0.5ml of extract, 1.0 ml of 0.12 M sodium carbonate, 0.4ml of 25µM NBT and 0.2ml of 0.1mM EDTA were added. The reaction was initiated by adding 0.4ml of 1.0mM hydroxylamine hydrochloride and incubated for 20 min. The absorbance was measured at 560nm using spectrophotometer. The super oxide radical scavenging activity was defined as reduction of NBT by 50%.

Determination of Hydrogen peroxide scavenging activity:
The ability of the U.indica extracts to scavenge hydrogen peroxide was determined by measuring the decrease in absorbance at 230nm spectrophotometrically using method of Ruch et al., (1989). Briefly extracts were mixed with 3.0 ml of 40 mM H$_2$O$_2$ solution prepared in 0.1M phosphate buffer, pH7.4 and incubated for 10 min. The absorbance of the solution was determined at 230 nm against blank solution containing the plant extract without H$_2$O$_2$.

\[
\text{H}_2\text{O}_2 \text{ scavenging activity } (\%) = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right) \times 100
\]

Inhibition of lipid peroxide formation:
The lipid peroxidation was induced using FeSO$_4$- ascorbate in sheep liver homogenate as described by Bishayee and Balasubramaniyam (1971) and the formed TBARS was estimated by the method of Okawa et al., (1979). The reaction mixture consisting of 0.1ml each of 25% (w/v) sheep liver homogenate in 40mM Tris-Cl buffer, pH 7.0, 30mM KCl, 0.16mM ferrous sulphate (FeSO$_4$), plant extract or positive control and 0.06mM ascorbic acid was incubated at 37°C for 1hr. After incubation, 0.4ml of this reaction mixture was treated separately with 0.2ml of 1% sodium dodecyl sulfate (SDS), 1.5ml of 1.0% TBA and 1.5 ml of 20% acetic acid and pH was adjusted to 3.5. The total volume was made up to 4.0 ml by adding distilled water and the reaction mixture was kept in water bath at 95°C for 1hr. After cooling 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1v/v) was added and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10min, the organic layer was taken and its absorbance at 532 nm was measured. Percent of inhibition of lipid peroxidation was (I) = [(A$_{333}$ of control - A$_{333}$ of sample / A$_{332}$ of control)] x 100.

In vitro cell toxicity of U.indica:
ER positive breast cancer cells (MCF7) were grown as monolayer cultures in Dulbecco’s Modified Eagle’s Medium (DMEM) and ER negative breast cancer cells (BT-549) were grown as monolayer cultures in RPMI-1640. Both media are supplemented with 5% foetal bovine serum, 100U/ml each of penicillin and streptomycin and maintained at 37°C in a 5% CO$_2$ incubator. The cell cytotoxicity of aqueous bulk extracts of white and red varieties of U.indica against MCF-7 and BT-549 was determined by MTT assay. Cells were seeded into 96-well plate at 1 x 10^4 cells/well density and treated with extracts (25mg/ml) for 48 h. After 48 h MTT was added to each well and solubilized the formazan crystals by DMSO. Then absorbance was measured at 570 nm in a microplate ELISA reader. Effect of extract was quantified as the percentage of control absorbance of reduced dye at 570nm. Percent of inhibition [100-(absorbance of test wells/absorbance of control wells) x 100] were calculated. All experiments were performed in triplicate.

In vitro inhibition of MMP-9 (Collagenase -1) activity:
Matrix metalloproteinase -9 activity assay was performed in 50mM Tris–HCl buffer, pH 7.5 containing 0.15M NaCl, 10 mM CaCl$_2$, 0.02% NaN$_3$, 0.05% Brij 35 and 50 µg ZnSO$_4$ as previously described (Netzel-Arnett et al., 1991). The inhibitory activity of plant extracts on MMP-9 (bacterial collagenase -1) was determined using synthetic fluorescent substrate. 0.5ml of extract was incubated with 0.3ml of 1M substrate and 0.2ml of bacterial collagenase-1 (Sigma, USA) at 37°C for 20h and the reaction was terminated by the addition of 1.0 ml of 3% acetic acid. The residual activity of MMP-9 was determined by measuring the fluorescence intensity at 495 nm (excitation) and 520 nm (emission) using fluorescence microplate reader. The inhibitory activity of plant extracts was expressed as percent control.

