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# INVESTIGATING THE EFFECT OF PHYTOCHEMICALS RICH WATERMELON SEEDS AGAINST HYPERTENSION

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Hypertension has become the most prevalent issue in Pakistan that has targeted 18% adults below 45 years of age and 33% of above 45 years. Watermelon seeds possess adequate quantity of cations (e.g. $K^{+}$, $Mg^{+}$ and $Ca^{+2}$) and phytochemicals (e.g. alkaloids, saponins, polyphenol and flavonoids) which exert potent antihypertensive effects due to their vasodilatory, antioxidant and anti-angiotensin converting enzyme activities. Present study was conducted to analyze the proximate composition, cations and phytochemical screening of watermelon seeds and their effect on hypertensive as well as normal men and women. The efficacy study was open labeled, single-arm and consisted of 40 days with one week of wash out. Male (n=16) and Female (n=16) of age 30-60years were divided into two groups; hypertensive (group-A) and normal (group-B). Each group was administered with 50g of watermelon seeds per day. Systolic and diastolic blood pressure was measured at different time interval. Overall, watermelon seeds show appreciable amount of Protein (34.73±0.15%), Fat (32.04±0.52%), Carbohydrate (21.20±0.97%).Whereas, cations including magnesium was found as 531±1mg/100g, Potassium is in the range of 743±1mg/100g. Whereas, level of Calcium is 134±0.93mg/100g. Phytochemicals including total phenols (553.93±1.10mg GAE/100g), Total flavonoids (386.12±1.09mgCE/100g), Saponins (0.83±0.09%) and Alkaloids (3.41±0.15%) was found to be in high amount. Moreover, significant decrease ($p$<0.001) in systolic i.e. 13-16mmHg and diastolic blood pressure of hypertensive patients i.e. 8-10mmHg was observed. Thus, study reveals effective usage of watermelon seeds for treatment of hypertensive patients due to presence of high amount of phytochemicals as well as cations.
**Keywords:** Cucurbitaceae, *Citrullus lanatus*, roasted seeds, antihypertensive properties, minerals, potassium deficiency.

## INTRODUCTION

Hypertension is one of the leading causes of stroke, heart attack and heart failure associated with decrease life expectancy. Systolic blood pressure (SBP) over 120 mmHg and diastolic blood pressure (DBP) over 80 mmHg are elucidated as pre-hypertension while SBP over 140mmHg and DBP over 90 mmHg are elucidated as hypertension. Risk of hypertension is associated with many factors such as stress, sedentary behavior, obesity, potassium deficiency, renal failure, rise of rennin level etc. (Rafieian-Kopaei *et al*., 2016). Hypertension is most prevalent global health issue adversely targeting approximately one billion individuals all around the world (Miranda, 2016). According to the data of WHO, hypertension ranked as one of the most prevalent health issue worldwide and responsible for about 12.8% of deaths (Yuliandra *et al*., 2017). National Heath Survey of Pakistan 1990-94 demonstrated that the 18% of adults between 15-45 years and 33% above 45 years of age are suffering from hypertension (PNHS, 1998).

Watermelon [*Citrullus lanatus* (*Thunb*.)] is a part of Cucurbitaceae family and locally known as tarbooz (urdu) in Pakistan and originate from tropical and subtropical regions (Achu *et al*., 2005). It is mostly cultivated in Africa, Asia, United States and Russia (Seidu and Otutu, 2016). Pakistan is at 20th rank and has a contribution of 0.5% in watermelon production of the world (Slominski, 2016). Dried seeds of watermelon fruit are consumed as snacks in salted and roasted form among Chinese, Israelis etc. (Gwana *et al*., 2014). In Pakistan there is a wide consumption of watermelon. However, large amount of seeds are wasted considering them as raw material. These seeds are discarded in spite of their high nutritive value. Watermelon seeds have high nutritional value as they contained appreciable amount of protein, fat and carbohydrates. Seeds contain numerous beneficial cations like magnesium, potassium, calcium and certain phytochemical components like alkaloid, saponin, polyphenols and flavonoid (Sonawan *et al*., 2016; Mehra *et al*., 2015). Due to these nutrients watermelon seeds possess the antihypertensive properties.

