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Evaluation of *In-vitro* cytotoxic and antioxidant activity of methanolic extracts of *Ipomoea carnea* and *Alternanthera sessilis*

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Abstract: *Ipomoea carnea* and *Alternanthera sessilis* are two common weeds in India which have shown to possess several medicinal properties. The present study pertains to assess the antioxidant and cytotoxicity activity of the leaf extracts of these plants. The extraction of leaf content of the plants was carried out by soxhlet method using methanol. The in vitro cytotoxicity assay was performed in vitro on vero cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay method. In vitro antioxidant activity was carried out by DPPH radical scavenging method. The cytotoxicity activity for *I. Carnea* i.e. IC50 (50% growth inhibition) value was found to be 1mg/ml and the IC50 of *A.sessilis* was found to be 6.5mg/ml. The antioxidant activity of *I. Carnea* i.e. IC50 value was found to be 1200ug/ml whereas that of *A. sessilis* was found to be 400ug/ml. The cytotoxic activity was higher for Ipomoea extracts as compared to Alternanthera extracts. However, the DPPH free radical scavenging activity was higher for *Alternanthera sessilis* extracts as compared to *Ipomoea carnea* extracts. Both showed high in vitro activity, which indicates the therapeutic potential of these plants.

Key words: Antioxidant; Cytotoxicity; DPPH radical; MTT; Weeds

Introduction

Weeds are the important and unused components of the agricultural ecosystem (Njoroge, *et al.,* 2004). The role of weeds, commonly found in disturbed areas, in traditional medicine floras has been overlooked (Stepp, *et al.,* 2001). However, weeds are useful to human beings as food, erosion control, medicines, aesthetic value, shelter, supply of organic matter and mineral nutrients to the soil. Consumption of agricultural weeds is a world-wide phenomenon as some of the plants are characterized by high nutritional value and medicinal properties (Maroyi, 2013).

Ipomoea carnea is one common weed popularly known as Besharam. It is a large diffuse shrub with milky juice which grows in dense populations along river beds, banks, canals and other waterlogged (wetland) areas. It has been identified as a useful material for several applications including medicinal purposes (Gaur, *et al.,* 2014).

Alternanthera sessilis is another weed that inhabits many areas of the world. It has been used widely for its medicinal uses, as well as for food. Young shoots and leaves are eaten as a vegetable in Southeast Asia. The leaf is very rich in iron, vitamin A and dietary fiber. *A. sessilis* is used internally against intestinal inflammation, externally to treat wounds, to treat hepatitis, tight chest, bronchitis, asthma, lung troubles, to stop bleeding and as a hair tonic (Singh, *et al.,* 2009).

Plants in general contain many polyphenolic compounds. Antioxidants are a class of such secondary metabolites of plants. Several isolated plant constituents as well as extracts have been recognized to possess antioxidant effects against free radicals in biological systems (Mervat, *et al.,* 2010). This activity is mainly due to the presence of phenolic compounds such as flavonoids, phenols, tannins etc. Natural antioxidants have a wide range of biochemical activities including inhibition of ROS generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health promoting effects in the prevention of degenerative diseases. Although several synthetic antioxidants are commercially available, they are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants (Patel, *et al.,* 2010). In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human diseases. Nowadays, natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. The present study was undertaken to evaluate the above selected weeds for their *In-vitro* properties such as cytotoxicity and antioxidant activity.

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Materials and Methods

Collection and identification: The leaves of *Ipomoea carnea* and *Alternanthera sessilis* were collected from local areas in Mumbai. The plants were identified as *Ipomoea carnea* and *Alternanthera sessilis* at the Blatter herbarium, St. Xavier's College, Mumbai.

Preparation of extracts of the plants: Mature leaves of both plants were removed from the stem. These leaves were first washed in sterilized distilled water, followed by washing in mercuric chloride solution (0.1 %) and again a washing with distilled water. The leaf material was air dried, weighed and transferred in a homogeniser where it was crushed into powder which was stored in a dark bottle at room temperature until further use.

