INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 14, No. 1, p. 194-203, 2019
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Antioxidant, antimicrobial and biochemical analysis of mutated and cultivated *Momordica charantia*

Komal Siddiqui, Noor-e-Saba Khaskheli*, Zaib-Un-Nisa, Mehtab Hussain Nizamani

Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan

Article published on January 31, 2019

Key words: Antioxidant, Antimicrobial, Biochemical Analysis, Mutated and Cultivated *Momordica charantia*

Abstract

Momordica charantia has long been regarded as a food and medicinal plant**.** It plays a major role as a source of carbohydrate, protein, vitamins minerals and other nutrients in human diet. The aim of the study was to analyse antimicrobial activity, biochemical and antioxidant contents of cultivated and mutated *Momordica charantia* plant extracts. Two types of extracts were made; aqueous and methanol based, from leaves and fruit of *Momordica charantia*. In the initial work *Momordica charantia* seeds were treated with different mutagens namely sodium azide, silver nitrate and ethidium bromide and were germinated in IBGE research garden. Statistical analysis was carried out along with chlorophyll and carotenoid testing during the growth phase. Ethidium bromide treated seeds showed best germination rate as well as highest shoot length as compared to other mutatated plants. Highest Chlorophyll contents and carotenoids were found in leaves of ethidium bromide treated plant. The fruit and leaves of mutated and control plants were examined for biochemical contents.

***Corresponding Author:** Noor-e-Saba Khaskheli ✉ nooresaba@hotmail.com

Introduction

The drugs which are already in application to treat infectious diseases is of concern because, drug safety is a huge Global issue due to its adverse effects. Thus Herbal and natural product are utilized in folks medicine for hundreds of years throughout the world. *Momordica charantia* is one of the plant that has been commonly used as medication (Nagasawa, Watanabe, & Inatomi, 2002; Takemoto, Jilka, Rockenbach, & Hughes, 1983). *Momordica charantia* usually referred to as bitter melon/gourd or karela, a member of Cucarbitaceae, is a slender, tendril climbing, annual vine. *M. charantia* is an energetic nutritious-compact plant composed of a complex arrangement of beneficial constitute which include primary metabolites such as carbohydrates, proteins and chlorophyll and secondary metabolites include alkaloids, flavonoids, tanins, saponins, disogenin, calcium, copper etc. Secondary metabolites of *M. charantia* are supposed to have health benefits (Daniel *et al.,* 2014). Researchers also found that bitter melon is full of antioxidants like carotenoids, including alpha and provitamin A, lycopene and zeaxanthin moreover it contain iron, beta carotene, potassium, vitamins (A,B1,B2,B3,B9,C), minerals, and dietary fibers. These pharmacological chemicals support in treating enormous range of diseases. Steroidal compounds are of importance and of interest because it is observed that steroidal structure might serve as potent beginning material in the synthesis of hormones. This may be the reason of *M. charantia* used as vegetable for pregnant women or breast feeding mothers to make sure their hormonal balance is normal. (Supraja & Usha, 2013). Other compounds reported in this vegetable include a cytotoxic ribosome binding terpenoid and several glycosides including charantin, charantoside, and momordicoside (Begum *et al.,* 1997; Harinantenaina *et al.,* 2006; Akihisa *et al.,* 2007; Chang *et al.,* 2008; Dhiman, Gupta, Sharma, Gill, & Goyal, 2012). Antioxidants activities of bitter gourd seeds peel and flakes exhibit striking DPPH free radical scavenging activity. The presence of antioxidants in the *M. charantia* result in the reduction of Fe^{3+} to Fe^{2+} thus it cease the radical chain reaction by donating an electron (Saeed, *et al.*, 2010).

The anti-leukemic and anti-cancerous activity of *M. cahrantia* against varied cell lines including the cancer of liver, human leukemia skin cancer and solid sarcomas have also been reported (Zhu, Zhong, Luo and Xiao 1990). *Momordica charantia* is well understood to have anti- hyperglycemia, anti-cholesterol, immunosuppressor, anti-ulcerogenic, anti-spermatogenic and androgenic activities, anti-HIV, antiulcer, anti-inflammatory, anti-leukemic, antimicrobial, anti- cholesterol, and anti-tumor activities (Scartezzini & Speroni, 2000; Pitipanapong *et al.,* 2007). The constituents that are responsible for this activity is a non-chemical element called charantin, which is composed of: sitosteryl glycoside and stigmasteryl glycoside. Charantin is used to treat diabetes mellitus and might be replaced with treatment by injection of insulin, that has not been successful in stimulating the exocrine gland of the diabetic patients to lower blood glucose to the require diabetic property like charantin and others are now being extensively approve as an alternate remedy for diabetes mellitus, and they are free from side effects (Lolitkar, Rajarama and Noteon, 1962; Olaniyi, 1974). The presence of phenolic compounds in the plants reveals that the plants have antimicrobial agent. *M. charantia* can be used in the treatment of the placenta and navel of newborn baby which not only heals fast but also prevent the formation of infections (Okwu, 2001; Okwu, 2003).

