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SCREENING OF ANTIOXIDANT AND ANTICANCER ACTIVITY IN *CROTON BONPLANDIANUM* LEAF AND STEM CRUDE EXTRACTS.

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ABSTRACT

The phytoconstituents were remarkably higher in the leaf than the stem of *Croton bonplandianum*. Antioxidant activity of *Croton bonplandianum* was elucidated by DPPH. The methanolic extracts of both the leaf and the stem has the elevated levels of scavenging property when compared to the other extracts. The cytotoxicity study was carried out for plant the leaf and stem extracts of *Croton bonplandianum*. These extracts were screened for its cytotoxicity against HepG2 cell lines at different concentrations to determine the % of Viability and % of toxicity by MTT assay. The results are graphically represented and the percentage of viability was found to decrease and the percentage of toxicity was found to be increasing with increasing concentration of the extracts.

KEYWORDS: Anticancer, Antioxidant, Plant extract, DPPH and MTT assay.

INTRODUCTION

The uses of plants in the treatment of certain human diseases is evidence of man's ingenuity. Currently the traditional medicine is widely practiced, especially in developing countries. The genus Croton belongs to the family Euphorbiaceae. It is a flowering plant. The common names for this genus are rush foil and croton. In South India, the name given to the plant is "Railpachilai" or "Rail poondu". Naturally, the Euphorbiaceae family is a native of southern Bolivia, Paraguay, Southwestern Brazil and Northern Argentina (Chakrabarty and Balakrishnan, 1992). Euphorbiaceae family consists of large family of flowering plants with

322 genera and around 8,000 species (Webster, 1994). Croton plant has been used traditionally for a variety of purposes such as medicine, food substances and as well as biofuels, fuel, detergents from ash, skin diseases, rashes, and bleeding wounds. In China, the medicinal uses include treating for constipation as the plant's seeds are a source for diarrhea and a chemical product called phorbol which induces tumor.

Due to antioxidant activity of naturally occurring substances in higher plants, attention has increased on the protective activity of these natural antioxidants against chronic disorders caused by oxidative process. *Croton bonplandianum* and *Uncariatomentosa* (UT) are plants from the Amazon River basin widely used for inflammatory disorders and with exhibited antioxidant and radical scavenging activity (Desmarchelier *et al*., 1997 and Goncalves *et al*., 2005).

The 2,2-diphenylpicrylhydrazyl (DPPH) assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. The method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant. Several protocols have been followed for this assay using different conditions such as different reaction times, solvents, pH and different compounds used as antioxidant standards (Krystyna Pyrzynska and Anna Pekal, 2013).

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was the first homogeneous cell viability assay developed for a 96-well format that was suitable for high throughput screening (HTS) (Mosmann, 1983). The MTT assay technology has been widely approved and remains popular in academic labs as supported by thousands of published articles. The MTT substrate is prepared in a physiologically balanced solution, added to cells in culture. Viable cells with active metabolism convert MTT into a purple coloured formazan product with an absorbance maximum near 570 nm. When cells die, they lose the ability to convert MTT into formazan, thus color formation serves as a useful and convenient marker of only the viable cells (Marshall *et al.,* 1995). Speculation in the early literature involving specific mitochondrial enzymes has led to the assumption mentioned in numerous publications that MTT is measuring mitochondrial activity (Berridge and Tan 1993 and Berridge *et al.,* 1996).

MATERIALS AND METHODS

Collection of plant materials

Healthy, disease free plant of *Croton bonplandianum* were collected from in and around Redhills, Thiruvellore District, Tamilnadu. Then the plant was identified and authenticated by Plant Anatomy Research Centre **(PARC/2012/1307)**. The fresh plant leaves and stem were used as material and then dried under shade. Dried plant material was powdered using mechanical grinder the powdered material was preserved in an air tight container.

Preparation of plant extracts

The coarse powder (500 gm) was subjected to maceration for 72 hours, followed by maceration for 48 hours by using solvents Hexane, Ethyl acetate and Methanol. The extracts were dried under desiccator. These extracts were subjected to antioxidant activity.

Materials required

DPPH,

Leaf extracts (1mg/ml),

Stem extracts (1mg/ml),

BHT,

Hexane,

Ethyl acetate and Methanol.

Methods

Antioxidant activity

Technique

The ability of the extracts to annihilate the DPPH radical (1, 1-diphenil-2-picrylhydrazyl) was investigated by the method described by (Blois 1958). Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. 100µg of each extracts were added, at an equal volume, to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula.

