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Analysis of biochemical responses in *Vigna radiata* varieties in vitro condition with medicinal plant extracts and their possible amelioration

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Abstract
The response of medicinal plant extracts in *Vigna radiata* L. wilzeck (in terms of physiological and Biochemical level) is shown *in Vitro* condition to become more efficient for human being. In *Vigna radiata* L. wilczek we use *Ocimum sanctum* L., *Calotropis procera* Ait. Ait (F), *Astragalous tribuloides* delile because these medicinal plants are easily available and has many medicinal properties. We use different extracts of these medicinal plants these extracts are alcoholic extract, acidic extract and alkaline extract. Among these extracts *Ocimum sanctum* L. alcoholic extract has great efficacy of bioactive compounds on SML-668 variety of mungbean. The pulse crop *Vigna radiata* L. is an important protein and mineral source that is grown in all over India. It also plays an important role in sustaining soil fertility by fixing atmospheric nitrogen. However, the productivity of mungbean is increased. In the present study, we use three varieties of *Vigna radiata* L. wilczek viz., IPM-02-03, RMG-492 and SML-668 is widely grown in northern India. We were evaluated for medicinal plant extracts response at early growth stage. These medicinal plants shows deterimental effect and modulation of several metabolic components in several biochemical parameters like cholorophyll content, phenolics and antioxidant enzymatic responses of superoxide dismutase, peroxidase were observed in extracts treatment.

Keywords: vigna radiata, vitro condition, *Ocimum sanctum* L., *Calotropis procera*

1. Introduction
The mungbean (*Vigna radiata*) is alternatively called moongbean, greengram, golden gram, Oregon pea, chickasano pea, chiroko lentil is a plant species in legume family (Purseglove, 1977; Sinha, 1977; Duke, 1983) [1, 2, 3]. Mungbean possesses antidiabetic properties and helps in reducing blood glucose levels, glycagon, triglycerides, plasma-c peptides and cholesterol. An important feature of the mungbean crop has high yielding potential of nutrient elements are needed for adequate plant growth, production to maintain their physiological and metabolic processes by using *Ocimum sanctum L*., *Calotropis procera*, and *Astragalous tribuloides* Delile. The extracts of *Ocimum sanctum L*. is used in the treatment of epilepsy, asthma or dyspnea, hiccups, skin, parasitic infection, neuralgia, headache (Chopra, 1993) [4] It is also used in gastric and hepatic disorders (Hebber S.S, Hursha V., 2004 *et al*) [5]. It is also called "Elixir of Life" or called as "Queen of Plants" means "the incomparable one" or "matchless one" because of its healing powers for the treatment of bronchitis, rheumatism and pyrexia (Nadkami K., 1982) [6]. *Calotropis procera* (Ait.) Ait f extracts has anticancerous properties in tumour cell lines (Umar *et al*. 2003; Choedon *et al*. 2006; Teicher, 2002) [7, 8, 9]. Its latex is used in cure of leprosy hairfall, toothache, dprosy, eczema and diarrohoea (Herbal monograph., 2002) [10]. Its extract is mostly used in relax of muscles of uterus or to increase uterine contractility in mothers to facilitate the safe childbirth or to induce abortion in women (Attah AF *et al*. 2012) [11]. *Calotropis procera* show allelopathic effects to replace *Chenopodium album*, *Melilotus alba*, *Melilotus induca*, *Saphaeranthus indicus* and *Phalaris minor* (Oudhia P, Tripathi RS., 1995) [12] In case of *Astragalous tribuloides* extracts have antiviral, anti inflammatory properties and regulate immune responses of body (Saleem S. 2013) [13]. It also helps to reduce the risk of liver cancer and prevent the diabetes (Dineva I, Krasteva I. 2010) [14]. They affect the anticancerous cell lines (HepG2) i.e. Hepatic carcinoma cell line, (5637) Human Bladder carcinoma and (L929) i.e. Mice fibroblast normal cell line. So, these medicinal plants extract help to give resistance of disease in human being with the help of these extracts.
The aim of the present work is to screen the varieties of *Vigna radiata* (IPM02-03, RMG 492 and SML-668) which are grown in Rajasthan for biochemical response *in Vitro* condition with medicinal plant extracts. This study will provide a theoretical basis for improving the *Vigna radiata* variety with the help of medicinal plant extracts *in Vitro* condition.