About 20 grams of coarsely powdered plant material was exhaustively extracted with 200 ml of methanol (Sigma-Aldrich) at its boiling point temperature, using a soxhlet apparatus. The extracts obtained were filtered and concentrated to remove methanol. These filtered extracts were kept at 4^{o}C till further use. Various concentrations of the extract prepared according to the assay. All the dilutions were prepared by dissolving the extract in 10% DMSO.

***In-vitro* cytotoxicity activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay**

The cytotoxic activity of Ipomoea and Alternanthera leaves was carried out using MTT assay. Vero cells according to Roy, *et al.,* 2016. The cytotoxic effect (50% inhibitory concentration [IC50]) was measured using following equation:

% Cytotoxic effect = [(Absorbance of control − Absorbance of sample)/ (Absorbance of control)] ×100.

Radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the fractions was measured *In-vitro* by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay as per Dahake, *et al.,* 2012. The control was prepared as without any sample. Ascorbic acid was used a standard whose absorbance was measured at 517nm. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%) = [(control absorbance - sample absorbance) / (control absorbance)] x 100.

The data was expressed as mean ± SD from three separate observations. The antioxidant assay (IC50) was calculated by logarithmic method using the Graph Pad Prism v5.0 software.

Results

Cytotoxicity assay

Different concentrations (0.1–10 mg/ml) of soxhlet extracts of Ipomoea and Alternanthera were added onto vero cell line (0.2 × 106 cells/ml) in the 96 well plate and were analyzed by MTT based cytotoxicity assay. The concentration of soxhlet extracts required to kill 50% of the cells i.e. IC50 of Ipomoea was found to be 1 mg/ml. Whereas, IC50 of Alternanthera extracts was found to be 6.5 mg/ml. The IC50 was calculated and the percent cytotoxicity was represented by using GraphPad Prism version 5.0 software (Figure 1). Also, as the concentration of the extract was increased, there was an increase in the cytotoxic effect displayed by the extract. Thus, the cytoxicity was directly proportional to the concentration of the extracts.

DPPH radical scavenging activity

Ipomoea carnea and *Alternanthera sessilis* extracts of different concentrations (50ug/ml-1200ug/ml) were used for the assay. The assay was carried out by the DPPH radical scavenging method. The absorbance was read at 517 nm. The IC50 was calculated and the percent inhibition was represented by using graphpad prism v5.0 software. The IC50 of *I. carnea* extract was found to be 1200ug/ml (figure 2). The IC50 of *A. sessilis* extract was found to be 400ug/ml (figure 2). However, it was noticed that like the cytoxicity effect, the antioxidant effect too was concentration dependent. There was a steady increase in the DPPH activity with the increase in the concentration of the extracts. Thus, the antioxidant activity was directly proportional to the concentration of the extracts.

Discussion

Nature has bestowed us with many different kinds of plants and all parts individually or totally exhibit therapeutic properties. The part may be leaf, bark, seed, stem, flowers, fruits, twigs and peel etc. each part showing different biological activity and antioxidant potency (Chanda, *et al.,* 2013).

Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in pharmaceutical and drug research. Unlike other free radicals, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition (Middha, *et al.,* 2012).

The antioxidant activity of *I.carnea* and *A.sessilis* were investigated by DPPH radical scavenging method. IC50 was calculated by graphpad prism software v-5.0. There was a steady increase in the antioxidant activity of the extracts as their concentration increased. The IC50 of *I. carnea* extract was found to be 1200ug/ml (figure 2). The IC50 of *Alternanthera sessilis* extract were found to be 400ug/ml (figure 2). We observed that the DPPH free radical scavenging activity (IC50) was higher in Alternanthera extracts (400ug/ml) as compared to Ipomoea extracts (1200ug/ml).

