Antioxidative, protein kinase inhibition and antibacterial potential of seven mango varieties cultivated in Pakistan

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Abstract: Peel, pulp and kernel extracts of seven mango fruit (varieties) were analyzedsubjected for antibacterial and antioxidative potential. Langra peel showed good activity against *B. subtilis*, *S. aureus* and *E. aerogenes*. Good zone of inhibition by chaunsa kernel (28mm) and pulp (22mm) against *Streptomyces* stipulate its potential as anticarcinogencancerous. Dosehri and almashil pulp, and sindhri peel asserted free radical scavenger (upto 79%) determined through DPPH assay. The peel and kernel of almashil contained maximum (total) flavonoids contents (58 & 43μgQE/100mg, respectively) while; total phenolics were higher in kernel of sindhri, chaunsa, langra and hujra and almashil pulp. Reducing power potential demonstrated variation from 300 to 554μgAAE/100mg. Total antioxidant potential was maximum in hujra pulp (512μg AAE/100mg). Concluded This study concludes that mango has vast beneficial potential for prone to human health and may also be used for isolation of antioxidative and antimicrobial as well as a protein kinase inhibition agent.

Keywords: Mango, phenolic, flavonoids, reducing power, antibacterial, antioxidant, protein kinase inhibition, DPPH

INTRODUCTION

Mango "King of all fruits" is known for its due to attractive colors, savoring smell, and delightful taste. Belonging to family Anacardiaceae and genus mangifera, about 500 classes of different mango types exist worldwide. Pakistan is blessed with has very different and tasty kinds of mangoes because of geographical location and compatiblesuitable environment. The major varieties include are Anwar Retol, Dasehri, Langra, Chaunsa, Sindhri, Fajri, Alamas, Gulab Khas, Saharni, Pairi and Fazli.

The main mango growing districts in the Punjab province are Multan, Bahawalpur, Muzzaffargarh and Rahim Yar Khan. While in Sindh province, that plant it is mainly grown in Mirpur Khas, Hyderabad and Thatta regions. Pakistan earns about \$24 million annually from mango by exporting which is almost 40% production (Ghafoor *et al.*, 2013).

Mango is rich in nutrients; phytochemicals; polyphenolic complexes; vitamin C, E and A; carotenoids; prebiotic dietary fiber; etc. Apart from these; varying quantities of vitamins B and other essential nutrients like amino acids, copper, potassium, vitamin K and vitamin B6, pyridoxine are also present in mango (Fowomola, 2010) possibly due to varietal difference (Rodriguez *et al.*, 2006). Bioactive complexes in mango assist human well being health by slowing carcinogenic, atherosclerotic, oxidative, angiogenesis and mutagenic actions (Cao and Cao, 1999). These compounds are also involved in curing mouth diseases, dysentery, disorders of gastrointestinal track,

typhoid fever, scurvy, diarrhoea and sore throat (Campbell et al., 2002). Crushed leaves and seeds of mango are used to cure stomach pain, and also recommended against bee's stings and scorpion poison (Doughari and Manzara, 2008). Different acids (i.e. tartaric acid, malic acid, citric acid, succinic acid, and oxalic acid) present in mango give sour taste to unripe mango hence; they have antiscorbutic, astringent and acidic nature in comparison to ripe mangos that are invigorating, laxative, diuretic and antiscorbutic in nature. Mango seed and peel also contain large amounts of organic compounds i.e. quercetin; quercetin O-glycosides; isoquercetin; quercetin galactoside; 4-dihydroxy benzoic acid; ellagic acid; mangiferin; isomangiferin and carotenoids (Ribeiro et al., 2007; Schieber et al., 2003; Abdalla et al., 2007a; Ajila et al., 2007) which play their role in different biological activities (Soong and Barlow, 2004: Maisathisakal and Gordon, 2009).

Considering the economic and medicinal importance of mango, the studies of on biological activities on mango species of cultured in Pakistan, are rather limited. Therefore, the objectives of this work was to finger print the extracts from pulp, peel and seed kernel of seven mango varieties cultivated in Pakistan; employing sophisticated analytical techniques to determine the antioxidant, total phenolics, total flavonoids, reducing power potential, antibacterial and protein kinase inhibition activities.

