JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

UTILIZATION OF MICROWAVE ASSISTED EXTRACTS OBTAINED FROM VARIOUS PARTS (WHOLE FRUIT, SEEDS, LEAVES AND ROOTS) OF CITRULLUS COLOCYNTHIS AS HYPOCHOLESTEROLEMIC AGENT IN ALBINO RATS

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doi: 10.15414/jmbfs.2021.10.4.541-545

ARTICLE INFO

Received 9. 6. 2020
Revised 23. 9. 2020
Accepted 24. 9. 2020
Published 1. 2. 2021

Regular article

OPEN ACCESS

ABSTRACT

The study was conducted to investigate the hypolipidemic properties of Microwave assisted extracts (MAE) obtained from different parts (whole fruit, leaves, seeds and roots) of *Citrullus colocynthis* on hyperlipidemic experimental rats. The trial consisted of 30 male rats that were divided into six groups each having 5 rats whereas, out of these 25 were hyperlipidemic and 5 were normal rats. After the induction of high cholesterol for 15 days, 20 rats were fed with microwave assisted extract of different parts at a concentration of 200mg/kg/B. W for 28 days. The administration of the extracts reported considerable reduction in the lipid profile parameters of the hypercholesterolemic rats. The MAE of seed of *Citrullus colocynthis* displayed the optimum results showing reduced levels of cholesterol, triglyceride and LDL-c by (10.33%), (22.50%) and (15.70%) while an elevation of (16.75%) in HDL-c content compared to the hypercholesterolemic control. Likewise, the percent increase in the concentrations of CAT and SOD by (6.92%) and (18.47%). The study concluded that the MAE of *Citrullus colocynthis* showed a positive effect on hypercholesterolemia thus providing therapeutic benefits.

Keywords: *Citrullus colocynthis*, Hyperlipidemia, Microwave Assisted Extracts, Cholesterol

INTRODUCTION

Medicinal plants have been known through centuries, due to its potential properties against different diseases and infections. Most of the time such plants are gigantic source of Antioxidants, Flavonoids, Phenolic compounds and Phytochemicals **(Demmig-Adams and Adams, 2002)**. These plants having medicinal properties have vital role throughout world in treating different ailments. As the synthetic medicines have been known to cause many side effects and less effective upon diseases, so the approach to cure diseases with natural medications has increased tremendously **(Nimruzi *et al*., 2013)**.
Citrullus colocynthis has been used as source of energy and remedy for many diseases since early times.it is commonly known as Colocynth, Bitter cucumber, Bitter melon. It is generally grown in desert regions of Asia and Mediterranean regions. Citrullus colocynthis looks like a vine having hard rind small fruits and leaves on it. Roots of the plant have been used to treat snake poison, urinanry tract infection, jaundice, pains in bones and diseases related to eyes. Leaves of Citrullus colocynthis were helpful in therapy for asthma, diuretics and jaundice. Fruit and seeds of Citrullus colocynthis has anti diuretics, anthelmintic effect **(Shahid *et al.*, 2019)**. It also cures tumor, urinary tract infection, joints inflammation, ulcers and asthma diseases. Extracts obtained from different parts of this plant such as stem, leaves, roots, seeds or fruit have positive effect on different ailments **(Qureshi *et al*.,2010)**.
The extraction of components from different plants has been done by numerous techniques. Conventional and non-conventional techniques are practiced since centuries. Non-conventional methods have many benefits such as environmentally friendly, as it consumes less chemicals, less operational time and yields excellent quality of extract as compared to conventional methods **(Uma and Sekar, 2014)**. Microwave assisted extraction (MAE) is one of the unique methods for extracting materials form plants by the help of microwave rays/energy. Microwave assisted extraction technique as numerous advantages such as increased extraction of components from plants due to quick heating, increase extraction yield and small equipment utilized. Due to immense qualities it has been used to extract organic and organo-metallic components from various plants **(Alupului *et al*., 2012)**. By considering advantages of MAE, this study was conducted to work on extracts obtained from different parts (whole fruit, leaves, seeds and roots) of *Citrullus colocynthis* by microwave assisted extraction.

MATERIAL AND METHOD

Plant Material

Various parts of *Citrullus colocynthis i.e.* roots, leaves, seed, & whole fruit was procured from local market of Lahore. These parts were washed, and air-dried at room temperature. Collected roots, leaves, seeds and whole fruit was dried in oven drier at 50 ± 5^0C to minimize the moisture content. After that they were grinded to reduce the particle size in order to facilitate extraction.

