"Effect Of Cooking Practices On Antioxidant And Antibacterial Activity Of Selected Vegetables"

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ABSTRACT: Assess antioxidant plus antibacterial activity of cooked vegetable dishes (common Pakistani recipes). Effects of Pakistani style cooking were investigated on antioxidant activity and radical scavenging activity of *P.sativum, B.campestris, S.tuberosum, D.carota, Trigonella foenum-graecum, B.rapa, S. oleracea, C.annuum, B.oleracea var. botrytis, S.melongena and B.oleracea var. capitata*, of raw, raw mixture and cooked form. Different concentrations of vegetables' samples were checked with DPPH. There was no rhythmic change in antioxidant activity at ranging concentration rising from 10 to 100 µg/ml. Checked that selected vegetables have scavenging activity but no anti-bacterial activity against selected bacterial strains while standard antibiotics have showed their inhibition zone. In DPPH assay, average antioxidant activity of raw vegetables ranged from -3.083±0.034 to 4.418±0.0160. Raw vegetable **mixture'**s average antioxidant activity ranged from -0.3025 ±0.0461 to -1.7651±.0565. Cooked vegetable'**s** average antioxidant activity ranged from -0.021±0.0060 to 2.242±0.114. Cooking influenced scavenging activity significantly ($P < 0.05$). IC 50 value ranged from -49.003 µg/ml to 24.595 µg/ml. Antibacterial activity of the entire extracts was evaluated by applying agar well diffusion technique.

MATERIALS AND METHODS: Different types of vegetable are consumed in cooked form in Pakistani dishes were selected and purchased from local market of Sialkot.

Chemicals: Entire chemicals utilized were of chromatographic grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH•), Agar, Antibiotic (for +ve response), ascorbic acid, Distilled water plus methanol were used.

Instruments used in the research work: Test tube, Test tube stands, Petri plates, Inoculating loop, Flasks, Laminar air flow, Spirit lamp, Stirrer (glass rod), Cotton plugs, Aluminum foil, Rubber bands, Borer, Micro pipette, DMSO (for -ve response), Incubator, Swab, Whattman No. 1 filter paper

Conclusion: Checked that raw mixture and cooked vegetables have antioxidant activity but no anti-bacterial activity against our selected bacterial strains while standard antibiotics have shown their inhibition zone.

Research Objective(s): Assess antioxidant activity of some vegetable dishes/recipes before and after cooking. Study the consequences of Pakistani cooking technique on antibacterial activity of selected vegetable-based dishes

Term Index: ROS (Reactive Oxygen Species), LDL (Low-Density Lipoproteins), FRAC (Ferric Reducing Antioxidant Capacity), ORAC (Oxygen Radical Absorption Capacity), TRAP (Total Radical Trapping Antioxidant Parameter), FRAP (Ferric Reducing Antioxidant Capacity), DMSO (Dimethylsulfoxide), TEAC (Trolox Equivalent Antioxidant Capacity), RNS (Reactive Nitrogen Species, AIDS (Acquired immunodeficiency syndrome), HIV (Human Immunodeficiency Virus), SA (Scavenging Activity), DPPH• (2, 2-diphenyl-1- picrylhydrazyl), ATP (Adenosine Triphosphate)

INTRODUCTION

Background: Plants are colossal source of drugs from ancient time. There are different sources like preserved monuments, written paper as well as original drugs which are plants based. Cure through plants is the results of several years of great effort against sickness. ALL over globe plants are utilized against infectious illness. Herbal medicines which are derived from plant base material and have beneficial against disease. That is used in pharmacy manufacturing. Free radicals and antioxidants they were well known in combustion technology, polymer as well as radiation etc. Free radicals were reported for the first time in the history in 1956 and their associated toxic effects were discussed. (Gutteridge, 2000)

Free radicals: Highly reactive molecules or free radicals have great frequency to react with DNA nucleotides, protein's sulfhydryl bonds as well as polyunsaturated fatty acids. Production of free radicals may be endogenous (as of regular metabolic reactions) and it can be exogenous (by exposure to radiations, pesticides, pollutions as well as tobacco smoke etc). Numerous health issues are due to these free radical's damage like cancer, cataracts, inflammatory plus cardiovascular disorder and emphysema etc. Vitamins have capability to scavenge free radicals are examples of antioxidants (Lobo, 2010).

Antioxidants: Antioxidants are considered as organic chemical complexes. They have capacity to slow down oxidation of additional organic molecules in human body. During cellular oxidation-reduction reactions, free radicals are generated which may go ahead and start chain reactions which is dangerous for cell. For the stability of oxidative state, living systems contain composite system of antioxidants which are taken consumed as dietary antioxidants (Krishnaiah, 2007).

Source of Natural Antioxidants: Antioxidants especially the vitamins are among the main regulators in human metabolism. A healthy lifestyle accompanied by intake of healthy foods enriched with antioxidant activity can save from diseases. Interestingly, from last few decades, natural antioxidants for example caffeic acid, carvacrol, plant extracts, α-tocopherol as well as quercetin have been integrated into foodstuff wrapping (Sanches-Silva, 2014).

Natural antioxidants and Edible materials: This field of research into antioxidant activity of dietary components has got huge interest in the present decade. In Pakistan, a huge majority of people suffer from deficiency of vitamins. For example, 13

% of people have less vitamin A level in serum. Such antioxidants can be obtained from vegetables and fruits. Cannot fulfill our nutritional needs without vegetables. For example, approximately 78%of vitamin A is obtained from vegetables and some other sources (Akhtar, 2013).

