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Studies on Phytochemical and Antioxidative Potential in *Ocimum basilicum* L. by inducing variability using Ethyl Methane Sulphonate in Uttarakhand

Neha Kumari[1], Suman Khanduri [1], Megha Sharma[1], Sweta Mathur[1], Neha Gosai[1] and Maneesha Singh[2]

[1,2]Department of Botany, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun 248 001, Uttarakhand, India

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ABSTRACT

Ocimum basilicum L. (Lamiaceae), popularly known as basil, is an aromatic plant that extensively used for medicinal uses and to add a distinctive flavor and aroma to food. The present study aims to evaluate the phytochemical and antioxidant activity of this commercially important herb, which have antimicrobial and antioxidant properties. The current investigation has been carried out to induce variations in *Ocimum basilicum* L. using Ethyl Methane Sulphonate (EMS), a chemical mutagen with different doses to determine the various parameters. The yield of various crude extract of screened mutant ranged from 1.12-3.22%. Phytochemical studies showed that protein, alkaloids, amino acid, carbohydrates, flavonoids, tannins, phenolics and saponin were present in all mutants. The total phenolic content of screened mutants of *Ocimum basilicum* L. showed large variation ranges in aqueous extract from 3.18 to 27.63 mg GAE/g and in ethanol extract 19.59 to 46.55mg GAE/g. The content of flavonoid varied in aqueous extract from 0.92 to 6.68 mg QE/g and in ethanol extract 9.12 to 34.9 mg QE/g. The study revealed that, in comparison to the standard ascorbic acid with an IC50 value of 229 µg/ml, all mutants exhibited IC50 values ranging from 84.66 to 496.7 µg/ml. These findings suggest that inducing mutagen causes variations, improves crops, evaluation of antioxidant potential and creates new genetic resources with desirable traits under diverse climatic conditions.

Key words: *Basil, Phytochemicals, Antioxidant potential, Essential oil*

Introduction

The extensive uses of phyto constituents extracted from plants are widely acknowledged, spanning applications in food preservation, adding value to products, and serving as therapeutic elements for addressing diverse health issues (Ranjha *et al.,* 2020). Abundant data supports the consideration of medicinal flora as a viable alternative and potential future for conventional and manufactured antibiotics (Ribeiro *et al.,* 2020). The confirmed proof highlighting the negative health effects of synthetic food additives, particularly when used without caution in the food industry, emphasizes the importance of exploring an increased involvement of natural ingredients in the domains of food processing and preservation. The growing body of evidence emphasizing health risks linked to the careless utilization of synthetic food additives suggests a need to limit their usage and investigate safer alternatives derived from natural sources (Tortosa *et al.,* 2020). In the last several decades, researchers and experts

([1]Research Scholar,[2]Professor)

have actively focused on polyphenols as promising additives with the possibility to enhance the quality of food and mitigate the potential for specific diseases such as inflammation, free radical damage, and cancer in humans (Nowak *et al*., 2021).

Sweet basil, a renowned culinary and medicinal herb from Lamiaceae family, scientifically identified as *Ocimum basilicum* L., originating from India and various Asian countries, but it has now achieved global cultivation (Rubab *et al*., 2017). Basil leaves, known for their traditional uses as a folk cure, have a long history of treating a broad spectrum of health issues, encompassing cancer, gastrointestinal issues, psychological ailments, swelling, liver disorders, dental decay, and respiratory inflammation (Janick *et al*., 1990). Pharmacological evidence robustly supports these traditional claims, demonstrating properties such as antioxidant defense, anticarcinogenic, analgeic and immunostimulatory effects (Naz *et al*., 2015).

These biochemical activities are attributed to phenolic acids, flavonoids, rosmarinic acid, aromatic compounds, and basil essential oils, including eugenol, linalool, chavicol, and α-terpineol (Purushothaman *et al*., 2018).

Basil finds widespread culinary use beyond mere seasoning, encompassing a diversity of applications such as enhancing the flavor of dips, tomato-flavored dishes, creamy pasta and various squashes (Miele *et al*., 2021). Furthermore, its incorporation into recipes provides valuable shelf-life stability properties, contributing to the preservation of nutrient-dense perishable goods (Zahran *et al*., 2020).