MTT assay measured the cell viability based on the reduction of yellow tetrazolium MTT to a purple formazan dye mitochondrial dehydrogenase enzyme. So, the amount of formazan produced reflected the number of metabolically active viable cells. MTT results showed that both extracts possessed cytotoxic effect against vero cell lines. The cytotoxicity results obtained showed a direct relation with the concentration of the extracts. The toxicity of the plants increased with an increase in its concentration. However, Ipomoea extracts showed higher cytotoxic effect as compared to Alternanthera extracts. The IC50 of Ipomoea was 1mg/ml whereas that of Alternanthera was 6.5mg/ml (figure 1).

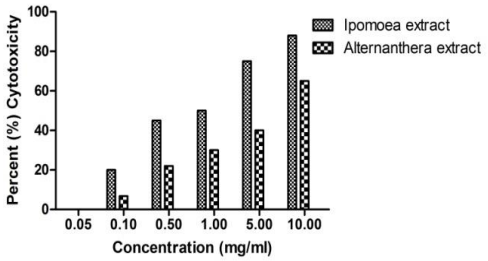

Figure 1: *In-vitro* cytotoxicity assay of Ipomoea and Alternanthera extracts of different concentration (0.1–10 mg/ml).

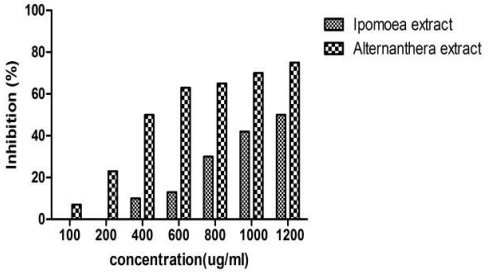

Figure 2: *In-vitro* antioxidant activity of Ipomoea and Alternanthera extracts of different concentrations (100-1200ug/ml).

A number of studies have reported antioxidant or cytotoxic activity of weeds commonly present in the world. For instance, Sanger, *et al.,* 2013, have studied antioxidant activity of 5 sea weeds in Indonesia and suggested that few of them which had good antioxidant properties can be used as potent natural antioxidants. Similarly, Rocha, *et al.,* 2007 have studied various sea weeds commonly found in Brazil and suggested that they can be exploited for further therapeutic use.

Previous studies on antioxidant activity of *Ipomea carnea* have been studied by Ambiga (2015) and Vaishali, (2012) however they have taken flowers of the plant and ethanol extracts of the plants respectively, for the study. Similarly, previous study on *Alternanthera sessilis* have been done by Murugan, *et al.,* 2013, however they have taken the vegetables of the plant for determination of its antioxidant property.

Conclusion

Our analysis demonstrates that methanolic extracts of *I.carnea* and *A.sessilis* possesses significant antioxidant and cytotoxicity properties. The plants antioxidant property may be attributed to the presence of high content of phytochemicals such as phenols and flavonoids. This, free radical scavenging activity of the weeds could provide substantial health benefits to humans by providing protection against free oxygen radicals and hence oxidative stress. The high cytotoxicity of these extracts, especially *Ipomoea carnea*, suggests the presence of certain cytotoxic compounds in these extracts. The presence of these compounds indicates that the above plants can be used as a natural resource for future bio-guided fractionation and isolation of these toxic compounds. Hence, weeds which are usually unwanted plants can now be exploited further to obtain maximum benefits from them, which was one of the objectives of this study. Since both the plants showed antioxidant property, the concerned phytochemicals responsible for the antioxidant properties like phenols, tannins and flavanoids, should be extracted, isolated and investigated further. Also, the toxic elements in the plants can be isolated and studied further towards the development of new anti-tumor or anti-cancer drugs. Since the antioxidant property of *Alternanthera sessilis* was higher, it is a better candidate as a source of natural antioxidants as compared to *Ipomoea carnea.* The weeds used in this study are easily available throughout India. Their growth requires little maintenance and the expense involved in acquiring these plants is negligible. These plants hence should be exploited for several therapeutic purposes.

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