MATERIALS AND METHODS

Collection and preparation of extracts

Seven mango varieties i.e. Chaunsa, Langra, Dosehri, Sindhri, Hujra, Almashil and, Dalasi were collected from

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Multan, Bahawalpur and Rahim Yar Khan regions of Pakistan with the help of Pakistan Horticulture Development and Export Board. These varieties are popular were selected among those popular in people. Approximately 3-4 mature, ready to market fruits of each variety were washed under running tap water and dehydrated on filter paper. After peeling, pulp was cut in long thin slices to increase surface area and to make easy drying. The kernel was also separated from the seed. The peel, pulp and kernal of all varieties were first measured to get fresh weight and then partially dried at room temperature under aseptic conditions under continuous airflow. Dried peel, pulp and seed kernel were crushed into small pieces by using pastel and mortar, and dipped in ethanol. After one week extracts were separated from residues by repeated simple filtration using Whatmann filter paper No 1 followed by vacuum filtration (if required) until the filtrate did not contain solid residue. The solvent was evaporated by rotary evaporator and the extracts were stored at 4°C till further analysis.

Antioxidative assays

Determination of total flavonoid contents

Total flavonoid contents were determined following the method of Ghasemi *et al.* (2009) with some modifications. A volume of 20µl of the sample (4mg/ml) was mixed with 10µl of 1M potassium acetate buffer and 10µl of 10% aluminum chloride in 160µl of distilled water and incubated for 30min at room temperature. After that, absorbance was recorded at 405nm wavelength on UV-Vis spectrophotometer. The total flavonoid content was quantified from a standard quercetin curve. A linear correlation at different concentrations (0.0 to 25.0µg/ml) was drawn to find regression coefficient as (y=0.0101x-0.004 and $R^2=0.991$).

Determination of total phenolic contents

The total phenolic contents were determined by the Folin-Ciocalteau method (Ebrahimzadeh *et al.*, 2008) with little modifications. A 20µl of each sample (4mg/ml) was mixed with 90µl diluted stock solution of Folin-Ciocalteu reagent and incubated for 5min at room temperature followed by addition of 90µl of 6% sodium carbonate solution. Absorbance was recorded at 630nm wavelength on using UV-Vis spectrophotometer. The concentration of total phenolic content in the samples was determined as µg of gallic acid equivalent (GAE) by calibration curve. Gallic acid was used as standard and a linear correlation at different concentrations (0.0 to 25.0µg/ml) was drawn to find regression coefficient as (y=0.0732x-0.0205 and R²=0.991).

Determination of total antioxidant capacity/potential

The antioxidant activity of the extracts was evaluated by phosphomolybdenum method according to the procedure of as per Prieto *et al.*, (1999). An aliquot of 100µL from stock solution of each extract (4mg/mL in DMSO) was

mixed with 900µL reagent solutions comprising of 0.6M sulfuric acid, 4mM ammonium molybdate and 28mM sodium phosphate. The reaction mixtures were incubated at 95°C for 90min followed by cooling at room temperature and absorbance was measured at 695nm by using microplate reader. DMSO (100µL) in place of test samples was used as control. For calibration curve, ascorbic acid was used as positive control. The resultant TAC was expressed as μg ascorbic acid equivalent per mg dry weight (μg AAE/mg extract).

Determination of reducing power assay

The reducing power assay was carried out by the method of Koleva *et al.* (2002). A 200μl of sample (4mg/ml) was mixed with 500μl of 0.2M phosphate buffer (pH 6.6) and 500μl of 1% potassium ferricyanide and the mixture was incubated at 1500°C for 20min. Afterwards, 500μl of 10% trichloroacetic acid (TCA) was added and centrifuged at 3000rpm for 10min. Finally, 500μl of the supernatant was mixed with 100μl of 0.1% ferric chloride. The absorbance was measured at 630nm wavelength using UV-Visible Spectrophotometer. The intensity of reducing power is directly proportional to the absorbance of the reaction mixture. Ascorbic acid was maintained as positive control and results were expressed as μg ascorbic acid equivalent per mg dry weight (μg AAE/mg extract).