To discover plant varieties with elevated antioxidant levels within this species, a breeding program is necessary to broaden its genetic diversity. The application of induced mutation serves as a breeding technique to enhance the genetic foundation in plant species. Following this process, individuals exhibiting heightened levels of desirable traits are subsequently observed. The experiment aims at using induced mutation to create genetic variation in *Ocimum basilicum* L. and then select for plant individuals with high levels of antioxidant potential, phenolic, flavonoid concentration and reducing power activity. Medicinal plant breeding has been given subdued priority. The present investigation endeavors to initiate and pave the way for advancing medicinal plant breeding practices.

Materials and Methodology

The seeds of *Ocimum basicilicum* L. (CIM- Saumya variety) were obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP, CSIR) in Lucknow, Uttar Pradesh (Lal, 2003). Subsequently, they underwent treatment with the chemical mutagen EMS at varying concentrations. These experiments were conducted in Department of Botany, School of Basic and Applied Sciences and at Agriculture field of School of Agricultural Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun. Dehradun is situated at latitude $30^{0}316'$ N and longitude $78^{0}03219'$ E, covering an area of 300 sq. Km with an elevation of 650 m above the sea level. The average annual rain fall is 2073 mm in Dehradun city.

Treatment

Seeds were pre-soaked in distilled water for a duration of 3 hours then treated with EMS at concentrations of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0 %. Each concentration of EMS was prepared in distilled water measuring 100 mL with Dimethyl sulphoxide (DMSO) and 100 seeds (M0) were treated for 1, 2, 3, and 4 hours separately to increase the probability of generating mutants. LD_{50} of EMS treatment was calculated to induce mutation on a large scale in *Ocimum basilicum* L.

Preparation of field and planting of treated seeds

The land was properly prepared by ploughing and application of farm yard manure and mutagen treated seeds alongside control was sown individually under suitable agro-climatic conditions to raise the M1 generation. 100 treated seeds from each concentration and treatment time combination were planted individually in raised beds of 1 m × 4 m size in a field of 10 - 15 cm height with irrigation channels. A fine layer of soil was used to cover the seeds. This treated population was designated as M1 population. 100 untreated seeds of CIM- Saumya variety of *Ocimum basilicum* L. were planted in separate block for comparison of survival mutants in the field.

Preparation of extract

After the screening of variability, collect and dry the leaves of the selected M1 generation plants in the shade. The plant sample were dried, crushed and

grinded into powder form and extracted with 150ml of ethyl alcohol solvent for 48 hours. After two days, the extract was filtered and evaporated on the water bath till it was reduced to get dry extract. The extract was then transferred to airtight container which was previously weighed and kept in the refrigerator until it was assessed for their phyto-constituents and antioxidant activity.

Phytochemical analysis

Chemicals obtained or derived from plants sources, such as betacarotene, that occur naturally in plants are called phytochemicals. The term is commonly employed to describe chemicals that might impact health but have not been conclusively identified as essential nutrients. Alkaloids, flavonoids, glycosides, saponins etc. are some groups of phyto-chemicals. They fulfill diverse functions within plants, exhibiting biological activity and serving in the treatment of various ailments. The ethanol extracts of plant sample with different chemicals were subjected to preliminary qualitative phytochemical investigation. The various tests performed were given below:

Test for protein

The test solution was prepared by dissolving the extract in distilled water.

- **Biuret test:** The test solution was treated with 40% NaOH and $CuSO_4$ solution, resulting in the formation of a blue color.
- **Xanthoproteic test:** The test solution was treated with concentrated HNO_3, followed by the addition of ammonia, resulting in the formation of a yellow color.

Test for amino acid: Test solution was prepared by dissolving the extract in distilled water.

- **Ninhydrin test:** Test solution was added with N inhydrin solution and boils it, violet colour was obtained.

Test for carbohydrates

Prepare test solution by taking the extract and adding water along with 1 volume of 1N HCl.

Molisch's test

The test solution, a gradual addition of 1 ml of concentrated H_2SO_4 from the sides, resulting in the formation of a purple ring at the junction of the two layers.

Test for glycoside

Prepare the test solution by mixing the extract in alcohol.

- **Bromine water test**

By adding the test solution with bromine water, yellow precipitate was obtained.

Test for tannin and phenolic compounds

Test solution was prepared by adding the extract with alcohol.

