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RESEARCH ARTICLE

Comparative study of effect of physical and chemical mutagens on phytochemical content and antioxidant activity of *Aegle marmelos* Corr.

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Abstract

Aegle marmelos Corr.(Bael) contains various important phytochemicals in all the parts of the plant which are responsible for its medicinal properties. Bael plantlets grown *in vitro* were treated by mutagenic agents namely colchicine, Diethyl sulphate (DES) and Gamma radiations and the effect of these treatments on total phenolic content, total flavonoid content and antioxidant activity was compared. The levels of phenolics in Colchicine and Gamma treated plants was found to be nearly two times the levels of in the control specimens, while the treatment of DES resulted in a significant reduction in the levels. The colchiploids were found to contain nearly double the concentration of flavonoids than the untreated controls followed by the Gamma treated samples. The exposure to DES lowered the concentration of total flavonoids than the untreated control plantlets. Free radical scavenging activity by DPPH showed an increase in colchiploids, the DES treated ones were similar to control while the Gamma irradiated specimens had poor activity than the control plantlets

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Introduction –

Aegle marmelos Corr. Family Rutaceae (Bael) is one of the most important medicinal plant as well as a fruit plant throughout the tropical countries. All parts of *Aegle marmelos*, such as the roots, bark, leaves, flowers, fruits and seeds are edible and possess medicinal properties. Leaves, stem, roots and fruits at all stages of maturity are used as ethano – medicines against various human ailments. The leaves of *Aegle marmelos* contain several bioactive compounds including essential oils, phenolics, alkaloids, condensed tannins, anthocyanins and flavonoid glycosides. The leaves are astringent, laxative and an expectorant and are useful in treatment of treatment of inflammations, diarrhoea, dysentery, heart palpitation, and asthmatic complications. It is proved to have antibacterial activity(Saradha Jyothi K and B Subba Rao, 2010), hypoglycemic activity (Sharmila Upadhya et al, 2004), antialcer, anti-hyperlipidemic, antioxidant, anticancer, radioprotective, anti-inflammatory antipyretic, analgesic and antispermatogenic effects against various animal models (Pallab Maity et al , 2009).

Aegle marmelos is propagated through seeds, but has low viability and it leads to variability. Varietal purity and induction of mutations to improve the medicinal properties of this important ethano – medicinal plant could be achieved through tissue culture of Bael. The plantlets grown *in vitro* were subjected to physical and chemical mutagens to induce mutations and to improve the medicinal properties of the said plant. The plantlets obtained in M_3 generation were screened for genetic variations induced by mutagenic treatments by Colchicine, Diethyl sulphate and Gamma radiations, to make an attempt to produce an improved variety having higher levels of bioactive compounds with increased medicinal potential The *in vitro* grown shoots of *Aegle marmelos* Corr. (Bael) due to the treatment with colchicines, Diethyl sulphate and Gamma radiations were screened to produce an improved variety having higher levels of bioactive compounds with increased medicinal potential. The levels of total phenolic

compounds and total flavonoids along with the DPPH free radical scavenging activity were compared in the control and treated specimens.

Polyphenols are a group of highly hydroxylated phenolic compounds present in the extractive fraction of several plant materials. Polyphenols in plant include hydroxycoumarins, hydroxycinnamate derivatives, flavanoids, flavonones, flavones, anthocyanins, tannins, hydroxystilbenes, aurones etc. Polyphenols are responsible for their antioxidant action as well as their marked effects in the prevention of various oxidative stress associated diseases, defence against ultraviolet radiation and aggression by pathogens, parasites as well as contribute to plant colours. Flavonoids constitute one of the most ubiquitous groups of plant phenolics that have two benzene rings separated by a propane unit. The flavones and flavonols are the most widely distributed of all the phenolics. A variety of dietary plant Flavonoids inhibit tumour development in experimental animal models. The biflavonoids have the pharmacological effects like their ability to inhibit the release of histamines, the adhesion of blood platelets and the action of lens aldose reductase, to block the inflammatory effects of hepatotoxins, and to act as a heart stimulant (Kumar Devendra, 2013).

Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors. Antioxidants may guard cells against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by the disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack (Nadeem Ahmad Siddique et al, 2010). Free radicals and reactive oxygen metabolites can react with proteins, nucleic acids and lipids, causing changes in genetic material and inactivation of enzymes. The scavenging of stable DPPH radical model is a widely used method to evaluate antioxidant activity in a short time (Gangadhara Anjala et al, 2013).

The M_3 plantlets of treated and control specimens of Bael were compared for the concentration of bioactive compounds and the antioxidant activity.

Materials and Methods –

Nodal explants of *Aegle marmelos* Corr. obtained from mature tree were grown *in vitro* using MS medium supplemented with 2 mg/lit of BAP and 0.5mg/lit NAA to obtain multiple shoots.The 60 days old shoots were used for the treatment with 0.8 % aqueous colchicine for 6 hours, 0.02% of Diethyl sulphate and 40G of Gamma radiations. The 90 days old M_3 plantlets obtained after each treatment were used to analyze the phytochemical profile.

a. **Preparation of plant extract** –

2 gm of dried and powdered plant material of control (untreated) and mutagen treated sample was extracted with 20 ml of methanol by hot extraction method. The residue was dissolved in 2 ml of methanol. This extract was used for further analysis of phytochemicals.

b. **Determination of Total phenolic content in control and treated samples:**

Total phenolic content of plant extracts are determined by the Folin-Ciocalteu method (Sadasivam and Manickam. 2008) with certain modifications. The method involves the use of Folin- Ciocalteau reagent which reacts with the reducing compounds i.e. polyphenols in the extracts under alkaline conditions there by producing a blue coloured complex. This is quantitatively estimated at 720nm against 100 to 1000 µg/ml of standard gallic acid solution.

The diluted extracts (100 µl) were pipetted into different test tubes to which 0.2 mL of Folin- Ciocalteau reagent and 1 mL of 20 % (w/v) Na_2CO_3 solution were added. The tubes placed in a boiling water bath for exactly 1 min and then were cooled under running tap water. The absorbance of the resulting blue solution was measured at 720 nm with a spectrophotometer. The experiment was carried out in triplicates. The amount of phenolics present in the sample was determined from a standard curve of Gallic acid and was expressed in mg per gram of the extract.

c. **Determination of total flavonoid content in control and treated samples –**

Aluminium chloride colorimetric technique was used for flavonoids estimation (Rao et al.,2011). Flavonoid content was determined in triplicates using 5 to 25µg/ml of standard Quercetin solution. 3 ml of 5% sodium nitrate and 0.3ml of aluminium chloride followed by 2ml of 1M NaOH were added to each tube of standard as well as plant extracts. The absorbance each tube was measured at 510 nm using UV –Visible spectrometer.

d. **Determination of antioxidant activity by DPPH Radical Scavenging Assay**:

The DPPH (2, 2-diphenyl-1- picrylhydrazyl) assay is based on the capability of stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with H-donors. DPPH, when acted upon by an antioxidant, is converted into diphenylpicryl

hydrazine. The degree of stable DPPH decolourization to DPPHH (reduced form of DPPH) which is yellow, indicated the scavenging efficiency. This method was reported by McCune and Johns (2002).

DPPH free radical scavenging assay was performed to determine the antioxidant activity of different extracts. DPPH (0.002%) was used as free radical donor, with standard ascorbic acid solution as the control. Equal volume of extracts (1 mg/ml) and DPPH were mixed and the tubes were incubated at room temperature in dark for 10 minutes. The optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH decolourization to DPPHH (reduced form of DPPH) which is yellow in color indicated the scavenging efficiency of the extract. The experiment was carried out in triplicates. The scavenging activity of the extract against the stable DPPH was calculated using the following equation -

Scavenging activity (%) = (A – B) / A x 100

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination (McCune and Johns. 2002).