Determination of DPPH assay

The DPPH free radical scavenging assay was carried out by the method of Tagashira and Ohtake (1998) with some alteration. A volume of 20µl of each sample (4mg/ml) was mixed with 180µl of DPPH reagent (3.2mg of DPPH reagent/100ml DMSO) followed by incubation for 1 hour in the dark. The reduction of the DPPH radical was determined by recording the absorbance at 517nm using UV-Vis spectrophotometer. DPPH free radical-scavenging activity was calculated by the following equation:

DPPH radical scavenging activity (% inhibition) = [(control OD - sample OD) / control OD)] ×100

Antibacterial activity

To conduct antibacterial assay, the procedure detailed of by Kabuki and others (2000) was followed with some modification (Rizvi *et al.* 2013). In short, the extracts of peel, pulp and kernel were prepared at 4mg/ml in DMSO. The bacterial strains *Bacillus subtilis, Pseudomonas septica, Micrococcus luteus, Enterobacter aerogenes* and *Staphylococcus aureus* were regrown refreshed overnight in nutrient broth (Merck). Prepared plates of nutrient agar medium were surface streaked with the respective bacterial strain followed by placing of 6mm diameter (mm) discs containing 5µl each of extract solution; negative and positive control. After incubation of plates at 37°C for 24h; the diameter of zone of inhibition was measured. Roxithromycin (3µg/ml) was used as positive control while pure DMSO as negative control.

The percentage growth inhibition was calculated by: Percentage inhibition = (TS-SC)/PC x 100 Where TS, SC and PC represents test sample, solvent control and positive control, respectively.

Protein kinase inhibition assay

The procedure described by Zhang et al. (2008) was followed for determination of protein kinase inhibition (the activity. Streptomyces largest genus Actinobacteria) was maintained on ISP4 medium (Difco Laboratories) for the preparation of spores and liquid TSB medium (Sigma-Aldrich) was used for propagation of mycelium. Streptomyces culture was refreshed on TSB medium in a shaker incubator at 28°C. An aliquot of 60ul of refreshed culture was taken in an eppendorf tube and mixed with 540µl of sterile TSB media. The ISP4 medium was autoclaved and approx. 20-25ml was poured into sterile Petri plates and allowed to solidify. Sterile cotton swabs were used to culture inoculum homogeneously over the entire surface of the Petri plates. The discs of (6mm diameter) were placed on the surface and 5µl each of each extract solution (20mg/ml in DMSO); and the control (DMSO) was poured carefully one the discs. After incubation of plates at 37°C for 24h; the diameter of zone of inhibition was measured.

STATISTICAL ANALYSIS

All of the tests were performed in triplicate and the results were analyzed statistically through SPS in order to determine average difference between the mean values. The means were further analyzed using one way analysis of variance (ANOVA) and least significant difference (LSD) at probability level p<0.05. Linear regression was applied for establishing and verifying the correlation between ascorbic acid and gallic acid for antioxidant activities.

RESULTS

Langra and Hujra bear have maximum average weight followed by Imashil and Sindhri (table 1). In all mango varieties, pulp weight was maximum; from 63% (for Ddosehri) to 80% (for langra and dalasi). Highest moisture content was s were observed in dosehri as the dry weight was only 13% of total pulp fresh weight while maximum dry weight was observed for chaunsa (61%). For other mango varieties, the pulp dry weight was approx. one third of fresh original weight. Dosehri mango variety produced maximum extract of peel (7.2g; 24.3% of peel dry weight) while chaunsa pulp produced maximum extract (10.4g).

Antioxidative potential of mango peel, pulp and kernel extracts

Total phenolic and total flavonoid contents

The peel, pulp and kernel showed varying concentration of total phenolics among mango verities. Pulp of almashil

contained the highest phenolic contents (7.2 μ g GAE/100mg of sample) following kernel of Chausa and Hhujra. The lowest values of total phenolics were found for Chaunsa and Sindhri; 2.4 and 3.4 fold less than almashil pulp. It was observed that kernel mostly contained significant amount of phenolics followed by peel and pulp. Peel also contained significant amount of phenolics with maximum concentration 6.1 μ g GAE/100mg in chaunsa (fig. 1A). Almashil peel bearded leading position containing total flavonoids contents (58.5 μ g QE/100mg of sample) and it was approximately thirty times higher than dosehri and Ddalasi peel (fig 1B).

It was observed that edible part of mango (pulp) contained low amount of total flavonoids except langra that contained 40.25µg QE/100mg of extract. The flavonoid contents of langra pulp were 13 time higher than dalasi pulp and 4.5 times higher than almashil pulp.

Kernel of almashil, hujra and sindhri also contained significant amount of flavonoids; 43.9; 33.7; and $32\mu g$ QE/100mg of extract, respectively. Comparing presence of total flavonoids among different mango parts almashil peel contained 6.5 times higher than pulp while peel and kernel of langra contained 3.2 and 2.9 fold less flavonoid contents than its pulp.