Preparation of Extract

Extraction of Bitter melon roots, leaves, seeds & whole fruit was carried in an adapted commercial kitchen microwave oven. The maximum output of this oven was 700 W. In the MAE procedure, a 25g aliquot of Bitter melon roots, leaves, seeds & whole fruit powder was individually placed in a 250 ml round bottom flask; 25 ml of distilled water was added to moisturize for 30 min. The flask was connected to a Clevenger apparatus and heated at powers of 150 W for varied

extraction time 1, 5, 10 and 15 minutes respectively. The volatile distillate was eluted out by n-hexane and dried through anhydrous sodium sulphate. The n-hexane was removed under vacuum conditions and the extract was refrigerated prior to analysis **(Liu *et al.*, 2013).**

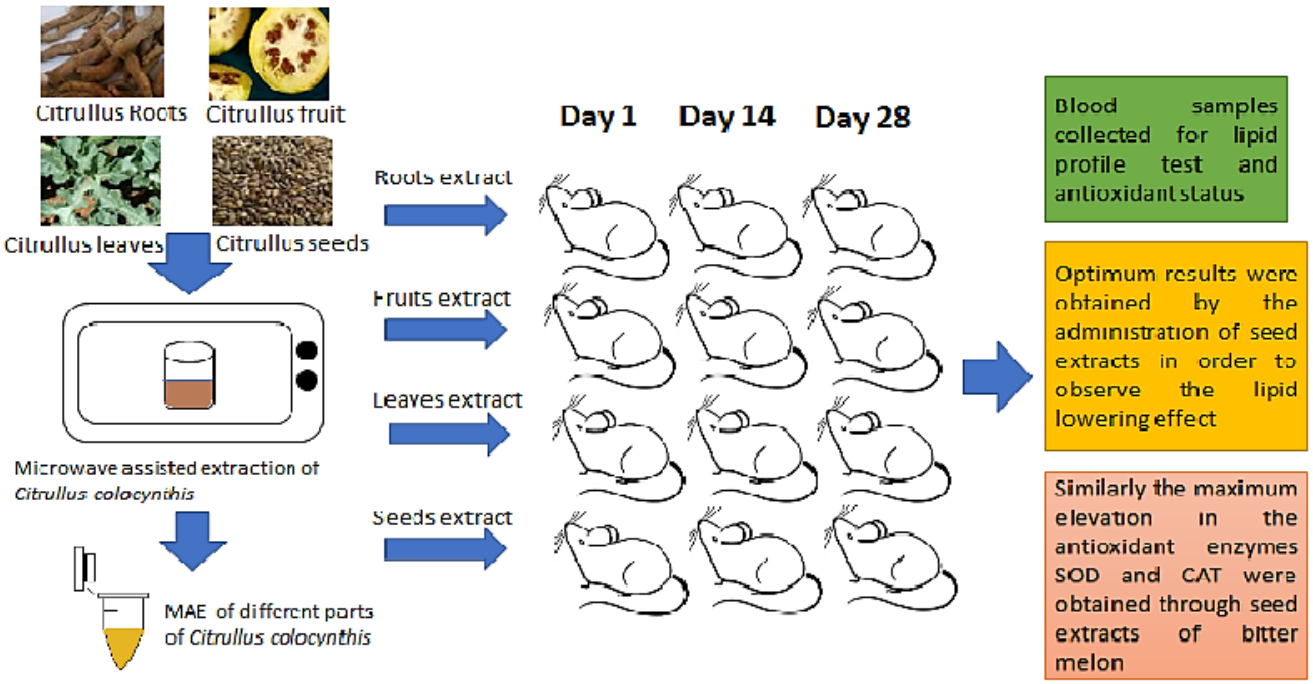

Figure 1 Graphical representation of experimental design. Microwave assisted extraction of different parts (roots, leaves, fruits and seeds) and their administration to the rats. Analysis of the blood samples after completion of study interval to verify the effect of each part in lowering of lipid

Experimental Animals

Male rats were purchased from animal house of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, having weight between 200g-250g. The rats were kept for 1 week on basal diet for acclimatization purpose. The environmental conditions were controlled throughout the trial like temperature (23 ± 2 °C) and relative humidity (55 ± 5%) along with 12-h light-dark period.