In vitro inhibition of cathepsin B:
Effect of plant extracts on human liver cathepsin B (Sigma, USA) activity
was determined using Na-CBZ-L-Lysine p-Nitrophenyl Ester (Sigma, USA) as substrate according to method of Bajkowski and Frankfurter (1975) with some modification. 2.84 ml of 20mM sodium acetate buffer, pH 5.0 containing 1mM EDTA and 5mM L-cysteine was mixed with 0.05ml of 5.2mM Na-CBZ-L-Lysine -p-Nitrophenyl Ester substrate, 0.1 ml of plant extract and mixed well by inversion. Then increase in absorbance at 326nm was monitored for two min and 0.01ml of cathepsin B enzyme (Sigma, USA) solution in sodium acetate buffer was added. For control, 0.1ml of sodium acetate buffer was added instead of plant extract. Immediately mixed by inversion and increase in A326nm was recorded for 3 min. The A326nm/min was obtained using the maximum linear rate for both the test and the control. Percent of inhibition was calculated as \[\frac{A_{326\text{control}} - A_{326\text{test}}}{A_{326\text{control}}} \times 100.\]

**Statistical analysis:**

The experiments were replicated thrice for each parameter and the standard deviation was calculated.

**RESULTS:**

Several inflammatory diseases are thought to be related to oxidative injury and oxygen free radicals have been proposed as an important causative agent of heart disease, aging and various types of cancers (Choi et al., 2005). In carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process (Nam et al., 2011). Antioxidants protect from radical induced cellular damage by terminating free radical intermediates. Epidemiological and in vitro studies have strongly supported that medicinal plant constituents with antioxidants exerting protection against oxidative stress in inflammatory, age related diseases and lung disorders (Fernando Holguin et al., 2005). In the present study, different parts of red and white varieties of *U. indica* were used to study their reducing ability, antioxidant activity, radical scavenging ability and inhibitory activity of cathepsin B and MMP-9.

**Total antioxidant activity of *U. indica***:

Several assays have been used for the determination of antioxidant activity of medicinal plants including ABTS, ORAC and FRAP. In present study, FRAP method was used to estimate antioxidant activity of *U. indica*, as it is simple, rapid, highly reproducible and sensitive method (Thaipong et al., 2006). Different concentrations (25, 50 and 100mg/ml) of aqueous buffer extracts of bulb, leaf, stem and root of both white and red varieties were prepared as described in “Materials and Methods”.

The total antioxidant activity of different parts of *U. indica* varieties was depicted in fig. 1a. The results showed that the total antioxidant activity was 16 and 21% in aqueous bulb extracts of white and red varieties respectively, compared to controls at 25mg per ml concentration. Antioxidant activity of aqueous leaf, root and stem extracts of both white and red varieties were 21.5, 15 and 15.5% respectively, at 25 mg/ml concentration. Further, total antioxidant activity of aqueous bulb, leaf, root and stem extracts of both varieties was increased 2 fold with 50mg/ml and 4 fold with 100mg/ml concentrations.

**Oxygen radical scavenging activity of *U. indica***:

Generation of oxygen radicals is directly related with oxidation of molecules in biological systems. Despite the existence of various methods, just one procedure cannot identify all possible mechanisms characterizing radical scavenging activity. Therefore, different oxygen radical scavenging activities of aqueous extracts of bulb, leaf, stem and root of both white and red varieties were evaluated. DPPH free radical scavenging method is an easy, rapid,

a) Aqueous buffer extracts of bulb, stem, leaf and roots of white and red varieties of *U.indica* (25,50 and 100mg/ml) were prepared as described in “Materials and Methods”. Total antioxidant activity was assayed by estimating reduction of ferric tripypyridyl trizine complex to ferrous tripipyridyl trizine complex in presence or absence of an extract and expressed as percent control. All data were expressed as mean± S.D. (n=3).

b) The reducing ability of white and red varieties of *U.indica* was assayed by estimating the reduction of ferricyanide to ferrocyanide in presence or absence of an extract and the results were expressed in terms of Ascorbic acid Equivalents. Each value represents mean ± S.D. (n=3).

