INTRODUCTION

Plants have been used as natural remedies for curing many physiological disorders, since ancient times. Use of plants as a source of medicine was found to be an important component of the health care system. India was found to be the largest producer of medicinal herbs and was appropriately called as the World’s largest botanical garden. [Ahmedull et al., 1999]. Dr. Stephen de Felice, coined” the term nutraceuticals” derived from the words “nutrition” and “pharmaceutical”, is none other than a food or food product that provides health and medical benefits, including the prevention and treatment of disease [Biesalski, 2001]. A nutraceutical was demonstrated to possess a physiological benefit or provide protection against chronic disease, through the bioactive compounds, the phytochemicals. It may sustain or promote health. Such substances may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products, processed foods and beverages (Kalra, 2003, Prakash et al., 2004).

Phytochemicals, are non-nutritive plant chemicals that show either defensive or disease protective properties. Plants produce these nonessential nutrients mainly to provide them protection. The intake of the phytochemical through diet may promote health benefits by protecting against chronic degenerative disorders, such as cancer, cardiovascular and neurodegenerative diseases. Most of the foods, such as whole grains, beans, fruits, vegetables and herbs contain phytonutrients/phytochemicals. These phytochemicals have tremendous therapeutic potential in curing various ailments, either alone and/or in combination. Phytochemicals with nutraceutical properties present in food are of great significance due to their beneficial effects on human health. They provide protection against numerous diseases
or disorders such as cancers, coronary heart disease, diabetes, high blood pressure, psychiatric diseases, ulcers, inflammation, microbial, viral and parasitic infections, spasmodic conditions, osteoporosis and associated disorders. Epidemiological and animal studies suggested that the regular consumption of fruits, vegetables and whole grains, reduces the risk of chronic diseases associated with oxidative damage (Scalbert et al., 2005, Kris-Etherton et al., 2002, Cieśluk et al., 2006).

**Reactive oxygen species** (ROS) are chemically reactive molecules containing oxygen. ROS are formed as a natural byproduct of the normal metabolism of oxygen and play an important role in cellular signalling and homeostasis (Devasagayam et al., 2004).

For proper gene expression and DNA replication the integrity of the genome is most important. When genome integrity is lost the normal cellular physiological activities would be altered and this leads to cellular pathological events such as senescence, apoptosis, and tumorigenesis (Wolters and Schumacher., 2013). The level of genotoxic reactive oxygen species (ROS) abnormally gets elevated under oxidative stress. ROS interact with and modify the chemical properties of biomolecules inside the cell, which causes oxidative insults such as oxidation of nucleic acids, peroxidation of lipids (Yin et al., 2011), and denaturation of proteins (Grimm et al., 2012).

Oxidative modification to DNA mainly occurs in Guanine base, which possesses the lowest oxidation potential of the DNA bases. This is the most frequent target of ROS. ROS-elicits changes in biomolecules (Dizdaroglu 2002). The oxidation of a DNA base, results in an increased risk of genome integrity, due to the alteration of the DNA repair process and base excision repair (BER), which could drastically increase the level of interrupted DNA strands resulting in indirect single strand break (SSB) (Maynard et al., 2009) and subsequently leads
to mismatched base pairing during DNA repair (Shaheen et al., 2010). This results in genome instability and accumulation of mutations leading to genetic heterogeneity in cancer cells (Jones et al., 2008).

ROS are ubiquitous and genotoxic. They include the superoxide anion radical (O2\(-\)), hydrogen peroxide (H2O2), the hydroxyl radical (OH\(-\)), and the nitric oxide radical (NO\(-\)). To maintain the genome integrity and physiological functions of normal cell, cells have developed strategies to control ROS levels. Such control is known as antioxidant defense (Birben et al., 2012). The natural antioxidant system is mainly classified into two categories namely in-vitro and in-vivo antioxidants. Free radical scavengers act as hydrogen donors, electron donors, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and non-enzymatic antioxidants exists in the intracellular and extracellular environment to detoxify ROS (Kondratyuk and Pezzuto., 2004).

The MTT assay is a colorimetric method of determining viable cell number in proliferation and cytotoxicity studies. This assay is based on the cleavage of the yellow tetrazolium salt, MTT, to form a soluble blue formazan product by mitochondrial enzymes. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Tetrazolium dye assay was used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. The amount of formazan produced is directly proportional to the number of living, not dead cells, present during MTT exposure. Since the MTT assay is rapid, convenient, and
economical, it has become a very popular technique for quantification of viable cells in culture. (Sylvester PW, 2011) (Berridge et al., 2005).