2. Materials and Methods

2.1 Preparation of extracts from leaves of the donor plants

Alcoholic Extract: 0.2 gm of leaves samples were crushed in 1 ml of 80% aqueous methanol. The samples were centrifuged at 5000rpm for 10 minutes and supernatent was collected which is concentrated with vaccum concentrator.

Acidic Extract: 1gm of leaves was boiled in 0.2 M HCL for 25-30 minutes. It was filtered with minutes. It was filtered with muslin cloth and separated out with ethyl acetate. Shake well and kept it for five minutes and concentrate with vaccum concentrator, this separation is done three times with ethyl acetate. Finally, it was dissolved in 80% aqueous methanol.

Alkaline Extract: 0.2 gm of the leaves was boiled in 0.2 M HCL for 25-30 minutes centrifuged it at 5000rpm for 10 minutes. Pellets kept in 2M NaOH for overnight. Then, again centrifuged it at 5000 rpm for 10 minutes. Filtered it with muslin cloth an adjust its pH 2.0 with concentrated 1 N HCL and separate it out with ethyl acetate and finally dissolve it in 80% methanol.

2.2 Planting material and Procedure

Uniformly mixed field soil was filled in well labelled pots with 19.4 cm and diameter 3.8cm. The varieties of genus *Vigna radiata* selected for the experiments viz, IPM02-03, RMG 492, SML-668 were obtained from Krishi Vigyan Kendra, Banasthali, Rajasthan.

Seeds of each variety were soaked in distilled water for 24 hr and imbibed in different medicinal plant extracts for 48 hr and kept in plant growth chamber in order to allow them to germinate. After 48 hr of soaking, seeds were transferred to autoclaved petridishes by using sterilized forceps having wet double layered filter paper. Petridishes were kept in plant growth chamber for providing suitable conditions for germination.

The plants were grown in pots for 21 days till the appearance of the second tri-foliate leaf (21 DAS). A set of biochemical experiment were done with the control plants. The plants were then subjected to different medicinal plant extracts for the next seven days.

2.3 Estimation of Chlorophyll Content

Fresh leaves (0.1 gm) were homogenized in cold conditions with 2 ml of chilled 80% aqueous acetone for the estimation of Chlorophyll pigments (Chlorophyll a, Chlorophyll b and Total Chlorophyll content) and determined spectrometrically following the method of Arnon *et al*., 1949 [15] The extract was centrifuged at 10000rpm at 4°C, diluted and the extinct coefficient of the supernatant was measured at two wave lengths of 663.645 nm using a UV-vis spectrophotometer (EC-UV2202SS).

2.4 Estimation of Phenolics

Total Phenolic Content was measured following Folin-Ciocalteau method with modifications (Singleton *et al*., 1999; Chakraborty *et al*., 2008) [16, 17] and expressed as gallic acid equivalent (GAE). Leaves were homogenized with 80% ethanol and the homogenate was used for the extraction and estimation of total phenolic content.

2.5 Antioxidant Enzyme assay (Protein, Superoxide dismutase, Catalase, Guiacol peroxidase

For the estimation of different enzymes, extraction was performed in extraction buffer prepared by dissolving 1mM EDTA, 2% PVP, 0.05 %Triton-X-100 and 1Mm ascorbic acid in phosphate buffer (50 mM, pH-7). Leaves were homogenized with extraction buffer in pre chilled mortar pestle and centrifuged at 10000 rpm for 20 minutes. The supernatent thus obtained was used for the estimation of different enzymes.