Induction of Hypercholesterolemia

Experimental hypercholesterolemic diet was prepared using corn oil (10%), corn starch (64.5%), cholesterol (1.5%), protein (10%), cellulose (10%), mineral (3%) and vitamins (1%). Groups were subjected to high cholesterol diet for first 15 days for induction of hypercholesterolemia. This was authenticated by examining their total cholesterol content at 15th day. Rats were anesthetized by exposure to isoflurane and the blood samples were collected through cardiac puncture **(Imran *et al.*, 2018)**.

Experimental design

30 rats were divided into groups, each comprising of 5 rats in them. Group N_0 normal control rats fed with their normal diet, Group C_0 was fed with only with high cholesterol diet. Group C_1 was administered with high cholesterol diet along with *Citrullus colocynthis* whole fruit extract. Group C_2 was administered with high cholesterol diet along with *Citrullus colocynthis* leaves extract. Group C_3 was administered with high cholesterol diet along with *Citrullus colocynthis* seeds extract. Group C_4 was administered with high cholesterol diet along with *Citrullus colocynthis* roots extract **(Kai *et al*.,2015)**.

Effect of extract on hyper cholesterol and safety assessment

Rats was anesthetized by exposure to isoflurane and the blood samples was collected in tubes by cardiac puncture and examined at 0 day (baseline trend), 15th day (post administration of cholesterol rich diet) and 21st day post induction of hypercholesterolemia along with administration of respective extracts to validate hypocholesterolemic effect **(Imran *et al.*, 2018)**.

Statistical analysis

Data were expressed as mean ±standard deviation and completely randomized design was conducted with two-way (Serum profiling & Antioxidant indices) ANOVA at a significance level of $p \leq 0.05$ **(Steel *et al.*, 1997)**.

RESULTS

Total Cholesterol

The statistical analysis demonstrated that there was significant ($p \leq 0.05$) effect of treatment and time intervals on the cholesterol concentrations of the rats. The effect of MAE obtained from different parts of *Citrullus colocynthis* on total cholesterol are mentioned in table 1. Results for the cholesterol content displayed highest percent reduction in treatment group C_3 (10.33%) followed by C_1 (9.84%), C_2 (9.26%) and C_4 (8.20%), respectively on the 28th day of administrating MAEs. In comparison to the control group Co (195.27±2.67 mg/dL), the cholesterol concentrations noted in treatment groups were 176.05±2.07 mg/dL (C_1), 177.18±2.38mg/dL (C_2), 175.08±1.83 mg/dL (C_3), and 179.24±2.87 mg/dL (C_4), respectively. The cholesterol content reduced from 187.38±2.99 mg/dL (0 day) to 180.63±2.20 mg/dL (14th day) and 173.49±1.81 mg/dL (28th day), respectively. The highest reduction was observed in in group C_3 as compared to C_0.

Total Triglycerides

The statistical analysis demonstrated that there was significant ($p \leq 0.05$) effect of treatment and time intervals on the triglyceride content of the hypercholesterolemic rats. The effect of microwave assisted extracts obtained from different parts of *Citrullus colocynthis* on total triglycerides have been displayed in figure 2. Results for total triglycerides observed in C_o (173.72±2.33mg/dL) was found to have reduced in C_1(137.96±3.21mg/dL), C_2 (139.09±2.10mg/dL), C_3 (134.33±1.09mg/dL) and C_4 (141.56±3.33mg/dL). The figure displayed a significant percent reduction in C_3 (22.50%) as compared to experimental group followed by C_1 (20.02%), C_2 (19.09%) and C_4 (18.18%) at the 28th day of administration of extracts.

