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ASSESMENT OF BIOCHEMICAL ATTRIBUTES OF *PRAECITRULLUS FISTULOSUS* TREATED WITH MUTAGENS

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A B S T R A C T

Plants are well known to have certain primary and secondary metabolites collectively are known as biochemicals that plays an important role for human health as their medicinal properties. The aim of present study was to enhance and evaluate biochemical profile of *Praecitrullus fistulosus* by induced mutagenesis to cause genetic variations, plant leaves were treated with different chemical and physical mutagens. Colchicine and Ethidium bromide were used as chemical mutagens. While Ultraviolet (UV) rays and X- rays were used as physical mutagens for the treatment of seeds. After the eleventh week of their growth, methanol extracts of dried leaves were prepared and further analyzed for the estimation of biochemicals. It was observed that total carbohydrates, total Proteins, phenolic compounds, antioxidant activity, reducing power, ascorbic Acid and Chlorophyll a, were found significantly ($p<0.05$) higher in Colchicine 0.02% treated plants, while reducing sugars were significantly ($p<0.05$) increases in Colchicine 0.01% treated plants as compared to control plants. Total flavonoids, total flavonol, Chlorophyll b and Carotenoids were increases significantly ($p<0.05$) in plants treated with 0.05% Ethidium bromide while tannin content was increased significantly ($p<0.05$) in 0.10% Ethidium bromide treated plants as compared to the control plants.

Keywords: Biochemical attributes, *Praecitrullus Fistulosus,* mutagens.

INTRODUCTION

Mutagenesis or mutation breeding is an interesting approach as it has been applied in a number of crops for yield improvement, creation of new varieties, tolerance to salinity and drought, disease resistance and for horticulture or floriculture purposes (Ali *et al*., 2007; Mensah and Obadoni, 2007; Skoric *et al*., 2008; Suzuki *et al*., 2008). It is widely used to improve and enhance the characteristics of plants (Micke, 1988). Mutagenesis is employed to create desirable mutation in plants for their improvement in order to modify their genetic basis that leads to better seed germination time, flowering time, fruiting time and many other potential traits for their survival (Tah and Roychowdhury, 2011). Plants provide novel products as well as chemicals for new drugs in future (Cox and Balick, 1994) and have played important role in maintaining human health and improving human life. One hundred twenty one useful drugs are derived from plants origin (Payne *et al*., 1991).

Tinda or round gourd *(Praecitrullus fistulosus)* is herbaceous plant having pointed hairy stem with curly long tendrils (Tindall, 1983), belongs to the family *Cucurbitaceae* and subfamily *Cucurbitoideae* (Tyagi *et al*., 2012). The seeds of *Praecitrullus fistulosus* are used as fodder and in medicinal purposes for curing of many diseases (Chadha and Tarsem, 1993). Its leaves are cooked as vegetables and are taken for maintaining blood pressure (Sultana, 2006). The mature fruit is used as a cooked vegetable. The fruits are also used to make pickles (Grubben and Denton, 2004). Among the group of medicinal plants tinda is an excellent plant having composition of all the essential nutrients good for the health (Kirtikar and Basu, 1998). *Praecitrullus fistulosus* contains carbohydrate, proteins, fiber and fats along with copper, nickel, zinc, lead, cobalt, cadmium, iron, chromium, calcium and sodium contents. (Hussain *et al*., 2010; Holland *et al*., 1991). *Praecitrullus fistulosus*

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possess tannins, cardiac glycosides, terpenoids, saponins and resins as phytochemicals (Ankita *et al*., 2012). Cardiac glycoside has been reported to have anti-inflammatory activity (Shah *et al*., 2011). Saponins have hemolytic property (Rao and Sung, 1995). Saponins possess anti-tumorous activities that lower the risk of human cancers by preventing the growth of cancer cells (Nafiu *et al*., 2011). Tannins have ability that enhances the healing of wounds (Njoku and Obi, 2009). The selection of an effective and efficient mutagenic agent is very important part in many mutation breeding programmes, to produce high frequency of useful and desired mutations. Many mutagens have been utilized to obtain useful variations in plant species (Singh and Singh, 2001). Colchicine is an alkaloid that inhibits microtubule polymerization and halts the mitotic cell division at metaphase stage by inhibiting spindle formation preventing the duplicated chromosomes to separate into their respective daughter cells; a series of polyploid cells appears (Eeckhaut *et al*., 2001; Eiselein, 1994). Ethidium bromide is an intercalating molecule having binding properties between pyrimidine bases are of particular importance in mutation breeding programmes (Hajduk, 1978). Ultraviolet radiations can induces gene mutations by causing photo chemical changes in DNA (Russell, 1994). X-rays treatment causes suppression in nuclear division (Marquardt, 1938). Another effect of X-rays involves production of typical chromosomal aberrations (Sax, 1941). The aim of present study was to enhance biochemical attributes of *Praecitrullus fistulosus* seeds treated with different physical and chemical mutagens.