- **Lead acetate solution**

By adding the test solution with lead acetate solution, white precipitate was obtained.

- **Acetic acid solution**

Adding the lead acetate solution to the test solution resulted in the formation of a white precipitate.

Test for flavonoids

Test solution was prepared by adding the 2MHCl with leaf extract heat it at 100^0C for 30-40 min. after it cool and ethyl acetate.

Alkaline test

By adding the test solution with NaOH solution, yellow color was obtained, for decolourization added dil. HCl.

Test for saponin

Vigorously mix 5.0 ml of distilled water with an aqueous crude plant extract in a test tube, and combine the resulting froth with a few drops of olive oil. This will exhibit a distinctive foam appearance indicative of the presence of saponins.

Determination of total phenolic content

The total phenolic content was determined using Folin-Ciocalteu reagent as per the method described by Singleton and Rossi (1965) with some modifications. 0.1 ml of sample and 50µl of 2N Folin-Ciocalteu reagents was added to a 5 ml volumetric. The solution was mixed and allowed to stand for 3-5 minutes at room temperature. Next, 0.3 ml of 20% sodium carbonate solution (w/v) was added, and the solution was mixed and kept aside for 15 minutes. Finally, after the addition of 5ml distilled water the blue colour was measured against blank at 725nm using a UV-spectrophotometer. The total

phenolic content of the samples was determined by comparison with the optical density values of different concentrations of the standard phenolic compound gallic acid. Each sample was analysed in triplicate, and a calibration curve of gallic acid was constructed by plotting absorbance versus concentration. The total phenolic content was expressed as gram of gallic acid equivalents (GAE) per 20 gm extract.

Determination of total flavonoid content

The total flavonoid content was determined by aluminum chloride ($AlCl_3$) method using quercetin as a standard. The extract (0.25 ml each) was mixed. with 1.25 ml double distilled water which was followed by addition of 75 µl of 5% $NaNO_2$ (Oriakhi *et al*., 2014). This reaction mixture was incubated for 5 minutes at room temperature and then 0.15ml of 10% $AlCl_3$ was added. The mixture was treated with 0.5ml of 1mM NaOH after incubation of 6 minutes at room temperature. Finally, the reaction mixture was diluted 5ml of double distilled water followed by an incubation of 20 min at room temperature. The absorbance was measured at 510 nm. The flavonoid content was calculated from a quercetin standard curve. The total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of samples.

DPPH radical scavenging assay

The scavenging activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The plant extract was redissolved in 70% ethanol. The 5 ml assay mixture contained 3.98 ml methanol, 20 µl extract (50 µl, 100 µl, 150 µl, 200 µl) and 1ml DPPH (0.15mM in methanol). After incubation at room temperature for 30 minutes, the decrease in absorbance was measured at 517nm using a spectrophotometer. Ascorbic acid was employed as reference. The IC50 value indicates the concentration of tested sample required to free radical concentration by 50%. The experiment was performed in triplicate.

DPPH scavenging activity (%) =A0-A1/ A0×100

Reducing power activity of different treated samples of *Ocimum basilicum* L.

The reducing power of the sample was determined by the Oyaizu (1986) method with some modification. Reducing power activity is based on the reduction of ferric cyanide (Fe^{3+}) in stoichiometric excess relative to the amount of antioxidants (Benzie and strain, 1996). Sample (50 µl) with different concentrations were mixed with 0.5 ml of 0.2M phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferric cyanide (w/v) and incubated at 50 °C for 20 minutes. After incubation, 2 ml of 10% trichloroacetic acid (w/v) were added to the mixture, followed by the centrifuge at 3000 rpm for 10 minutes. The upper layer (2.5 ml) was mixed with 2 ml of deionized water and 0.5 ml of 0.1% ferric cyanide (w/v), and the absorbance of the resultant solution was measured at 700 nm. Ascorbic acid was used as reference.

Extraction of oil

The essential oil was extracted from 100 g of fresh leaves of all selected mutant plants by hydro-distillation method using a Clevenger apparatus. The extracted essential oil was stored at 4 ºC in dark airtight bottles for oil profiling.