Collection and authentication of plant material

The white Ipomoea batatas and brown Solanum tuberosum were obtained from plant cultivator. It was authenticated at the National Institute of Herbal Science; Plant Anatomy Research Center Chennai as the tubers of sweet potatoes and brown potatoes and was confirmed as Ipomoea batatas Lam and Solanum tuberosum under the family Convoluaceace and Solanaceae respectively.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents were purchased from Sigma – Aldrich, USA, Ltd.

Extraction

Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles. About 30 grams of powder was added to 90 ml of solvent (ethyl acetate, ethanol, hydroethanol, and aqueous 1:3 ratio) in a dry flask. The flask was then incubated for 24 hrs in a shaker. After incubation, the extract was collected using Whatman No. 1 filter paper and evaporated below 40°C. To the residual mixture, another 50 ml of solvent was added and incubated in shaker for 12 hours. The extract was collected again using Whatman filter paper No. 1 and evaporated below 40°C, which was used for further phytochemical analysis.
Phytochemical Screening

Qualitative Analysis

Chemical tests were carried out on all the eight extracts of *Ipomoea batatas* and *Solanum tuberosum* powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1995).

❖ **Test for Carbohydrates**

To 2ml of plant extracts, 1ml of Molisch’s reagent and few drops of concentrated sulphuric acid were added. Purple colour formation indicated the presence of carbohydrates.

❖ **Test for Tannins:**

To 1ml of the plant extract, 2ml of 5% ferric chloride was added. Formation of greenish black colour indicated the presence of tannins.

❖ **Test for Saponins**

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

❖ **Test for Flavonoids**

5ml of dilute ammonia solution was added to a portion of aqueous filterate of plant extract followed by concentrated sulphuric acid. Appearance of yellow coloration indicated the presence of flavonoids.
Test for Alkaloids

To 2ml of the plant extracts, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer’s reagent were added. Presence of green color indicated the presence of alkaloids.

Test for Anthocyanins and Betacyanins

To 2ml of the plant extracts, 1ml of 2N sodium hydroxide was added and heated for 5 minutes at 100\(^0\) C. Formation of yellow color indicated the presence of betacyanins.

Test for Quinones

To 1ml of the plant extracts, 1ml of concentrated sulphuric acid was added. Formation of red color indicated the presence of quinones.

Test for Glycosides

To 2ml of the plant extracts, 3ml of chloroform and 10% ammonia solution was added. Pink color formation indicated the presence of glycosides.

Test for Cardiac Glycosides

To 0.5ml of the plant extracts, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was layered with 1ml of concentrated sulphuric acid. Brown ring formation at the interface indicated the presence of cardiac glycosides.
Test for Terpenoids

To 0.5ml of the plant extracts, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Red brown color formation at the interface indicated the presence of terpenoids.

Test for Triterpenoids

To 1.5ml of the plant extract, 1ml of Libermann – Buchard Reagent (acetic anhydride + concentrated sulphuric acid) was added. Blue or green color formation indicated the presence of triterpenoids.

Test for Phenols

To 1ml of the plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of green color indicated the presence of phenols.

Test for Coumarins

To 1 ml of the plant extract, 1ml of 10% sodium hydroxide was added. Formation of yellow color indicated the presence of coumarins.

Test for Proteins and Aminoacids

To 2ml of the plant extract, few drops of 0.2% Ninhydrin was added and heated. Purple color formation indicates the presence of aminoacids and proteins.
Test for Steroids and Phytosteroids

To 2ml of the plant extract, 5ml of chloroform was added and filtered, 2ml of acetic anhydride was added to 2ml of the filtrate with 2ml of sulphuric acid. The color changes from violet to blue or green and this indicates the presence of steroids.

Test for Phlobatannins

To 1ml of the plant extract, few drops of 10% ammonia solution was added. Appearance of pink colour indicates the presence of phlobatannins.

Test for Anthraquinones

To 1 ml of the plant extract, few drops of 2% HCL were added. Appearance of red colour precipitate indicates the presence of anthraquinones.

QUANTITATIVE ANALYSIS

Estimation of Flavonoids

Reagents

- Aluminium chloride
- Potassium acetate
- Quercitin
- Methanol
Procedure

Aluminium chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). Each plant extract (10mg/ml) was prepared and 0.5 ml of each sample was separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate and 2.8 ml of methanol was added. It was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The total flavonoids was expressed in mg/g (quercitin was used as standard).