Protein was estimated by the Bradford method (1976). Absorbance was taken at 595nm with the help of spectrophotometer and estimation of protein done by comparison with a standard curve of BSA.

Superoxide dismutase, SOD (E.C.1.15.1.1.) activity was determined by measuring the inhibition of photo reduction of NBT (Nitroblue tetrazolium) by the method given by Beauchamp *et al*., 1971. One unit of the enzyme activity is defined as the amount of enzyme required to inhibit the photoreduction of NBT by 50%.

Catalase, CAT (E.C.1.11.1.6) activity was determined spectrophotometrically by measuring the rate of disappearence of H_2O_2 at 240nm; taking extinction coefficient of $3.4Mm^{-1}cm^{-1}$ (Miyagawa *et al*., 2000) [19]. Peroxidase (E.C.1.11.1.7) activity was determined spectrophotometrically by measuring the breakdown of H_2O_2 using guaiacol as a substrate following the method of (Kar and Mishra 1976) [20]. The activity of peroxidase and catalase is expressed in terms of nkat mg^{-1} protein. The activity of superoxide dismutase is expressed as U mg^{-1} protein.

2.6 Statistical Analysis

For the experimental set up, a randomized block design was used. The data are represented as mean± Standard deviation of three biological replicates wherever applicable. Analysis of Variance (ANOVA)-Duncan multiple range test (DMRT) was conducted to detect significant differences between means ($p< 0.05$) using SPSS software (20.0, SPSS Inc.).

Table 1: Effect of *O. sanctum* L. Extracts on Chlorophyll Content (mg/g) in *V. radiata* plant (IPM-02-03, RMG-492, SML-668).

S. No	Treatment	Duration (h)	Chlorophyll a	Chlorophyll b	Total Chlorophyll
	Control	24	1.122 ± 0.015	0.625 ± 0.008	2.623 ± 0.019
		48	1.133 ± 0.007	0.654 ± 0.009	2.687 ± 0.013
		72	1.158 ± 0.010	0.687 ± 0.012	2.719 ± 0.015
		96	1.162 ± 0.009	0.701 ± 0.009	2.761± 0.020
		Sig., LSD	0.004, 0.095	0.000, 0.085	0.000, 0.150
	Ocimum sanctum L. (Alcoholic extract)	24	1.075 ± 0.014	0.662 ± 0.012	2.053 ± 0.010
		48	1.041 ± 0.013	0.634 ± 0.011	2.044 ± 0.011
IPM-02-03		72	1.003 ± 0.009	0.607 ± 0.008	2.031 ± 0.010
		96	0.992 ± 0.009	0.594 ± 0.007	2.016 ± 0.005
		Sig., LSD	0.000, 0.103	0.000, 0.087	0.005, 0.081
	Ocimum sanctum L. (Acidic extract)	24	0.783 ± 0.006	0.472 ± 0.009	1.562 ± 0.012
		48	0.745 ± 0.012	0.413 ± 0.006	1.556 ± 0.014