Oil profile through Gas Chromatography and Mass Chromatography

The analysis involved utilizing an Agilent Technology 8890 gas chromatograph with a 7693A Auto sampler data handling system. This system was equipped with a split/splitless injector and featured FID, using N2 as the carrier gas, for both Mass Spectrometry and GC analyses. A HP-5 capillary column (30 m × 0.32 mm x 0.25 µm film thickness) was employed in the chromatographic analysis. The temperature program consisted of an initial temperature of 60°C (held for 2 minutes), followed by a programmed increase at a rate of 3 °C/min until reaching a final temperature of 240 °C (held for 5 minutes). The injector and detector temperatures were maintained at 210 °C and 250 °C, respectively. The injection volume was 0.5 µl. The analyses of the oils were conducted using an Agilent Technology 8890 gas chromatograph with PAL RSI 85 Auto sampler. The system was equipped with a split/splitless injector (split ratio 1:50) and a data handling system for gas chromatography-mass spectrometry (GC-MS). The column was HP-5MS UI capillary columns (30 m × 0.25 mm x 0.25 µm film thickness). The carrier gas used helium, flowing at a rate of 0.080 ml/min. The gas chromatograph (GC) was connected to the mass detector (Agilent GC-MS/MS_7010 B system), which operated in the EI + mode. The temperature program employed was identical to the one detailed previously for GC analysis. Maintain the temperatures of the injector, transfer line, and ion source at 280 °C. Take mass spectra over m/z 40-450

amu to reveal the total ion current, utilizing an ionizing voltage of 70 eV. The process of identifying the components involved matching their recorded mass spectra with the MS libraries (NIST 2.3 and Wiley FFNSC) and relevant literature.

Statistical analysis

Mean (˜ *X*), Standard Error (SE), and Standard Deviation (SD), were done using R 3.1.0 and IBM SPSS statistics 20 to assess the intra- and inter-population (mutagen) variations in different quantitative traits.

Results and Discussion

Basil, commonly known as *Ocimum basilicum* L. (Lamiaceae), possesses various medicinal activities for treating headache, diarrhea, kidney malfunctions, diabetes, cancer, and several pharmacological activities (Naz *et al*., 2015). These activities primarily result from the presence of various chemically diversified constituents in it. After studying the available literature about basil importance revealing its phytoconstituents, antioxidant potential and essential oil for phytochemical analysis of ethanol and aqueous solvents method of inducing mutagen (Ethyl Methane sulphonate) was by studied. In this study, findings of crude extract yield, quantification of phenol, flavonoid, DPPH assay and reducing power activity and oil profiling have been mentioned under the following heads:

Phyto-chemical analysis and yield of crude extracts

In the present study, crude plant extract obtained from different treated sample of chemical mutagen showed colour variation in extract appearance varied from light green to dark green in ethanol and aqueous varied from light brown to dark brown. Total percentage of crude extract yield varied from 1.22% to 3.22% and in aqueous extract varied from 1.20 to 1.96%. The highest crude ethanol extract yield was in OB4a in treatment of 0.4% and in aqueous extract in OB8b in treatment of 0.8%. Whereas the lowest ethanol extract yield was in OB1c in treatment of 1% and in aqueous extract 1.20% in OB5b in

Table 1. Yield and appearance of crude extracts of aqueous and ethanol solvent of different dose concentration of EMS in *Ocimum basilicum* L. of M1 generation