MATERIALS AND METHODS

The experiments were carried out during April 2014 to June 2014. The experimental field of Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Sindh, Pakistan in Randomized Complete Block Design (RCBD) manner. For mutagenesis, the seeds of *Praecitrullus fistulosus* were pre-soaked with different concentrations of chemical mutagens, including Ethidium bromide (0.05% and 0.10%), (ACROS) and Colchicine (0.01% and 0.02%) of (ACROS) for one hour. The treated seeds were washed in running water to remove excess chemical mutagens. Then the seeds of were also treated with different physical mutagens for different time periods; including UV radiations (1 hour and 2 hours) and X-rays (5 seconds and 10 seconds). After this the seeds were sowed in the soil.

Preparation of plant leaves extracts: The leaves from each plant of *Praecitrullus fistulosus* were collected after 11th week of their growth and were dried for about 15 days under shade, and were ground to fine powder by electric grinder. 1.0 g of leaves powder was added to 10 ml of 70% methanol (AnalaR) at room temperature. After 24 hours the mixture was centrifuged in refrigerated centrifuge machine (Kubota 8700, Japan) at 7000 rpm for 15 minutes and the supernatant was separated out by filtration, through Whatman No.1 filter paper. Finally, the filtrate of leaves extract was stored in air tight bottles at - 40°C and was used for the different phytochemical analysis.

Determination of total carbohydrates: Total carbohydrate contents of prepared samples were determined according to the Phenol-Sulphuric acid method reported by Montgomery (1961).

Determination of total protein: Total protein contents of prepared samples were determined according to the method reported by Lowry *et al*., (1951).

Determination of reducing sugars: The reducing sugar contents of prepared samples were determined by the method reported by Miller (1959).

Determination of total phenolic compounds: Total phenolic compounds of methanol extracts of *Praecitrullus fistulosus* leaves, were determined by using Follin-Ciocalteau method reported by (Yasoubi *et al*., 2007).

Determination of total flavonoid contents: The total flavonoid contents of methanol extracts determined by using Aluminium chloride colorimetric method (Kim *et al*., 2003).

Determination of tannin contents: The tannin contents in the methanol extracts of leaves of *Praecitrullus fistulosus* were determined by using the Folin and Ciocalteu method (Tamilselvi *et al*., 2012). **Determination of antioxidant activity:** The antioxidant activity of methanol extracts of *Praecitrullus fistulosus* leaves, were determined by using the Phosphomolybdenum method described by (Prieto *et al*., 1999).

Determination of reducing power: The reductive capability of the methanol extracts of leaves of *Praecitrullus fistulosus* was quantified by the method of (Oyaizu, 1986).

Determination of total flavonol contents: The method of Kumaran and Karunakaran (2007) and Mbaebie *et al*., (2012), was used with slight modification to measure the total flavonol contents.

Determination of ascorbic acid: Ascorbic acid from the methanol extracts of *Praecitrullus fistulosus* leaves was determined spectrophotometrically by Bajaj and Kaur method (1981).

Determination of total chlorophyll and carotinoids: For the extraction of chlorophyll and carotenoids 0.1 g of fresh newly arising leaf samples of *Praecitrullus fistulosus* from each control and plants treated with chemical and physical mutagens were added separately in 80% acetone (AnalaR) 10 ml per each 0.1 gram, in test tubes and then crushed by using glass rod. The test tubes were sealed with parafilm tape to prevent the evaporation and were refrigerated at 4°C for 24 hours.

The chlorophyll contents were determined after 5 weeks of germination. The acetone extract was separated out into another test tube and the maximum absorbance of chlorophyll a, chlorophyll b and total carotene were taken at 662, 645 and 470 nm respectively by using UV visible spectrophotometer against a blank solution of 80% acetone (v/v) (Kumar *et al*., 2010). The experiment was repeated twice for statistical analysis. The amount of pigments *Chl a, Chl b* and carotenoids present in leaf samples was calculated according to the formula reported by (Lichtentaler and Wellburn, 1985).

$$C_a = 11.75\ A_{662} - 2.350\ A_{645}.$$

$$C_b = 18.61\ A_{645} - 3.960\ A_{662}.$$

$$C_c = 1000\ A_{470} - 2.270\ C_a - 81.4\ C_b/230.$$

Where C_a is chlorophyll a, C_b is chlorophyll b and C_c is total carotenoids.

STATISTICAL ANALYSIS

Analysis was done on all data collected and the mean and standard deviation was calculated by using Microsoft excel 2010 and used to assess differences between treatments, while the *p*-value was calculated by using IBM SPSS Statistics 22.