Mutation breeding now a days is generally used to produce useful traits in crops like larger seeds, new colors, or sweeter fruits that either cannot be found in nature or have been missing during evolution (Roose & Williams, 2007). Mutation breeding is a method of exposing seeds to chemicals or radiation so as to get mutants with interesting traits. (Oliva *et al.,* 2014; Schouten & Jacobsen, 2007).A vast range of characters that are altered through mutation breeding include plant structure, yield, flowering and ripeness time, quality and toleration to biotic and abiotic stresses. This study was aimed to investigate the potential of *Momordica charantia* plant after treatment with different chemical mutagens and evaluate the biochemical present in fresh and dry extracts of cultivated *Momordica charantia* fruit and their mutated leaves.

Materials and methods

This study was carried out at Medical and Environmental Research Laboratory, Institute of Biotechnology and Genetic Engineering.

Induced Mutation

Seeds of *Momordica charantia* (bitter gourd) plant were purchased from market. Prepared a solution of 0.01% and 0.02% of ethidium bromide, silver nitrate and sodium azide. Soaked seeds in solution for an hour. For control soaked seeds in distilled water. After an hour seeds were sowed in IBGE research garden. Their growth was observed weekly.

Preparation of 10% Aqeous and Methanol extracts

Fresh leaves of mutated and control *Momordica charantia* (bitter gourd) were collected from IBGE research garden. 3g of fresh leaves and fruit (peel,pulp & seeds)crushed in pestle morter. For dry extracts leaves of mutated and control *Momordica charantia* plus fruit (peel, pulp and seeds) were dried in oven at 50°C then finely powdered distilled water was added for aqueous extracts and methanol was added for methanol extracts and centrifuged at 6000 rpm for 20 minutes at 4°C, this procedure was repeated twice. Then supernatants of extracts were collected and filtered by using what man filter paper. The final volume was made. The aqueous and methanol extracts are ready for further analysis.

Determination of Chlorophyll A, Chlorophyll B and Carotenoids

For chlorophyll a, b and carotenoids determination 50mg fresh leaves were crushed. Leaves were placed in individual test tubes and 5ml of 80% acetone was added. The test tubes were covered and refrrigerated at 4°C for 24 hours. After 24 hours, The absorbance was taken at 662nm for chlorophyll A, 644nm for chloroplyll B and 470nm for carotenoids for each sample.

Chlorphyll A, Chlorophyll B and carotenoids were calculated by using the following formulae.

Chlorophyll A= $11.75(A_{662})-2.350(A_{644})$

Chlorophyll B= $18.61(A_{644})-3.960(A_{662})$

Carotenoids= $1000(A_{470})-2.270$ (Chlorophyll A)-81.4(Chlorophyll B) / 227.

Antimicrobial Activity

The antimicrobial activity of mutated leaves extracts of *Momordica charantia* was determined. For determination of antimicrobial activity agar plate method was used. The medium was prepared by dissolving 4g dextrose, 2g peptone and 4g agar in 100ml distilled water in 250ml flask closely capped with cotton plug. The media along with petriplates were autoclaved for 15 minutes at 121C and 15psi. The sterilized nutrient agar medium was allowed to cool at 50C and poured into sterilized petriplates under aseptic conditions. When media was solidified, small holes were made with the help of sterilized pipette tips. Then sterilized ear bud was dipped into the suspension of serial diluted fungal cultures and was gently spread onto the entire area of plate. The holes were filled with different leaf extracts and labeled accordingly. The petriplates were covered with parafilm and incubated at 27°C. The antimicrobial activity was observed after 48 hours of incubation. Antimicrobial activity was measured in terms of inhibition zone formed around the hole where leaf extract was present.

Biochemical analysis of extracts

Both water and methanol extract of fresh and dried mutated plant leaves extracts and cultivated bitter gourd fruit extracts were subjected under following tests:

Determination of pH

pH from 10% aqueous and methanol extract of fresh and dried samples were determined with the help of pH meter.

Determination of total sugar

Total sugar content from 10% aqueous and methanol extract of fresh and dried samples were determined by phenol sulfuric acid method (Montgomery, 1961). Glucose standard curve was used for calculation of total sugar contents from test sample

Determination of reducing sugar

The reducing sugar from 10% aqueous and methanol extracts of fresh and dried samples were determined by Dinitrosalicylic acid (DNS) method (Miller, 1959). Results were calculated from glucose standard curve.

Determination of total protein

Total protein content from 10% aqueous and methanol extracts of fresh and dried samples were determined by (Lowry, Rosebrough, Farr, & Randall, 1951) method. Total protein contents from test sample were calculated by albumin standard curve.

Determination of Total antioxidant

The antioxidants from 10% aqueous and methanol extract of fresh and dried samples were determined by using spectrophotometer method (Voces *et al.,* 1999) with slight modification. The results were calculated from Alpha Tocoferol standard curve.