Watermelon seeds have prophylactic and therapeutic properties against several diseases. Extract of watermelon seeds have therapeutic and antioxidant effect against hypertension, blood pressure, diabetes, cardiovascular disorders and cancer (Yadav *et al*., 2011; Patel and Rauf, 2017). Beneficial cations such as potassium, magnesium and calcium exert antihypertensive effect through endothelial dependent vasorelaxation via several mechanisms like calcium channel blockage, constraining of calcium self-influx into cells, constrained sodium reabsorption, suppression of angiotensin II secretion and stabilization of vascular cell membranes (Jee *et al*., 2002; Bo and Pisu, 2008; Houston and Harper, 2008; Haddy *et al*., 2006). Phytochemical such as alkaloids, saponins, polyphenols and flavonoids reduce high BP via numerous mechanisms like vasorelaxation and antioxidative effect through nitric oxide synthesis, inhibition of ACE (angiotensin converting enzyme), $Ca^{+2}$ channels blockage and sympathicolytic activity (depression of sympathetic nerve impulse transmission) (Persson *et al*., 2006; Lee *et al*., 2016; Li *et al*., 2005; Rubbo *et al*., 2000; Victorio *et al*., 2009; Geleta *et al*., 2016; Bunkar, 2017). Thus, watermelon seeds were suspected to be useful against herpertension due to its phytochemical contents. Therefore, primary objective of proposed study was to reduce systolic and diastolic blood pressure via loading phytochemical rich watermelon seeds. Thus, present study was planned to check proximate composition, mineral and phytochemical analysis of watermelon. Moreover, antihypertensive potency of water melon was checked for normal and hypertensive test subjects.

## MATERIALS AND METHODS

***Food sample*:** Watermelon [*Citrus lanatus* (*Thunb*.)] seeds were procured from local market of Faisalabad. One batch of food sample was purchased for sample preparation as well as food analysis.

***Sample preparation*:** Sun dried watermelon seeds were weighed and stored in air tight plastic bags in order to prevent moisture reabsorption at room temperature. Some portion of seeds were powdered well with the help of an electric grinder in order to utilize for subsequent analysis including proximate composition, mineral and phytochemicals.

***Proximate analysis*:** Watermelon seed powder was subjected for proximate analysis by following method described in AOAC (AOAC, 2007).

Moisture content was estimated by hot air oven. Sample agitation was done via muffle furnace for ash estimation. Protein content of watermelon seeds were estimated by Kjeldahl's method. Crude fat of samples was determined by refluxing in the Soxhlet. However, crude fiber estimation involves digestion of fat free sample with 1.25% $H_2SO_4$ and 1.25% NaOH solution followed by agitation through muffle furnace (AOAC, 2006).

***Mineral analysis*:** Minerals i.e. magnesium (Mg) and calcium (Ca) were determined by using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) While minerals e.g. potassium (K) and sodium (Na) were determined by flame photometer (Jenway PFP 7) following method described in AOAC (1990).

***Phytochemicals analysis*:** Phytochemicals including polyphenols, flavonoids, saponins and alkaloids was determined from watermelon seeds by following methods.

***Estimation of polyphenols*:** Total phenol contents of watermelon seeds were estimated by method illustrated by Hasan *et al*. (2015).

Gallic acid standard was used for quantification of total phenol. Different concentrations of gallic acids i.e. 0.01 – 0.10mg/ml was prepared in methanol for formation of calibration curve. 1ml aliquot of gallic acid standard was mixed in 5mL of 10% Folin-ciocalteu and 4mL of 20% sodium carbonate solution. Solution was kept for 1 hr. and absorbance was checked at 765nm. Moreover, 1ml of watermelon seeds extract was incubated for 1 hour after the addition of 5mL of 10% Folin-Ciocalteu and 4mL of 20% sodium carbonate. After 1 hr., blue color complex was formed. Later on, absorbance was checked at 765nm via UV/Visible light Spectrophotometer (CECIL-CE7200). Calibration curve was plotted according to the concentration related absorbance. Total polyphenol of watermelon seeds was expressed as gallic acid equivalent.