RESULTS AND DISCUSSIONS

Presence of phytochemicals and bioactive compounds in fruits or vegetables witness their medicinal values. Recent studies have shown that phenolic compounds derived from plants are more effective antioxidants due to the presence of vitamin C and vitamin E (Rice-Evans *et al*., 1997). As the plants of Praecitrullus fistulosus were grown in direct sun exposure so the amount of total carbohydrates and proteins in the leaves of different plants is significantly higher in sun exposure or high light intensity (Lichtenthaler *et al*., 1981). The results of different biochemicals from the leaves of *Praecitrullus fistulosus* treated with chemical and physical mutagens were compared with control plants mention in Figure 1 to Figure 11. It was observed that total carbohydrates (198 mg/g dry weight or D.W of leaves), total Proteins (245 mg/g D.W), phenolic compounds (197 mg/g D.W), antioxidant activity (6.3 g/g D.W), reducing power (5.63 mg/g D.W), ascorbic Acid (0.23 mg/g D.W) and Chlorophyll a (8.719 μg/g F.W), were significantly ($p<0.05$) higher in Colchicine 0.02% treated, while reducing sugars (54.1 mg/g D.W) were significantly ($p<0.05$) increases in Colchicine 0.01% treated plants as compared to control plants. Total flavonoids (66 mg/g D.W), total flavonol (2.55 mg/g D.W), Chlorophyll b (3.1005 μg/g F.W) and Carotenoids (1.432 μg/g F.W) were increases significantly ($p<0.05$) in plants treated with 0.05% Ethidium bromide while tannin contents (12.3mg/g D.W) was increased significantly ($p<0.05$) in 0.10% Ethidium bromide treated plants as compared to the control plants. Among the chemical mutagens treated plants Colchicine (0.02% & 0.01%) and ethidium bromide (0.05% & 0.01%) produced significant role in the enhancement of biochemicals in plants leaves as compared to control plants. In present study the amount of almost all biochemicals were lowest in case of X-rays 10 seconds because X-rays are always more or less destructive depending upon the environment and tend to retard the growth of many plant species (Shull and Mitchell, 1933). Ascorbic acid not only acts to regulate defense mechanism and survival but also act on plant hormones to regulate plant growth (Pastori *et al*., 2003). In X-rays 10 seconds treated plants lowest amount of ascorbic acid was observed which may be the reason to retard its growth. The amount of total carbohydrates, reducing sugar, total proteins, phenolic compounds, antioxidant activity and reducing power was found to be highest in case of plants treated with colchicine, similar results were obtained in *Aegle marmelos* Corr. (Bael) a medicinal plant when treated with colchicine, Diethyl sulphate (DES) and Gamma radiations, among which the level of phenolic compounds, flavonoids and antioxidant activity was highest in colchicine treatment as compared with control, DES and Gamma radiation treatments (Walvekar and Kaimal, 2014). The characteristics of plants can be enhanced or improved by the technique of induced mutagenesis.

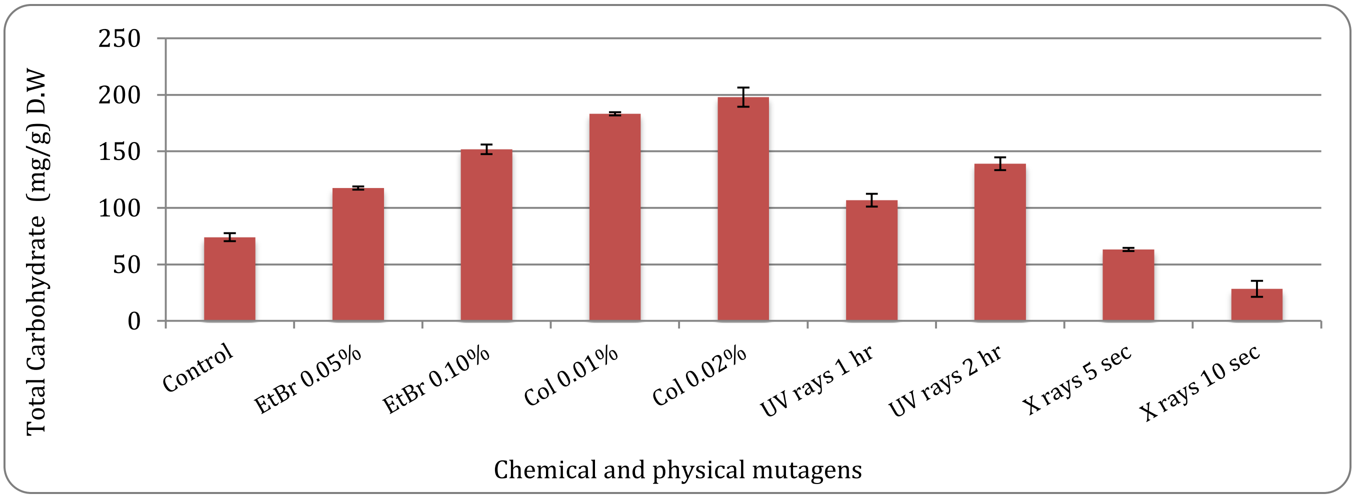

Figure 1. Effect of chemical and physical mutagens on the amount of total carbohydrates of *Praecitrullus fistulosus* leaves (Mean±S.D, p<0.05).