Determination of phenolics

Total phenolic content from 10% aqueous and methanol extract of fresh and dried samples were determined by using spectrophotometer by Follin-ciocalteu method (Yasoubi, Barzegar, Sahari, & Azizi, 2010). The results were calculated from Gallic acid standard curve.

Determination of flavonoids

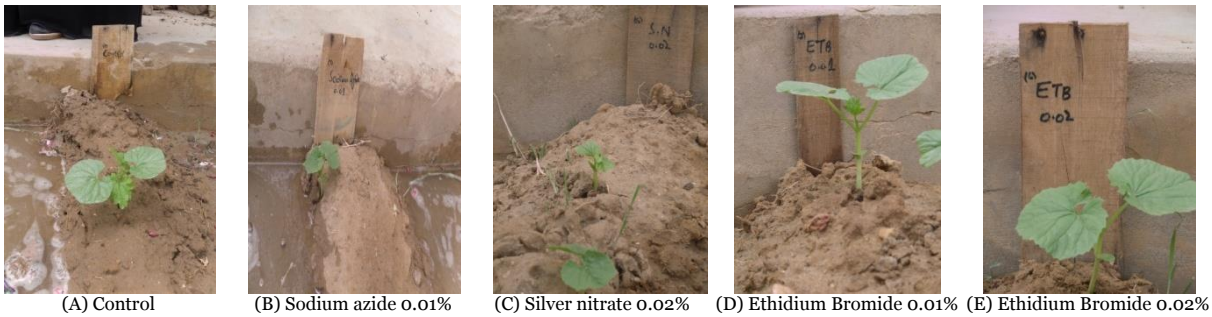
Total flavonoid content from 10% aqueous and methanol extract of fresh and dried samples were determined by aluminum chloride colorimetric method (Kim, Moon, Lee, & Choi, 2004). The result was calculated from Quercetin standard curve.

Results

The fruit and leaves of mutated and control plants were examined for biochemical contents. The highest level of total sugar (36.1mg/ml) was observed in methanol extract of fruit (dry pulp) while lowest sugar content (5.08mg/ml) was found in methanol extract of fresh seeds.

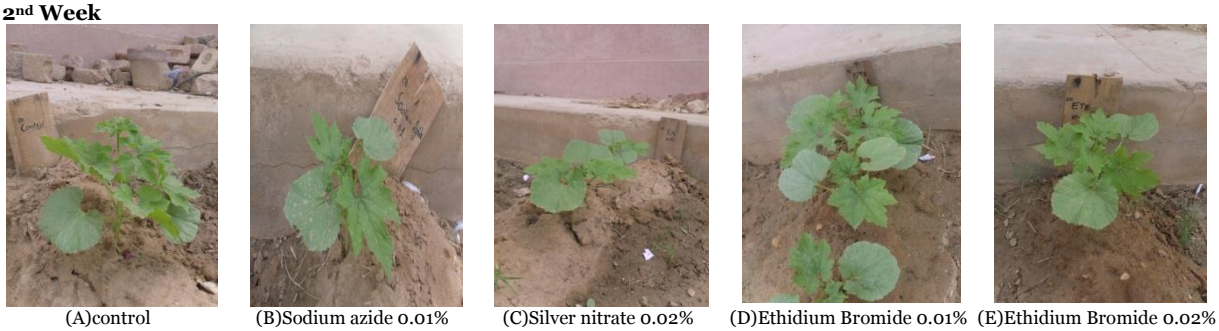
Growth observation

1st Week



(A) Control (B) Sodium azide 0.01% (C) Silver nitrate 0.02% (D) Ethidium Bromide 0.01% (E) Ethidium Bromide 0.02%

2nd Week



(A)control (B)Sodium azide 0.01% (C)Silver nitrate 0.02% (D)Ethidium Bromide 0.01% (E)Ethidium Bromide 0.02%

3rd Week

(A) Control (B) Sodium azide 0.01% (C) Silver nitrate 0.02% (D) Ethidium Bromide 0.01% (E) Ethidium Bromide 0.02%

Fig. 1. Weekly growth observation of mutated and control plant of *Momordica charantia*.

In *Momordica charantia* fruit highest reducing sugar (10.56mg/ml) was observed in aqueous extract (dry pulp) while lowest reducing sugar (1.03mg/ml) was found in aqueous extract of dry seeds. Highest total protein contents (5.62mg/ml) were observed in aqueous extract of dry pulp while minimum protein contents (0.68mg/ml) were observed in methanol extract of fresh seeds. In *Momordica charantia* fruit the highest antioxidant content (7.15mg/ml) was found in methanol extract of dry peel and pulp while lowest antioxidant content (0.37mg/ml) in aqueous extract of fresh pulp.