Vegetables are Source of Antioxidants: Vegetables have minerals, bioactive compounds as well as fibers. Such types of components are very useful against photo-oxidation and pathogens etc. Bioavailability of some antioxidants including beta-carotene from vegetables also depends on other things which require making in cooked form. It reflects that cooking can enhance bioavailability through discharging protein complex (Bernhardt, 2006). Only few vegetables are eaten as raw in the form of salad, but mostly vegetables are used after cooking for enhancement of digestibility. The availability of nutrients like vitamins plus minerals is enhanced by cooking. Thermal treatment, duration and cooking style may cause alternation in nutritive values. In brisk style cooking of vegetables, folic acid, ascorbic acid and some other nutrients become vulnerable to oxidation or readily oxidized by such type of cooking methods. Minerals are extremely affected by high thermal treatments. Extreme cooking may also be the reason of unpleasant results on digestion (Alvi, 2003).

Microbes: Microbes especially bacteria are big reason of disease and even death of all over the world. Main reason of different infectious diseases is pathogenic bacteria. Pathogenic microbes extensively found in our surroundings responsible for diseases and even death. Pus forming infections plus some severs meningitis infections pneumonia as well as urinary tract infection are due to *S. aureus. B. subtillus* and *E. coli* are reason behind food poisoning (Sapkota, 2012).

Antibiotic Resistance: Approximately from 60 years antibiotic are effectively utilized against different diseases. But bacterial genes are showing resistance against antimicrobial drugs. Expansion in occurrence of such genes in bacteria colonize and ultimately effect living organism especially humans by two ways, emergence as well as dissemination (Istúriz, 2000).

MATERIALS AND METHODS

Collection of Vegetables: *Pisum sativum, Brassica campestris, Solanum tuberosum, Daucus carota, Trigonella foenum-graecum, Brassica rapa, Spinacia oleracea, Capsicum annuum, B. oleracea var. botrytis, Solanum melongena, B. oleracea var. capitata, Allium cepa, Coriandrum sativum, Zingiber officinale* of family Solanaceae, Cucurbitaceae, Liliaceae as well as Brassicacae etc which are consumed in cooked form in Pakistani dishes were selected and purchased from local market of Sialkot.

Chemicals: Entire chemicals utilized were of chromatographic grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH•), Agar, Antibiotic (for +ve response), ascorbic acid, Distilled water plus methanol were used.

Instruments used in the research work: Test tube, Test tube stands, Petri plates, Inoculating loop, Flasks, Laminar air flow, Spirit lamp, Stirrer (glass rod), Cotton plugs, Aluminum foil, Rubber bands,

Borer , Micro pipette, DMSO (for -ve response), Incubator, Swab, Whattman No. 1 filter paper

Table 3.1 List of vegetables

Sr. No.	Plant species	Family name	Common name
1	*Brassica campestris*	Brassicaceae	Mustard plant
2	*Solanum tuberosum*	Solanaceae	Potato
3	*Daucus carota*	Apiaceae	Carrot
4	*Pisum sativum*	Fabaceae	Pea
5	*Brassica rapa*	Brassicaceae	Turnip
6	*Spinacia oleracea*	Amaranthaceae	Spinach
7	*Capsicum annuum*	Solanaceae	Capsicum
8	*Brassica oleracea var. botrytis*	Brassicaceae	Cauliflower
9	*Solanum melongena,*	Solanaceae	Egg plant
10	*T. foenum-graecum*	Fabaceae	Fenugreek
11	*Brassica oleracea var. capitata*	Brassicaceae	Cabbage
12	*Zingiber officinale*	Zingiberaceae	Ginger
13	*Coriandrum sativum*	Apiaceae	Coriander
14	*Cucurbita pepo*	Cucurbitaceae	Pumpkin
15	*Allium cepa*	Amaryllidaceae	Onion

Sample preparation

Two groups of samples were prepared, that are given below;

a) Uncooked vegetables

All vegetables were washed, cleaned, cut and grind.

b) Cooked vegetables

Vegetables were cooked in laboratory, after cleaning as well as washing with tap water. Were separated nonedible portion of vegetables. Nonedible portion were discarded while edible portions of vegetables where be cut into small pieces. Vegetables samples were divided into two groups, one portion was as control (raw or uncooked, were kept at normal room temperature). Second sample were subjected to treatment as domestic Pakistani style cooking.

b.1 Cloves of garlic (30grams) as well as ginger (30grams) were crushed then some chopped red chilli (10grams) was added and crushed together to make a paste.

b.2 Oil added in pan (50ml), onion (100 grams) heated over medium flame and then crushed mixture of ginger and garlic paste (prepared in equal

proportion) were added and fried until it turns aromatic.

b.3 Then 50 grams of tomato paste (peels removed) were added.

b.4 Red chilli powder (0.5gram), salt (1 gram) and turmeric (0.3 gram) were added. Saute until mixture was started to leave sides of saucepan.

b.5 Respective drained veggies were added and fried for 2 to 3 minutes. Cover and cook on a low heat for another 2 minutes

b.6 A little water (50 ml) was added and the mixture where be cooked on a medium to low heat until vegetables are tender.

b.7 Coriander (5 gram) and garam masala (1 gram) powder were added and let it simmer for two minutes.

b.8 The stove turned off.

Preparation of extracts: Samples of vegetables were prepared before and after cooking. Watery extracts where be ready with maceration technique, using to some extent modified technique illustrated by (Walia, 2011). Cooked and raw vegetable samples of 05 gram were dissolved in 50 ml of water. Strain this sample then mixture where be filtered by using Whatman filter paper 40. 3ml of extract both raw as well as cooked were taken in test tube. Added 1ml DPPH in each test tube and checked it on spectrophotometer.

Determination of free radical scavenging activity: DPPH stable free radical SA technique (Choi, 2002) was applied for this purpose. Extract sample of 2.5mL each (i.e. dilution of cooked as well as raw vegetables extracts plus standards), a total of 1 mL of DPPH in water (0.3 mM) was added plus left at normal human body temperature for half hour. Absorbance of all reaction mixture was observed at 518 nm. DPPH in methanol was as negative control while methanol and were taken as blank in 1:1 ratio. All samples were replicated thrice, and optical density of the samples was read spectrophotometer.

