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 since early times.it is commonly known as Colocynthis, 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 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 placed in a microwave oven drier at 50±5°C 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 placed in a microwave oven drier at 50±5°C and was washed, and air-dried at room temperature. Collected roots, leaves, seeds and whole fruit was dried in oven drier at 50±5°C to minimize the moisture content. After that they were grinded to reduce the particle size in order to facilitate extraction.

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°C 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-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 (normal control rats fed with their normal diet), Group C0 was fed with only with high cholesterol diet. Group C1 was administered with high cholesterol diet along with Citrullus colocynthis whole fruit extract. Group C2 was administered with high cholesterol diet along with Citrullus colocynthis leaves extract. Group C3 was administered with high cholesterol diet along with Citrullus colocynthis seeds extract. Group C4 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 21th 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≤0.05 (Steel et al., 1997).

RESULTS

Total Cholesterol

The statistical analysis demonstrated that there was significant (p≤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 C4 (10.33%) followed by C1 (9.84%), C2 (9.26%) and C0 (8.20%), respectively on the 28th day of administering 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 (C1), 177.18±2.38 mg/dL (C2), 175.08±1.83 mg/dL (C3), and 179.24±2.87 mg/dL (C4), 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 C4 as compared to C0.

Total Triglycerides

The statistical analysis demonstrated that there was significant (p≤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 C1 (173.72±2.33 mg/dL) was found to have reduced in C4(137.96±3.21 mg/dL), C2 (139.09±2.10 mg/dL), C3 (134.33±1.99 mg/dL) and C0 (141.56±3.33 mg/dL). The figure displayed a significant percent reduction in C4 (22.50%) as compared to experimental group followed by C3 (20.02%), C2 (19.09%) and C1 (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 | 0 day | 14th Day | 28 day | Mean |
|-----------|-------|-------|----------|--------|------|
| HDL (mg/dL) | N₀ | 94.21±0.40 | 96.78±0.25 | 99.35±1.76 | 96.78±0.80 |
| | C₀ | 185.34±2.23 | 194.60±2.66 | 205.88±3.12 | 195.27±2.67<sup>a</sup> |
| | C₁ | 187.11±3.21 | 176.44±1.41 | 164.61±1.16 | 176.05±2.07<sup>b</sup> |
| | C₂ | 188.21±3.12 | 177.10±2.71 | 166.32±1.33 | 177.18±2.38<sup>b</sup> |
| | C₃ | 186.84±2.48 | 175.25±1.52 | 163.15±1.51 | 175.08±1.83<sup>bc</sup> |
| | C₄ | 189.40±3.94 | 179.74±2.74 | 168.59±1.95 | 179.24±2.87<sup>d</sup> |
| Mean | 187.38±2.99<sup>a</sup> | 180.63±2.20<sup>b</sup> | 173.49±1.81<sup>bc</sup> | 180.29±2.33 |

N₀: Normal group; C₀: Control group (HCD); C₁: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C₂: HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C₃: HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C₄: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

N₀ Normal group; C₀: Control group (HCD); C₁: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C₂:HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C₃:HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C₄:HCD+Microwave Assisted *Citrullus colocynthis* roots extract

HCD=High Cholesterol Diet

**LDL**

The Statistical analysis revealed that there was significant (p<0.05) effect on treatments and intervals on LDL 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 C₃ (15.70%) followed by C₁ (13.95%), C₂ (11.77%) and C₀ (11.66%), respectively on the 28<sup>th</sup> 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 C₁ (95.27±2.48mg/dL), followed by (C₀(97.89±3.77mg/dL), C₃(100.36±1.94mg/dL) and C₄(100.49±1.70mg/dL), respectively. Furthermore, the mean value for LDL level at 0 day was 106.20±1.87mg/dL which reduced to 101.44±1.66mg/dL at 14<sup>th</sup> day and to 97.03±1.94mg/dL at 28<sup>th</sup> day, respectively.

![Figure 2 Effect of MAE of different parts of *Citrullus colocynthis* on Total Triglycerides](image)

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

| Parameter | Group | 0 day | 14th Day | 28 day | Mean |
|-----------|-------|-------|----------|--------|------|
| LDL (mg/dL) | N₀ | 38.44±1.22 | 42.63±1.24 | 45.76±1.34 | 42.27±1.26 |
| | C₀ | 108.11±2.13 | 112.93±1.39 | 120.25±2.52 | 113.76±2.01<sup>a</sup> |
| | C₁ | 105.92±2.11 | 97.64±1.57 | 90.11±1.04 | 97.89±1.57<sup>b</sup> |
| | C₂ | 107.56±1.75 | 101.49±2.18 | 92.04±1.91 | 100.36±1.94<sup>bc</sup> |
| | C₃ | 102.49±1.28 | 95.12±2.19 | 88.22±2.27 | 95.27±2.48<sup>c</sup> |
| | C₄ | 106.91±2.11 | 100.01±1.01 | 94.55±1.99 | 100.49±1.70<sup>d</sup> |
| Mean | 106.20±1.87<sup>a</sup> | 101.44±1.66<sup>b</sup> | 97.03±1.94<sup>bc</sup> | 101.44±1.66<sup>b</sup> |