Biochemical contents of mutated and control plant leaf extracts are summarized below:

The highest total sugar (10.8mg/ml) were observed in sodium azide mutated methanol extract of dry leaves while minimum total sugar (2.51mg/ml) observed in silver nitrate aqueous extract of fresh leaves. Highest reducing sugar (2.48mg/ml) observed in ethidium bromide methanol extract of dry leaves while lowest reducing sugar (0.44mg/ml) observed in ethidium bromide aqueous extract of fresh leaves. In leaves highest protein contents (1.12mg/ml) were observed in silver nitrate methanol extract of fresh leaves and lowest protein contents (0.33mg/ml) were observed in ethidium bromide aqueous extract of fresh leaves. In *Momordica charantia* leaves highest antioxidant contents (2.2mg/ml) were observed in silver nitrate methanol extract of dry leaves while lowest antioxidant contents (0.45mg/ml) were observed in ethidium bromide aqueous extract of fresh leaf.

The antimicrobial activity of leaf extracts of mutated and control plant was checked against *Aspergillus niger*. Maximum inhibition zone was observed in the aqueous extract of *M. charantia* treated with ethidium bromide and aqueous as well as methanol extract of control. Very small inhibition zone was also observed in the aqueous extract of *M. charantia* treated with sodium azide and silver nitrate, though their methanol extract showed medium inhibition zone against *Aspergillus niger*.

Highest Chlorophyll contents and carotenoids were found in leaves of ethidium bromide treated plant.

Table 1. Biochemical analysis of Aqueous & Methanol extracts of fresh and dry *Momordica charantia* fruit.

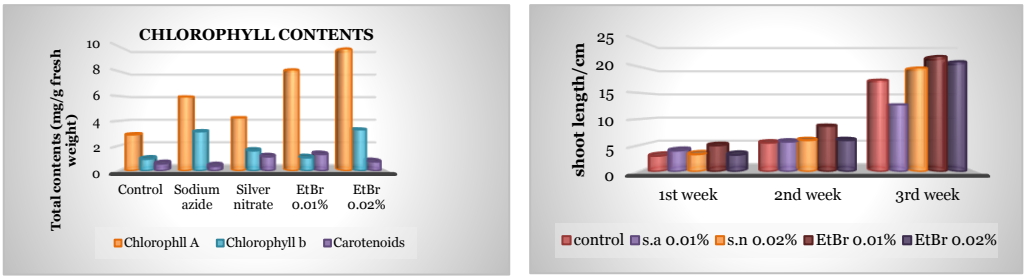
Sample	Extracts	Total sugar mg/ml		Reducing sugar mg/ml		Total protein mg/ml		Antioxidant mg/ml		Phenolics mg/	
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Peel	Aqueous	5.58	15.6	1.85	4.34	2.43	4.74	0.60	3.8	0.02	0.23
	Methanol	6.22	13.4	2.27	4.19	1.2	4.13	0.76	7.15	0.11	0.26
Pulp	Aqueous	6.40	25.5	2.50	10.5	2.54	5.62	0.37	3.36	0.04	0.22
	Methanol	6.58	36.1	2.29	10.2	2.25	5.14	0.68	7.15	0.09	0.25
Seeds	Aqueous	6.09	10.6	2.05	1.03	0.74	2.69	0.68	3.84	0.07	0.3
	Methanol	5.08	7.85	1.69	1.08	0.68	2.39	0.84	2.45	0.08	0.21

Table 2. Biochemical analysis of Aqueous & Methanol extracts of fresh and dry *Momordica charantia* mutated and control leaf extracts.

Samples	Moisture content/ 10g	Extracts	PH of extracts		Total sugar mg/ml		Reducing sugar mg/ml		Total protein mg/ml	
			DL	FL	DL	FL	DL	FL	DL	FL
Control	8.22	Aqueous	8.7	8.3	4.24	3.08	0.94	1.01	0.74	0.60
		Methanol	8.8	7.9	5.33	3.81	1.39	1.26	0.93	0.77
Silver nitrate 0.02%	8.45	Aqueous	8.7	8.7	5.52	2.51	1.32	0.66	0.70	0.54
		Methanol	8.8	8.8	10.4	3.23	1.29	1.02	0.83	1.12
Sodium azide 0.01%	8.37	Aqueous	8.8	8.0	3.57	3.68	0.79	1.18	0.53	0.46
		Methanol	8.9	8.7	10.8	4.15	1.81	1.28	0.91	0.97
Ethidium bromide 0.01%	8.28	Aqueous	8.9	8.8	3.60	3.40	0.63	0.51	0.37	0.36
		Methanol	8.9	9.0	10.6	3.45	1.98	0.77	0.86	0.77
Ethidium bromide 0.02%	8.36	Aqueous	9.0	8.9	3.59	3.14	0.83	0.44	0.57	0.33
		Methanol	8.7	8.9	6.0	3.48	2.48	1.40	0.76	0.82