***Estimation of flavonoids*:** Total flavonoid contents of watermelon seeds were estimated by the method illustrated by Imbenzi *et al*. (2014).

0.5mL extract of watermelon seed was combined with 2mL distilled water and 0.15mL solution of 5% sodium nitrate ($NaNO_2$). Solution was kept for 6min incubation. 0.15mL solution of 10% $AlCl_3$ was added in the mixture and again allowed to incubate for 6minutes followed by the addition of 4% NaOH into mixture solution. Methanol was added to make volume of mixture up to 5mL and mix vigorously. Mixed solution was allowed to incubate for 15minutes and the absorbance was taken at 510nm. The results were illustrated as catechin equivalent.

***Estimation of saponins*:** Saponins concentration in watermelon seeds was determined through method illustrated by Mehra *et al*. (2015).

5g watermelon seeds powder was taken in a conical flask with 50ml of aqueous ethanol. Solution was heated at 55°C for 4 hr. Then, flask content was filtered and residues were passed re-extraction by 50ml solution of 20% ethanol. Extract solution was concentrated over hot plate at 90°C till 10ml sample volume was left. Concentrated solution was taken in a separate funnel with 20ml of diethyl ether and then vigorously shaken till the aqueous layer obtained. After addition of 15ml of n-butanol, extract was allowed to wash (twice) with 10ml solution of 5% NaCl and residual solution was allowed to heat in water bath. Sample was transferred into crucible and oven dried till constant weight obtained.

***Estimation of alkaloids*:** Total alkaloids were estimated through the method illustrated by Mehra *et al*. (2015).

2.5g of watermelon seeds were added in a beaker along with 100ml solution of 10% ethanol ($C_2H_5OH$) based acetic acid ($CH_3CO_2H$) solution. Sample solution was kept at room temperature for approximately 4 hr. Mixture was subjected for filtration. Sample solution was concentrated until ¼ remnant was obtained. Concentrated $NH_4OH$ was added to form precipitation of sample solution. Sample precipitates were separated and filtered after rinsing with diluted $NH_4OH$. Sample solution was dried and weighed for alkaloid measurement.

***Efficacy study*:**

***Test subjects*:** Males and females of age 30-60 years were target population for study. Test subjects were recruited from surrounding of Faisalabad city. Total of 16 men and 16 women were selected for trial. Hypertensive patients with mean SBP > 120 but < 159 and mean DBP > 80 but< 99 mmHg during initial screening were selected for present study.

***Study design*:** Trial was open labeled, single-arm and consisting of 40 days with 1 week of washout followed by the clinical assessment on daily bases at different time intervals. Test subjects were divided into 2 groups as described in Fig. 1. Both groups were administered with same dose (50g) of watermelon seeds.

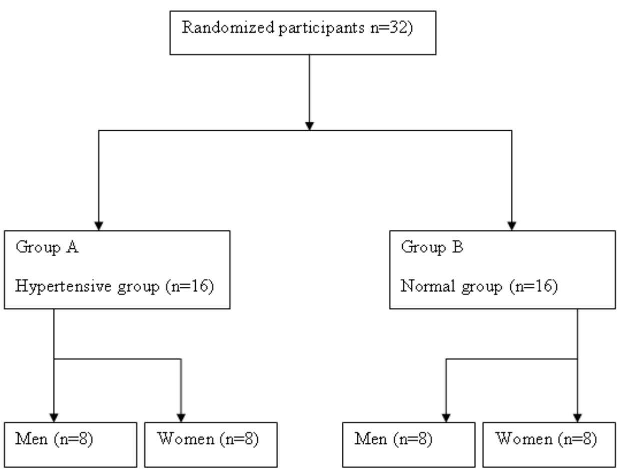



**Figure 1. Selection and recruitment of test subjects for intervention trial.**

***Screening of blood pressure*:** Mercury sphygmomanometer apparatus was used for the assessment of Systolic and Diastolic blood pressure (Taksande *et al*., 2015).

***Volunteer diaries*:** To record intake of polyphenol and flavonoids, volunteers were asked to keep diaries on daily basis during study. Diaries include all detail of food eaten during intervention trial. Intake of total polyphenol and flavonoid content were calculated from daily intake of food via using phenol-explorer data base that described the data on the polyphenol contents of 452 foods (Perez-Jimenez *et al*., 2010).