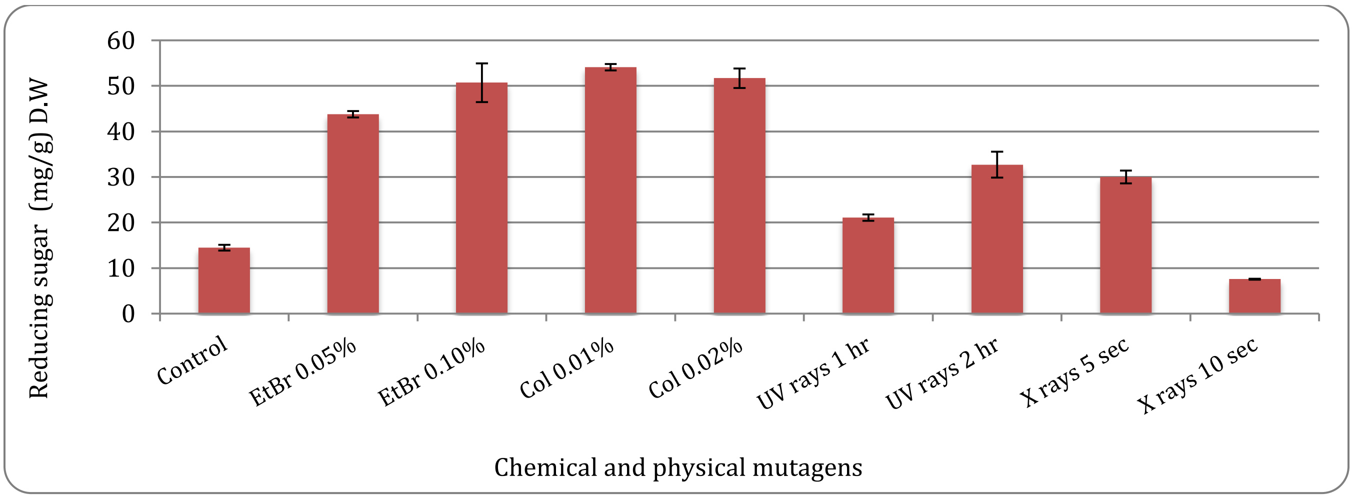

Figure 2. Effect of chemical and physical mutagens on the amount of reducing sugar of *Praecitrullus fistulosus* leaves (Mean±S.D, p<0.05).

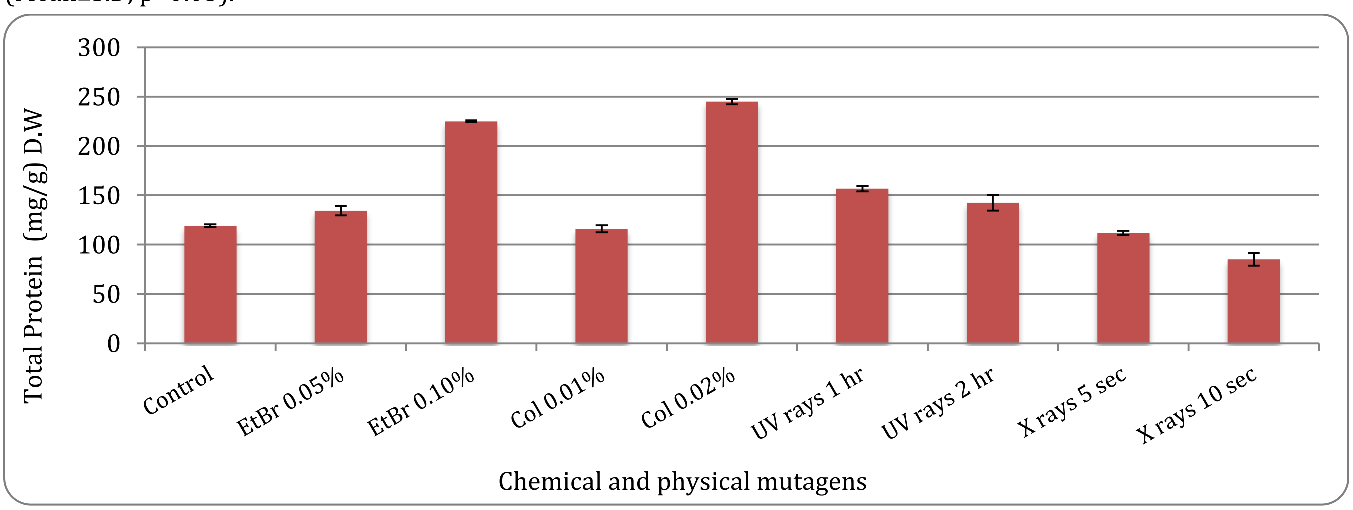

Figure 3. Effect of chemical and physical mutagens on the amount of total protein of *Praecitrullus fistulosus* leaves (Mean±S.D, p<0.05).