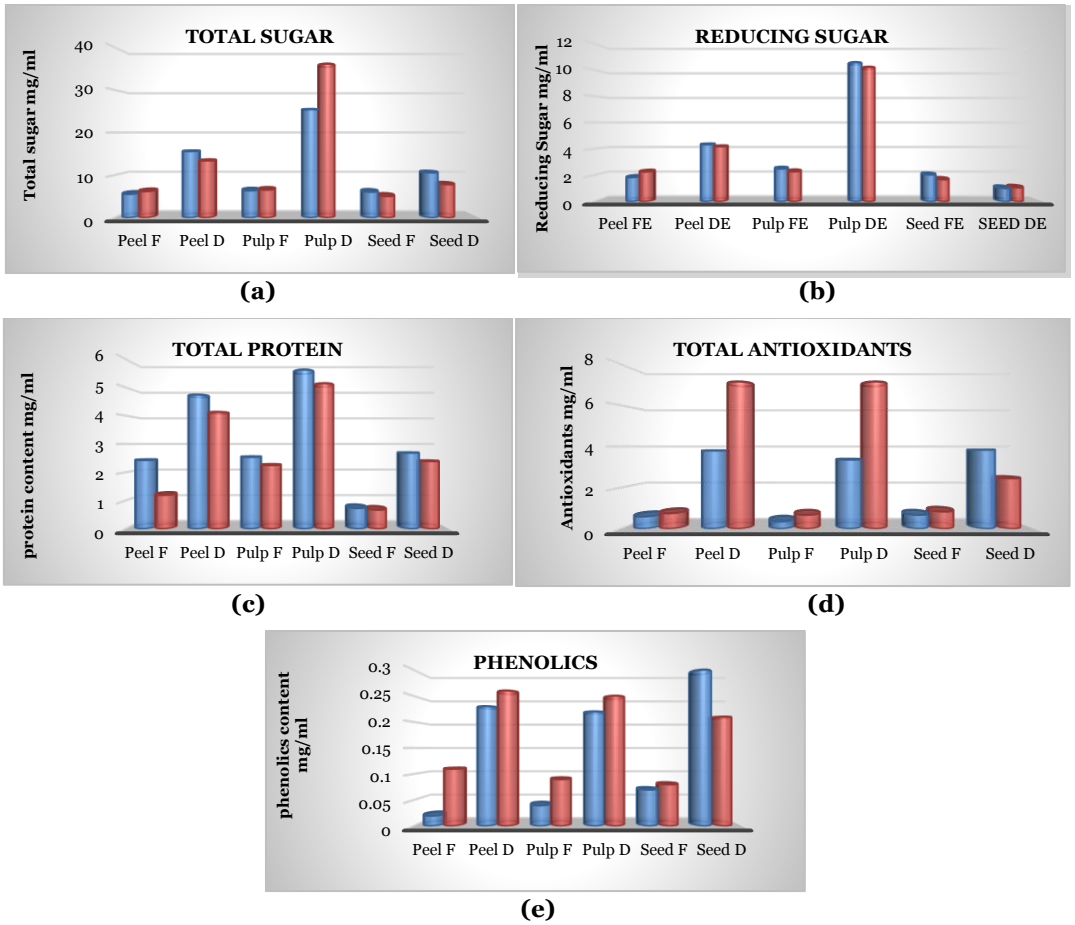
Table 3. Antioxidant and Phenolic contents of Aqueous & Methanol extracts of fresh and dry *Momordica charantia* mutated and control leaf extracts.

Samples	Extracts	Antioxidants mg/ml		Phenolics mg/ml	
		Fresh	Dry	Fresh	Dry
Control	Aqueous	0.6	1.3	0.15	0.13
	Methanol	1.28	1.5	0.15	0.2
Sodium azide 0.01%	Aqueous	0.77	0.89	0.21	0.28
	Methanol	0.94	1.74	0.22	0.16
Silver nitrate 0.02%	Aqueous	0.69	1.82	0.12	0.31
	Methanol	0.87	2.2	0.13	0.08
Ethidium bromide 0.01%	Aqueous	0.71	0.64	0.12	0.15
	Methanol	0.8	1.5	0.09	0.13
Ethidium bromide 0.02%	Aqueous	0.45	0.92	0.17	0.2
	Methanol	0.72	1.16	0.13	0.16



(a) **(b)**

Fig. 2. (a) Graphical representation showing Chlorophyll a, b and carotenoids contents of mutated plant of bitter gourd. **(b)** Shoot length of mutated *Momordica charantia* plant.



(a) **(b)**

(c) **(d)**

(e)

Fig. 3. Biochemical analysis of 10% Aqueous & Methanol extracts of fresh and dry bitter gourd fruit.