		72	0.733 ± 0.012	0.383 ± 0.011	1.544 ± 0.007
		96	0.717 ± 0.007	0.373 ± 0.011	1.531 ± 0.010
		Sig., LSD	0.000, 0.085	0.000, 0.084	0.035, 0.098
	Ocimum sanctum L. (Alkaline extract)	24	0.672 ± 0.010	0.312 ± 0.011	1.453 ± 0.007
		48	0.652 ± 0.008	0.303 ± 0.010	1.438 ± 0.005
		72	0.621 ± 0.007	0.274 ± 0.011	1.432 ± 0.011
		96	0.615 ± 0.009	0.241 ± 0.009	1.420 ± 0.004
		Sig., LSD	0.000, 0.067	0.000, 0.093	0.003, 0.065
		Sig., LSD	0.032, 0.099	0.001, 0.074	0.001, 0.087
S. No	**Treatment**	**Duration (h)**	**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophyll**
Vigna radiata cv. RMG	Control	24	1.341 ± 0.018	0.703 ± 0.007	2.733 ± 0.020
		48	1.396 ± 0.011	0.732 ± 0.012	2.800 ± 0.016
		72	1.416 ± 0.009	0.767 ± 0.013	2.850 ± 0.028
		96	1.436 ± 0.008	0.793 ± 0.008	2.903 ± 0.011
		Sig., LSD	0.000, 0.108	0.000, 0.092	0.000, 0.178
	Ocimum sanctum L. (Alcoholic extract)	24	1.153 ± 0.010	0.732 ± 0.012	2.242 ± 0.010
		48	1.130 ± 0.009	0.7120± 0.011	2.235 ± 0.010
		72	1.114 ± 0.006	0.695 ± 0.009	2.215 ± 0.014
		96	1.096 ± 0.010	0.675 ± 0.006	2.204 ± 0.006
		Sig., LSD	0.000, 0.078	0.000, 0.085	0.007, 0.092
	Ocimum sanctum L. (Acidic extract)	24	0.826 ± 0.009	0.495 ± 0.007	1.736 ± 0.013
		48	0.812 ± 0.012	0.468 ± 0.008	1.720 ± 0.009
		72	0.801 ± 0.007	0.451 ± 0.010	1.711 ± 0.006
		96	0.785 ± 0.007	0.443 ± 0.014	1.706 ± 0.007
		Sig., LSD	0.003, 0.081	0.001, 0.089	0.014, 0.080
	Ocimum sanctum L. (Alkaline extract)	24	0.754 ± 0.011	0.387 ± 0.010	1.665 ± 0.011
		48	0.746 ± 0.011	0.364 ± 0.009	1.654 ± 0.012
		72	0.733 ± 0.009	0.353 ± 0.009	1.632 ± 0.009
		96	0.722 ± 0.012	0.338 ± 0.004	1.617 ± 0.007
		Sig., LSD	0.032, 0.099	0.001, 0.074	0.001, 0.087
S. No	**Treatment**	**Duration (h)**	**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophyll**
	Control	24	1.833 ± 0.020	0.974 ± 0.017	2.964 ± 0.012
		48	1.897 ± 0.009	1.004 ± 0.006	3.024 ± 0.021
SML-668		72	1.913 ± 0.008	1.023 ± 0.008	3.062 ± 0.016
		96	1.945 ± 0.013	1.044 ± 0.009	3.095 ± 0.010
		Sig., LSD	0.000, 0.118	0.000, 0.098	0.000, 0.137
	Ocimum sanctum L. (Alcoholic extract)	24	1.218 ± 0.009	0.805 ± 0.008	2.417 ± 0.012
		48	1.202 ± 0.007	0.793 ± 0.010	2.406 ± 0.009
		72	1.193 ± 0.008	0.764 ± 0.009	2.396 ± 0.008
		96	1.184 ± 0.010	0.743 ± 0.010	2.375 ± 0.014
		Sig., LSD	0.007, 0.079	0.000, 0.081	0.008, 0.098
	Ocimum sanctum L. (Acidic extract)	24	0.983 ± 0.011	0.569 ± 0.012	1.943 ± 0.016
		48	0.964 ± 0.011	0.544 ± 0.009	1.924 ± 0.012
		72	0.956 ± 0.010	0.535 ± 0.015	1.915 ± 0.007
		96	0.944 ± 0.013	0.511 ± 0.009	1.898 ± 0.004
		Sig., LSD	0.016, 0.101	0.002, 0.101	0.006, 0.097
	Ocimum sanctum L. (Alkaline extract)	24	0.864 ± 0.009	0.474 ± 0.015	1.855 ± 0.013
		48	0.833 ± 0.006	0.464 ± 0.008	1.840 ± 0.004
		72	0.826 ± 0.012	0.431 ± 0.010	1.822 ± 0.011
		96	0.820 ± 0.007	0.412 ± 0.011	1.806 ± 0.004
		Sig., LSD	0.001, 0.078	0.000, 0.099	0.001, 0.079