N₀ Normal group; C₀: Control group (HCD); C₁: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C₂:HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C₃:HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C₄:HCD+Microwave Assisted *Citrullus colocynthis* roots extract

HCD=High Cholesterol Diet

**HDL**

The Statistical analysis revealed that there was significant (p<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%) C₁ followed by (9.74%) C₀, (9.64%) C₂ and (4.50%) C₄, respectively on the 28<sup>th</sup> day of administrating MAE. In comparison to the control group Co (32.90±0.69mg/dL), the highest HDL concentrations observed in C₁ (39.52±1.56mg/dL) then C₃(36.41±0.83mg/dL), C₂(36.02±0.88mg/dL) and C₄(34.45±0.92mg/dL). Similar, mean values of day intervals were, 0 day (34.04±1.18mg/dL), 14<sup>th</sup> day (36.08±1.16mg/dL) and 28<sup>th</sup> day (37.66±0.59mg/dL).

![Figure 2 Effect of MAE of different parts of *Citrullus colocynthis* on Total Triglycerides](image)

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

| Parameter | Group | 0 day | 14th Day | 28 day | Mean |
|-----------|-------|-------|----------|--------|------|
| HDL (mg/dL) | N₀ | 39.11±1.12 | 41.39±0.56 | 43.98±1.21 | 41.49±0.96 |
| | C₀ | 35.03±1.23 | 32.76±0.45 | 30.91±0.39 | 32.90±0.69<sup>a</sup> |
| | C₁ | 32.71±0.51 | 35.26±1.85 | 41.26±0.15 | 36.41±0.83<sup>b</sup> |
| | C₂ | 33.81±0.48 | 36.07±1.89 | 38.19±0.28 | 36.02±0.88<sup>c</sup> |
| | C₃ | 36.39±1.78 | 39.89±1.05 | 42.30±1.87 | 39.52±1.56<sup>d</sup> |
| | C₄ | 32.29±1.91 | 34.42±0.56 | 36.65±0.29 | 34.45±0.92<sup>e</sup> |
| Mean | 34.04±1.18<sup>a</sup> | 36.08±1.16<sup>b</sup> | 37.66±0.59<sup>c</sup> | 37.66±0.59<sup>c</sup> |

N₀ Normal group; C₀: Control group (HCD); C₁: HCD+ Microwave Assisted *Citrullus colocynthis* whole fruit extract; C₂:HCD+Microwave Assisted *Citrullus colocynthis* leaves extract; C₃:HCD+Microwave Assisted *Citrullus colocynthis* seeds extract; C₄:HCD+Microwave Assisted *Citrullus colocynthis* roots extract

HCD=High Cholesterol Diet
**Superoxide Dismutase (SOD)**

The statistical analysis observed significant (p<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 escalated 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 C0 (11.78±0.18IU/L) were not noticed to have elevated in C1 (13.19±0.07IU/L), C2 (13.50±0.18IU/L), C3 (14.45±0.30IU/L), C4 (14.30±0.03IU/L), respectively. The percentage elevation in the treatment groups showed highest increase in C4 (18.47%).

| Table 4 Effect of MAE of different parts of Citrullus colocynthis on SOD (IU/L) |
|-----------------------------------------------|
| Parameter | Group | 0 day | 14th Day | 28th Day | Mean |
|-----------|-------|-------|----------|----------|------|
| SOD(IU/L) | N0    | 13.22±0.20 | 13.78±0.11 | 14.01±0.22 | 13.67±0.17 |
|           | C0    | 12.62±0.25 | 11.71±0.12 | 11.02±0.19 | 11.78±0.18 |
|           | C1    | 12.09±0.08 | 13.60±0.15 | 14.71±0.22 | 13.99±0.07 |
|           | C2    | 12.12±0.19 | 13.60±0.15 | 14.71±0.22 | 13.50±0.18 |
|           | C3    | 13.14±0.50 | 14.44±0.22 | 15.79±0.19 | 14.45±0.30 |
|           | C4    | 13.34±0.02 | 14.23±0.02 | 15.33±0.05 | 14.30±0.03 |
|           | Mean  | 12.66±0.22 | 13.46±0.11 | 14.22±0.13 |       |

N0: Normal group; C0: Control group (HCD); C1: Microwave Assisted Citrullus colocynthis whole fruit extract; C2: Microwave Assisted Citrullus colocynthis leaves extract; C3: Microwave Assisted Citrullus colocynthis seeds extract; C4: 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<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 C0 (14.11±0.21IU/L), C1 (14.79±0.14IU/L), C2 (15.89±0.22IU/L) and C3 (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.