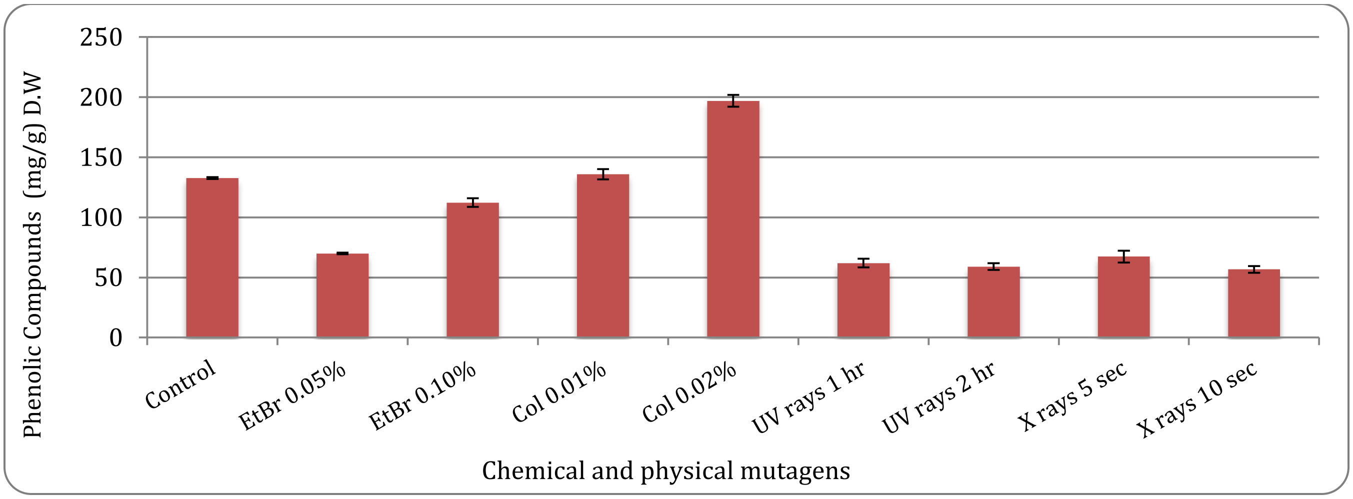

Figure 4. Effect of chemical and physical on the amount of phenolic compounds of *Praecitrullus fistulosus* leaves (Mean±S.D, $p<0.05$).

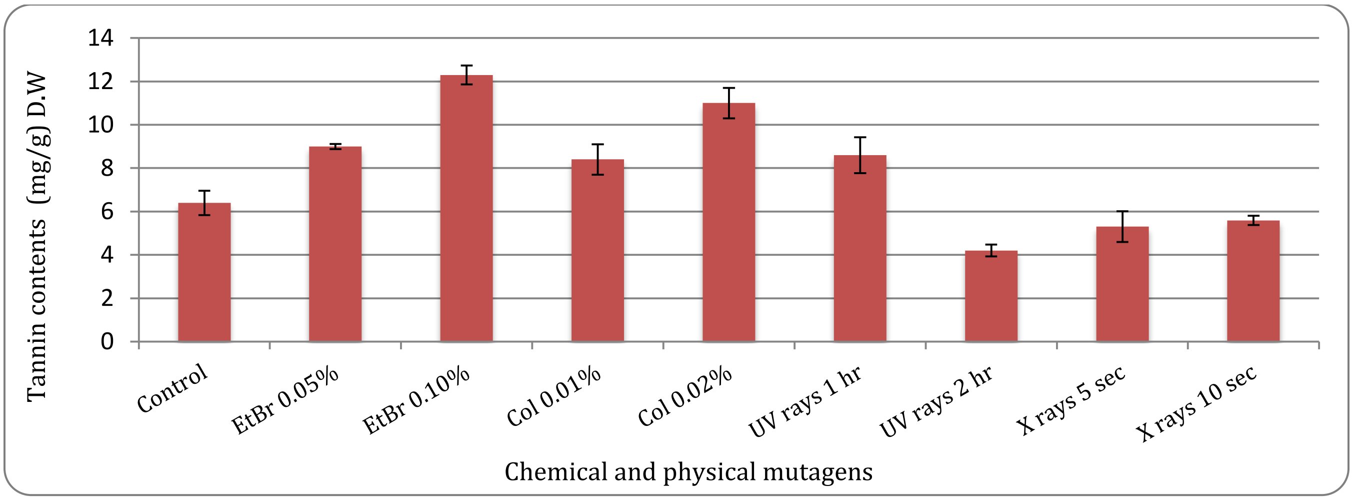

Figure 5. Effect of chemical and physical mutagens on the amount of tannin contents of *Praecitrullus fistulosus* leaves (Mean±S.D, $p<0.05$).