***Statistical analysis*:** Data were analyzed by using Statistix (Version 8.1). Tukey test was used to find out relation between dependent and independent variables. Statistical differences between the means of groups were evaluated by analysis of variance (ANOVA) (Steel *et al*., 1997).

## RESULTS

***Proximate composition*:** In present study, proximate composition of water melon seeds is presented in Table 1. Result showed that watermelon seeds comprised of significant amount of protein, (34.73±0.15%), fat (32.04±0.52%) and carbohydrate (21.20±0.97%). Whereas, moisture content of sun dried water melon seed was found to be 3.39±0.09%. On the other hand, Ash contents was reported as 3.67±0.13%. Crude fiber was recorded as 4.97±0.97%.

**Table 1. Proximate composition of sundried water melon seeds.**

| Sr. | Nutritional Components | Results± SE (%) |
|---|---|---|
| 1 | Crude protein | 34.73±0.15 |
| 2 | Crude fat | 32.04±0.52 |
| 3 | Crude fiber | 4.97±0.46 |
| 4 | Ash | 3.67±0.13 |
| 5 | NFE (Nitrogen Free Extract) | 21.20±0.97 |
| 6 | Moisture | 3.39±0.09 |

***Level of minerals*:** Current study measured level of different minerals including magnesium, potassium, calcium and sodium in watermelon seeds. Potassium was present in high amount among other minerals i.e. 742.81±1.04 mg/100g. Whereas, amount of magnesium and calcium was found to be 531.6±1.07mg/100g and 134.31 ±0.93mg/100g respectively. Sodium was reported as least among analyzed minerals i.e. 106.03 ± 1.19mg/100g.

***Level of phytochemicals*:** Different phytochemicals including total phenols, flavonoids, saponins and alkaloids were determined from water melon seeds. Total phenol was reported as 553.93±1.10mgGAE/100g. However, flavonoids level was found 386.12±1.09mgCE/100g. Whereas, saponins and alkaloids level was found as 0.83±0.09 and 3.41±0.15%, respectively.

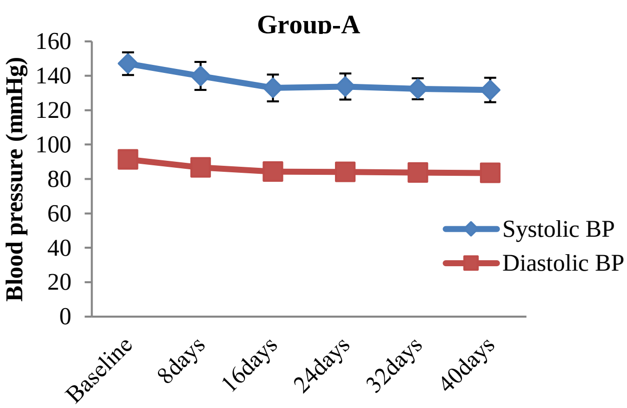
***Intervention trial against hypertension*:** Intake of watermelon seeds for controlling of systolic and diastolic blood pressure shows significant reduction. Data illustrated in Table 2 shows a significant ($p$<0.001) decrease in systolic blood pressure (147.04±6.60 to131.75±7.1mmHg, 136.79±14.48 to 124.00±6.56mmHg) and diastolic blood pressure (91.38±3.56 to 83.46±3.45, 90.50±3.10 to 80.71±2.44) among hypertensive males and females respectively. Similar decrease pattern was observed in normal males and females ranges from 79.67±0.56 to 77.57±3.67mmHg in systolic blood pressure as well as 79.50±0.75 to 75.63±4.43mmHg in diastolic blood pressure.

Variation in systolic and diastolic blood pressure of group A and B at different time interval was illustrated in Figure 2. After washout period, significant ($p$<0.001) increase of diastolic blood pressure was observed in hypertensive (84.50±3.04to89.56±5.53mmHg) and normal test subjects (77.53±1.35 to 78.00±2.83mmHg) as compare to intervention trial time. However, significant increase of systolic blood pressure was only observed in hypertensive test subjects (130.22±9.53to 136.06±9.79mmHg). Whereas, it remains unchanged in normal test subjects i.e. 112.88±3.59 to 114.88±4.19mmHg.