Concentration	Samples	Appearance of the extract		Quantity of plant material (gm)		Weight of extract (gm)		Percentage Yield	
		Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract	Aqueous Extract	Ethanol Extract
Control	OB	Brown	Dark Green	50g	50g	0.63	0.81	1.26	1.62
0.1%	OB1a	Brown	Dark green	50g	50g	0.66	0.78	1.32	1.56
	OB1b	Brown	Dark green	50g	50g	0.79	0.69	1.58	1.38
	OB1c	Dark Brown	Green	50g	50g	0.62	0.56	1.24	1.12
	OB1d	Brown	Green	50g	50g	0.71	1.59	1.42	3.18
0.2%	OB2a	Dark Brown	Green	50g	50g	0.72	1.25	1.44	2.50
	OB2b	Dark Brown	Dark Green	50g	50g	0.99	1.34	1.98	2.68
	OB2c	Brown	Dark green	50g	50g	0.76	1.02	1.52	2.04
0.3%	OB3a	Brown	Dark green	50g	50g	0.79	0.76	1.58	1.52
	OB3b	Light Brown	Green	50g	50g	0.95	1.17	1.90	2.34
	OB3c	Light brown	Dark green	50g	50g	0.73	0.75	1.46	1.50
0.4%	OB4a	Dark Brown	Dark green	50g	50g	0.88	1.61	1.76	3.22
	OB4b	Dark Brown	Green	50g	50g	0.70	1.12	1.40	2.24
0.5%	OB5a	Light Brown	Green	50g	50g	0.63	0.99	1.26	1.98
	OB5b	Light Brown	Green	50g	50g	0.60	0.91	1.20	1.82
0.6%	OB6	Dark Brown	Green	50g	50g	0.73	1.18	1.46	2.36
0.7%	OB7a	Brown	Light green	50g	50g	0.81	1.04	1.62	2.08
	OB7b	Brown	Dark green	50g	50g	0.72	1.01	1.44	2.02
0.8%	OB8a	Dark Brown	Green	50g	50g	0.96	1.47	1.92	2.94
	OB8b	Light Brown	Dark green	50g	50g	0.98	1.11	1.96	2.22
0.9%	OB9a	Light Brown	Dark green	50g	50g	0.81	1.22	1.62	2.44
	OB9b	Dark Brown	Green	50g	50g	0.75	1.03	1.50	2.06
1.0%	OB10	Brown	Green	50g	50g	0.97	0.76	1.94	1.52

treatment of 0.5% (Table 1).

Phytochemical screening is important process to identifying different classes of phytoconstituents present in various parts of the plants which is useful for the synthesis of drugs, and the active components could be further taken for investigation and research. Ethanolic and aqueous extract of *O. basilicum* L. were initially screened for qualitative phytochemical test that confirms the presence of carbohydrate, protein, amino acid, phenol, flavonoid, tannin and saponin (Table 2). Findings of the present studies has been supported by the studies conducted by Gebrehiwot (2015) and Hamad (2017). In an earlier investigation involving aqueous extracts of *Ocimum basilicum* L., a plant commonly utilized in traditional medicine, scientists documented the existence of diverse phytoconstituents. These compounds were present in fluctuating quantities, spanning from elevated to minimal levels, encompassing alkaloids, saponin, flavonoids, steroids, terpenes, tannins, coumarins, and carbohydrates. These findings align with more recent research outcomes (Azam and Irshad, 2016; Sanni *et al*., 2008).

Phenolic compounds in plants enables them to function as reducing agents, providers of hydrogen, and quenchers of singlet oxygen (Zheng, 2001; Miliauskas *et al*., 2004). Present findings on the total phenolic content were assessed from the regression equation of the calibration curve ($y = 0.0012x + 0.0178$, $R^2 = 0.9916$) expressed in GAE as milligram per gram of extract (mg/GAE/g/ extract). The value of phenolic content in M1 generation was varied from 3.18-27.63 µg/ml in aqueous extract and 19.59-46.55 µg/ml in ethanol extract. The highest phenolic content was observed in M1 generation in aqueous OB1d and in ethanol Ob4a, while the lowest content of phenol content was found in M1 generation in aqueous OB5a and in ethanol OB3c (Table 3).

Total Flavonoid Content

One of the most diverse and widespread groups of natural compounds is represented by flavonoids. A wide array of chemicals and biological functions, encompassing properties such as radical scavenging, is possessed by these compounds, with such

Table 2. Preliminary phytochemical screening of crude extract of *Ocimum basilicum* L. of M1 Generation

Concentration	Mutants	Carbohydrates		Protein		Flavonoid		Amino acid		Phenol		Saponin		Tannin	
		Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et	Aq	Et
Control	OB	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.1%	OB1a	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB1b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB1c	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB1d	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.2%	OB2a	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB2b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB2c	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.3%	OB3a	+	+	+	+	+	+	+	+		+	+	+	+	+
	OB3b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB3c	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.4%	OB4a	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB4b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.5%	OB5a	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB5b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.6%	OB6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.7%	OB7a	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB7b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.8%	OB8a	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB8b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.9%	OB9a	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	OB9b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.0%	OB10	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: + and - sign indicate the presence and absence of compounds respectively, aq- aqueous, et- ethyl alcohol

properties being especially distinct for flavonols (Tung, 2007; Miliauskas *et al.*, 2004).