% of Inhibition = (A of control – A of Test)/A of control * 100

MTT ASSAY – CRUDE EXTRACT

The MTT assay (Mossman, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. C e lls were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37^{o}C in humidified atmosphere with 5%CO2. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately1.2 X 104 cells/well and allowed to attach overnight at 37^{o}C. The medium was then discarded and cells were incubated with different concentrations of the extract for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) X 100

Cytotoxicity % = 100 – Viability%

RESULT

Antioxidant activity: DPPH free radical scavenging activity

The antioxidant activity of the leaf and stem extracts of *Croton bonplandianum* was assessed DPPH assay, which is often used to compare the activity of plant extracts. The free radical scavenging of the leaf and stem extracts of the *Croton bonplandianum* has been performed. When an antioxidant scavenges free radicals by hydrogen donation, the DPPH assay solution becomes lighter in color (Molyneux, 2004 and Villao *et al*., 2007). The result shows the scavenging potential to increase with respect to the concentration of the extracts.

The antioxidant potential of the stem is comparatively high when compared to the leaf extracts. The methanol extract of the stem possess the maximum antioxidant potential of 83.30%. The hexane and ethyl acetate extracts have 25.47% and 35.41% of antioxidant potential respectively. The methanol extract of the leaf contains 74.23% of antioxidant activity when compared to 27.23% and 41.74% of antioxidant activity in the hexane and ethyl acetate extracts (Figure 1 and Figure 2). The percentage of scavenging is shown in comparison with BHT as the standard control.

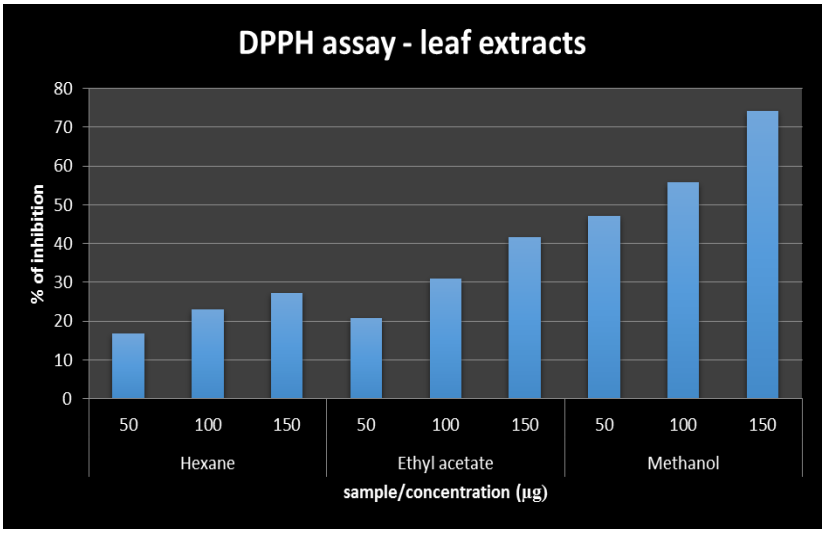

Figure 1: Scavenging potential of leaf extracts

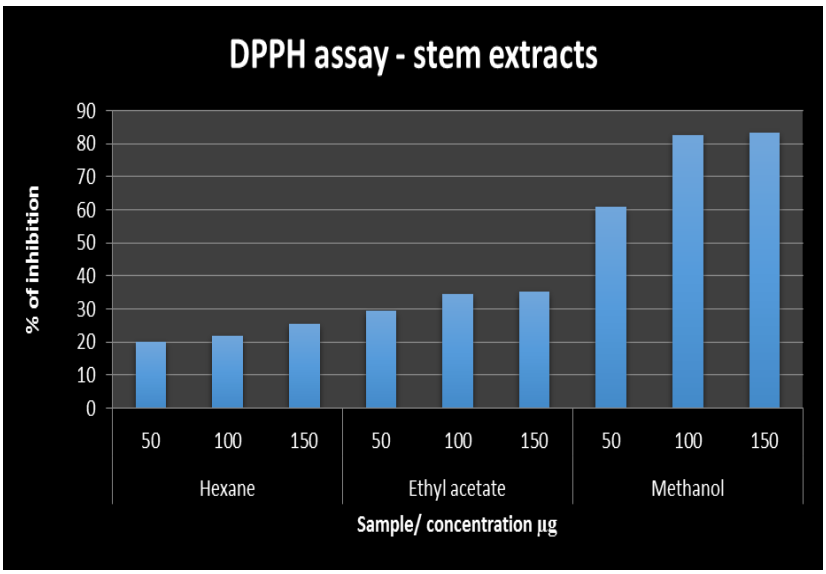

Figure 2: Scavenging potential of stem extracts

MTT assay for cell viability

The cytotoxicity study was carried out for plant the leaf and stem extracts of *Croton bonplandianum*. These extracts were screened for its cytotoxicity against HepG2 cell lines at different concentrations to determine the % of Viability and % of toxicity by MTT assay. Results are graphically represented in Figure 3 and Figure 4. The percentage of viability was found to decrease and the percentage of toxicity was found to be increasing with increasing concentration of the extracts. The methanol leaf extract of *Croton bonplandianum* shows the least % of viability of 41.51% and highest % of toxicity of 58.48%. The hexane and the ethyl acetate leaf extract show the % of viability to be 73.95% and 59.38% respectively. Similarly the hexane and the ethyl acetate leaf extract show the % of toxicity to be 26.04% and 40.61%