DPPH scavenging assay through evaluation of % scavenging activity of cooked vegetables at varying concentrations of methanol extracts. Another mixture was prepared of cooked sample via same method. Filtration procedure was repeated thrice for entire withdrawal of phyto-chemicals from solution. Supernatants, which was obtained collect from all samples then dehydrated beneath sterilized circumstances. Consequential sticky gummy accumulation was stored at 4^0C for additional use. After that, varying concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/mL) of resultant mixtures of vegetable samples, were checked with DPPH.

Statistical analysis: Percent S.A. was calculated through following method,

$$S.A.(\%) = \frac{As - Ac}{Ac} \times 100$$

S.A. place for Scavenging Activity, absorbance sample is ‘As’ plus absorbance of control is ‘Ac’. Standard deviation of percent free radical scavenging activity was determined in support of the entire three replicas. Regression models of DPPH SA were built in support of their average values. Values of IC50 were determined by means of regression equations created during scatter plot investigations.

Antibacterial Activity: For anti-bacterial activity vegetable samples were selected.

Collection and Identification of Plant Samples: Different vegetables which were show high antioxidant abilities of family *Solanaceae, Cucurbitaceae, Liliaceae as well as Brassicacae* etc which are consumed in cooked form in Pakistani dishes were selected.

Extract Preparation: Cook vegetables which were showed high antioxidant scavenging activity (*Solanum tuberosum, Solanum melogena, Pisum sativum, Capsicum annuum, Brassica oleracea var. botrytis* and *Cucurbita pepo*) were ground in fine particles. Extracts were prepared using water. For this purpose, 50g of each cooked and raw mixture vegetables sample were extracted with water (100 ml). Shake each sample and then filtered. The extracts were used for antibacterial assays (Behera, 2017).

Antibacterial Activity Preparation of Bacterial Inoculate: Inoculums of bacterial isolates were arranged in autoclaved L.B. media plus incubated this media intended for 24 hours at 37ºC. Used four bacterial isolates; *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, plus *E. coli.*

Antibacterial Susceptibility experiment: Agar well diffusion technique was used to evaluate antibacterial activity on behalf of entire vegetables extracts. L. B. media was arranged, autoclaved for 15 minutes at 121o C plus fixed in Petri dishes. All dishes consisted 20ml media. Inoculums of bacterial strain were evenly extended lying on media within different dishes by means of sterile swab. On solid media were bored 6mm well with the help of sterile borer. DMSO, Vegetable samples plus antibiotic were inserted within wells. Gentamicin, Meropenem and Ciprofloxacin were used as positive plus DMSO at the same time as negative control. All vegetables extract where be replicated thrice. Dishes were incubated on behalf of 24 hours at 37ºC.

Evaluation of zone or region of inhibition: Zone of inhibition for all vegetables were checked, calculated plus articulated in mm (Munazir *et al*., 2012). Afterward on activity index (A.I.) and Percent Inhibition (P.I.) were determined on behalf of each one solvent extract achieved at 50 µg/ml using the following formula:

Percent scavenging activity (S.A.) was determined through with subsequent prescription,

$$A.I = \frac{\text{Mean zone of inhibition of each solvent extract}}{\text{Zone of inhibition obtained for standard antibiotic}}$$

P.I = Activity index

RESULTS: Antioxidant activity of cooked and raw vegetable samples was assessed by DPPH.

***DPPH as Free constant Radical SA (Spectrophotometric method)*:** DPPH free constant radical (2,2-diphenyl-1- picrylhydrazyl). It did not dimerize and delocalization on this molecule determined the amount of purple color by absorption band which is around 517 nm (Molyneux, 2004). It is very effective process to evaluate antioxidant activity of samples in extremely short time. It determines the absorbance of DPPH radical to measure intrinsic capability of sample to provide electrons or hydrogen atoms to the oxidants. Stable DPPH-H which is formed by diminution of DPPH (in the form of solution) within

existence of hydrogen contributing antioxidant is main step of this method. DPPH is very stable radical at room temperature. It shows very dark purple color during its free radical sate. When reaction starts it, dark purple color modifies to yellow on getting proton from phenolics substances (Sultana, 2008). Modification in purple dye showed as decrease in absorbance, which point out free radical scavenging power of plants extracts (Dubey, 2010).

Vegetables extracts were prepared in water. DPPH radical SA within cooked plus raw mixture of *Cucurbita pepo* (486.45%) is highest than *Solanum tuberosum* plus *Capsicum annuum* (80.24%), *Solanum tuberosum* plus *Brassica oleracea var. botrytis* (63.73 %), *Solanum tuberosum* plus *Pissum sativum* (58.50%), *Solanum melogena* (53.65%), *Solanum tuberosum, Pissum sativum* and *Daucus carota,* (6.30%), *Solanum tuberosum* plus *Spinacia oleracea* (-61.80%) *Brassica rapa* plus *Spinacia oleracea* (-72.84 %), *Solanum tuberosum plus Trigonella foenum-graecum,* (-98.68%), *Brassica oleracea var. capitata* (-198.93%). While lowest SA is observed in *Brassica campestris* (-234.43%).

Table 4.1. Mean values of antioxidant activity of raw vegetables, mixtures of raw vegetable and cooked vegetables