Table 2: Effect of *O. sanctum* extracts on H_2O_2 content, Total phenolics and SOD, CAT in *V. radiata* plants infected with *M. phaseolina*

S. No.	Treatment	Duration (h)	H_2O_2 umol/g f.wt.	Total Phenolics mg/ g f.wt	SOD U/mg	CAT Nkat/mg
Vigna radiata Cv.IPM-02-03	Control	24	7.006 ± 0.020	0.149 ±0.010	8.07 ± 0.023	0.129 ± 0.002
		48	7.087 ± 0.011	0.153 ± 0.011	8.123 ± 0.022	0.153 ± 0.005
		72	7.059 ± 0.014	0.164 ± 0.008	8.142 ± 0.019	0.175 ± 0.007
		96	7.038 ± 0.012	0.177 ± 0.011	8.170 ± 0.020	0.193 ± 0.006
		Sig., LSD	0.001, 0.131	0.031, 0.088	0.003, 1.906	0.000, 0.044
	Ocimum sanctum L. (Alcoholic extract)	24	12.759 ±0.104	0.186 ± 0.007	8.31 ± 0.084	0.263 ± 0.006
		48	13.861 ±0.080	0.281 ± 0.016	9.553 ± 0.065	0.339 ± 0.003
		72	13.240 ±0.080	0.229 ± 0.013	9.189 ± 0.088	0.310 ± 0.005
		96	12.992 ±0.179	0.206 ± 0.011	9.030 ± 0.023	0.280 ± 0.006
		Sig., LSD	0.000, 1.058	0.000, 0.109	0.000, 0.629	0.000, 0.044
	Ocimum sanctum L. (Acidic extract)	24	14.036 ±0.063	0.294 ± 0.015	9.743 ± 0.148	0.316 ± 0.006
		48	14.966 ±0.138	0.386 ±0.012	10.39 ± 0.156	0.400 ± 0.004
		72	14.719 ±0.067	0.336 ± 0.014	9.913 ± 0.101	0.363 ± 0.008
		96	14.340 ±0.076	0.300 ± 0.015	9.859 ± 0.119	0.328 ± 0.007
		Sig., LSD	0.000, 0.819	0.000, 0.124	0.001, 1.193	0.000, 0.058