Results and Discussion –

The result of this experiment shows that the mutagen treatments have altered the concentration of bioactive components in *Aegle marmelos* Corr. The treatment of colchicine and gamma radiations has resulted in a significant increase in the levels of total phenolics and flavonoids, while the exposure to DES resulted in reduction in the concentration of these bioactive compounds. The concentration of total phenolics in control specimens was 1.62 ± 0.11 mg/gm of while the colchiploids were found to contain 2.36 ± 0.05 mg/gm which is significantly high, followed by Gamma irradiated plantlets, having 2.28 ± 0.02 mg/gm of total phenolic content. The DES treated plantlets on the other hand showed 1.18 ± 0.05 mg/gm of total phenolics which is lower than the control plantlets.

The concentration of total flavonoids in the control specimens was calculated to be 53.2 ± 0.11 µg/gm, while the colchicines treated plantlets had 96 µg/gm ± 0.4, which is nearly two times of the control specimens. DES treatment showed 48.6 ± 0.23 µg/gm of total flavonoids, showing a marginal reduction in the flavonoid concentration. The Gamma treatment showed 73.2 ± 0.23 µg/ gm of total flavonoid content which is higher than the control but lower than the colchiploids.

The effect physical and chemical mutagens on the free radical scavenging activity of *Aegle marmelos* was measured in terms of hydrogen donating or free radical scavenging ability by using the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH). The untreated control and DES treated plantlets showed similar DPPH free scavenging activity, with 39.24 ± 1.09% in case of control and 39.21 ± 1.09% in the DES treated samples. The colchicines treated plantlets were found to be more efficient having 54.63% ± 0.86 % inhibition, which is highest among all the four observations. The Gamma irradiated plantlets showed a marginal reduction in the ability to scavenge the free radicals than the control specimens, having percentage inhibition equal to 36.66 % ± 1.27%.

Table – 1. The concentration of bioactive compounds and antioxidant activity in control and mutagen treated samples.

Sample	Total Phenolics mg/gm	Total Flavonoids µg /gm	% Antioxidant Activity
Control (Untreated)	1.62 ± 0.11	53.2 ± 0.11	39.24 ± 1.09
Colchicine treated	2.36 ± 0.05	96.6 ± 0.4	54.63 ± 0.86
DES treated	1.18 ± 0.05	48.6 ± 0.23	39.21 ± 1.09
Gamma treated	2.28 ± 0.02	73.2 ±0.23	36.66 ± 1.27

Note - All observations are in triplicates ± SE of Mean

Figure – 1. Comparative study of phenolic content of *Aegle marmelos* (L) Corr. in treated and control specimens

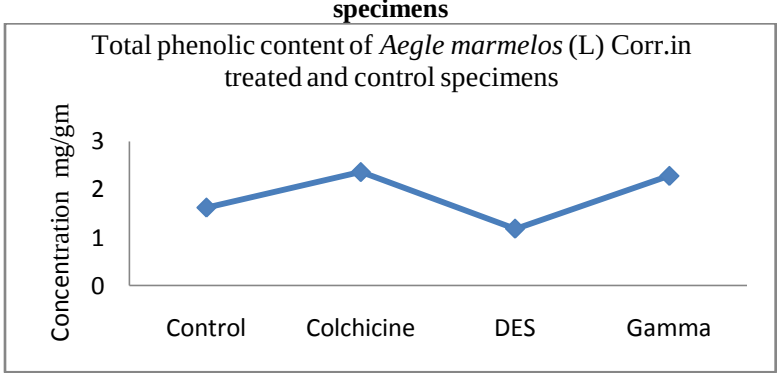


Figure – 2. Estimation of flavanoids from *Aegle marmelos* Corr. - Control and treated samples