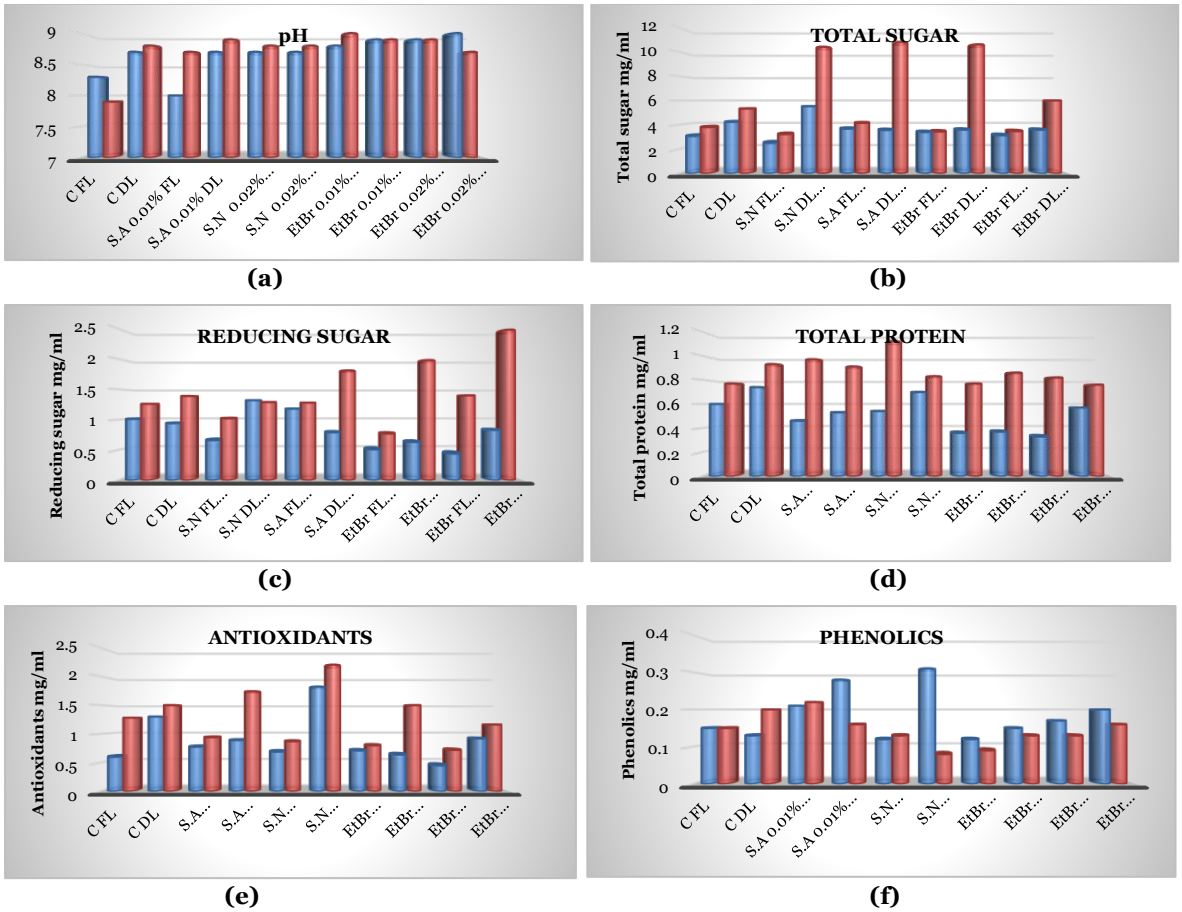
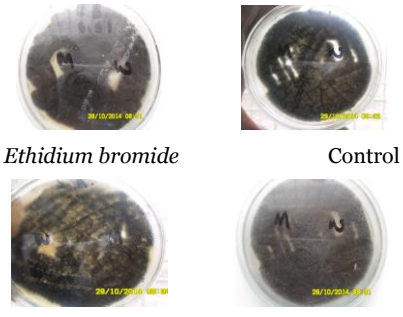


Fig. 4. Biochemical analysis of 10% Aqueous & Methanol extracts of fresh and dry *Momordica charantia* mutated plant leaves (DL; dry leaves, FL; fresh leaves)

Table 1. Antifungal activity of *Momordica charantia* mutated and control plant leaf extracts against *Aspergillus Niger*.

Extracts	Aqueous	Methanol
Control	++ve	++ve
S.A 0.01%	+ve	+ve
S.N 0.02%	+ve	+ve
Etbr 0.01%	++ve	++ve
Etbr 0.02%	+ve	++ve



Ethidium bromide Control

Sodium azide *Silver nitrate*

Fig. 5. Antifungal activity of *Momordica charantia* mutated and control plant leaf extracts against Aspergillus Niger. (Size of inhibition zone: +ve= Small, ++ve= Medium, +++ve Large zone and –ve= No zone).

Discussion

Now a day's usage of ionizing radiation, like X-rays, gamma rays and neutrons and chemical mutagens for inducing diversity, is well established. Induced mutations have also been used to improve major crops such as wheat, rice, barley, cotton, peanuts, and beans, which are seed propagated (Ahloowalia & Maluszynski, 2001). Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms.

Momordica charantia has long been regarded as a food and medicinal plant**.** It plays a major role as a source of carbohydrate, protein, vitamins minerals and other nutrients in human diet.