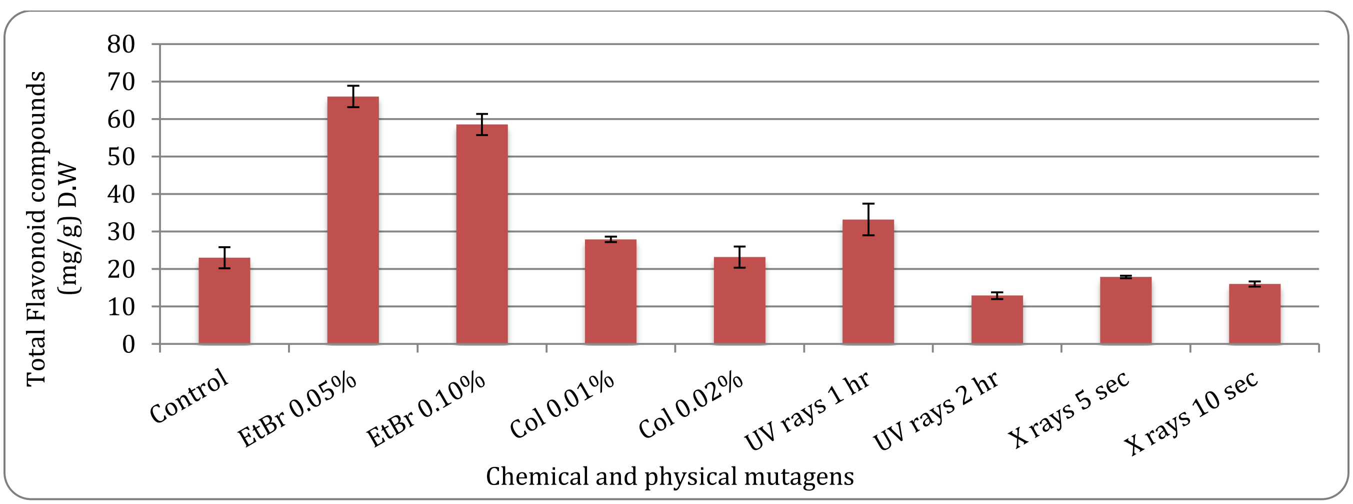

Figure 6. Effect of chemical and physical mutagens on the amount of total flavonoid compounds of *Praecitrullus fistulosus* Leaves (Mean±S.D, $p<0.05$).

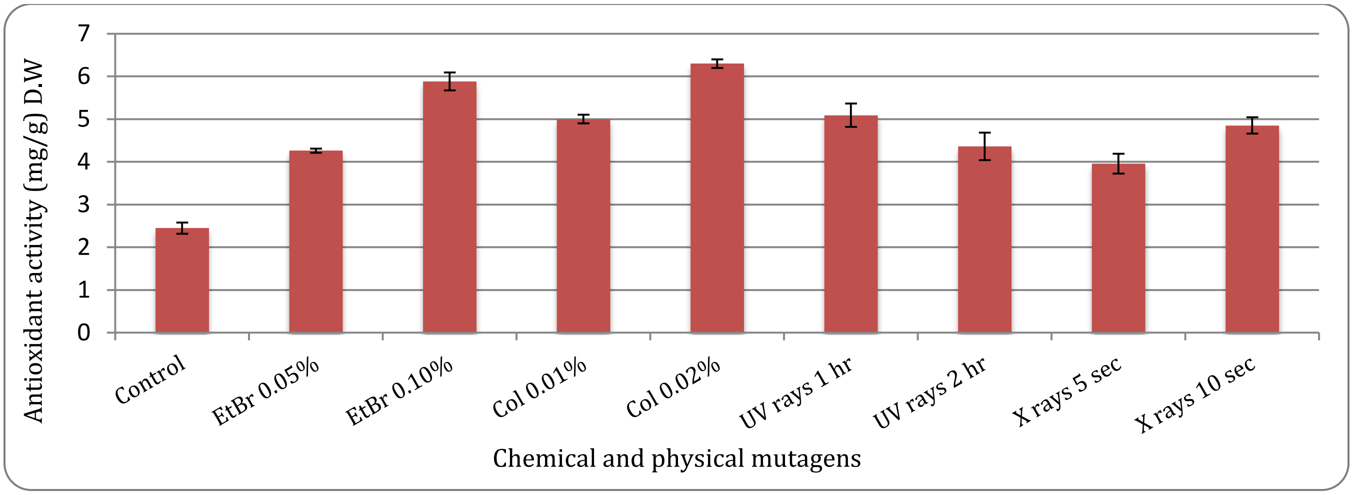

Figure 7. Effect of chemical and physical mutagens on the amount of antioxidant activity of *Praecitrullus fistulosus* leaves (Mean±S.D, p<0.0 5).