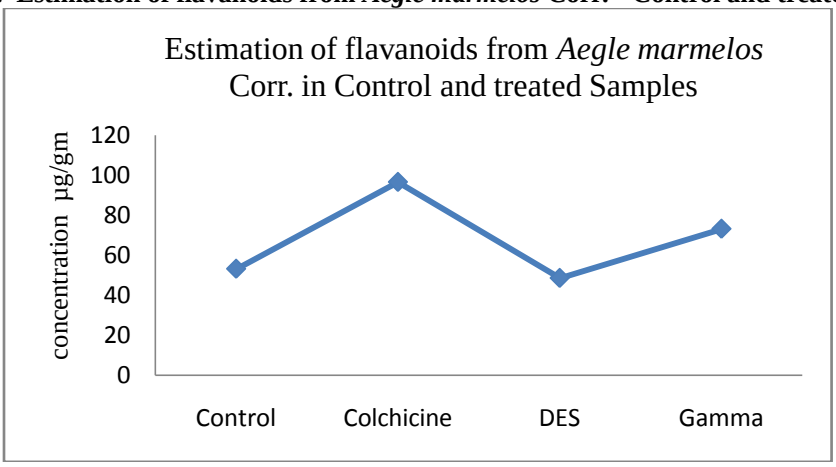
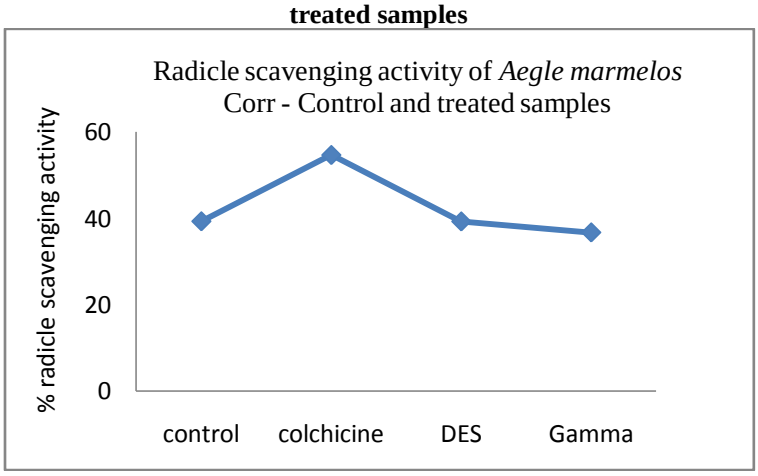


Figure – 3. Comparative study of % Radicle scavenging activity of *Aegle marmelos* Corr in Control and treated samples



Conclusion –

The result of the present study revealed that the exposure of *Aegle marmelos* Corr. to the physical and chemical mutagens has altered its concentration of bioactive compounds and the antioxidant activity. The treatment with colchicines, that results in doubling of the number of chromosomes shows increased levels of phenolics and flavonoids. The antioxidant activity of the colchicines treated plantlets was also found to be higher than the untreated control plantlets. This treatment was found to improve the phytochemical profile of Bael.

The Gamma irradiation treatment also shows increase in the levels of bioactive compounds, but it was not as efficient as the colchicines. This treatment resulted in reduction in the antioxidant activity of Bael in comparison to the control plantlets.

Diethyl Sulfate, an alkylating agent was found to have an adverse effect in the concentration of bioactive principles of Bael, reducing the levels of phenolics as well as flavonoids. The free radical scavenging activity however, was found to be same as the control plantlets. It can be concluded that the exposure of *Aegle marmelos* Corr. to colchicines made it more medicinally active. The Gamma irradiation also increased the phytochemical concentration compared to the control specimens. The DES treatment was not found to be suitable to improve the concentration of active principles of Bael, as well as its antioxidant activity. The *in vitro* grown rooted plantlets with colchicine treatment were proved to be better than the control, increasing its efficiency as an ethano – medicinal plant.

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Annexure –

1. Table – 1 The concentration of bioactive conpounds and antioxidant activity in control and mutagen treated samples.
2. Figure – 1. Comparative study of phenolic content of *Aegle marmelos* (L) Corr.in treated and control specimens
3. Figure – 2. Estimation of flavanoids from *Aegle marmelos* Corr. - Control and treated samples

4. Figure – 3. Comparative study of % Radicle scavenging activity of *Aegle marmelos* Corr in Control and treated samples