Figure 1. Antioxidant activity of white and red verities of *U.indica*:
a) DPPH radical scavenging activity of white and red varieties of *U. indica*. Aqueous buffer extracts of bulb, stem, leaf and roots of white and red varieties of *U. indica* (25mg/ml) were prepared and DPPH radical scavenging activity was determined in presence or absence of an extract as described in “Materials and Methods”. The results were expressed as percent DPPH radical scavenging activity of extract. All data are expressed as mean± S.D. (n=3). b) Hydroxyl radical scavenging activity. Hydroxyl radical scavenging activity of *U. indica* extracts were assayed by determining the degradation of deoxy ribose into fragments and their reactivity with thiobarbituric acid in presence or absence of an extract. The results were expressed as percent of hydroxyl radical scavenging activity against the extracts of different parts of white and red varieties of *U. indica*. All data were expressed as mean± S.D. (n=3). c) Superoxide radical scavenging activity. The superoxide radicals were generated using NBT-hydroxy-amine –EDTA system and radical scavenging activity in presence or absence of an extract  was determined as described in “Materials and Methods”. The results were expressed as percent of superoxide radical scavenging activity against the extracts of different parts white and red varieties of *U. indica*. All data were expressed as mean± S.D. (n=3). d) Inhibition of lipid peroxide formation. Lipid peroxidation was induced using FeSO$_4$- ascorbate in sheep liver homogenate and the inhibition of lipid peroxidation in presence or absence of an extract was estimated by measuring absorbance at 532 nm. The results were expressed as the percent of inhibition of lipid peroxidation against the extracts of different parts of white and red varieties of *U. indica*. All data were expressed as mean± S.D. (n=3).

Figure 2. Oxygen radical scavenging activity of white and red verities of *U. indica*:

sensitive and widely used method for determination of radical scavenging activity of a specific compound or plant extracts (RamaRao *et al.*, 2007). The percent of inhibition of DPPH radical was 16.5, 18.0, 14.0, 11.5% with aqueous extracts of bulb, leaf, stem and root of white variety respectively, whereas 18, 18, 16, 14% respectively, with red variety at 25 mg/ml (Fig.2a). Among the reactive oxygen species, hydroxyl radical is highly reactive and physiologically harmful, suspected in carcinogenesis (Stephanson Cory *et al.*, 2003). Therefore, inhibition of hydroxyl radical-mediated deoxyribose degradation in Fe$^{2+}$-EDTA-Ascorbic acid and H$_2$O$_2$ reaction mixture by *Urgenea* extracts (25mg/ml) was determined (Hazra *et al.*, 2008). The results of figure 2b, indicates that the aqueous bulb extract of white variety exhibited 23% and red variety exhibited 31% hydroxyl radical scavenging activity. Whereas, aqueous leaf extracts of both white and red varieties exhibited 21% hydroxyl radical scavenging activity. Further, hydroxyl radical scavenging
activity of aqueous root extract of white variety was 16% and red variety was 14%, whereas 20 and 22% with aqueous stem extracts of both white and red varieties, respectively.

Superoxide anion radical (O$_2^-$) is one of the strongest reactive oxygen species among the free radicals and changed to other harmful reactive oxygen species and free radicals such as hydrogen peroxide and hydroxyl radical (Mohammad Al-Mamun et al., 2007). Therefore, superoxide radical scavenging activity of U.indica extracts (25mg/ml) was determined using hydroxylamine as a source of super oxide as described in “Materials and Methods”. The results indicate that the superoxide radical scavenging activity was 17% with aqueous bulb extract of white variety and 22% with red variety. The aqueous leaf extract of white variety showed 16% and red variety showed 18% and aqueous root extracts of the both white and red varieties showed 17%, Whereas, aqueous stem extract of white variety exhibited 12% and red variety exhibited 15% (Fig.2c)