Determination of Coumarins

TLC method was used for coumarin determination Pharmacopeial Forum: 26(6). 1 gm of the extract was mixed with 5ml of methanol and boiled for 30 sec, cooled, filtered and used as test solution. 5mg of coumarin, mixed in 10 ml of methanol was used as reference solution and was run in TLC silica gel F 254 plate. Chromatogram was obtained and the zones were read at 65nm in UV Spectrophotometer. The coumarin content present in the sample was calculated.

Determination of Saponins

To 0.5 g of the extracts, 5ml of a mixture of alcohol and 5ml water was added and heated to boiling. It was centrifuged and the supernatant was used. Then 10μl of the supernatant was applied on the TLC plate as bands of 20mm by 2mm. The plate was developed using a mixture of (1:30,40,40 ratio) 1ml of glacial acetic acid, 30 ml of water, 40 ml of ethyl acetate and 40 ml of proponal. The plate was allowed to dry in air. Then 20 per cent V/V solution of nitric acid was sprayed and heated at 120⁰C for 10 min. It was cooled and 50 per cent V/V solution of potassium hydroxide in alcohol was sprayed until the zones
appear. Then after spraying anisaldehyde solution the chromatogram exhibits spots with an Rf value of about 0.4. The zones are cut and is taken for estimation.

**Determination of Quinones**

Mobile Phase was prepared by filtering and degassing the mixture of methanol and dehydrated alcohol (13.7). Standard was prepared by weighing accurate quantity of Quinone in dehydrated alcohol and heated at 50\(^{0}\) for 2 minutes if necessary, to obtain a known concentration of about 1.0 mg/ml.

**Assay preparation:** 5.0 gm of the extract was accurately weighed and transferred to a 50-mL volumetric flask, dissolved in dehydrated alcohol and heated at 50\(^{0}\) for 2 minutes. Then it was cooled and diluted with alcohol upto the volume and mixed well. The container was tightly closed and protected from light.

**Chromatographic system:** The liquid chromatography was equipped with a 275 nm detector and a 5 mm X 15 cm column was packed and maintained at a temperature of 35\(^{0}\). The flow rate was adjusted to obtain a retention time of about 11 minutes. Equal volumes of about 5\(\mu\)l of the standard and extract were injected into the chromatograph and the recorded chromatograms were measured with the major peaks. The quantity of quinone present in the sample was measured. [Pharmacopeial Forum: Volume No. 26(6) Page 1601].

**ANTI OXIDANT ACTIVITY**

**Determination of free Radical Scavenging activity**

**DPPH Radical Scavenging activity**

**Reagents**

- DPPH (2,2-diphenyl-1-picrylhydrazyl)
- BHT (Butylated hydroxytoluene) was purchased from Sigma, USA.
All the other chemicals used were of analytical grade.

Principle

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with purple color (absorbed at 517 nm). If free radicals have been scavenged, DPPH will degenerate to yellow color. This assay uses this principle to show the free radical scavenging activity.

Procedure

Antioxidant activity or free radical scavenging activity of the aqueous extract against DPPH (2,2-diphenyl-1-picrylhydrazyl) was measured according to Mensor et al., (2001). Different concentrations of the extract was added to the methanolic solution of (2,2-diphenyl-1-picrylhydrazyl) DPPH. After 5 minutes at room temperature the absorbance was recorded at 517nm. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with purple color (absorbed at 517nm). If free radicals have been scavenged, DPPH will degenerate to yellow color. The experiment was repeated. BHT (Butylated hydroxytoluene) was used as standard control.

The percentage inhibition of DPPH radical by the sample was calculated using the following formula:

\[
\text{Inhibition\%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

Determination of Reducing Power

Reagents

- Acetate buffer: 300 mM, pH 3.6
- TPTZ (2,4,6-tri (2-pyridyl)-s-triazine): 10 mM in 40 mM Dilute Hcl
- Ferric chloride: 20 mM
The FRAP reagent was prepared by mixing 1, 2 and 3 in the ratio of 10:1:1

**Standard**: Ferrous sulphate 1 mM

**Principle**

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996). FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has intense blue color) can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is therefore, directly related to the total reducing power of the electron donating antioxidants present in the reaction mixture.

**Procedure**

Various concentrations of the extract diluted with distilled water was mixed with 1.5 ml of working FRAP reagent and incubated at 37°C for 4 minutes. After incubation the absorbance was measured at 593 nm. Ferrous sulphate standard was processed in the same way and the FRAP value was calculated. The change in absorbance is therefore, directly related to the total reducing power of the electron donating antioxidants present in the reaction mixture.

**Nitric Oxide Radical Scavenging Assay**

**Reagents**

- Sodium Nitroprusside
- Greiss reagent (1% sulfanilamide, 2%H₃PO₄ and 0.1% napthylethlenediamine
dihydrochloride).