Total antioxidant and reducing power potential

Most of the mango verities showed good total antioxidant results but few showed low activities (fig 1C). Hujra pulp showed maximum total antioxidant potential ($512\mu g$ AAE/100mg) followed by kernel of Aalmashil ($507\mu g$ AAE/100mg) and peel of Hhujra ($494\mu g$ AAE/100mg).

Dalasi pulp contained least total antioxidant potential and it was approx 20 fold less then Hujra pulp. Sindhri, Chausa and Hujra mango parts (peel, pulp and kernel) showed total antioxidant capacity with minor variation while significant variation was observed in Dosehri and dalasi mango parts. All mango verities parts showed reducing power potential between AAE/100mg of sample except pulp of dalasi and sindhri, and peel of sindhri (fig. 1D). Langra pulp demonstrated maximum reducing potential power $(554.2 \mu g)$ AAE/100mg of extract).

It was observed that peel and /or kernel of most of the mango verities contained more reducing power potential as compared with pulp. Dalasi pulp showed two fold less reducing power potential as compared with its own parts following sindhri peel and pulp that showed approx 1.5 times less activity than kernel.

DPPH radical scavenging activity

Dosehri and almashil pulp showed highest DPPH scavenging activity; 79.3 and 78.4%, respectively (fig. 2).

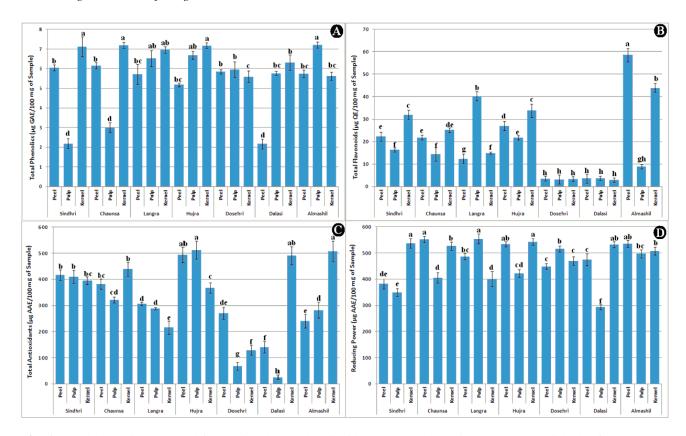


Fig. 1: Total phenolics (a); total flavonoids (b); total antioxidants (c); and reducing power potential (d) of peel, pulp and kernel of seven mango varieties. Data is presented as mean \pm standard error of three values. For each parameter different letters indicate significant differences at $P \le 0.05$ between parts and species.

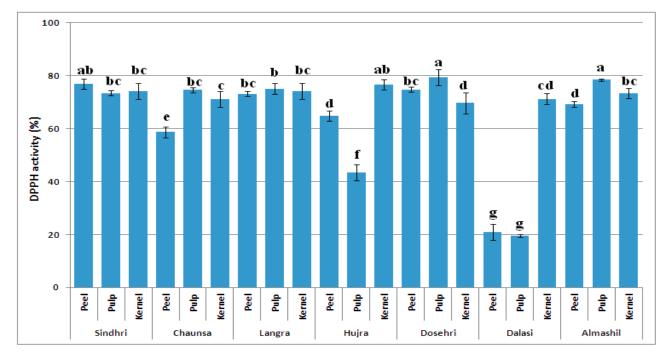


Fig. 2: Radical scavenging activity of peel, pulp and kernel of seven mango varieties. Data is presented as mean \pm standard error of three values. For each parameter different letters indicate significant differences at $P \le 0.05$ between parts and species.