**Table 2. Phytochemicals and level of minerals in sun dried water melon seeds.**

| Sr. | Nutritional Components | mg/100gm |
|---|---|---|
| 1 | Magnesium (Mg) | 531.62±1.00 |
| 2 | Potassium (K) | 742.81±1.00 |
| 3 | Calcium (Ca) | 134.31±0.93 |
| 4 | Sodium (Na) | 106.03±1.00 |
| 5 | Polyphenols | 553.93±1.00 |
| 6 | Flavonoids | 386.12±1.00 |
| 7 | Saponins (%) | 0.83±0.09 |
| 8 | Alkaloids (%) | 3.41±0.15 |

**(a)**

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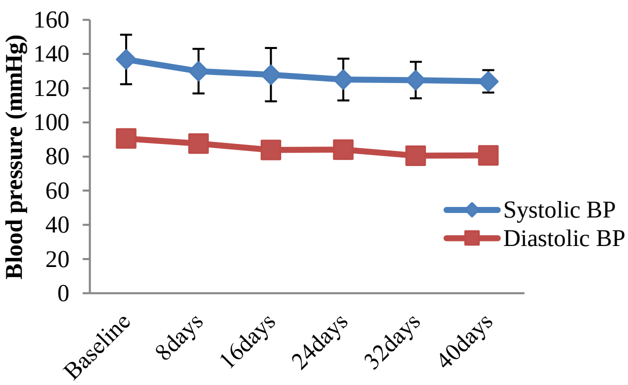
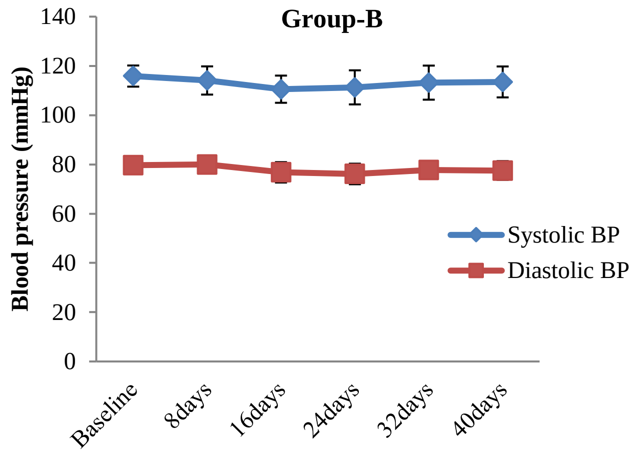



**Figure 2. Systolic and diastolic blood pressure of normal test subjects i.e. group-A (a-males, b-females) after intake of water melon seeds at different time intervals.**

**(a)**

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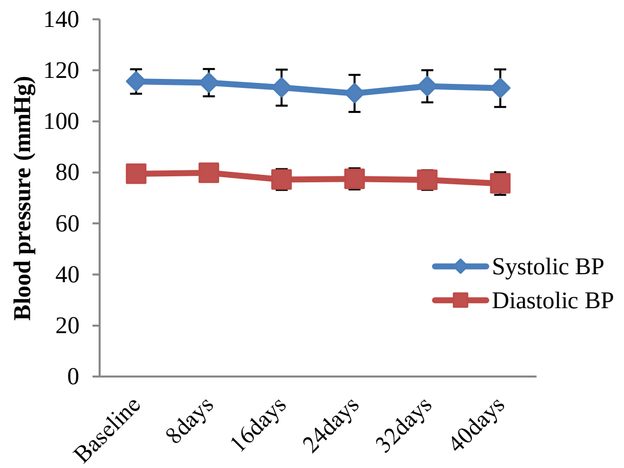


**Figure 3. Systolic and diastolic blood pressure of hypertensive test subjects i.e. group-B (a-males, b-females) after intake of water melon seeds at different time intervals.**