Samples	Average assessment's Antioxidant activity (OD) of RAW vegetables	Average assessment's Antioxidant activity (OD) of RAW vegetable mixture **AC**	Average assessment's Antioxidant activity (OD) of cooked vegetables **AS**	$S.A.(\%) = \frac{As - Ac}{Ac} \times 100$
Cucurbita pepo	-.2456 ± .0412	-.3025 ±0.0461	-1.774±0.1901	486.45%
Allium cepa	1.2050±0.3494			
Allium sativum	1.8508±0.1535			
Capsicum annum	1.543±0.0741			
Brassica campestris	3.200 ± 0.227	-1.6679 ±1572	2.242±0.114	-234.43 %
Pissum sativum	-.4546 ±0.0918	-1.7651 ± .0565	-1.877± 0.052	6.30 %
Daucus carota	-2.618±0.039			
Solanum tuberosum	2.211±0.014			
Brassica Rapa	1.267±0.041	-1.1807± .0654	-0.3207±.0659	-72.84 %
Spinacia oleracea	4.418±0.0160			
Capsicum annuum	-3.083±0.034	-1.5207 ± .0280	-2.741±0.029	80.24%
Solanum tuberosum	2.211±0.014			
Brassica oleracea var. botrytis	1.870±0.042	-1.2166 ± .0345	-1.992±0.250	63.73 %
Solanum tuberosum	2.211±0.014			
Solanum melongena	-2.365±0.050	-1.2431 ± .2756	-1.910±0.184	53.65%
Trigonella foenum-graecum	4.469±0.078	-1.5860 ± .0865	-0.021±0.0060	-98.68%
Solanum tuberosum	2.211±0.014			
Pissum sativum	-2.4546±0.0918	-1.6143 ± .0329	-2.5587±.2410	58.50 %
Solanum tuberosum	2.211±0.014			
Spinacia oleracea	4.418±0.016	-1.7158 ± .0410	-0.6553±0.0112	-61.80
Solanum tuberosum	2.211±0.014			
Brassica oleracea var. capitata	1.293±0.123	-1.4915 ± .0583	1.4756±0.2237	-198.93

Difference during DPPH radical scavenging actions (%inhibition) with respect to raw and cooked vegetables species was not constant. Results and trends of antioxidant activity in raw, raw mixture and cooked vegetables varied in each sample. In our study, the most common thing which observed in all results was that antioxidant activity of individual raw vegetables was much higher as compared to raw mixtures or cooked vegetables.

DPPH SA: Evaluation of % SA of Cooked Vegetables at changeable concentration of Methanol extract.

Another mixture was prepared of cooked sample via same method. Filtration procedure was repeated thrice for entire withdrawal of phyto-chemicals from solution. Supernatants, which was obtained collect from all samples then dehydrated beneath sterilized circumstances. Consequential sticky gummy accumulation was stored at 4^0C for additional use. After that, varying concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/mL) of resultant mixtures of vegetables' samples, were checked with DPPH. There was no rhythmic change in antioxidant activity at ranging concentration rising from 10 to 100µg/ml. It can be seen in Table no. 4.2 values of optical density almost random at different concentrations.

Table 4.2. Comparison of % Scavenging activities of cooked vegetables at varying concentrations of methanol extracts.

%	*B.campestris*	*S.tuberosum, D.carota & p. sativum*	*B. rapa & S.oleracea*	*C. annum & S. tubersum*	*B. oleracea* var. botrytis	*S. melongena*	*S. tuberosum & T. foenum-graecum*	*P. sativum & S. tuberosum*	*S.oleracea & S.* tuberosum	*B. oleracea* var. capitata
10	1.8683± 0.1788	1.1219± 0.0592	0.7578± 0.0778	1.0579± 0.0249	0.1442± 0.0105	0.8730± 00830	0.9402± 0.1024	0.1830± 00077	0.9462± 0.0255s	0.5059± 0.1782
20	2.1358± 0.3058	1.2284± 0.0687	0.7198± 0.1664	1.3985± 0.2547	0.6539± 0.0043	0.7425± 00201	1.1032± 0.0903	0.2277± 00259	0.8382± 0.0936	0.9519± 0.0708
30	1.8218± 0.1245	0.9772± 0.1121	0.8164± 0.0268	1.0579± 0.0249	0.1442± 0.0105	0.8730± 0.0830	0.9402± 0.1024	0.1830± 0.0077	0.9462± 0.0255	0.5060± 0.1782
40	2.4044± 0.0915	1.4092 ±0.3582	0.8164± 0.0930	0.9270± 0.1426	0.6832± 0.0093	0.8854± 0.1148	0.9071± 0.0706	0.4851± 0.0111	0.9461± 0.0267	0.8625± 0.0684
50	1.7497± 01712	1.1497 ±0.0197	0.8625± 0.0380	1.1608± 0.0958	0.4090± 00549	0.7853± 0.1625	1.0249± 0.0723	0.1276± 0.0107	0.8596± 0.0254	0.8117± 0.3785
60	2.3773± 0.1607	1.1574 ±0.1460	1.2078± 0.0106	1.1749 ±0.0848	0.3703± 0.0569	0.8759± 0.0675	1.1216± 0.0462	0.3590± 0.0259	1.0011± 0.0596	0.5612± 0.0809
70	2.3053± 04698	1.0895 ±0.1238	0.7621± 0.0345	1.0315± 0.0910	0.5152± 0.0892	0.7453± 0.1388	09276±0 .2047	0.5606± 0.0165	0.9187± 0.2337	0.6038± 0.0397
80	2.4451± 0.4332	1.0665 ±0.0204	0.6774± 0.0180	1.0053± 0.1372	0.6222± 0.0261	0.6614± 0.0895	1.0026± 0.1779	0.5292± 0.0123	0.9896± 0.0753	0.6921± 0.1180
90	2.3109± 0.2278	1.1013 ±0.1242	0.7764± 0.0232	1.0972± 00146	0.6082± 0.0084	0.6614± 0.0895	0.9052± 0.0783	0.5292± 0.0123	1.0040± 0.0428	0.6376± 0.0918
100	2.0810± 0.2417	1.0804 ±0.0679	0.5860± 0.0234	1.1465± 0.0140	0.4134± 0.0135	0.6394± 01143	1.0116± 0.0068	0.0910± 0.0049	1.1861± 0.0941	0.5556± 0.1546

Statistical analysis: Percent S.A. was calculated through following method,

$$S.A.(\%) = \frac{As - Ac}{Ac} \times 100$$

S.A. place for Scavenging Activity, absorbance sample is 'As' plus absorbance of control is 'Ac'. Standard deviation of percent free radical scavenging activity was determined in support of

the entire three replicas. Regression models of DPPH SA were built in support of their average values. Values of IC50 were determined by means of regression equations created during scatter plot investigations. IC50 symbolizes for concentration of sample which can carry out as regards 50 % free radical SA. Assessments of regression coefficient (R2) were viewed in the direction of explain strength of dependent plus independent variables. Two-way Analysis of Variance (ANOVA) intended effects of raw mixture plus cooked vegetables. Checked concentrations plus interaction between these two independent variables which were all less than 0.05.