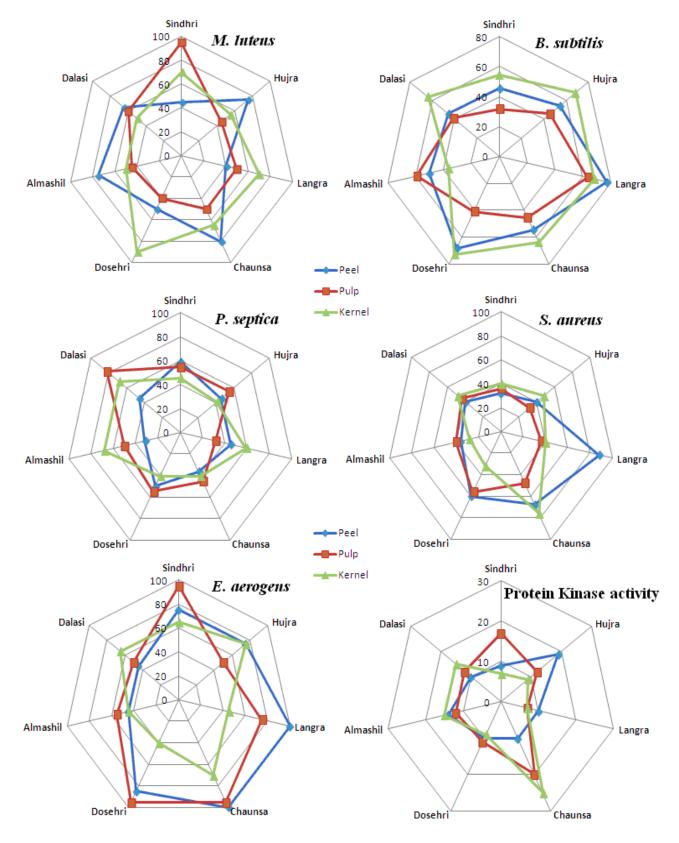


Fig. 3: Percentage inhibition of mango extracts against M. luteus, B. subtilis, P. septica, S. aureus, E. aerogens and protein kinase inhibition activity.

Table 1: Fresh and dried weight and extract weight of peel, pulp and kernel of seven mango varieties

| Mango | Fresh Weight: (g) | | | Dried Weight: (g) | | | Weight of extract (g) | | |
|----------|-------------------|---------|---------|-------------------|---------|----------|-----------------------|------|--------|
| Variety | Peel | Pulp | Kernel | Peel | Pulp | Kernel | Peel | Pulp | Kernel |
| Chaunsa | 161.9±2 | 110.2±4 | 102.2±2 | 45.0±0.5 | 67.2±1 | 74.4±0.2 | 4.5 | 10.4 | 0.6 |
| Langra | 140.2±3 | 788.6±3 | 64.3±3 | 48.3±0.1 | 187.6±2 | 40.8±0.4 | 2 | 2.5 | 1 |
| Sindhri | 137.2±2 | 531.3±5 | 89.0±1 | 41.2±0.3 | 84.6±1 | 49.0±0.1 | 5.2 | 2.2 | 0.7 |
| Dosehri | 96.5±4 | 520.6±4 | 30.5±2 | 29.6±0.2 | 28.8±2 | 15.6±0.5 | 7.2 | 5 | 0.5 |
| Almashil | 177.8±3 | 587.2±5 | 40.4±1 | 56.9±1 | 222.8±1 | 17.5±0.2 | 3.5 | 3.1 | 1.3 |
| Hujra | 192.8±2 | 744.3±3 | 58.8±3 | 60.2±0.5 | 242.7±2 | 32.1±0.3 | 5 | 5.2 | 0.5 |
| Dalasi | 85.2±1 | 444.0±4 | 27.3±1 | 23.3±1 | 143.8±1 | 14.5±0.7 | 2.4 | 6.6 | 0.85 |

Table 2: Antibacterial activity of mango extracts against bacterial species tested by disc diffusion method