respectively. The MTT results for the stem extracts indicate that the methanol extract of *Croton bonplandianum* shows the least % of viability of 44.46% and highest % of toxicity of 55.53%. The hexane and the ethyl acetate stem extract show the % of viability to be 80.65% and 57.88% respectively. Similarly the hexane and the ethyl acetate stem extract show the % of toxicity to be 19.34% and 42.11% respectively. The comparative results of the leaf and the stem shows that the leaf extracts possess more cytotoxicity effect than the stem extracts.

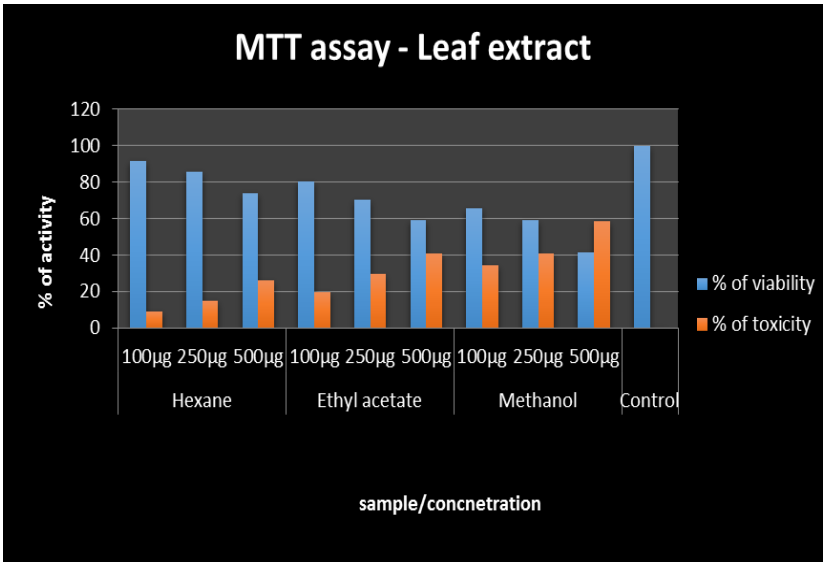

Figure 3: Anticancer activity of leaf extracts on HepG2 cell lines

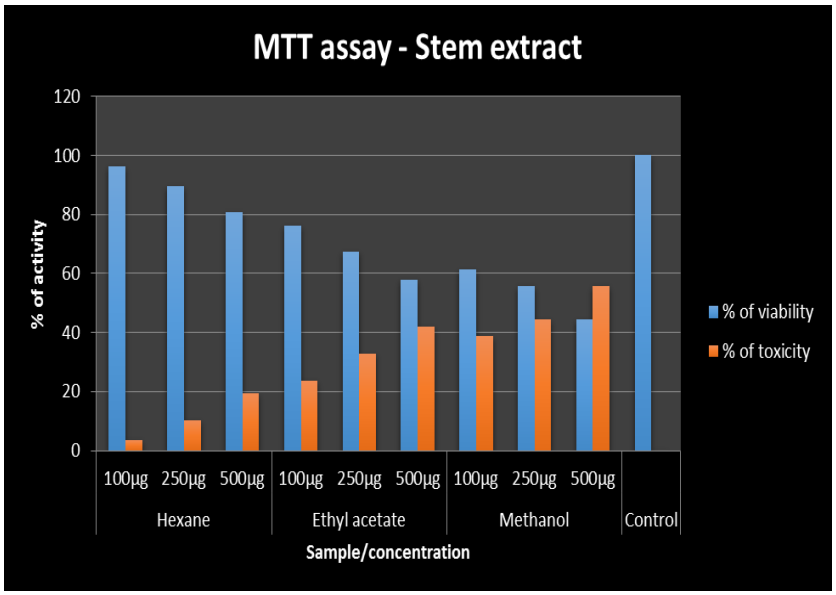

Figure 4: Anticancer activity of Stem extracts on HepG2 cell lines

CONCLUSION

The phytoconstituents were remarkably higher in the leaf than the stem of *Croton bonplandianum*. Antioxidant activity of *Croton bonplandianum* was elucidated by DPPH.

The methanolic extracts of both the leaf and the stem has the elevated levels of scavenging property when compared to the other extracts. Cytotoxicity analysis of the crude extracts of the leaf and stem of *Croton bonplandianum* was carried out HepG2 cancer cell lines. Results showed that the methanolic extract of the leaf had the highest activity against HepG2 cell line.

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