Table 4.3. DPPH assay, regression equation, regression coefficient and IC50 for Vegetable samples.

Vegetables	**R.E.(y=ax+b)**	**R^2**	**IC_{50}**	**$IC_{50(\mu g/ml)}$**
B. campestris	y=0.039+1.930	0.217	1232.564	1.23256
P. sativum, S. tuberosum& D.carota	y=-0.010x+1.197	0.078	-4880.300	-4.8803
B. Rapa & S. oleracea	y=0.009x+0.852	0.033	-5460.889	-5.4609
C. annuum & S. tuberosum	y=-0.007x+1.147	0.032	-6979.000	-6.979
B. oleracea var. botrytis & S. tuberosum	y=0.023x+0.324	0.134	2159.826	2.15983
Solanum melongena	y=0.002x+0.810	0.002	24595.000	24.595
Trigonella foenum-graecum & S. tuberosum	y=-0.001x+0.997	0.004	-49003.000	-49.003
Pissum sativum & S. tuberosum	y=0.021x+0.211	0.118	2370.905	2.3709
S. oleracea & S. tuberosum	y=0.023x+0.826	0.487	2138.000	2.138
Brassica oleracea var. capitata	y=-0.011x+0.730	0.046	-4427.400	-4.9274

Scatter plot plus regression analysis of all vegetables are in figure 4.36 to figure 4. 43. The table 4.3 represents IC_{50} value of regression coefficients. The Lowest value examined in *Trigonella foenum-graecum & S. tuberosumvv* - $49.003_{\mu g/ml}$ and highest value in *S. melongena* $24.595_{\mu g/ml}$. ($p<0.01$) was illustrated by Two-Way ANOVA and DPPH free radical SA random with rising concentrations described that contrary relationship took place between antioxidant activity as well as IC_{50} values in vegetables samples. They took seven vegetables before and after 7 minutes of cooking. IC_{50} values of vegetables increased while antioxidant capacity reduced. In our study antioxidant activity of vegetables are also reduced after cooking and IC_{50} values of is between -$49.003_{\mu g/ml}$ to $24.595_{\mu g/ml}$ (Srianta, 2012)explained that leafy vegetables have higher antioxidant activity with IC_{50} values of 4.53 mg/ml as well as 8.46 mg/ml while in our results leafy vegetables after cooking have IC_{50} values 2.138 mg/ml, -49.003 mg/ml, -5.4609 mg/ml and 1.23256 mg/ml.

Antibacterial activity: This investigation was planned to explore antibacterial activity of raw mixture plus cooked vegetables. Humans have incredible influence on edible vegetations since

before civilization. Vegetables are cheap source of food for humans. Vegetables are affluent source of antioxidants and nutrients. Green leafy vegetables considered balance diet as well (Kamble, 2013). Bacteria are giving very high resistance to antibiotics, so natural antimicrobial agents may be best alternative to these conventional medicines. Majority of medicines are plant derived in recent pharmacy industry (Dubey, 2010).

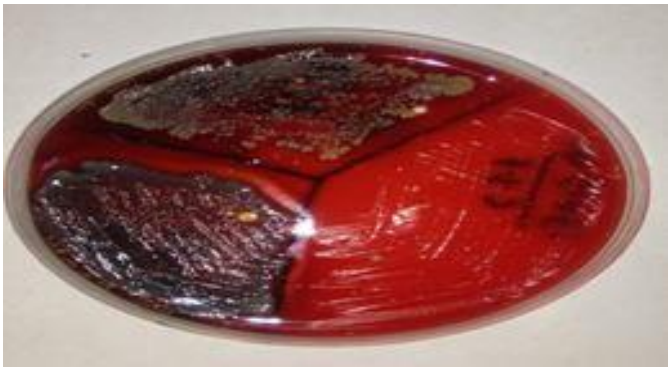


Figure 1 A

Figure 3 C

Figure 2B

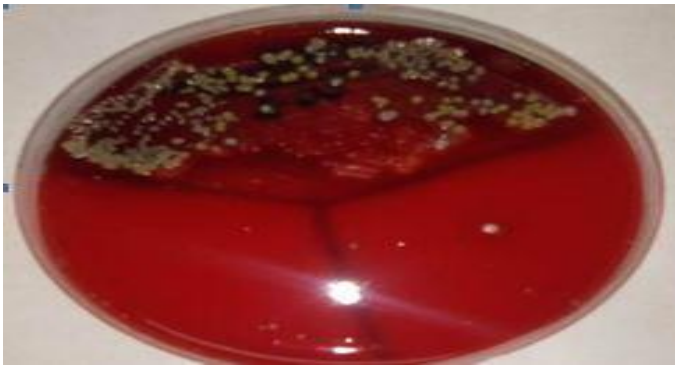
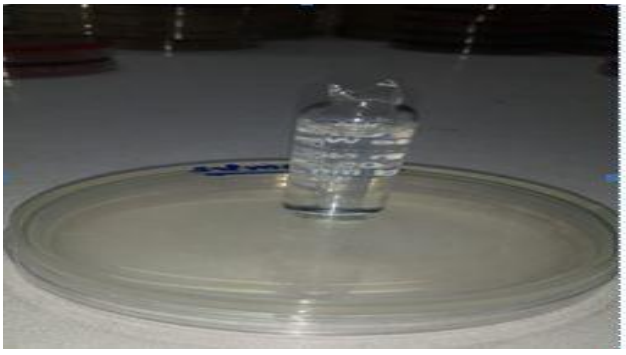