Lipid peroxidation is a collective effect of reactive oxygen species, which leads to deterioration of biological systems. The importance of inorganic iron as a catalyst for the induction of lipid peroxidation in both *in vitro* and *in vivo* models has been indicated by several studies (Wills et al., 1969). In the present study, sheep liver homogenate was used as a model to determine the effect of U.indica extracts (25mg/ml) on iron induced lipid peroxidation, as it is easily available. From the figure 2d, it is evident that inhibition of iron induced lipid peroxidation was 26.5 % with aqueous bulb extract of white variety and 29% with red variety, whereas 19.5% with aqueous leaf extract of white variety and 26.4% with red variety compared to controls. The aqueous root extracts of both white and red varieties have shown 20% iron induced lipid peroxidation compared to control. The percent of inhibition of iron induced lipid peroxidation by aqueous stem extracts of white and red varieties were 17% and 23% respectively, compared to controls.

**Non-radical reactive oxygen scavenging activity of U. indica:**
Hydrogen peroxide is highly toxic non-radical reactive oxygen species. Figure 3 showed the hydrogen peroxide scavenging ability of aqueous bulb extract of white variety was 4.73% and red variety showed 3.8% at 25 mg/ml concentration, whereas aqueous root extract of both white and red varieties showed 6.0% and aqueous stem extract of both white and red varieties showed 2.5% at 25mg/ml concentration. Whereas, the hydrogen peroxide scavenging ability of aqueous leaf extracts of white and red varieties were 8.0% and 12.0% respectively, compared to controls at 25mg/ml concentration.

**In vitro cell cytotoxic activity of U. indica:**
In order to evaluate the cytotoxic effect of aqueous bulb extracts from white and red varieties of U.indica, an anti-proliferative assay with ER positive breast cancer cell line (MCF-7) and ER negative breast cancer cell line (BT-549) was performed. The results were depicted in the figure 4a. The aqueous bulb extract of red variety exhibited 82% of anti-proliferative activity against MCF-7 compared to control, whereas white variety inhibited 62%. Further, the results also show that aqueous bulb extract of red variety exhibited 88% of anti-proliferative activity against BT-549 cells compared to control, whereas white variety inhibited only 42% activity.

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Inhibition of MMP-9 & cathepsin B activities by U.indica: A growing body of evidence indicates that oxygen derived radicals play significant role in intracellular signaling of cancer cells. In addition, ROS mediated signaling is involving in the activation of matrix metalloproteinase-9 (Collagenase -1) and cathepsin B in various human diseases including tumors and inflammation (Zeng et al., 2006). Therefore, we performed the in vitro inhibition assays of MMP-9 and cathepsin B to explain possible anticancer activity of U.indica. The aqueous bulb extract of red variety inhibited 78% of MMP-9 activity compared to control, whereas white variety inhibited 42%. Further, the results also show that aqueous bulb extract of red variety inhibited 86% of cathepsin B activity compared to control, whereas white variety inhibited only 28% activity (Fig.4b). The results of the present study indicates that aqueous bulb extract of U.indica red variety have high potential of inhibiting tissue remodeling enzymes compared to white variety.

DISCUSSION:

In living systems, free radicals are constantly generating and causing extensive damage to tissues and biomolecules leading to various disease conditions (Halliwell et al., 1998). Many synthetic drugs are known to protect against oxidative damage, but they reported to have adverse side effects. An alternative to synthetic drugs are antioxidants from natural sources (Yazdanparast et al., 2007; Yazdanparast and Bahramkias; 2008). Hence, considerable attention has been focusing on utilization of natural antioxidants as potential disease preventing agents. Recently, many natural antioxidants have been isolated from different plant materials (Packer et al., 1997; Jovanovic, 2000).

In the present study, a comparative study on antioxidant, antiradical and cathepsin B and matrix metalloproteinases inhibitory activities of U.indica red and white varieties was carried out.