Table 1 Effect of MAE of different parts of *Citrullus colocynthis* on Total Cholesterol

Parameter	Group	Days			Mean
		0 day	14th Day	28 day	
Cholesterol (mg/dL)	N_0	94.21±0.40	96.78±0.25	99.35±1.76	96.78±0.80
	C_0	185.34±2.23	194.60±2.66	205.88±3.12	195.27±2.67^{a}
	C_1	187.11±3.21	176.44±1.41	164.61±1.16	176.05±2.07^{bc}
	C_2	188.21±3.12	177.10±2.71	166.23±1.33	177.18±2.38^{bc}
	C_3	186.84±2.48	175.25±1.52	163.15±1.51	175.08±1.83^{c}
	C_4	189.40±3.94	179.74±2.74	168.59±1.95	179.24±2.87^{b}
	Mean	187.38±2.99^{a}	180.63±2.20^{b}	173.49±1.81^{c}	180.29±2.33

N_0: Normal group; C_0: Control group (HCD); C_1: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C_2: HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C_3: HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C_4:HCD+Microwave Assisted *Citrullus colocynthis* roots extract
HCD=High Cholesterol Diet

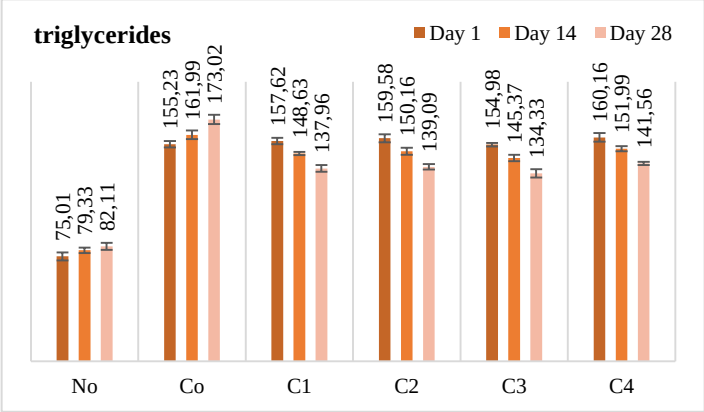

Figure 2 Effect of MAE of different parts of *Citrullus colocynthis* on Total Triglycerides
N_0: Normal group; C_0: Control group (HCD); C_1: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C_2:HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C_3:HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C_4:HCD+Microwave Assisted *Citrullus colocynthis* roots extract
HCD=High Cholesterol Diet

LDL

The Statistical analysis revealed that there was significant ($p \leq 0.05$) effect on treatments and intervals (0, 14 & 28 days) on LDL levels of experimental rats. The values of MAE from various parts of *Citrullus colocynthis* and their effect on total LDL levels have been mentioned in table 2. Results for the LDL content displayed peak reduction in treatment group C_3 (15.70%) followed by C_1 (13.95%), C_2 (11.77%) and C_4 (11.66%), respectively on the 28th day of administrating MAE. In comparison to the control group Co (113.76±2.01mg/dL), the highest reduction in LDL concentrations were observed in C_3 (95.27±2.48mg/dL) followed by C_1(97.89±1.57mg/dL), C_2(100.36±1.94mg/dL) and C_4(100.49±1.70mg/dL), respectively. Furthermore, the value for LDL level at 0 day was 106.20±1.87mg/dL which reduced to 101.44±1.66mg/dL at 14th day and to 97.03±1.94mg/dL at 28th day, respectively.

Table 2 Effect of MAE of different parts of *Citrullus colocynthis* on LDL

Parameter	Group	Days			Mean
		0 day	14th Day	28 day	
LDL	N_0	38.44±1.22	42.63±1.24	45.76±1.34	42.27±1.26
	C_0	108.11±2.13	112.93±1.39	120.25±2.52	113.76±2.01^{a}
	C_1	105.92±2.11	97.64±1.57	90.11±1.04	97.89±1.57^{c}
	C_2	107.56±1.75	101.49±2.18	92.04±1.91	100.36±1.94^{bc}
	C_3	102.49±1.28	95.12±2.19	88.22±2.27	95.27±2.48^{d}
	C_4	106.91±2.11	100.01±1.01	94.55±1.99	100.49±1.70^{b}
	Mean	106.20±1.87^{a}	101.44±1.66^{b}	97.03±1.94^{c}	

N_0: Normal group; C_0: Control group (HCD); C_1:HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C_2:HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C_3:HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C_4:HCD+Microwave Assisted *Citrullus colocynthis* roots extract
HCD=High Cholesterol Diet

HDL

The Statistical analysis revealed that there was significant ($p \leq 0.05$) effect on treatments and intervals on HDL of experimental rats. The values of MAE from various parts of *Citrullus colocynthis* and their effect on total HDL levels have been mentioned in table 3 Results for the HDL content displayed peak percent elevation in treatment group (16.75%) C_3 followed by (9.74%) C_1, (9.64%) C_2 and (4.50%) C_4, respectively on the 28th day of administrating MAE. In comparison to the control group Co (32.90±0.69mg/dL), the highest HDL concentrations observed in C_3 (39.52±1.56mg/dL) then C_1(36.41±0.83mg/dL), C_2(36.02±0.88mg/dL) and C_4(34.45±0.92mg/dL). Similar, mean values of day intervals were, 0 day (34.04±1.18 mg/dL). 14th day (36.08±1.16mg/dL) and 28th day (37.66±0.59mg/dL).