***Volunteer diaries*****:** Volunteer diaries were analyzed for total intake of polyphenol as well as flavonoids during their daily intake of food. Analysis of diaries revealed that there is significant mean difference in the dietary intake of total phenol among hypertensive males and females (606.03±67.01mg & 564.47±51.93mg, respectively) as compared to normal males and females i.e. 636.83±54.74mg & 583.57±60.13mg, respectively. Data in Table 2 also shows that intake of polyphenol was higher among men as compared to the women of both groups. Significant mean differences in the dietary intake of total flavonoids among men and women of hypertensive (186.49±29.00mg & 160.28±27.57mg, respectively) and normal group (209.58±28.56mg & 178.08±21.33mg, respectively) are also shown in Table 2. Present data also reveals that intake of total flavonoids was higher in men as compared to women. Moreover, food diaries

**Table 3. Intake of water melon seeds and its response against blood pressure in normal and hypertensive test subjects along with daily intake of phytochemicals via using food diaries.**

| | Hypertensive group | | Normal group | | |
|---|---|---|---|---|---|
| | **Men** | **Women** | **Men** | **Women** | **p-value** |
| Systolic blood pressure (mmHg± SD) | | | | | |
| At baseline | 147.04±7 | 136.79±15 | 115.88±4 | 115.62±5 | <0.001 |
| After 40 days | 131.75±7 | 124.00±7 | 113.50±6 | 113.00±7 | <0.001 |
| Diastolic blood pressure (mmHg± SD) | | | | | |
| At baseline | 91.38±4 | 90.50±3 | 79.67±0.56 | 79.50±0.75 | <0.001 |
| After 40 days | 83.46±3 | 80.71±2 | 77.57±4 | 75.63±4 | <0.001 |
| Dietary Assessment (mean± SD) | | | | | |
| Total phenol intake | 606.03±67 | 564.47±52 | 636.83±55 | 583.57±60 | |
| Total flavonoid intake | 186.49±29 | 160.28±28 | 209.58±29 | 178.08±21 | |

| | **Hypertensive group** | ***p*-value** | **Normal group** | ***p*-value** |
|---|---|---|---|---|
| Systolic blood pressure (mmHg± SD) | | | | |
| At baseline | 141.90±12 | | 115.75±4 | |
| After 40 days | 130.22±10 | <0.001 | 112.88±4 | >0.05 |
| After washout | 136.06±10 | | 114.88±4 | |
| Diastolic blood pressure (mmHg± SD) | | | | |
| At baseline | 90.98±3 | | 79.58±0.64 | |
| After 40 days | 84.50±3 | <0.001 | 77.53±1 | <0.001 |
| After washout | 89.56±6 | | 78.00±3 | |

also show that food items including fruits, cereals, vegetable and tea were major contributors of total polyphenols and flavonoids intake.

## DISCUSSION

Watermelon seeds have prophylactic and therapeutic properties against several diseases due to its antioxidant, cardio-protective, hypotensive and anticancer properties. Its usage as home remedy agent for urinary tract infection as well as edema has been reported. (Patel and Rauf, 2017; Sonawane *et al*., 2016; Parmar and Kar, 2009). The present study used sundried watermelon seeds against hypertension due to presence of phytochemicals alongside cations. Nutritional composition of sundried watermelon seeds shows high amount of protein (34.73±0.15%), fat (32.04±0.52%), carbohydrate (21.20±0.97%) along with considerable amount of numerous beneficial cations such as Mg (531.6±1.07 mg/100g), K (742.81±1.04 mg/100g), Ca (134.31±0.93 mg/100g) and phytochemicals including total phenols (553.93±1.10 mgGAE/100g), total flavonoids (386.12±1.09 mgCE/100g), saponins (0.83±0.09%) and alkaloids (3.41±0.15%).