Figure 4 D

Figure 4.39 A: *Staphylococcus aureus*, **B**: Raw mixture of vegetable extracts (**A**: *C. annuum* + *S. tuberosum*,**B:** *B. oleracea var. botrytis* + *S. tuberosum*,**C:** *P. sativum* + *S. tuberosum*, D: *S. melongena*) against *S. aureus.* **C:** Cooked vegetables extracts (**A**: *C. annuum* + *S. tuberosum*,**B:** *B. oleracea var. botrytis* + *S. tuberosum*,**C:** *P. sativum* + *S. tuberosum*, **D:** *S. melongena* ***E:*** *C.pepo* ***F:***Raw mixture of *C.pepo*) against *S. aureu*

A 1

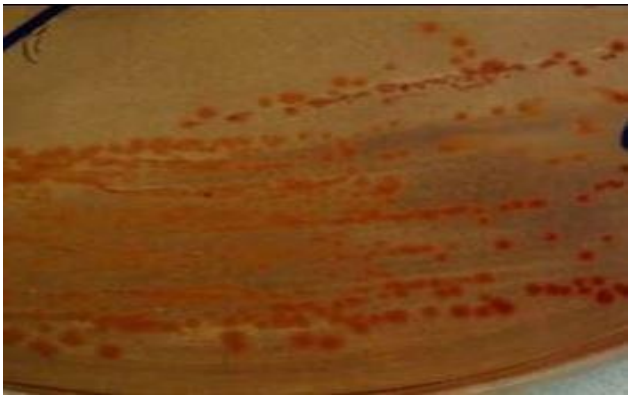


B 1

C 1

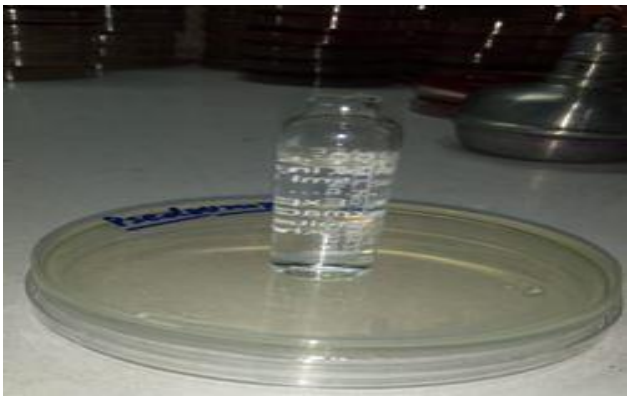
D 1

Figure 4.40 **A, B:** *Salmonella typhi*, **C:** Raw mixture of vegetable extracts (**A**: *C. annuum* + *S. tuberosum*,**B:** *B. oleracea var. botrytis* + *S. tuberosum*,**C:** *P. sativum* + *S. tuberosum*, D: *S. melongena*) against *S. typhi* **D:** Cooked vegetables extracts (**A**: *C. annuum* + *S. tuberosum*,**B:** *B. oleracea var. botrytis* + *S. tuberosum*,**C:** *P. sativum* + *S. tuberosum*, **D:** *S. melongena* ***E:*** *C.pepo* ***F:***Raw mixture of *C.pepo*) against *S. typhi.*



A 2

C 2

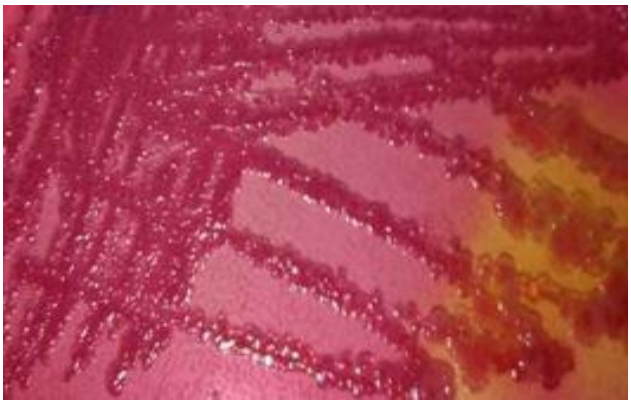


B 2

D 2

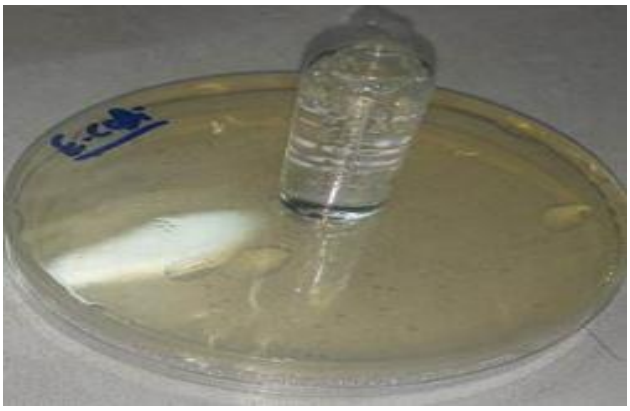
Figure 4.41 **A, B:** *Pseudomonas aeruginosa*, **C:** Raw mixture of vegetable extracts (**A**: *C. annuum* + *S. tuberosum*,**B:** *B. oleracea var. botrytis* + *S. tuberosum*,**C:** *P. sativum* + *S. tuberosum*, D: *S. melongena*) against

P. aeruginosa, **D:** Cooked vegetables extracts (**A**: *C. annuum + S. tuberosum*,**B:** *B. oleracea var. botrytis + S. tuberosum*,**C:** *P. sativum + S. tuberosum,* **D:** *S. melongena* ***E:*** *C.pepo* ***F:***Raw mixture of *C.pepo*) against against *P. aeruginosa***.**