- Ascorbic acid
- (5 mM) in PBS- Phosphate Buffered Saline

**Procedure**

Nitric Oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium Nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. (Marcocci *et al.*, 1994). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in PBS was mixed with different concentrations of *Ipomoea batatas, Solanum tuberosum* extracts and incubated at 25°C for 150 min.

The samples were reacted with Greiss reagent (1% sulfanilamide, 2%H₃PO₄ and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the pink colored chromphore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with napthylethylenediamine was read at 540 nm and compared with that of standard solutions treated in the same way. Ascorbic acid was used as a standard.

\[
\text{Inhibition\%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

**Superoxide Radical Scavenging Assay**

**Reagents**

- Nitro blue tetrazolium (NBT)
- Nicotinamide adenine dinucleotide (NAD)
Phosphate Buffer, pH-7.4
- Phenazine Methosulfate (PMS)

**Procedure**

Measurement of superoxide radical scavenging activity was done by using method of Nabavi *et al.*, (2008). The reaction mixture contained 1ml of Nitro blue tetrazolium (NBT) solution (312 µM prepared in phosphate buffer, pH-7.4), 1ml of Nicotinamide adenine dinucleotide (NAD) solution (936 µM prepared in phosphate buffer, pH-7.4) was mixed with different concentrations of the extract. Finally the reaction was accelerated by adding 100 µl phenazine methosulfate (PMS) solution (120 µM prepared in phosphate buffer, pH-7.4). The reaction was incubated at 25°C for 5 minutes and absorbance was measured at 560 nm. The standard Quercitin was treated similarly.

\[
\text{Inhibition\%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

**Hydrogen Peroxide Radical Scavenging Activity**

**Reagents**

- Hydrogen Peroxide
- PBS- Phosphate Buffered Saline

**Procedure**

Hydrogen peroxide radical scavenging activity was determined according to the method of Nabavi *et al.*, (2008). The solution of hydrogen peroxide (40mM) was prepared in PBS (pH 7.4). Various concentrations of extract was added to 2 ml of H₂O₂ and absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing
the PBS without H₂O₂. The percentage of H₂O₂ scavenging of sample and standard compound Quercitin was calculated.

\[
\text{Inhibition\%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

**Cell Viability Assay**

Standardization, maintenance and storage of hepatic carcinoma cell lines were done as per standard procedure. MTT assay was performed to select the best extract and to estimate the optimum dosage for the treatment of hepatocellular carcinoma.

**Reagents**

- DMEM medium
- 10% Fetal Bovine Serum
- MTT
- DMSO
- HepG2 cells (liver cancer) were purchased from NCC Pune.
- 3T3 (normal) Cells were purchased from NCC Pune.

**Principle**

Mitochondrial succinate dehydrogenase enzyme in living cells reduce the yellow water soluble substrate 3- (4,5 – dimethyl thiazol -2- yl ) – 2,5 diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically (Mosamann, 1983). Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

**Procedure**
HepG2 (liver cancer cell lines) & 3T3 (normal) Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO2. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10^4 cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the aqueous & ethyl acetate plant extracts for 24 hours. After incubation, the medium was discarded and 100 μl fresh medium was added with 10 μl of MTT (5 mg/ml). After 4 hours, the medium was discarded and 100 μl of DMSO was added to dissolve the formazan crystals. The absorbance was read at 570 nm in a microtitre plate reader.

**Cell survival was calculated by the following formula**

Viability % = (Test OD/ Control OD) X 100 [Cytotoxicity % = 100 – Viability%].

Untreated cells were used as control and Cyclo-90 (90μg/ml) was used as positive control (PC).

Controls were maintained throughout the experiment (Untreated wells as cell growth control and diluents treated wells as diluents control). The assay was performed in triplicates for each of the extracts. The mean of the cell viability values were compared to the control to determine the effect of the extract on cells. A graph was plotted against the % cell viability Vs dilution of the extract. The minimum concentration of extract that was toxic to HepG2 cells was recorded as the effective concentration when compared to positive control (PC- Cyclophosphamide).
RESULTS AND DISCUSSION

Qualitative phytochemical analysis

In the present study of phytochemical screening four solvents namely ethyl acetate, ethanol, hydroethanol and aqueous were used for extraction of *Ipomoea batatas*. Each of the extract was analysed by specific reactions, as described by Harbone 1995 and Sofawara 1993 Trease 1989. Based on change in the colour and intensity of the colour formation of the specific reaction the identification of the phytochemicals present in the extracts was confirmed.