	Ocimum sanctum L. (Alkaline extract)	24	14.927 ±0.048	0.353 ± 0.011	10.03 ± 0.114	0.392 ± 0.004
		48	15.844 ±0.082	0.434 ± 0.007	10.88 ± 0.095	0.479 ± 0.007
		72	15.423 ±0.069	0.405 ± 0.013	10.19 ± 0.064	0.419 ± 0.008
		96	15.057 ±0.057	0.381 ± 0.013	9.993 ± 0.091	0.409 ± 0.005
		Sig., LSD	0.000, 0.587	0.000, 0.102	0.000, 0.832	0.000, 0.052
S.NO.	**Treatment**	**Duration (h)**	**H_2O_2 umol/g f.wt.**	**Total Phenolics mg/ g f.wt**	**SOD U/mg**	**CAT Nkat/mg**
	Control	24	7.092 ± 0.032	0.165 ± 0.009	8.474 ± 0.051	0.182 ± 0.004
RMG 492		48	7.147 ± 0.012	0.172 ± 0.011	8.524 ± 0.016	0.197 ± 0.006
		72	7.108 ± 0.023	0.183 ± 0.009	8.541 ± 0.042	0.211 ± 0.007
		96	7.082 ± 0.018	0.198 ± 0.007	8.597 ± 0.008	0.224 ± 0.005
		Sig., LSD	0.033, 0.201	0.009, 0.79	0.015, 0.309	0.000, 0.049
	Ocimum sanctum L. (Alcoholic extract)	24	12.472 ±0.468	0.226 ± 0.009	9.18 ± 0.148	0.347 ± 0.007
		48	13.777 ±0.259	0.333 ± 0.016	9.92 ± 0.028	0.477 ± 0.006
		72	13.131 ±0.110	0.286 ± 0.007	9.44 ± 0.032	0.416 ± 0.007
		96	12.959 ±0.079	0.254 ± 0.012	9.22 ± 0.036	0.371 ± 0.003
		Sig., LSD	0.003, 2.477	0.000, 0.104	0.000, 0.708	0.000, 0.054
	Ocimum sanctum L. (Acidic extract)	24	13.771 ±0.135	0.328 ± 0.010	10.33 ± 0.211	0.420 ± 0.004
		48	14.851 ±0.162	0.425 ± 0.012	11.18 ± 0.096	0.508 ± 0.009
		72	14.546 ±0.103	0.364 ± 0.011	10.76 ± 0.28	0.454 ± 0.011
		96	14.048 ±0.063	0.348 ± 0.013	10.52 ± 0.086	0.425 ± 0.006
		Sig., LSD	0.000, 1.093	0.000, 0.101	0.003, 1.688	0.000, 0.072
	Ocimum sanctum L. (Alkaline extract)	24	14.171 ±0.144	0.451 ± 0.019	11.17 ± 0.125	0.520 ± 0.009
		48	15.253 ±0.070	0.573 ± 0.014	11.77 ± 0.251	0.624 ± 0.008
		72	15.101 ±0.048	0.514 ± 0.009	11.51 ± 0.013	0.547 ± 0.006
		96	14.329 ±0.326	0.483 ± 0.007	11.22 ± 0.090	0.505 ± 0.006
		Sig., LSD	0.000, 1.646	0.000, 0.119	0.004, 1.322	0.000, 0.069
S.NO.	**Treatment**	**Duration (h)**	**H_2O_2 umol/g f.wt.**	**Total Phenolics mg/ g f.wt**	**SOD U/mg**	**CAT Nkat/mg**
SML-668	Control	24	8.059 ± 0.037	0.189 ± 0.009	9.025 ± 0.030	0.267 ± 0.017
		48	8.165 ± 0.013	0.191 ± 0.004	9.088 ± 0.008	0.278 ± 0.003
		72	8.083 ± 0.017	0.206 ± 0.013	9.107 ± 0.002	0.299 ± 0.006
		96	8.047 ± 0.023	0.212 ± 0.005	9.115 ± 0.004	0.314 ± 0.005
		Sig., LSD	0.001, 0.219	0.024, 0.076	0.000, 0.144	0.001, 0.085
	Ocimum sanctum L. (Alcoholic extract)	24	10.693 ± 0.514	0.299 ± 0.016	10.407 ± 0.56	0.417 ± 0.116
		48	11.713 ± 0.405	0.360 ± 0.027	11.239 ± 0.10	0.461 ± 0.013
		72	11.150 ± 0.071	0.312 ± 0.017	11.08 ± 0.037	0.433 ± 0.012
		96	10.494 ± 0.346	0.306 ± 0.014	10.95 ± 0.060	0.418 ± 0.003
		Sig., LSD	0.016, 3.338	0.017, 0.172	0.034, 2.562	0.003, 0.093
	Ocimum sanctum L. (Acidic extract)	24	11.018 ± 0.084	0.401 ± 0.019	12.06 ± 0.049	0.518 ± 0.006
		48	12.026 ± 0.475	0.530 ± 0.012	12.82 ± 0.097	0.587 ± 0.006
		72	11.867 ± 0.134	0.513 ± 0.014	12.54 ± 0.254	0.536 ± 0.005
		96	11.314 ± 0.098	0.474 ± 0.011	12.21 ± 0.077	0.523 ± 0.004
		Sig., LSD	0.004, 2.291	0.000, 0.129	0.001, 1.289	0.000, 0.047
	Ocimum sanctum L. (Alkaline extract)	24	12.998 ±0.150	0.510 ± 0.016	14.04 ± 0.053	0.615 ± 0.008
		48	13.331 ± 0.127	0.644 ± 0.019	14.81 ± 0.107	0.705 ± 0.011
		72	13.153 ± 0.115	0.582 ± 0.011	14.25 ± 0.040	0.643 ± 0.007
		96	13.050 ± 0.080	0.543 ± 0.008	14.11 ± 0.093	0.625 ± 0.007
		Sig., LSD	0.041, 1.083	0.000, 0.126	0.000, 0.702	0.000, 0.073