The present study was carried out to investigate the unexplored area of this plant for that purpose *Momordica charantia* seeds were exposed to different chemical mutagens that is ethidium

bromide, sodium azide and silver nitrate and compared their growth rate and phytochemical analysis with control. Ethidium bromide treated seeds showed best germination rate. Levy and Ashiri studied the effects of ethidium bromide (EB) compared to ethyl methane-sulfonate (EMS) mutagen in peanuts and they concluded that the mutagenic efficiency of EB was much higher than that of EMS (Levy & Ashiri, 1975).

In present study the phytochemical screening and quantitative estimation of fresh and dried extracts of *Momordica charantia* germinated fruit and mutated plant leaves showed that the fruit and leaves were rich in carbohydrates, proteins, antioxidants in all the extracts. Qualitative phytochemical analysis of *Momordica charantia* was reported by Prarthna Daniel *et al.,* which confirms the presence of phytochemicals such as flavanoids, saponins, terpenoids, coumarins, emodins, alkaloids, proteins, cardiac glycosides, anthraquinones, anthocyanins, steroids etc (Daniel, Supe, & Roymon). The chemical prospection of *M. charantia* fruit and leaf extracts have indicated the presence of various secondary metabolites that are known to present different therapeutic applications, for example, tannins (antimicrobial, antiviral, moluscicidal and antitumoral), flavonoids (anticarcinogenic, antiviral, antihemorrhagic and antioxidant) (Okuda, Yoshida, & Hatano, 1989; 199; Marston & Hostettmann, 1985; Scalbert, 1991).

Plant-derived antioxidants, especially, the phenolics have gained considerable importance due to their potential health benefits. Epidemiological studies have revealed that utilization of plant foods containing antioxidants is advantageous to health because it down-regulates many degenerative processes and is capable of lowering the occurrence of cancer and cardio-vascular diseases (Arabshahi-Delouee & Urooj, 2007). Ronny Horax *et al.,* (2005), studied the total phenolic contents of oven dried and freeze dried tissues of *M. charantia* and their antioxidant activity. The total phenolic contents of the oven dried tissues were higher than those of the freeze-dried tissues, which might be due to an increase in the amount of simple phenols during oven-drying. Though in antioxidant content they observed no significant difference in oven dried and freeze-dried sample while the antioxidant activities of the methanolic extracts from peel and pulp were higher than that from seed (Horax, Hettiarachchy, & Islam, 2005). Similar results were obtained in present studies in which oven dried fruit and leaves of *M. charantia* show high phenolic contents than fresh extracts. Also the methanol extracts had high antioxidant content.

The antimicrobial activity of leaf extracts of mutated and control plant was checked against *Aspergillus niger*. Maximum inhibition zone was observed in the aqueous extract of *M. charantia* treated with ethidium bromide as well as methanol and aqueous extract of control. Very small inhibition zone was also observed in the aqueous extract of *M. charantia* treated with sodium azide and silver nitrate, though their methanol extract showed medium inhibition zone against *Aspergillus niger*. Similar results have been reported by Roopashree *et al.,* (2008) that aqueous extracts are more effective against all tested bacterial strains. The antibacterial activity of leaf and fruit extracts of *Momordica charantia* with different solvents was check against microorganisms. Among the various extracts, methanol extracts have shown better antibacterial activity (Supraja & Usha, 2013). Similar results obtained in methanol extracts of *M. charantia* treated with sodium azide and silver nitrate.

Conclusion

The findings in present study of *M. caharantia* reveal that the fruit and leaf extracts could be used as biopreservative because of its high antimicrobial and antioxidant activity and has potential health benefits. In addition further evidence regarding the efficacy, safety and appropriate dosage of antioxidants in relation to chronic diseases is needed.

References

Ahloowalia B, Maluszynski M. 2001. Induced mutations–A new paradigm in plant breeding. Euphytica **2**, 167-173.

Akihisa T, Higo N, Tokuda H, Ukiya M, Akazawa H, Tochigi Y, Kimura Y, Suzuki T. 2007. Cucurbitane-type triterpenoids from the fruits of *Momordica charantia* and their cancer chemopreventive effects. Journal of Natural Products **70**, 1233-1239.

Arabshahi-Delouee S, Urooj A. 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. Food Chemistry **4,** 1233-1240.

Begum S, Ahmed M, Siddiqui BS, Khan A, Saify ZS, Arif M. 1997. Triterpenes, a sterol and a monocyclic alcohol from *Momordica charantia*. Phytochemistry **7,** 1313-20.

Chang CI, Chen CR, Liao YW, Cheng HL, Chen YC, Chou CH. 2008. Cucurbitane-type triterpenoids from the stems of *Momordica charantia*. Journal of Natural Products **8**, 1327-30.

Daniel P, Supe U, Roymon MG. 2014. A review on Phytochemical analysis of *Momordica charantia*. Int. J. Adv. Pharm. Biol. Chem **1,** 214-20.