| Mango | Part | B. subtilis | M. luteus | P. septica | S. aureus | E. aerogens | | | |
|---------------|--------|-------------------------|-----------------------|-----------------------|---------------------|----------------------|--|--|--|
| variety | | Zone of inhibition (mm) | | | | | | | |
| | Peel | 10±1 ^e | 9±0.5 ^g | 13±0.25° | 8±1 ^h | 15±0.5° | | | |
| Sindhri | Pulp | $7\pm1^{\rm h}$ | 10±1 ^f | 12±1 ^{cd} | 9±0.5 ^{gh} | 19±1 ^{ab} | | | |
| | Kernel | 12±0.25 ^d | 14±0.75° | 10±1 ^e | 10±0.5 ^g | 13±1 ^d | | | |
| Hujra | Peel | 12±0.5 ^d | 15±1° | 10±1 ^e | 10±0.4 ^g | 15±0.5° | | | |
| | Pulp | 10±0.3 ^e | 9±0.1 ^g | 12±0.2 ^{cd} | 8±0.4 ^h | 10±1 ^{ef} | | | |
| | Kernel | 15±0.5 ^{bc} | 11±1 ^e | 9±1 ^f | 10±1 ^g | 15±0.5° | | | |
| Langra | Peel | 17±1 ^a | 8±0.5 ^h | 10±0.2 ^e | 22±0.5° | 20±0.3° | | | |
| | Pulp | 14±1° | 10±0.25 ^f | 7±0.7 ^h | 9±0.25gh | 15±1° | | | |
| | Kernel | 15±1 ^{bc} | 14±0.75° | 13±0.5° | 10±0.5 ^g | 9±0.2 ^f | | | |
| Chaunsa | Peel | 12±1 ^d | $16\pm0.5^{\rm b}$ | 8 ± 0.3^{g} | 17±0.5° | 20±0.7° | | | |
| | Pulp | 10±0.6 ^e | 10±1 ^f | 10±1 ^e | 12±1 ^f | 19±0.8 ^{ab} | | | |
| | Kernel | 14 ± 0.9^{c} | 13±0.5 ^d | 9±1 ^f | 19±0.4 ^b | 14±1 ^{cd} | | | |
| Dosehri | Peel | 15±0.4 ^{bc} | 10±1 ^f | 11±1 ^d | 15±0.9 ^d | 17±0.10 ^b | | | |
| | Pulp | 9±1 ^f | 8±0.7 ^h | 12±0.75 ^{cd} | 14±0.4 ^e | 19±0.7 ^{ab} | | | |
| | Kernel | 16±1 ^b | 18±0.9 ^a | $9\pm0.75^{\rm f}$ | 8±1 ^h | 8±1 ^g | | | |
| Almashil | Peel | 11±0.9 ^{de} | 15±0.3° | 7±0.5 ^h | 9±0.5gh | 9±0.10 ^f | | | |
| | Pulp | 13±0.8° | 9±1 ^g | 11±1 ^d | 10±0.7 ^g | 11±0.8 ^e | | | |
| | Kernel | 8±1 ^g | 10±0.5 ^f | 15±0.2 ^b | 7 ± 0.6^{i} | 9±0.5 ^f | | | |
| Dalasi | Peel | 10±1 ^e | 13±0.75 ^d | 10±0.7 ^e | 10±0.6 ^g | 9±1 ^f | | | |
| | Pulp | 9±0.7 ^f | 12±0.75 ^{de} | 18±0.5 ^a | 11±1 ^{fg} | 10±0.4 ^{ef} | | | |
| | Kernel | 14±0.75° | 10±1 ^f | 15±0.6 ^b | 12±0.9 ^f | 13±1 ^d | | | |
| Roxithromycin | | 22 | 20 | 22 | 25 | 27 | | | |
| DMSO | | 0 | 0 | 0 | 0 | 0 | | | |

These activities were four times greater than dalasi pulp and approx. 1.9 fold than hujra pulp. Chaunsa and langra pulp also showed significant activity. Peel of many mango verities also showed good percent scavenging activity i.e. sindhri, langra, dosehri, and same case for kernel. Dalasi peel and pulp showed least scavenging activity and it was about three fold less than present in others.

Antibacterial activity

All mango parts extracts showed varying zone of inhibition against bacterial strains. It was observed that most extracts were active against E. aerogenes (zone of inhibition ≥ 15 mm) followed by B. subtilis > S. aureus > M. leuteus and P. septica. Maximum zone of inhibition

(22mm) was observed against *S. aureus* by Langra peel (table 2). This extract also showed good activity against *E. aerogenes* (20mm) and *B. subtilis* (17mm). Maximum percent inhibition was observed against *E. aerogens* followed by *B. subtilis* and *M. leutus* (fig. 3). *P. septic* and *S. aureus* were least inhibited by mango parts extracts.

Protein kinase inhibition assay

Protein kinase inhibition assay results demonstrate that chaunsa pulp and kernel, and hujra peel are good candidates for isolation of anticancerous agents. Chaunsa kernel exhibited maximum inhibition (22mm zone of inhibition) of *Streptomyces* following pulp (20mm) (fig. 3). The zone of inhibition for other mango varieties and parts varied from 7 to 15mm.

DISCUSSION

All the mango varieties vary in size and shape and other physiological characteristics i.e. thickness of peel, size and weight of kernel, water content etc. Therefore variation in dry weight and extract were observed. The pulp mostly contain moisture contents and undissolved portion (fiber etc). The extracts weights were quite less as compared with fresh and dry weight indicating that soluble portion is very less amount.