3. Results and Discussions

Mungbean respond with different extracts of *Ocimum sanctum* L. acclimatize through various physiological and biochemical changes. But the alcoholic extract of *Ocimum sanctum* L. is highly active extract in SML-668 variety of Mungbean as comparison to other extracts of *Ocimum sanctum* L. This extract shows maximum amount of bioactive compounds.

All varieties of mungbean subjected to medicinal plant extracts in the present study shows a rapid increase of chlorophyll a content (Table 1).Incidentally, the content of chlorophyll b decrease the change significantly. Overall the total content of chlorophyll in the present study showed a increasing trend in SML-668. Which was statistically significant for the variety of SML-668 indicating that these photosynthetic pigments are sensitive.

A diverse response in phenolic content was observed in present study. High phenolic content is observed in SML-668 in extracts condition while in varieties IPM-02-03 and in RMG-492 *in Vitro* condition. There is essential metabolites pathway under extracts condition and mitigation maybe through the antioxidant enzymes. Phenolic compounds are secondary metabolites and have specific role in plant defense (Mandal *et al*., 2010) [21]. They are also known to have high antioxidant properties and thus, may be involved in reducing stress due to reactive oxygen species (Chakraborty *et al*., 2008, Jha *et al*., 2013) [17, 22].

Antioxidant defense mechanism plays an important role in enzyme activity. A statistically significant increase in superoxide dismutase enzyme activity was noted in 48 hr of all varieties of mungbean then it decrease in 72 hr. when we gave the extracts of *Ocimum sanctum* L under experiment. It is to be noted that SOD generates H_2O_2 which acts as signalling compound, but also it increases cell damage and has high level of H_2O_2 concentration in the plants.

Peroxidase activity remains increased in 48 hr then 72hr to maintain a balance between the ROS and antioxidant machinery which is essential to provide a proper biochemical environment within the cell. Under the extracts gave to the *Vigna radiata* L. the activity of CAT is found to increase in 48 hr of SML-668 variety of Mungbean. It shows the activating the detoxification mechanism. It functions to catalyze the decomposition of hydrogen peroxide to water and oxygen and this reaction is important because if the cells did

not breakdown the hydrogen peroxide, they would be poisoned and die. The activity of the catalase enzyme remains increased. The activities of superoxide dismutase, peroxidase and catalase are well known as protective mechanism. This type of modulation of the antioxidant response in the different varieties of Vigna under study effectively work towards the extracts of *Ocimum sanctum* L. as comparison to other extracts of medicinal plants.

4. Conclusion

In the present study, It was observed that the pulse cultivars studied significantly affected by extracts of *Ocimum sanctum* L. in terms reduction in chlorophyll and carbohydrate content, increase in lipid lipid peroxidation which indicates membrane damage and is most likely to be triggered by H_2O_2. The modulation of antioxidant enzyme activity in an effort to combat this extract of *Ocimum sanctum* L. was also observed. Increase phenolic compounds may also indicate a protective mechanism as they act as antioxidants in plant tissue.

During the extracts of *Ocimum sanctum* L. gave variations were noted in different varieties and the variety of SML-668 in 48 hr better than other time intervals and other varieties of mungbean. The present study would facilitate the application of optimal methods for increasing extracts condition of *Ocimum sanctum* L.