The semi – quantitative determination of the phytochemicals showed the presence of high quantity of carbohydrates, saponins, flavonoids, quinones, cardiac glycosides, coumarins in the aqueous extract and ethyl acetate extract of *Ipomoea batatas* compared to the ethanolic and hydroethanolic extract of *Ipomoea batatas*. Table 1 shows the presence of various phytochemicals present in *Ipomoea batatas* extract.

Extraction of *Solanum tuberosum* was done using four solvents namely ethyl acetate, ethanol, hydroethanol and aqueous. Out of all the four extracts ethyl acetate extract was found to posses major phytochemicals compared to the ethanol, hydroethanol and aqueous extracts of *Solanum tuberosum*. Thus upon screening of phytochemicals the ethyl acetate extract of *Solanum tuberosum* showed the presence of phytochemicals like carbohydrates, saponins, flavonoids, cyanins, quinones, cardiac glycosides, coumarins acids etc. Table 2 shows the presence of various phytochemicals in *Solanum tuberosum*.
Table 1: Qualitative analysis of phytochemicals of *Ipomoea batatas*

<table>
<thead>
<tr>
<th>Test</th>
<th>Ethyl Acetate</th>
<th>Ethanol</th>
<th>HydroEthanol</th>
<th>Aqueous</th>
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<tbody>
<tr>
<td>Carbohydrates</td>
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<td>Tannins</td>
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<td>Saponins</td>
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<td>Flavonoids</td>
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<td>Alkaloids</td>
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<td>Cyanins</td>
<td>Betacyanin (+)</td>
<td>Betacyanin (+)</td>
<td>Betacyanin (+)</td>
<td>Betacyanin (+)</td>
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<td>Quinones</td>
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<td>Glycosides</td>
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<td>Cardiac glycosides</td>
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<td>Terpenoids</td>
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<td>Triterpenoids</td>
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<td>Phenols</td>
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<td>Coumarins</td>
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<td>Acids</td>
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<td>Proteins and amino acids</td>
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<td>Steroids and phytosteroids</td>
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<tr>
<td>Phylobatannins</td>
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<td>Anthraquinone</td>
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[+++] Strongly present,  [+] Present,  [-] Absent.
Table 2: Qualitative analysis of phytochemicals of *Solanum tuberosum*

<table>
<thead>
<tr>
<th>Test</th>
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[+++] Abundantly present, [++] Present, [-] Absent.
In modern medical science Phytomedicines are increasingly being utilized for human health. As fruits and vegetables are rich in nutraceuticals, the importance and awareness of food consumption has increased in human health (Nile and Park, 2014).

The terpenes, are the largest class of phytonutrients present in green foods, and grains. It is also known as isoprenoids. In plants it is used to fix carbon through photosynthetic reactions using photosensitizing pigments. Terpenes were found to possess a unique antioxidant activity when they interact with free radicals (Prakash et al., 2004, Prakash and Kumar., 2011, Nichenametla et al., 2006, Stahl, 2005)

Different extracts of Ipomoea carnea plant was reported to possess anti-bacterial, anti-fungal, anti-oxidant, anti-cancer, anti-convulsant, immunomodulatory, anti-diabetic, hepatoprotective, anti-inflammatory, anxiolytic, sedative and wound healing activities (Fatima et al., 2014).

Polyphenols are the most important antioxidants in human diets. Polyphenols are categorized into different classes as phenolic acids, flavonoids, lignans, and stilbenes. Phenolic acids are naturally occurring compounds found in plants with peculiar structural similarities, presence of carboxylic group as in caffeic acid, gallic acid, p-coumaric acid, vanillic acid, ferulic acid and protocatechuic acid (Robbins, 2003).

Berries are a rich source of a wide variety of non-nutritive, nutritive and bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic acids, stilbenes and tannins, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins and minerals (Nile and Park, 2014). The shrub Ipomoea carnea has been used traditionally for thousands of years, potentially providing health benefits due to the presence of polyphenols,
flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates and fibers (Robbins, 2003).

The chief constituent of tea polyphenols are flavonols (catechin, epicatechin, catechingallate and epigallo-catechingallate), flavanols (quercetin, kaempferol and their glycosides), flavones (vitexin, isovintexin) and phenolic acids (gallic acid, chlorogenic acid). In apples, pears, and grapes Caffeic acid in the form of caffeoyl esters and coumaric acids are common. Apart from that apples and pears were found to be rich in chlorogenic acid and grapes in gallic acid. Apples contain high levels of quercetin among fruits.

Grain-derived products have higher concentration of phenolic acids in the outer layers of kernel that constitute the bran. Most of the phenolic acid derivatives are hydrolysable tannins and are usually esterified with glucose. Citrus fruits are major sources of flavonones and hesperidin is found in abundance (Anagnostopoulou et al., 2006).