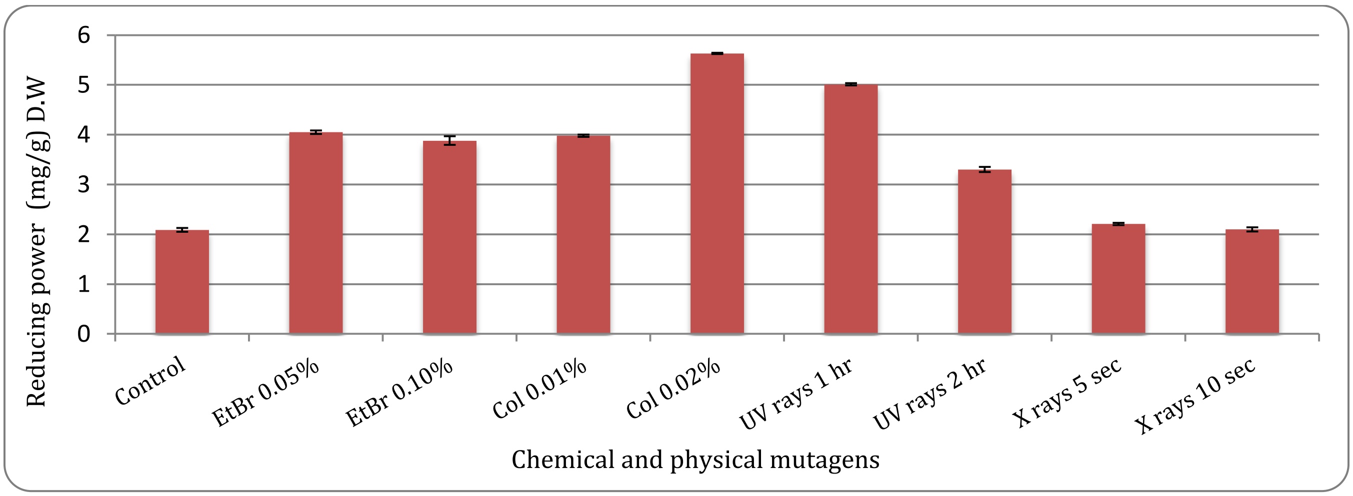

Figure 8. Effect of chemical and physical mutagens on the amount of reducing power of *Praecitrullus fistulosus* leaves (Mean±S.D, p<0.05).

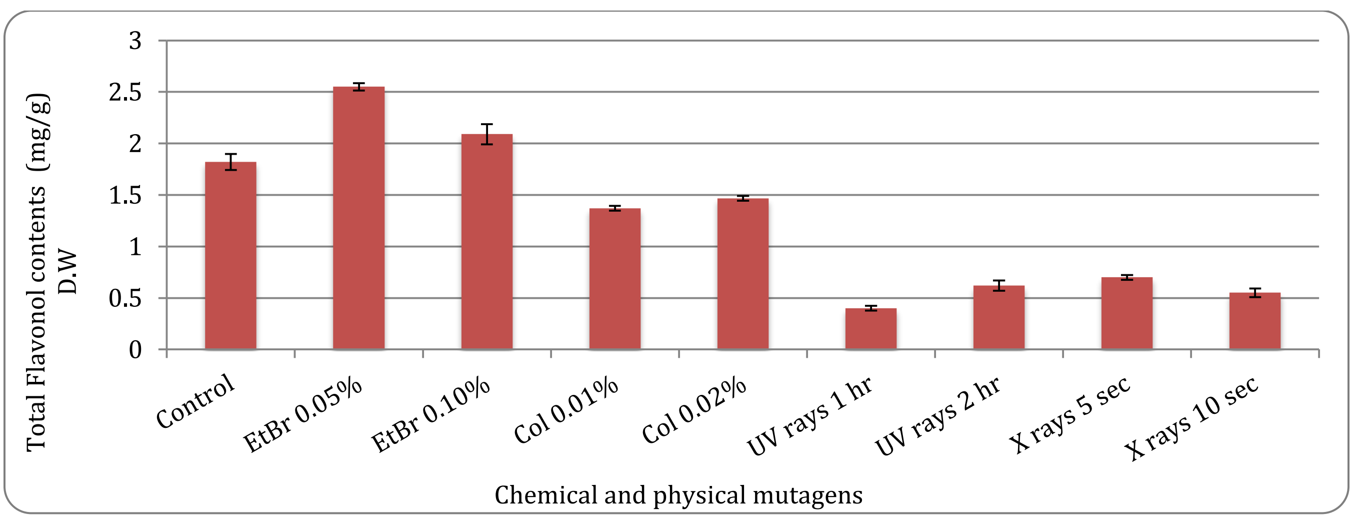

Figure 9. Effect of chemical and physical mutagens on the amount of total flavonol contents of *Praecitrullus fistulosus* leaves (Mean±S.D, p<0.05).