The content of flavonoid was evaluated from the regression equation of the calibration curve (y=0.0049x-0.0687; R^2 = 0.9925) expressed in QUE as milligram per gram of the extract (mg/GAE/g extract). The value of flavonoid content in M1 generation was varied from 0.92-6.68 µg/ml in aqueous extract and 9.12-34.93 µg/ml in ethanol extract. The highest content of flavonoid was observed in M1 generation in aqueous OB8a and in ethanol OB8a while the lowest content of flavonoid was found in M1 generation in aqueous OB9b and in ethanol OB2a (Table 3).

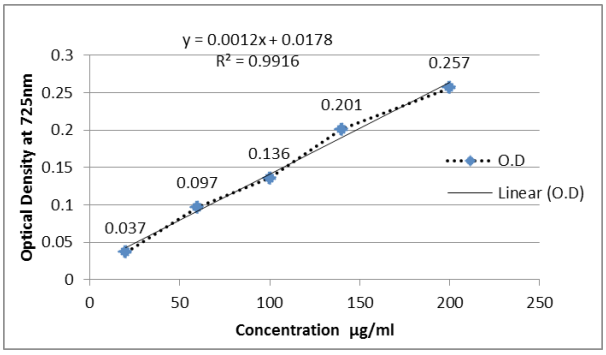

Fig. 1. Standard curve represents the concentration of gallic acid (µg/ml) against the absorbance

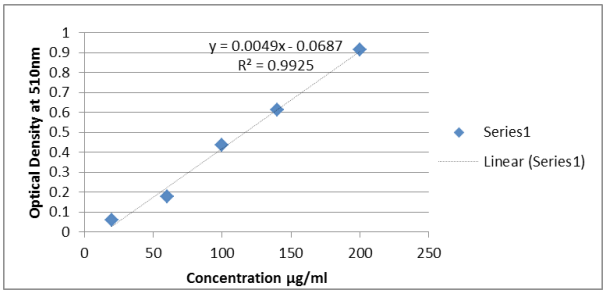

Fig. 2. Standard curve represents the concentration of quercetin (µg/ml) against the absorbance

DPPH radical scavenging assay

In the present investigation the scavenging effect of the extract was assessed by evaluating DPPH reduction against the positive control, ascorbic acid.The sensitive DPPH-stable free radical method is employed to assess the antioxidant activity of the plant extract (Koleva and Beek, 2002). A strong absorption maximum at 517nm is given by the odd electron in the DPPH free radical, and it is purple in colour

Table 3. Total Phenol and Flavonoid Concentration of different variants of *Ocimum basilicum* L. in M1 generation

Concentration	Mutants	Total Phenol content(mg GAE/g)		Total. Flavonoid content(mg QE/g)	
		Aqueous	Ethanol	Aqueous	Ethanol
0.1%	OB1a	15.0±0.07	28.1±0.35	4.5±0.42	23.6±0.52
	OB1b	11.0±0.09	27.3±1.15	3.1±0.48	19.2±0.0
	OB1c	9.2±0.08	32.2±0.41	1.8±0.15	24.4±0.27
	OB1d	27.6±0.03	33.8±0.50	2.61±0.51	25.0±0.13
0.2%	OB2a	23.7±0.29	26.3±0.50	3.83±0.51	9.12±0.49
	OB2b	14.8±0.51	41.8±0.81	4.63±0.49	11.9±0.50
	OB2c	11.8±0.02	23.7±0.55	4.02±0.09	23.2±0.55
0.3%	OB3a	22.2±0.37	0.18±0.08	6.08±0.51	33.6±0.49
	OB3b	24.2±0.03	41.9±0.72	5.64±0.48	29.5±0.33
	OB3c	17.8±0.18	19.5±0.26	5.46±0.51	31.1±0.49
0.4%	OB4a	27.8±0.18	46.5±0.55	6.18±0.41	32.6±0.50
	OB4b	20.9±0.83	36.4±0.45	3.93±0.05	20.0±0.16
0.5%	OB5a	3.1±0.52	38.8±0.35	5.35±0.60	22.6±0.15
	OB5b	6.2±0.60	40.6±0.52	3.01±0.50	24.4±0.49
0.6%	OB6	15.5±0.37	40.5±0.55	0.92±0.05	31.5±0.50
0.7%	OB7a	6.6±0.19	36.3±0.35	4.63±0.49	25.7±0.40
	OB7b	4.0±0.58	32.8±1.02	5.22±0.06	26.1±0.05
0.8%	OB8a	27.0±0.21	43.7±0.26	6.68±0.50	34.9±0.18
	OB8b	9.6±0.33	41.9±0.14	5.92±0.04	28.6±0.45
0.9%	OB9a	26.5±0.50	30.5±0.41	2.54±0.44	27.5±0.50
	OB9b	14.7±0.45	23.2±0.55	2.81±0.30	21.8±0.84
1.0%	OB10	8.1±0.48	26.4±0.49	5.10±0.55	9.18±0.49
Control	OB	11.7±0.10	29.7±0.44	5.10±0.55	21.1±0.13