The qualitative analysis of phytochemicals confirmed that high number of phytochemicals were obtained in the aqueous extract and ethyl acetate extract of *Ipomoea batatas* and ethyl acetate extract of *Solanum tuberosum* and these three extracts were subjected to Quantitative Analysis.

**Quantitative Analysis of Phytochemicals**

Mahmood *et al.*, 2014 stated that medicinal plants are used by about 80% of the world population in developing and developed countries for their primary and basic health care needs owing to better tolerability, superior compatibility with human body and having lesser side effects. Latest research investigation observed that the bioactive and antioxidant
potentials of these plants are attributed to the presence of polyphenols, flavonoids, lignins, alkaloids, terpenoids, carotenoids and vitamins (Vayalil., 2002, Vinson et al., 2005, Agbor et al., 2011).

**Table 3** shows the quantitative analysis of the phytochemicals present in the extract. It was observed from the table that saponins (23.46 mg) were found to be in the higher concentration compared to other three phytochemicals. Quinones (10.05 mg) content was found to be two times higher than flavonoids (3.7 mg) and coumarin content (13.46 mg) was found to be 4 times higher than flavonoids level in aqueous extract of *Ipomoea batatas*. The ethyl acetate extract of *Ipomoea batatas* shows saponins (10.3 mg), quinones (4 mg), coumarins (3 mg) and flavonoids (2.8 mg). When we compared the quantity of all the four phytochemicals in these two extracts, the aqueous extract of *Ipomoea batatas* was found to be the best compared to the ethyl acetate extract of *Ipomoea batatas* extract.

Ethyl acetate extract of *Solanum tuberosum* was found to have higher concentration of quinones (15.2 mg) compared to other three phytochemicals. Saponins (8.8 mg) followed by flavonoids (8.3 mg). The coumarins concentration was found to be two times lesser than quinones concentration.

Quinones play a vital role in the electron transport chain of most organisms either in the form of ubiquinones or menaquinones. The quinones involved in the electron transport chain are hydrophobic and lipid soluble. Water soluble quinones are cytotoxic and they do have the ability to covalently modify DNA and proteins and undergo redox cycling. Plants and fungi use polyphenol oxidases (PPOs) enzymes to generate quinones (Cerenius et al., 2008). Bacteria produce quinones as antibiotics e.g. doxorubicin from Streptomyces peucetius
Table 3 Quantitative Estimation of Phytochemicals

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous extract of <em>Ipomoea batatas</em> mg/gm</th>
<th>Ethylacetate extract of <em>Ipomoea batatas</em> mg/gm</th>
<th>Ethyl acetate extract of <em>Solanum tuberosum</em> mg/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>23.46±0.6</td>
<td>10.3±0.21</td>
<td>8.8±0.12</td>
</tr>
<tr>
<td>Quinones</td>
<td>10.50±0.5</td>
<td>4.0±0.2</td>
<td>15.2±0.3</td>
</tr>
<tr>
<td>Coumarins</td>
<td>13.46±0.5</td>
<td>3.0±0.04</td>
<td>6.7±0.02</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>3.7±0.02</td>
<td>2.8±0.01</td>
<td>8.3±0.03</td>
</tr>
</tbody>
</table>
Arcamone., (1969). Shiao et al., (2001) stated that the generation of quinones by PPO in plants and invertebrates leads to melanisation at the point of infection.

Saponins are vast group of biologically functional products in plants. Soyasaponins are usually glycosylated, which give rise to a wide diversity of structures and functions. The molecular mechanism of soyasaponins Aa and Ab was identified in regulating adipocyte differentiation and expression of adipogenic marker genes in 3T3-L1 adipocytes (Yang et al., 2014). In addition to industrial applications as foaming and surface active agents, saponins have been extensively used as detergents, pesticides and molluscicides & also have beneficial health effects (Arunasalam, 2004)