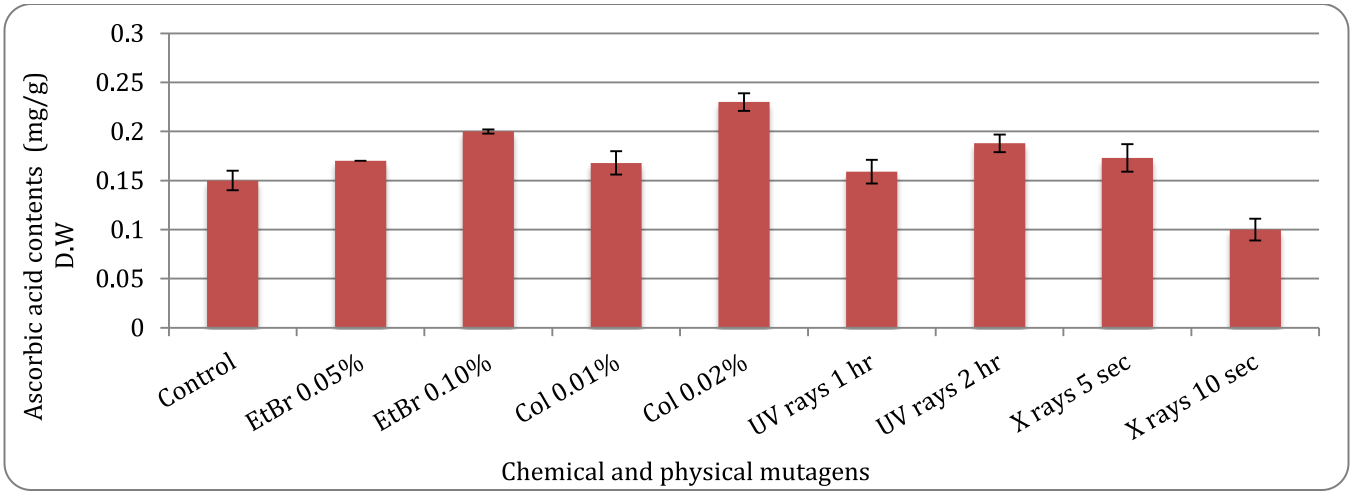

Figure 10. Effect of chemical and physical mutagens on the amount of ascorbic acid of *Praecitrullus fistulosus* leaves (Mean±S.D, p<0.05).

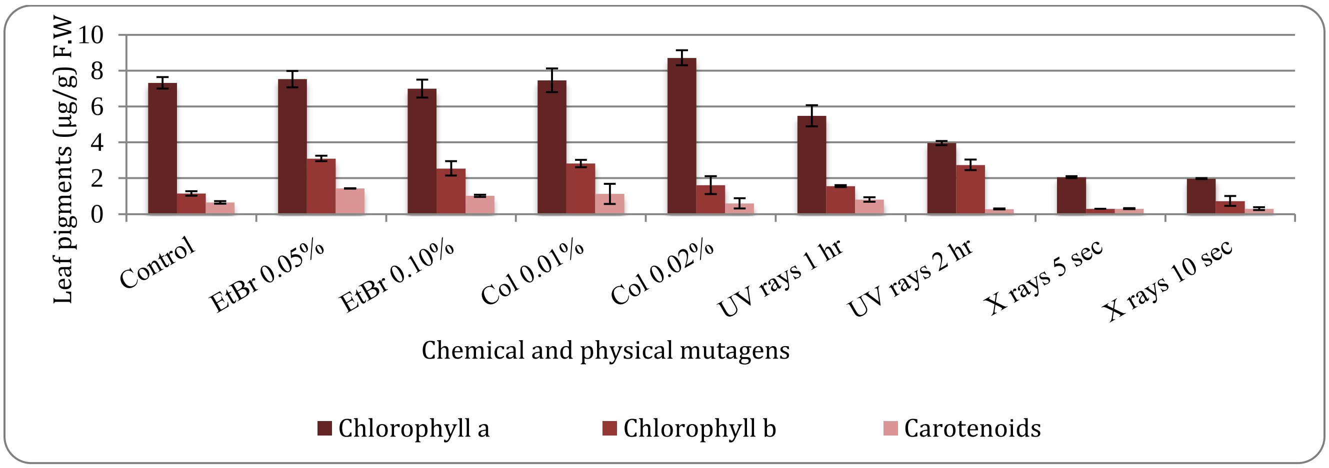

Figure 11. Chlorophyll a, chlorophyll b and carotenoids contents (µg/g F.W) in *Praecitrullus fistulosus* leaves, treated with different mutagens against control (Mean ± S.D, p<0.05).

As plants are very important source of bioactive compounds and possess medicinal values due to the presence of these secondary metabolites or phytochemicals. The plants treated with chemical mutagens showed better and enhanced biochemicals production especially colchicine 0.02% treated plants as compared to control while physical mutagenic treated plants were unable to grow properly and showed poor production of biochemicals especially the plants treated with X-rays. The physical mutagens including X-Rays and UV – Light did not produce any effective role for the improvement the biochemicals.

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