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6. References

1. Purseglove JW. Tropical Crops: Dicotyledons, Vols 1 and 2. The English Language Book Society and Loongman Publishers, London. 1977; 273-6, 290-4, 318-21.
2. Sinha SK. Food Legumes: Distribution, Adaptibility and Biology of Yield. FAO Plant Production and Protection Paper no.3. Food and Agriculture Organization of the United Nations, Rome, 1977.
3. Duke JA. Handbook of Legumes of World Economic Importance. Plenum Press, New York, 1983, 102-6:275-8, 293-6.
4. Chopra RN, Chopra IC, Handa KL, Kapoor LD. Indegenious drugs of India (Published by UN Dhar, Pvt. Ltd., Calcutta, 1993.
5. Hebbar SS, Hursha VH, Shripathi V, Hedge GR. Ethnomedicine of Dharwad district in Karnatka, India-plants in oral healthcare. Journal of Ethnopharmacology. 2004; 94:261-266.
6. Nadkami K, Nadkarni: Indian Materia Medica with Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home Remedies, Popular Prakashan Private Ltd., Bombay, India. 1982-1999, 2.
7. Umar A, Viner JL, Anderson WF, Hawk E. Development of COC inhibitors in cancer prevention and therapy. American Journal of Clinical Oncology. 2003; 26:S48-57.
8. Choedeon T, Mthan G, Arya S, Kumar VL, Kumar V. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. World Journal of Gastroenterology. 2006; 12:2517-22.
9. Teicher BA. Tumor models in cancer research. Humana press. New Jersey, 2002.
10. Himalaya. Herbal monograph, Himalaya herbal healthcare, 2002. www.himalayahealthcare/research paper/index.hlm.
11. Attah Alfred F, Margaret o' Brien, Christian Gruber W. Medical University of Vienna, Center for Physiology and Pharmacology, Schwarzspanierstr. 17, A-1090 Vienna, Austria Journal of Ethnopharmacology. 2012; 30:143(1):377-382.
12. Oudhia P, Kolhe SS, Tripathi RS. Allelopathic effect of *Ageratum conyzoides* on germination of linseed var. kiram: Weed News. 1995; (1-2):15-18.
13. Saleem S, Hafiz Muhamad Aslam, Maheen Anwar *et al*. Fahr's Syndrome: Literature review of current evidence. Orphat Journal of Rare Disease. 2013; 8:156.
14. Dineva I, Krasteva I, Berger M, Konstantinov S. *In Vitro* antineoplastic activity of some cytoreductive drugs versus new compounds of plant origin. International Journal of Current Chemistry. 2010; 1(4):281-290.
15. Arnon DI. Copper enzymes in isolated chloroplasts: polyphenol oxidases in Beta Vulgaris. Plant Physiology. 1949; 24:1-15.
16. Singleton VL, Orthofer R, Lamuela-Ravntos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteau Reagent. Method Enzymology. 1999; 299:152-178.
17. Chakraborty D, Mandal SM. Fractional changes in phenolic acids composition in root nodules of *Arachis hypogea*. Plant Growth Regulation. 2008; 55(3):159-163.
18. Bradford MM. A Rapid and sensitive method for the quatitation of microgram quatities of protein utilizing the principles of protein dye binding. Analytical Biochemistry. 1976; 72:248-254.
19. Miyagawa Y, Tamori M, Shigeoka S. Evaluation of the defense system in chloroplasts to photooxidative stress caused by paraquat using transgenic tobacco plant expressing catalase from Escherichia coli. Plant Cell Physiology. 2000; 41:311-320.
20. Kar M, Mishra D. Catalase, Peroxidase and Polyphenol oxidase activities during the leaf senesence, Plant Physiology. 1976; 57:315-319.
21. Mandal SM, Chakraborty D, Dey S. Phenolic acids act as signalling molecules in plant microbe symbioses. Plant Signaling and Behaviour. 2010; 5(4):359-368.
22. Jha B, Kumar M, Reddy CRK. The ameliorating effect of Acadian marine plant extract against ionic liquids-induced oxidative stress an DNA damage in marine *macroalga* Ulva lactuca. Journal of applied Phycology. 2013; 25(2):369-378.