Coumarins are phytochemicals with a vanilla like flavour. It could exist either free or combine with glucose. The Benzopyrones are a group of compounds whose members include coumarins and flavonoids. Dietary exposure to benzopyrones is quite significant, as these compounds are found in vegetables, fruit, seeds, nuts, coffee, tea and wine (Aoife Lacy and Richard O’Kennedy., 2004). Recently researchers have made use of Coumarin as the new chemoassay-utilizing coumarin-based fluorescent probe 1 offers a cost- and time-effective method to identify the biomarkers in CNS patients (Yap et al., 2014). Coumarins were found to be natural substances possessing anti-tumour activity in vivo, with the effect believed to be due to its metabolites (e.g. 7-hydroxycoumarin). A recent study has shown that 7-hydroxycoumarin inhibits the release of Cyclin D1, which is overexpressed in many types of cancer. Coumarins were identified to be physiological, bacteriostatic and anti-tumour activity which actually made this compound to be a novel therapeutic agent (Aoife Lacy and Richard O’Kennedy., 2004).
Flavonoids are naturally occurring diphenylpropanoids that appear in animal and human cells due to the consumption of vegetables, fruits and beverages such as tea and wine. Flavonoids can be classified into six major subgroups: flavonols (e.g., quercetin, kaempferol), flavones (e.g., apigenin, luteolin), flavanones (e.g., hesperidin, naringenin), flavan-3-ols (e.g., catechin, theaflavin, and gallic esters of catechin and theaflavins), anthocyanidins (e.g., pelargonidin, cyanidin) and isoflavones (e.g., genistein, daidzein) (Mandel et al., 2008). In recent times, plant flavonoids have attracted attention as potentially important dietary cancer chemo-protective agents (Hertog et al., 1993, Okwu DE and Okwu ME, 2004). In addition, the possible antitumor action of certain flavonoids has also generated interest (Kandaswami et al., 1991, Elangovan et al., 1994).

Antioxidant assay

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. (Langseth, 1993; Halliwell, 1994).

Figure 1 shows the DPPH radical scavenging activity of various concentrations of Ipomoea batatas at increasing time period. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm (Ganapaty, 2007).

From the Figure 1 it is very clear that increase in the concentration of the extract showed increased radical scavenging. At 10μg concentration of aqueous extract of Ipomoea batatas <10% inhibition was observed at 5th minute period, there was drastic increase in the %
Figure 1

DPPH Radical Scavenging Activity of *Ipomoea batatas* - aqueous extract with increase in time and concentration.
inhibition to 70% as the concentration of *Ipomoea batatas* increased to about 100 μg. It proved clearly that the % inhibition gets gradual increased with increase in time period as well as with increase in the concentration, thus the % inhibition got increased to 82% at 30th minute at 100 μg concentration of the extract.

**Figure 2** shows the DPPH radical scavenging activity of various concentrations of *Solanum tuberosum* at increasing time period.

In the similar way the % inhibition of ethyl acetate extract of *Solanum tuberosum* increased gradually with increase in the concentration and with time. At 100 μg concentration at 5th minute the % inhibition was 20, were as at 1000 μg concentration the % inhibition was at 56, thus it got increased to about 80% at 30th minute. This proved that the DPPH radical scavenging activity is directly propotional to the time and the concentration of the extracts.

**Figure 3** shows nitric oxide radical scavenging activity, **Figure 5** shows superoxide radical scavenging activity of Aqueous extract of *Ipomoea batatas*.

NO is believed to participate in the regulation of the oxidation/reduction potential of various cells and may be involved in “either the protection against or the induction of oxidative stress within various tissues, depending upon its concentration (Bland., 1995). Emerging evidence suggests that some diseases are related to either an inadequate or excessive production of NO₃ (Moncada, S. and Higgs., 1993). Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity (Hagerman *et al.*, 1998). Superoxide dismutases (SOD) are enzymes that catalyze the conversion of two superoxides into hydrogen peroxide and oxygen. The benefit here is that
Figure 2

DPPH radical scavenging activity of *Solanum tuberosum* - ethyl acetate extract with increase in time and concentration.
hydrogen peroxide is substantially less toxic than superoxide. SOD accelerates this detoxifying reaction roughly 10,000-fold over the non-catalyzed reaction.

\[
\text{SOD} \quad \text{O}_2^- + \text{O}_2^- \rightarrow \text{O}_2 + \text{H}_2\text{O}_2
\]

SODs are metal-containing enzymes that depend on a bound manganese, copper or zinc for their antioxidant activity.

In the present investigation of \textit{Ipomoea batatas} the Nitric oxide scavenging activity was about 16% inhibition at 10 μg as the concentration and it increased to 41% as the concentration increased to 150 μg. Similarly the % inhibition of super oxide radical increased from 54% to 69% with increase in the concentration from 10 μg to 100 μg of the extract.

\textbf{Figure 4} shows nitric oxide radical scavenging activity, \textbf{Figure 6} shows superoxide radical scavenging activity of Ethyl acetate extract of \textit{Solanum tuberosum}.

Nitric oxide scavenging activity of ethyl acetate extract of \textit{Solanum tuberosum} was 22% at 100 μg concentration then it increased gradually to 40% at 2000 μg concentration. Then 100 μg of ethyl acetate extract scavenged 16% of superoxide radical which gradually elevated to 62% at 2000 μg concentration of the extract.