(Sarla *et al.*, 2011). The DPPH free radical scavenging activity of *Ocimum basilicum* L. compared with the standard (ascorbic acid) by evaluating their antioxidant efficiencies, referred to as Inhibition concentration IC. The IC50 is the concentration at which 50% inhibition of free radical activity is observed for an antioxidant. The greater the overall effectiveness of the antioxidant of the mutant sample, the lower the IC50 number.

The value of IC50 of M1 generation mutants varied from 84.66 µg/ml - 496.7µg/ml in aqueous extract and 141.22 µg/ml – 219.22 µg/ml in ethanol extract and standard (ascorbic acid) shown in the

Table 4. DPPH activity of Mutants of *Ocimum basilicum* L. in M1 generation

Concentration	Mutants	DPPH Assay-IC50(µg/ml) in Aqueous Extract	DPPH Assay-IC50(µg/ml) in Ethanol Extract
		M1 Generation	M1 Generation
0.10%	OB1a	167.29	156.76
	OB1b	162.24	209.54
	OB1c	292.45	177.43
	OB1d	96.51	146.76
0.20%	0B2a	231.34	219.22
	OB2b	84.66	181.64
	OB2c	282.99	167.87
0.30%	OB3a	263.47	196.74
	OB3b	328.39	169.54
	OB3c	396.48	175.32
0.40%	OB4a	299.12	183.65
	OB4b	346.96	156.75
0.50%	OB5a	289.32	193.43
	OB5b	352.23	167.35
0.60%	OB6	333.11	183.76
0.70%	OB7a	376.27	152.45
	OB7b	465.25	201.34
0.80%	0B8a	245.5	189.49
	OB8b	403.34	196.63
0.90%	OB9a	496.7	182.47
	OB9b	311.42	141.22
1.00%	OB10	366.1	185.35
control	OB	237.36	208.56
Ascorbic Acid Standard		229	229

Table 4.

Reducing Power Activity of *Ocimum basilicum* L.

The determination of *Ocimum basilicum* L. extract revealed noticeable colour change, ranging from yellow to green and blue at 700nm. These changes depend upon the reducing power of the sample concentration. High absorbances indicates high reducing power of the resultant solution. In this assay, the reducing power exhibited a direct correlation with the absorbance of light in the reaction mixture. For the ethanol extract, the highest absorbance was noted in the 0.8% treatment, while the lowest was observed in the 1.0% treatment. In the case of the aqueous extract, the maximum absorption occurred in the 0.3% treatment, whereas the minimum was noted in the 0.1% treatment. The dose-response curve for the reducing power of the plant extract is depicted in Table 5.

Essential Oil Profile of *Ocimum basilicum* L.

On the basis of GC-MS analysis dominant chemical component were linalool and Methyl Chavicol whereas others Z-Citral, Geraniol, E-Citral, Trans-Caryophyllene, Cis- alpha Bisabolene, were the minor constituents. Linalool component was varied from 30.40 to 47.81%. 36.26 to 61.05 % Methyl Chavicol, 0.51 to 1.21% Z-Citral, 0.16 to 0.42% Geraniol, 0.85 to 1.98, 0.85 to 1.98 E-Citral, 0.29 to 0.50% Trans-Caryophyllene, 1.04 to 1.22 Cis- alpha Bisabolene were reported in selected mutant along with control.