Table 3 Effect of MAE of different parts of *Citrullus colocynthis* on HDL

Parameter	Group	Days			Mean
		0 day	14th Day	28 day	
HDL	N_0	39.11±1.12	41.39±0.56	43.98±1.21	41.49±0.96
	C_0	35.03±1.23	32.76±0.45	30.91±0.39	32.90±0.69^{c}
	C_1	32.71±0.51	35.26±1.85	41.26±0.15	36.41±0.83^{b}
	C_2	33.81±0.48	36.07±1.89	38.19±0.28	36.02±0.88^{b}
	C_3	36.39±1.78	39.89±1.05	42.30±1.87	39.52±1.56^{a}
	C_4	32.29±1.91	34.42±0.56	36.65±0.29	34.45±0.92^{b}
	Mean	34.04±1.18^{c}	36.08±1.16^{b}	37.66±0.59^{a}	

N_0: Normal group; C_0: Control group (HCD); C_1: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C_2:HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C_3:HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C_4:HCD+Microwave Assisted *Citrullus colocynthis* roots extract
HCD=High Cholesterol Diet

Superoxide Dismutase (SOD)

The statistical analysis observed significant ($p \leq 0.05$) effect of variables (time intervals and treatments) on the SOD levels in hypercholesterolemic experimental rats. The results for the effect of MAE on the levels of SOD are mentioned in table 4. The levels of SOD were elevated from (12.66±0.22IU/L) at 0 day, to 13.46±0.11IU/L at 14th day and to 14.22±0.13IU/L at 28th day respectively. However, the values observed for C_o (11.78±0.18IU/L) were noticed to have elevated in C_1 (13.19±0.07IU/L), C_2 (13.50±0.18IU/L), C_3 (14.45±0.30IU/L), C_4 (14.30±0.03IU/L), respectively. The percentage elevation in the treatment groups showed highest increase in C_3 (18.47%).

Table 4 Effect of MAE of different parts of *Citrullus colocynthis* on SOD (IU/L)

Parameter	Group	Days			Mean
		0 day	14th Day	28 day	
SOD(IU/L)	N_0	13.22±0.20	13.78±0.11	14.01±0.22	13.67±0.17
	C_0	12.62±0.25	11.71±0.12	11.02±0.19	11.78±0.18[d]
	C_1	12.09±0.08	13.24±0.04	14.25±0.10	13.19±0.07[c]
	C_2	12.12±0.19	13.69±0.15	14.71±0.22	13.50±0.18[b]
	C_3	13.14±0.50	14.44±0.22	15.79±0.19	14.45±0.30[a]
	C_4	13.34±0.02	14.23±0.02	15.33±0.05	14.30±0.03[b]
	Mean	12.66±0.22[c]	13.46±0.11[b]	14.22±0.13[a]	

N_0: Normal group; C_0: Control group (HCD); C_1: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C_2: HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C_3: HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C_4: HCD+Microwave Assisted *Citrullus colocynthis* roots extract
HCD=High Cholesterol Diet

Catalase

The statistical analysis showed the effect of time interval and treatment to be significant ($p \leq 0.05$) on the levels of catalase in hypercholesterolemic experimental rats. The means for the values of CAT are reported in the figure 3. The levels of catalase observed in C_o (14.79±0.13IU/L) was shown to have elevated in C_1 (14.11±0.21IU/L), C_2 (15.56±0.13IU/L), C_3 (15.89±0.24IU/L) and C_4 (14.44±0.03IU/L), respectively on the 28th day of administration. The percent elevation observed in experimental groups were 4.60%, 4.94%, 6.92%, 2.36%, respectively.