Antioxidants are known to scavenge free radical by donating hydrogen or electron and by chelating metal ion, which is required for radical generation (Olufunmiso et al., 2011). FRAP assay is reliable, rapid and sensitive assay routinely used to evaluate total antioxidant activity of biological samples (Maisalshisakul et al., 2008; Klimczak et al., 2007). This assay depends upon the reduction of ferric tripyridyl triazine [Fe (III)-TPTZ] complex to the ferrous tripyridyl triazine [Fe (II)-TPTZ] (Benzie and Strain, 1996). The results of the present study showed that significantly high percentage of FRAP activity was observed with aqueous extracts of both bulb and leaves of red variety compared to white variety. However, less significant activity was noticed with aqueous extracts of stem and root of both varieties. This assay indicates that antioxidants present in the extracts might be act as reducing agent, which reduced ferric tripyridyl triazine [Fe (III)-TPTZ] complex in a redox reaction manner (Hou et al., 2003). Further, high reducing ability of the aqueous extracts of both bulb and leaves manifests the reducing power of the U.indica red variety as a potential source of antioxidants.

The reducing ability of plant extract can serve as a significant indicator of its potential antioxidant activity (Koki et al., 2010). This method measures the ability of the phytochemicals that transforms potassium ferricyanide (Fe3+) to potassium ferrocyanide (Fe2+) (Nayam et al., 2011). The results obtained in this study revealed that the aqueous bulb extracts of white and red varieties of U.indica had significant reducing ability.

Oxygen derived reactive oxygen species such as super oxide, hydroxyl radical and hydrogen peroxide induce oxidative damage to biomolecules and as such implicated with variety of pathological conditions including cancer, diabetes, atherosclerosis, neurodegenerative disorders and arthritis (Olga et al., 2003). Antioxidants of plant origin are believed to protect the cells from oxygen derived reactive oxygen species induced damages (Sarvajeet Singh et al., 2010). DPPH method, the most versatile assay used for assessing oxygen radical scavenging ability of U. indica in the present study. The results obtained in this study revealed that different parts of U. indica red and white varieties exhibited different levels of oxygen free radical scavenging abilities. However, significant DPPH radical scavenging activity was noticed with aqueous leaf extract of white variety and aqueous extracts of both bulb and leaf of red variety. It was also evident that the aqueous leaf extract of white variety and aqueous extracts of both bulb and leaf of red varieties high hydrogen donating ability to act as antioxidants.

Superoxide radical is formed in viable cells during various metabolic reactions (Fridovich, 2003) and its effect can be magnified because it produces other types of free radicals and oxidizing agents that can induce cell damage (Lui and Ng, 1999). Super oxides are produce from molecular oxygen by oxidative enzymes as well as via non-enzymatic reactions such as auto oxidation of amines (Mega jha et al., 2010). Super oxide produced in autoxidation of hydroxylamine, reduces nitroblue tetrazolium in the presence of EDTA. The inhibition of nitroblue tetrazolium reduction by medicinal plant extracts is an index of its super oxide anion radical scavenging ability. In this study, highest inhibition of nitro blue tetrazolium reduction was observed with aqueous bulb extract of U. indica red variety compared to white variety indicating its super oxide radical scavenging ability. However, moderate activities were noticed with aqueous root and leaf extracts of both red and white varieties. The observed super oxide radical scavenging activity of U. indica extracts may be due to the neutralization of super oxide anion character of NBT either by transfer of electron or hydrogen atom.