Maximum linalool content was found in OB4a

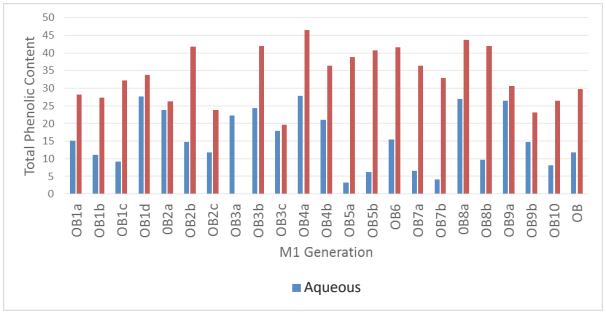

Fig. 3. Total phenolic content in M1 Generation

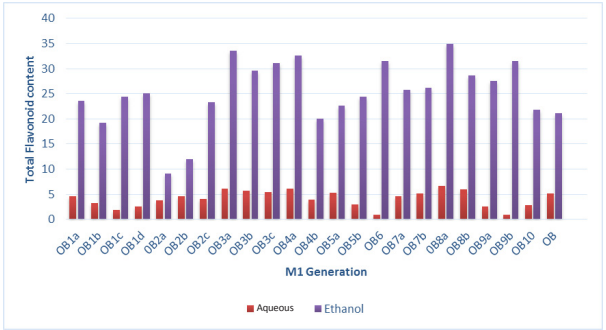

Fig. 4. Total Flavonoid content in M1 generation

Table 5 . The essential oil composition of selected mutants of *Ocimum basilicum* L.

S.No.	Component	(OB)(control)(In%)	(OB4a)(In%)	(OB8a)(In%)	(OB2b)(In%)
1.	Linalool	47.81	35.40	34.34	30.40
2.	Methyl Chavicol	36.26	55.53	54.15	61.05
3.	Z-Citral	1.21	0.92	0.94	0.51
4.	Geraniol	0.42	0.22	0.32	0.16
5.	E-citral	1.98	1.48	1.72	0.85
6.	Trans-Caryophyllene	0.50	0.38	0.33	0.29
7.	Cis- alpha Bisabolene	1.31	1.04	1.07	1.22

(35.40%) while the minimum in OB2b 30.40%) and in control 47.81%. Maximum chavicol content was found in OB2b (61.05%) while the minimum in OB8a (54.15%) and in control (36.26%). Maximum Z-Citral content was found in OB4a (0.92%) and minimum in OB2b **(**0.51%**)** and control (1.21%). Maximum Geraniol content was found in OB4a (0.32%) and minimum in OB2b (0.16%) and in control (0.42%). Maximum E-citral content was found in OB4a (1.48%) and minimum in OB2b (0.85%) and control (1.98%). Maximum Trans-Caryophyllene content was found in OB4a (0.38%) while minimum in OB2b (0.29%) and in control (0.50%). Maximum Cis- alpha Bisabolene content was found in OB2b (1.22%) while in minimum OB4a (1.04%) and along control (1.31%). As a result, EMS treatment increases methyl chavicol in comparison to control.

The investigation demonstrated that the action of the chemical mutagen EMS, administered at different doses, induces biochemical and metabolic disturbances. These disturbances result in a significant effect on the quantification of phenol, flavonoid, DPPH, and reducing power activity. EMS treatment has positive effects on yield contributing traits in *Ocimum basilicum.*

Conclusion

The outcomes of this study confirm presence of medicinally important chemical constituents in basil herb. The study encompassed both qualitative and quantitative analyses to determine the chemical components in the extracts from basil leaves. GC-MS analysis reveals the exact composition of different dominant chemical components of linalool and Methyl Chavicol whereas others Z-Citral, Geraniol, E-Citral, Trans-Caryophyllene, Cis- alpha Bisabolene, were the minor constituents. The findings indicates that the ethanol extract is the most suitable solvent compare to aqueous extract of the basil leaf, different doses of mutants of essential oil with high yield of chemical constituents observed. Further investigation will help to isolate mutants and characterize these bio active compounds. Exploring their pharmacological activities will lead to the contribution of the novel therapeutic drugs and mutagens.

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Conflict of Interest-The authors declare that there is no conflict of interest

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