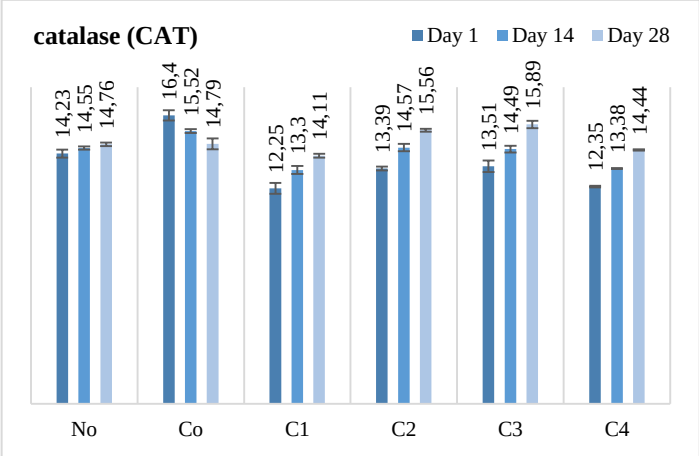

Figure 3 Effect of MAE of different parts of *Citrullus colocynthis* on Catalase (IU/L)
N_0: Normal group; C_0: Control group (HCD); C_1: Microwave Assisted *Citrullus colocynthis* whole fruit extract; C_2: Microwave Assisted *Citrullus colocynthis* leaves extract; C_3: Microwave Assisted *Citrullus colocynthis* seeds extract; C_4: Microwave Assisted *Citrullus colocynthis* roots extract
HCD=High Cholesterol Diet

DISCUSSION

Citrullus colocynthis has been known as traditional medicine since ages, due to presence of major nutrients and flavonoids. It has been used in many countries to treat ailments, specially diabetes mellitus **(Errajraji *et al*., 2010)**. Our findings are not in harmony to the findings of Adam et al, because the dosage prescribed was 0.25g/kg, which was orally administered to sheep for less than two weeks **(Adam *et al*., 2001)**. Studies have shown that fruit and leaves of *Citrullus colocynthis* have many constituents such as cucurbitacin (A, B, C and D) **(Nayab *et al*., 2006)**. According to **Khouri et al. (2007)** reduction was seen in cholesterol and triglycerides in hyperlipidemic patients that were fed on 500mg/kg/BW of powdered seeds of Citrullus colocynthis. The antihyperlipidimic influence of *Citrullus colocynthis* fruit is accredited due to occurrence of bioactive components i.e. isoflavoninis, two cucurbitan triterpene glycosides, colocynthosides A and B **(Yoshikawa *et al*., 2007)**. According to Zamani et al., extract obtained from seeds and fruit of *Citrullus colocynthis* has significantly reduced LDL and triglycerides and HDL levels were increased **(Subhan *et al*., 2008)**. The reduction in cholesterol is basically because of high amount of saponins in different parts of *Citrullus colocynthis*, that combines with cholesterol and excretes out of body **(Ren *et al*., 2001)**. According to Agarwal et al., reported that aqueous extract of roots has significantly reduced the serum blood profile as compared to extracts obtained from other solvents (benzene, chloroform and ethyl alcohol) **(Agarwal *et al*., 2012)**.
Different parts of *Citrullus colocynthis* are enriched with flavonoids and phenolic compounds due to which ii possess antioxidantative properties. The studies have shown that fruit and fruit pulp extracts have effect on enzymes (catalase and SOD) that protects from oxidative damage **(Dallak *et al*., 2010)**. According to studies, reduction in these enzymes in body is due to less synthesis of enzymes due to high accumulation of radicals **(Xiong *et al*., 2015)**. According to Nessa and Khan methanolic extract of Citrullus colocynthis leaves have the highest phenolic content so it inhibits the highest effect on oxidative enzyme, as compared to extract obtained from cholorform **(Nessa and Khan, 2014)**. Similar roots extract of *Citrullus colocynthis* have positive effect on enzymes and protects the body from going under stress condition **(Agarwal *et al*., 2012)**.

CONCLUSION

The study concluded that different parts of Citrullus colocynthis possess lipid lowering properties. In addition to this, MAE extracts can be used to treat the disease related to altered lipid profile. Further experiments are also required to investigate the therapeutic influence to validate its curative use.

Acknowledments: The authors would like to acknowledge University Institute of Diet and Nutritional Sciences, The University of Lahore for their cooperation.

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