**DISCUSSION**

Citrullus colocynthis has been known as traditional medicine since ages, due to presence of major nutritions 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 antiatherosclerotic influence of Citrullus colocynthis fruit is accredited due to occurrence of bioactive components i.e. isolavonins, two cucurbitane triterpene glycosides, colocynthisides 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).

**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.

**ACKNOWLEDGMENTS**

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

**REFERENCES**

Adam, S. E. I., Al-Yahya, M. A., & Al-Farhan, A. H. (2001). Response of Najdi sheep to oral administration of Citrullus colocynthis fruits, Nerium oleander leaves or their mixture. *Small Ruminant Research*, 40(3), 239-244. https://doi.org/10.1016/s0921-4888(01)00184-5

Alphular, A., Calinescu, L., & Lavric, V. (2012). Microwave extraction of active principles from medicinal plants. *JUPB Science Bulletin, Series B*, 74(2), 129-142.

Demmig-Adams, B., & Adams, W. W. (2002). Antioxidants in photosynthesis and human nutrition. *Science*, 290(5601), 2149-2153. https://doi.org/10.1126/science.1078602

Errajraji, A., Ouldouch, F., & El-Ansari, N. (2010). Use of medicinal plants for type 2 diabetes treatment, in *Morocco. Medicine of Metabolic Diseases*, 4 (3), 301-304.

Kai, N. S., Nee, T. A., Ling, E. L. C., Ping, T. C., Kamariah, L., & Lin, N. K. (2015). Anti--hypercholesterolemic effect of kenaf (Hibiscus cannabinus L.) seed on high-fat diet Sprague dawley rats. *Asian Pacific journal of tropical medicine, 8*(1), 6-13. https://doi.org/10.1016/j.aptm.2015.06.107

Khouri, N. A., El-Akawi, Z., & Daradka, H. (2007). Effect of Short-Term Treatment with Citrullus colocynthis L on the Lipid Profile and Other Blood Biochemical Parameters in Albino Rats. *Journal of Asian Chemistry*, 19(2), 1468. https://doi.org/10.4103/0974-7645.146019

Nessa, F., & Khan, S. A. (2014). Evaluation of antioxidant and xanthine oxidase inhibitory activity of different solvent extracts of leaves of Citrullus colocynthis. *Pharmacognosy Research*, 6(3), 218. https://doi.org/10.4103/0974-8490.135359.

Nimuru, M., Salehi, A. R., & Imamhi, M. H. (2013). Impotence and Iranian traditional medicine. *Journal of Islamic and Iranian traditional medicine, 3*(4), 435-442.

Qureshi, R., Bhatti, G. R., & Memon, R. A. (2010). Ethnomedicinal uses of herbs from northern part of Nara desert, *Pakistan Journal of Botany*, 42(2), 839-851.
Ren, M. Q., Kuhn, G., Wegner, J., & Chen, J. (2001). Isoflavones, substances with multi-biological and clinical properties. European Journal of Nutrition, 40(4), 135-146. https://doi.org/10.1007/pl00007388.

Shahid, Q., Khalil, A. A., Faiz-ul-Hassan, S., Khan, A. A., Hina, G., Khan, M. A., ... & Sameen, A. (2019). Proximate and mineral nutrient composition of various parts of Citrullus colocynthis an underutilized plant. Pakistan Journal of Food Sciences, 29(2), 10-14.

Subhan, N., Alam, M. A., Ahmed, F., Shahid, I. J., Nahar, L., & Sarker, S. D. (2008). Bioactivity of Excoecaria agallocha. Revista Brasileira de Farmacognosia, 18(4), 521-526. https://doi.org/10.1590/s0102-695x2008000400004.

Uma, C., & Sekar, K. G. (2014). Phytochemical analysis of a folklore medicinal plant Citrullus colocynthis L. (bitter apple). Journal of Pharmacognosy and Phytochemistry, 2(6).

Xiong, Z. E., Dong, W. G., Wang, B. Y., Tong, Q. Y., & Li, Z. Y. (2015). Curcumin attenuates chronic ethanol-induced liver injury by inhibition of oxidative stress via mitogen-activated protein kinase/nuclear factor E2-related factor 2 pathway in mice. Pharmacognosy Magazine, 11(44), 707. https://doi.org/10.4103/0973-1296.165556.

Yoshikawa, M., Morikawa, T., Kobayashi, H., Nakamura, A., Matsuhira, K., Nakamura, S., & Matsuda, H. (2007). Bioactive saponins and glycosides. XXVII. Structures of new cucurbitane-type triterpene glycosides and antiallergic constituents from Citrullus colocynthis. Chemical and Pharmaceutical Bulletin, 55(3), 428-434. https://doi.org/10.1248/cpb.55.428.