A 3

C 3



B 3

D 3

Figure 4.42 **A, B:** *E.coli* **C:** Raw mixture of vegetables extracts (**A**: *C. annuum + S. tuberosum*,**B:** *B. oleracea var. botrytis + S. tuberosum*,**C:** *P. sativum + S. tuberosum,* D: *S. melongena*) against *E.coli***, D:** Cooked vegetables extracts (**A**: *C. annuum + S. tuberosum*,**B:** *B. oleracea var. botrytis + S. tuberosum*,**C:** *P. sativum + S. tuberosum,* **D:** *S. melongena* ***E:*** *C.pepo* ***F:***Raw mixture of *C.pepo*) against *E. coli*

Investigation have tried to measure antibacterial activity of such vegetables (*solanum tuberosum, Daucus carota, Pisum sativum, Capsicum annuum, Brassica oleracea var. botrytis* and *Cucurbita pepo*) which were showed highest scavenging activity after cooking. For this purpose, used four strains *S. aureus, S. typhi, P. aeruginosa,* and *E. coli.* Were used well diffusion method to inject our samples. Our samples have not showed any antibacterial activity while they were show high SA activity. Observed that raw mixture plus cooked vegetables have antioxidant activity but no anti-bacterial activity against our selected bacterial strains while standard antibiotic has showed their inhibition zone.

Discussion: Vegetables are cheap source of food for humans. These vegetables are fountainhead of antioxidants and nutrients. Green leafy vegetables are considered balance diet as well. Mostly health conscious people like to eat more vegetables and fruits and less quantity of red meat. Green vegetables are great source of minerals and vitamins for People who have low income (Kamble, 2013). Plants are extensive source of medicine from ancient time. Relationship of human with exploration of drug through plants vegetation is very old. There are plenty of proofs from different sources like preserved monuments, written paper as well as original drugs which are plants based. Cure through plants is the results of several years of great effort against sickness. All over globe plants are utilized against infectious illness. Herbal medicines are such medicines which are derived from plant base material and have beneficial properties against disease. Plants are used in a variety of home remedies as well as raw stuff in pharmacy manufacturing. Likely 7,500 plants are applicable in health only in India. Peoples who are vegetarian are healthier because their foods include very high quantity of different "supernutrients" such as micronutrients, phytochemicals and defensive antioxidants. Anticancer, antimicrobial, anti-parasites and antiviral are major pharmacological roles of plants. Free radical scavenging agents are also present in plants. Risk of major diseases is reduced due to the phytochemicals of plants (Shakya, 2016). Use of vegetations in food like cereals different fruits and vegetables are very beneficial for human health. Major bioactive compounds in plant extracts like phenolic as well as polyphenolics have great antioxidant activity. Polyphenols also have repair system in the favor of plant from oxidative polymerization by different enzymes. These compounds also have major role in reproduction as well as plants development. They also provide sensory characteristics, nutritional properties and excellent defense against pathogens as well as predators (Naczk, 2006).

In the present study have tried to check antioxidants plus anti-bacterial activity of vegetables which cannot consume raw. Mostly vegetables are not utilized as raw; use it after cooking i.e. boiling microwaving as well as grilling. In Pakistan, vegetables are used after proper cooking or cooked with other vegetables; often single vegetable is not cooked. Tried to analyze vegetables before and after cooking by applying spices, oil plus thermal treatment. Several assays as well as techniques to evaluate antioxidant activity have been developed but used DPPH• method. It is very stable free radical. Delocalization of this molecule give rise to purple color by absorption band which is around 518nm (Molyneux P. , 2004).

Selected famous Pakistani vegetables' dishes and have checked their antioxidant plus antibacterial activity. All vegetables extracts were showed good antioxidant plus scavenging activity. In subcontinent especially in Punjab and Rajasthan *B. campestris* which is locally known as sarsoo ka saag is very famous dish. Different ingredients are used in this recipe. Main component is *B. campestris* and

S. oleracea is also added to get better color and taste. Different spices i.e *Zingiber officinale, A. sativum* paste and *A. cepa* may be added. It is topped with butter or ghee. Traditionally people eat it with Corn bread (Bhat, 2014). Checked SA of *B. campestris* which was very low -234.43% (Jiménez-Monreal, 2009) investigated different home cooking methods like baking, griddling, pressure cooking, microwaving, frying as well as boiling on antioxidant activity of twenty vegetables by applying various antioxidant assays. *Cynara scolymus* kept its extreme high scavenging lipoperoxyl radical capability during all cooking processes and highest losses observed in *B. oleracea var. botrytis* following by microwaving as well as boiling. There is lowest loss of antioxidant in microwave cooking, baking and grilling. Boiling as well as Pressure cooking illustrates the way of high loss. Frying has intermediate position. Water is also not considered good for vegetables' cooking. While our results showed that, raw vegetables have higher antioxidant activity than raw mixtures and cooked Pakistani dishes (with high thermal treatment and spices) (Miglio, 2007) evaluated effects of frequent cooking methods of vegetables like frying, boiling as well as steaming on total antioxidant capacity. Water based cooking methods better in conserved antioxidant activity of vegetables. With respect to texture quality as well as discoloration steamed vegetables were better than boiled while in case of frying antioxidant compounds were not fully retained. In our study, found that *Trigonella foenum-graecum* in raw form had highest antioxidant activity while in mixture raw and cooked form it was decreased. Its scavenging activity was -98.68% (Wachtel-Galor, 2008) found that antioxidant contents were enhanced by microwaving plus boiling but decreased with longer cooking no matter which ever method was applied. In our result, also found that high thermal treatment effected antioxidant activity of vegetables (Azizah, 2009) found that different cooking styles strongly changed pyhtochemicals especially antioxidants. A study was conducted on *C. pepo.*