Hydroxyl radicals can be generated by ferric ions without any oxidizing agent. As poorly chelated iron have adverse effects on human body, numerous natural products containing iron binding agents are essential in the maintenance of human health. Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and also damage to living cells. The cell damaging action of hydroxyl radical is well known, as it is the strongest among free radicals (Koppenaal and Liebman, 1984). In the present study, hydroxyl radical scavenging activity of U. indica was evaluated using the deoxyribose assay (Hou et al., 2003). This is very convenient assay to determine the reaction of water-soluble compounds with hydroxyl radicals (Valentao et al., 2003). In this study, Fe3+-EDTA- hydrogen peroxide-ascorbic acid system was used to generate hydroxyl radicals (Fenton’s reaction). This degrades the deoxyribose into fragments, which further reacts with thiobarbituric acid upon heating at a low pH forming pink chromogen, which can be detected. In addition, inhibition of hydroxyl radicals indicates the ability to prevent the lipid peroxide formation (Aurand, 1977). In the present study, a significant hydroxyl and lipid peroxide radical scavenging activities were observed with aqueous bulb extract of red variety when compared to the white variety. This lipid peroxidation inhibitory activity of U. indica may be due to scavenging of hydroxyl radicals.

Hydrogen peroxide itself is not very reactive but together with other reactive oxygen species can damage several cellular components (Perumal et al., 2010). Hydrogen peroxide is considering as weak oxidizing agent and can inactivate some enzymes directly by promoting the oxidation of essential thiol groups. H₂O₂ can diffuses through the cell membrane directly and can reacts with Fe²⁺ and Cu²⁺ ions and forms hydroxyl radicals which initiates toxic effects (Miller, et al., 1993 ). In this study, aqueous extracts of leaves and roots of U.indica showed the maximum hydrogen peroxide scavenging activities.

In the present study, the cytotoxicity activity of U.indica on MCF-7 and BT-549 cells was evaluated by MTT assay. MTT assay is a well-established in vitro method for cytotoxicity against cancer cell lines and hence it was utilized to determine the selective activity of the extracts. Cytotoxicity screen-
models provide important preliminary data to help selecting plant extracts with potential anti-neoplastic properties for future work and provide scientific support to the use of *U. indica* for the treatment of cancer patients by traditional healers.

Histamine B and MMP-9 are key tissue remodeling enzymes required for metastasis of solid tumors. *U. indica* is a perennial geophyte reported to have anti-cancer activity (Harini et al., 2008). In one study, they demonstrated that aqueous extract of bulb contained a novel glycoprotein with potent anti-angiogenic and pro-apoptotic activity against mouse mammary carcinoma (Deepak and Salimath, 2006). Recent studies have demonstrated that ROS activates MMP-9 and cathepsin B to mediate the breakdown of extracellular matrix (Liu et al., 2005). The present study demonstrated that aqueous extract of *U.indica* bulb have significant inhibition on proliferation of breast cancer cell lines as well as both cathepsin B and MMP-9 activities. This study indicates that the anticancer activity of *U.indica* is partly due to its inhibitory activity on proliferation and also inhibition of tissue remodeling enzymes, cathepsin B and MMP-9 probably by scavenging oxygen free radicals.

CONCLUSION:
In summary, our study demonstrated that both red and white varieties of *U. indica* are potential source of natural antioxidants as confirmed DPPH, superoxide and hydroxyl radical and hydrogen peroxide scavenging activities. This study also demonstrated the possible anti-cancer activity of *U. indica* as aqueous extracts showed significant inhibitory effect on tissue remodeling proteases, such as MMP-9 and Cathepsin B. Further studies are required to identify the antioxidant compounds and to understand its anti-cancer activity.

List of abbreviations:
ROS: Reactive oxygen species, TBA:Thiobarbituric acid, DPPH: 1,2-diphenyl-1-1-picyrhydrazyl, TPTZ: 2,4,6-tripyridyl-s-triazine, NBT: Nitroblue tetrazolium, SDS: Sodium dodecyl sulphate, NaN₃: Sodium azide, EDTA:Ethyle diamine tetra acetate, K₃[Fe (CN)₆]:Potassium ferrocyanide, TCA:Trichloro acetic acid, TARS:Thiobarbituric acid reactive substances, SDS:Sodium dodecyl sulfate, AscAE: Ascorbic acid Equivalents.

ACKNOWLEDGEMENT:
We thank authorities of GITAM University for continuous support and encouragement. We also thank authorities of Raghu Degree and P.G College for providing facility for conducting this project.

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Source of support: Nil, Conflict of interest: None Declared