Dhiman K, Gupta A, Sharma DK, Gill NS, Goyal A. 2012. A review on the medicinally important plants of the family Cucurbitaceae. Asian Journal of Clinical Nutrition **1,** 16-26.

Harinantenaina L, Tanaka M, Takaoka S, Oda M, Mogami O, Uchida M, Asakawa Y. 2005. *Momordica charantia* constituents and antidiabetic screening of the isolated major compounds. Chemical and Pharmaceutical Bulletin **7,** 1017-21.

Horax R, Hettiarachchy N, Islam S. 2005. Total phenolic contents and phenolic acid constituents in 4 varieties of bitter melons (*Momordica charantia*) and antioxidant activities of their extracts. Journal of food science **4,** 275-80.

Kim HY, Moon BH, Lee HJ, Choi DH. 2004. Flavonol glycosides from the leaves of *Eucommia ulmoides* O. with glycation inhibitory activity. Journal of Ethnopharmacology **2-3,** 227-30.

Levy A, Ashiri A. 1975. Ethidium bromide—an efficient mutagen in higher plants. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **3,** 397-404.

Lolitkar MM, Rao MR. 1962. Note on a hypoglycaemic principle isolated from the fruits of *Momordica charantia*. Journal of the University of Bombay **29,** 223-4.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry **1**, 265-275.

Marston A, Hostettmann K. 1985. Review article number 6: Plant molluscicides. Phytochemistry **4,** 639-652.

Miller G. 1959. Modified DNS method for reducing sugars. Analytical Chemistry **3,** 426-428.

Montgomery R. 1961. Further studies of the phenol-sulfuric acid reagent for carbohydrates. Biochimica et biophysica acta **3,** 591-593.

Nagasawa H, Watanabe K, Inatomi H. 2002. Effects of bitter melon (*Momordica charantia* L.) or ginger rhizome (*Zingiber offifinale* rosc) on spontaneous mammary tumorigenesis in SHN mice. The American journal of Chinese medicine **02n03,** 195-205.

Okuda T, Yoshida T, Hatano T. 1989. Ellagitannins as active constituents of medicinal plants. Planta medica **02,** 117-22.

Okwu DE. 2003. The potentials of *Ocimum grattisium*, *Pergularia extensa* and *Tetrapleura tetraptera* as spice and flavouring agents. Nigeria Agriculture Journal **35,** 143-148.

Okwu DE. 2001. Evaluation of the chemical composition of indigenous species and flavouring agents. Global Journal of Pure and Applied Science **7,** 455-459.

Olaniyi A. 1974. A neutral constituent of Momordica foetida. Lloydia **4,** 361-362.

Oliva CF, Vreysen MJ, Dupé S, Lees RS, Gilles JR, Gouagna LC, Chhem R. 2014.Current status and future challenges for controlling malaria with the sterile insect technique: technical and social perspectives. Acta tropica **132,** 130-9.

Pitipanapong J, Chitprasert S, Goto M, Jiratchariyakul W, Sasaki M, Shotipruk A. 2007. New approach for extraction of charantin from *Momordica charantia* with pressurized liquid extraction. Separation and Purification Technology **3,** 416-22.

Roose ML, Williams TE. 2007. Mandarin tree named 'Tango', Google Patents.

Saeed MK, Shahzadi I, Ahmad I, Ahmad R, Shahzad K. 2010 Nutritional analysis antioxidant activity of bitter gourd (*Momordica charantia*) from Pakistan. Pharmacologyonline **1,** 252-260.

Scalbert A. 1991. Antimicrobial properties of tannins. Phytochemistry **12,** 3875-3883.

Scartezzini P, Speroni E. 2000. Review on some plants of Indian traditional medicine with antioxidant activity. Journal of ethnopharmacology **1,** 23-43.

Schouten HJ, Jacobsen E. 2007. Are mutations in genetically modified plants dangerous?" BioMed Research International.

Supraja P, Usha R. 2013. Antibacterial and phytochemical screening from leaf and fruit extracts of *Momordica charantia*. International journal of Pharma & Bio sciences 4 **1.**

Takemoto DJ, Jilka C, Rockenbach S, Hughes JV. 1983. Purification and characterization of a cytostatic factor with anti-viral activity from the bitter melon. Preparative biochemistry **4,** 371-93.

Voces J, Alvarez AI, Vila L, Ferrando A, De Oliveira CC, Prieto JG. 1999. Effects of administration of the standardized Panax ginseng extract G115 on hepatic antioxidant function after exhaustive exercise. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology **2,** 175-84.

Yasoubi P, Barzegar M, Sahari MA, Azizi MH. 2010. Total phenolic contents and antioxidant activity of pomegranate (*Punica granatum* L.) peel extracts. Journal of Agricultural Science and Technology **9,** 35-42.

Zhu ZJ, Zhong ZC, Luo ZY, Xiao ZY. 1990. Studies on the active constituent of *Momordica charantia* **25**, 898 -903.