It was stirred fried later boiled for 2, 4 and 6 minutes and the result were 18 to 45% removal of its entire phenolic compounds. On the other hand, lycopene plus beta carotene was enhanced 02 to 40.6 times upon stir frying plus boiling. 2 minutes boiling of *C. pepo* enhanced antioxidant activity many times. In this study cooked vegetables with traditional Pakistani cooking method in which frying, and, in some cases, boiling was also done e.g. *Brassica campestris* (Chuah, 2008) found effect of various cooking techniques on antioxidants activity of colored capsicum. *Capsicum annum* is known as source of antioxidant, Phyto-chemicals as well as ascorbic acid. Six varieties used with mixture of cooking method for example stir frying, microwave, boiling in water as well as heating for five minutes. Raw and cooked capsicum was analyzed. Outcomes suggest that no noteworthy difference occur ($P > 0.05$) in five minutes when cooked but show major differences ($P < 0.05$) for five minutes in boiling water, more decreased occur

in next thirty minutes. While in our study there is significant difference in antioxidant activity before and after cooking while SA is 80.24% which is 2nd highest in all vegetables (Perla, 2012) worked antioxidant properties of *S. tuberosum* pertaining to six-month depot. He observed five cultivars of and nine advanced selections with Varity of flesh, color and skin. He subjected familial and domestic cooking methods and observed that they could retard various compounds plus % of DPPH radical SA in all samples. In boiling antioxidants are reduced to a lesser extent. Purple *S. tuberosum* had high contents of flavonols while red had flavonoids. Due to over cooking high loss of compounds occurred. Purple as well as red fleshed *S. tuberosum* showed higher radical scavenging properties than white as well as yellow after all above mentioned cooking techniques. Pigments as well as polyphenols in *S. tuberosum* are reduced in microwaving, boiling as well as baking. *S. tuberosum* are great source of antioxidants; can't use it without cooking. In this case cooked *Solanum tuberosum* along with other vegetables like *Trigonella foenum-graecum, Capsicum annum, Pissum sativum, Brassica oleracea var. botrytis* and *Spinacia oleracea.* Results vary in all mixtures.

S. melongena is important member of family Solanaceae, it is well known vegetable in Asia especially in subcontinent and Europe. It is considered storehouse of phytonutrients, phenolic compounds, dietary fibers and vitamins (Dubey, 2010). Mostly it is not used in raw form. Boiling, grilling or frying is used for its cooking. In Pakistan mostly *S. melongena* are cooked with *S. tuberosum.* Mashed form of *S. melongena* is cooked and known as baingan ka barta in Punjab. Its roasted directly on flame thus has a smoky flavor. It is a pure vegetarian dish as *A. cepa*, *Zingiber officinale*, *A. sativum*, red chili, salt, oil, garam masala as well as parsley added to it. This dish is traditionally eaten with roti, paratha (Indian flat bread) or rice. Tried to estimate the antioxidant activity of traditionally cooked and raw mixture of *S. melongena.* SA of S. melongena was 53.65%.

Vegetables extracts were prepared in water. DPPH radical SA in cooked and raw mixture of *C. pepo* (486.45%) is highest than *S. tuberosum* plus *C. annuum* (80.24%), *S. tuberosum* plus *B. oleracea var. botrytis* (63.73 %), *S. tuberosum* plus *P. sativum* (58.50%), *S. melogena* (53.65%), *S. tuberosum, P. sativum* and *D. carota,* (6.30%), *S. tuberosum* plus *S. oleracea* (-61.80%) *B. rapa* plus *S. oleracea* (-72.84 %), *S. tuberosum plus Trigonella foenum-graecum,* (-98.68%), *B. oleracea var. capitata* (-198.93%) while lowest SA is observed in *B. campestris* (-234.43%). Difference during DPPH radical scavenging actions (%inhibition) with respect to raw and cooked vegetables species was not constant. Results and trends of antioxidant activity in raw, raw mixture and cooked vegetables vary in each sample. In our study, the most common thing which observed in all results was that antioxidant activity of individual raw vegetables was much higher as compared to raw mixtures or cooked vegetables.

Microbes especially bacteria are big reason of diseases and even death in all over the world. Main reason of different infectious diseases is pathogenic bacteria. Pathogenic microbes are extensively found in our surroundings. *S. aureus* is pus forming infections microbe which causes pneumonia and sever urinary tract infection. Food *poisoning is caused by B. subtillus* and *E. coli*. (Sapkota R. D., 2012)

Since more than 70 years have been using antibiotics against bacteria to save human health. In the beginning antibiotics were very useful against bacteria, but with the passage of time, new phenomenon pertaining to antibiotic resistance in bacteria was observed. Antibiotics resistance in bacteria is a natural phenomenon and due to this resistance, many strong antibiotics are declared useless now. (D'Costa, 2011)

Conclusion: Plants protect human body against harmful effects of free radicals and disease-causing micro-organisms. Vegetables are rich source of antioxidants as well as antibacterial agents.

Antioxidant activity of these vegetables was determined by applying DPPH assay and it was observed that scavenging potential of these vegetable extracts was of varying degrees.

Two-way Analysis of Variance (ANOVA) intended effects of raw mixture plus cooked vegetables. Were checked concentrations plus interaction between these two independent variables which were $P < 0.05$ (Excluded of P. sativum & S. tuberosum and Trigonella foenum-graecum & S. tuberosum).

Difference during DPPH radical scavenging actions (%inhibition) with respect to raw and cooked vegetables was not constant. Results and trends of antioxidant activity in raw, raw mixture and cooked vegetables varied in each sample. In our quantitative study, the most common thing which observed in all results was that antioxidant activity of individual raw vegetables was much higher as compared to raw mixtures or cooked vegetables.

Antioxidant activity of raw vegetables > Raw mixture + cooked vegetables' antioxidant activity.

At varying concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL) of resultant mixtures of vegetables' samples, were checked with DPPH. There was no rhythmic change in antioxidant activity at ranging concentration rising from 10 to 100 µg/ml. The bacterial species were cultured in Lauria Bertaini (L.B.) medium and vegetable samples and standard antibiotics were co-cultivated with their respective bacterial strains to see how effectively they control their growth in the given medium. The zone of inhibition of entire vegetables extract was checked. In results checked that raw mixture and cooked vegetables have antioxidant activity but no anti-bacterial activity against our selected bacterial strains while standard antibiotics have shown their inhibition zone.

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