Phenolic compounds either alone or attached with cell wall mainly present as aglycones, glycosides or esters in plants. It has been reported that in mango kernel antioxidant agents are mostly present in free form instead of conjugate form. Mango contains mangiferin, a special polyphenol of nutraceutical and pharmaceutical significance. However, other polyphenols such as gallic acids, gallotannins, quercetin, isoquercetin, ellagic acid, and β -glucogallin have been identified in the mango pulp (Schieber et al., 2000). It also has been reported that the total antioxidant activity of mango peel extract established higher activity than that of standard mangiferin and quercetin 3-oglucoside, indicating that the antioxidative capacity is synergistic effect of all the compounds present in the mango pulp. A variety of polyphenols were have also been quantified in mango seed kernel i.e. tannin, gallic acid, coumarin, caffeic acid, vanillin, mangiferin, ferulic acid, cinnamic acid, and many others with total polyphenolic contents 112mg (GAE)/ 100 g in seed kernel (Abdalla et al., 2007b). The color of mango pulp and skin; yellowish to radish tint; is due to the presence of anthocyanins, a group of phenolic compounds with good antioxidant properties (Rice-Evans et al. 1997). The mango fruit contains a variety of flavonoids including catechin, epicatechin, quercetin, isoquercetin, fisetin, and astragalin. These flavonoids increase the neutracetical value of mango. Quercetin, a promising flavonoid was has been identified in unripe and ripemature mango fruits however, it disappears on ripening (El-Ansari et al., 1969).

Dalasi pulp contained least total antioxidant potential and it was approx 20 fold less then Hujra pulp. Sindhri, Chausa and Hujra mango parts (peel, pulp and kernel) showed total antioxidant capacity with minor variation while significant variation was observed in Ddosehri and dalasi mango parts. Soong and Barlow (2006) demonstrated that freeze dried and heated mango kernel showed better antioxidant activities as compared with normal conditions and such variation also found in species; and even environmental and cultivation techniques after fluctuatione in the values (Scalzo *et al.*, 2005). The reducing power of the mango extracts was measured by direct electron donation in the reduction of [Fe (CN)6]3 to [Fe(CN)6]4. The product was visualized by addition of free Fe3+ ions after the reduction reaction.

by forming the intense Prussian blue color complex, (Fe3+)4[Fe2+(CN)6]3, and quantified by absorbance measurement. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating ability (von Gadow et al., 1997). Phenolics, carotenoids and anthocyanins present in the peel, pulp and kernel are good electron donors and could reduce Fe3+/ferricyanide complex to ferrous form, which indicates the antioxidant activity (Chung et al., 2002; Yen and Chen, 1995). However, the total activities of mango parts extract is not due to single class of compounds but other types also influence the results with remarkable increase. Ajila et al. (2007) demonstrated that raspuri mango variety extract showed low IC₅₀ values compared to that of Badami peel extracts. Ma et al. (2011) found that the reducing power of eight mango extracts decreased in the order Tainong 1 > Xiaoji > Fengshunwuhe >Mallika > Jinhuang > Irwin >Ao > Guifei. Ribeiro et al. (2007) reported that values of DPPH radical-scavenging activities of pulp of four mango varieties varied ranging from 39.6% to 94.2%. However, variations in antioxidant properties have been observed disregarding mango varieties, parts used and extraction methodologies. Vage-Vage et al. (2013) reported that seed of mango contained higher DPPH EC50 values following pulp and similar situation for phenolics and flavonoids. DPPH activity varies depending upon fruit ripeness and inclined upon mango fruit genotype (Ma et al., 2011).

It has been reported that extracts of different mango parts inhibited the bacterial growth with zone of inhibition upto 17.5mm (Kabuki and others, 2000; Masibo and He, 2009). However, in our results maximum zone of inhibition was observed upto 22mm (95% inhibition in comparison with positive control). The variation in zone of inhibition for Gram negative and Gram positive bacterial strains might be due to structural difference of cell envelop and cell wall (Bibi *et al.* 2011) and variation in the metabolite contents of mango varieties. Mango contains different metabolites such as mangiferin, those possess antimicrobial activity, however, concentrations of such metabolites differ in mango varieties and mango parts (Stoilova *et al.*, 2005).