Amount of protein and fat in watermelon seeds reported by Raziq *et al*. (2012) were 21-35% and 28-35%, respectively. Thus, reported values are in correspondence to the results obtained in present study. Several studies reported level of magnesium, potassium and calcium of watermelon seeds as 509 mg/100g, 705.35±5.28 mg/100g and 147.28 mg/100g which are found to be in accordance to the resulted value from current study (Egbuonu, 2015; Ibeanu *et al*., 2012; Gwana *et al*., 2014). Moreover, total phenol and flavonoid contents of watermelon seeds from the current study are comparable to the value obtained by Mehra *et al*. (2015) which were 422.2 mgGAE/100g and 3.066CE/100g, respectively. The amount of saponins and alkaloid of watermelon from current study are comparable to the value obtained by Braid *et al*. (2012) and Gwana *et al*. (2014) which were 0.72 and 2.58%, respectively. The compositional variation between crops usually occurs due to several factors such as difference in crop variety such as genetic variation, difference in climate, soil variety, growing conditions, cultivation methods, and use of different solvents for analysis.

High consumption of fruit and vegetable rich diet ensures the high magnesium and potassium intake which significantly resulted in the reduction of diastolic blood pressure (-3.1/-2.1 mmHg) among test subject with normal or stage 1 hypertension and also exert significant reductive effect on clinical blood pressure (Appel *et al*., 1997). Intake of table salt sensitivity related blood pressure depends on the adequacy of magnesium, potassium and calcium (Mccarron *et al*., 1997). However, Polyphenols and flavonoids analyzed from red wine has been reported to reduce blood pressure via exerting an in-vivo vasorelaxation mechanism and enhancing the synthesis of nitric oxide (Benito *et al*., 2002).

Certain bioactive compounds such as polyphenol, flavonoids, alkaloids and saponins exhibits in-vitro potency to inhibit ACE (Angiotensin converting enzyme) response (Park *et al*., 2003). *Moringa oleifera* leaves reported to have antihypertensive activity due to the presence of saponins and alkaloids as their major bioactive components (Fahey, 2005). According to the current study, watermelon seeds exert significant (*p*<0.001) reductive effect on systolic and diastolic blood pressure of hypertensive (13-16 mmHg, 8-10 mmHg) and normal (2-3mmHg, 2-4 mmHg) test subjects. Significant decline (p<0.001) of about 12/8 mmHg in blood pressure of hypertensive patients was reported by Dyckner and Waster (1980) after magnesium supplementation with dosage of 170 mg/day. Madhavi *et al*. (2013) also observed a significant decline in mild and moderate hypertension resulting from the consumption of celery seeds extract. Moreover, Akrami *et al*. (2018) demonstrated a significant fall of 14mmHg in the systolic blood pressure of hypertensive patients (n=30) aged between 30-60 years resulting from the dietary intake of flaxseed oil.

Dash diet rich in magnesium, potassium, calcium and low in saturated fat and sodium usually induce significant (p<0.001) reduction of systolic and diastolic blood pressure among normal population (Sacks *et al*., 2001). A study on potassium supplementations reported a significant fall of 1.8 mmHg/ 1.0 mmHg in blood pressure of normal population (Whelton *et al*., 1996).

Lastly, in present study; test subjects kept record of daily food intake via using food diaries. Volunteer diaries were calculated for daily intake of phytochemical including total phenols as well as flavonoids. It was observed in current study that high intake of polyphenol was observed among men as compare to women in hypertensive (606.03±67.01mg & 564.47±51.93mg, respectively) and normal group (636.83±54.74mg & 583.57±60.13mg, respectively). Significant mean differences in the dietary intake of total flavonoids among the men and women of hypertensive (186.49±29.00mg & 160.28±27.57mg, respectively) and normal group (209.58±28.56mg & 178.08±21.33mg, respectively) were observed. Zhang *et al*. (2014) observed the flavonoid intake of 65-225mg/day among the middle age and elderly Chinese men and women. The mean intake of flavonoids worldwide ranged between 150-600mg/day (Escobar-Cevoli *et al*., 2017). Similar difference in the polyphenols intake from various food items was observed between men and women by Taguchi *et al*. (2015). Difference in intake of total phenol and flavonoid among men and women could be due to their food preference, difference of dietary habits and high food serving consumption from five food group.

***Conclusion*:** Present study reported that watermelon seeds are rich in macronutrients like protein, fat and carbohydrate. Seeds also contain an adequate amount of minerals especially potassium, magnesium, calcium and certain phytochemicals including polyphenols, flavonoids, saponins and alkaloids which exerts reductive as well as therapeutic effects against hypertension.

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