Superoxide can cause oxidation or reduction of solutes depending on their reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyse the breakdown of superoxide radical (Shirwaiar \textit{et al.}, 2007).

\textbf{Figure 7} and \textbf{Figure 8}, shows Hydrogen peroxide radical scavenging effect of \textit{Ipomoea batatas} and \textit{Solanum tuberosum} respectively.
Figure 3

Nitric Oxide Radical Scavenging activity of *Ipomoea batatas* - aqueous extract

% inhibition

Concentration

10 µg
25 µg
50 µg
100 µg
150 µg
Std Ascorbic acid 100 µg

Figure 4

Nitric Oxide Radical Scavenging Activity of *Solanum tuberosum* - ethyl acetate extract

% inhibition

Concentration

100 µg
250 µg
500 µg
1000 µg
2000 µg
Std Ascorbic acid 100 µg
Figure 5

Superoxide Radical Scavenging activity of *Ipomoea batatas* - aqueous extract

Concentration

% inhibition

10 µg, 25 µg, 50 µg, 100 µg, 150 µg, Std Quercitin 100 µg

Figure 6

Superoxide Radical Scavenging activity of *Solanum tuberosum* - ethyl acetate extract

Concentration

% inhibition

100 µg, 250 µg, 500 µg, 1000 µg, 2000 µg, Std Quercitin 100 µg
10 μg of the aqueous extract of *Ipomoea batatas* was able to scavenge 59% of hydrogen peroxide radical, it was found to get increased to 70% at 150 μg of the extract. Thus the hydrogen peroxide scavenging activity of *Ipomoea batatas* extract was found to be increasing with increase in the concentration.

In case of ethyl acetate extract of *Solanum tuberosum* the hydrogen peroxide radical scavenging activity was found to be 18% inhibition, but the increase in the concentration of the extract didn’t show increasing scavenging activity instead it was found to get saturated at 500 μg concentration itself so the same 50% inhibition was observed at 2000 μg concentration too.

**Figure 9** and **Figure 10**, shows Ferric Reducing Antioxidant Power of *Ipomoea batatas* and *Solanum tuberosum* respectively.

Reducing power was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996). The FRAP assay showed that there was an increase in reducing power from 0.038 to 1.158 absorbance at 593nm with increase in concentration of *Ipomoea batatas* from 10μg to 150μg. Similarly FRAP reducing power was observed in *Solanum tuberosum* also. At 100μg concentration the absorbance was 0.042 whereas at 2000μg concentration it was increased to 1.220.

**Cytotoxicity Assay**

The criterion for cytotoxicity for the crude extracts, as established by the National Cancer Institute (NCI), is IC50 value lower than 30 μg mL-1 (Suffness *et al.*, 1990).

Tissue culture technology has found wide application in the field of cell biology. Cell cultures are utilized in cytogenic, biochemical and molecular laboratories for diagnostic as well as research studies. In most cases, cells or tissues were grown in culture for days or
weeks to obtain sufficient numbers of cells for analysis. Since the introduction of Hep G2 cell line in (Aden et al., 1979) more than 250 studies using this resource have been published.

**Figure 11** shows the effect of *Ipomoea batatas* extract on HepG2 cell lines. In all the three concentrations namely 100 μg/ml, 250 μg/ml, and 500 μg/ml the cytotoxicity was found to higher on Hep G2 cell lines which proved to show very good antiproliferative property.

**Figure 12** shows the effect of *Solanum tuberosum* extract in HepG2 cell lines. The antiproliferative property was determined using MTT assay. *Solanum tuberosum* extract was able to exert antiproliferative effects in HepG2 cell line in dose dependent manner. The results showed that HepG2 cell lines treated with 100μg/ml of *Solanum tuberosum* extract showed 62% cytotoxicity.
Figure 11

MTT Assay of *Ipomoea batatas* -aqueous extract on HepG2 cell lines

- 100µg
- 250µg
- 500µg
- PC Cyclo-90

Concentration vs. cytotoxicity %

Figure 12

MTT Assay of *Solanum tuberosum* -aqueous extract on HepG2 cell lines

- 100µg
- 250µg
- 500µg
- PC Cyclo-90

Concentration vs. cytotoxicity %
<table>
<thead>
<tr>
<th>Reference</th>
<th>Text</th>
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<tbody>
<tr>
<td>8</td>
<td>Benzie IFF, Strain JJ. 1996. Ferric reducing ability of plasma (FRAP) as a measure of</td>
</tr>
</tbody>
</table>


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