The *Streptomyces* exhibits a mycelia growth habit on solid media. Under nutrient limitation starvation, differentiation occurs resulting in the formation of septatic aerial hyphae to form spores. This regulation is exerted at the level of transcription and most probably at post-translational covalent modification of proteins (Stowe *et al.*, 1989). Protein phosphorylation is a regulatory mechanism in cellular differentiation, developmental process and metabolism in *Streptomyces*. Based on these studies a number of protein kinase inhibitors have been isolated involved in regulatory networks for signal transduction and cell differentiation (Kikkawa *et al.*, 1989; Hong *et al.*, 1993). Protein kinases

catalyze the transfer of the phosphoryl group from adenosine triphosphate to specific residues of their protein substrate. Because phosphorylation of proteins is a keyregulator of their activity, protein kinases are involved in cell signaling and any dysfunction engender deregulation of the cellular processes (Meijer and others, 2004). For these reasons, kinases constitute very important therapeutic targets and the development of kinase inhibitors has become of major importance nowadays (Nehme et al., 2013; Talcott et al., 2006).

CONCLUSION

A significant genotypic difference was found among mango verities when assessed through for total phenolics, total flavonoids, DPPH scavenging activity, total antioxidant and reducing power potential and even at for antibacterial and protein kinase inhibition assay. In addition, it was also found that the mango peel is also a potential agent demonstrating these properties so can be used as sole agent for isolation of bioactive constituents. Although the pulp is mostly consumed but mango peel and kernel, thrown as waste; are also functional food added ingredients in food and pharmaceutical industries.

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REFERENCES

- Abdalla AEM, Darwish SM, Ayad EHE and El-Hamahmy RM (2007a). Egyptian mango by-product 1. Compositional quality of mango seed kernel. *Food Chem.*, **103**(4): 1134-1140.
- Abdalla AEM, Darwish SM, Ayad EHE and EL-Hamahmy RM (2007b). Egyptian mango by-product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. *Food Chem.*, **103**(4): 1141-1152.
- Ajila CM, Naidu KA, Bhat SG, Prasada and Rao UJS (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chem.* **105**(3): 982-988.
- Bibi Y, Nisa S, Chaudhary MF and Zia M (2011). Antibacterial activity of some selected medicinal plants of Pakistan. *BMC Comp. Alt. Med.*, **11**: 52.
- Campbell RJ, Ledesma N and Campbell CW (2002). Tropical Mangos "How to grow the world's most delicious fruit".1sted. Fairchild Tropical Garden Miami, Florida., pp.222-507.
- Cao YH and Cao RH (1999). Angiogenesis inhibited by drinking tea. *Nature*, **398**(6726): 381-398.
- Chung YC, Chang CT, Chao WW, Lin CF and Chou ST (2002). Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus*

- subtilis IMR-NK1. J. Agri. Food Chem., **50**(8): 2454-2458.
- Doughari JH and Manzara S (2008). *In vitro* antibacterial activity of crude leaf extract of *Mangifera indica* Linn. *Afr. J. Micrbiol. Res.*, **2**: 67-72.
- Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR (2008). Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *Afr. J. Biotechnology*, **7**(18): 3188-3192.
- El-Ansari MA, Reddy KK, Sastry KNAS and Nayudamma Y (1969). Polyphenolic components of mango (*Mangifera indica*). Leather Sci., **16**: 13-14.
- Fowomola MA (2010). Some nutrients and antinutrients contents of mango (*Magnifera indica*). seed. *Afr. J. Food Sci.*, **4**(8): 472-476.
- Ghafoor A, Mustafa K, Zafar I, Mushtaq K and Hussain M (2013). Determinants and margins of exporting mango from Pakistan to UAE market. *Sarhad. J. Agri.*, **29**(3): 474-484.
- Ghasemi K, Ghasemi Y and Ebrahimzadeh MA (2009). Antioxidant activity, phenol and flavonoid contents of 13 Citrus species peels and tissues. *Pak. J. Pharm. Sci.*, **22**(3): 277-281.
- Hong SK, Atsushi M, Sueharu H and Teruhiko B (1993). Effects of protein kinase inhibitors on *in vitro* protein phosphorylation and cellular differentiation of *Streptomyces griseus*. *Mol. Gene Genetics*, **236**(2-3): 347-354.
- Kabuki T, Nakajima H, Arai A, Ueda S, Kuwabara Y and Dosako S (2000). Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L). kernel seeds. *Food Chem.*, **71**(1): 61-66.
- Kikkawa U, Kishimoto A and Nishizuka Y (1989). The protein kinase C family, heterogeneity and its implications. *Ann. Rev. Biochem.*, **58**: 31-44.
- Koleva II, Van TA, Beek JP, Linssen AD and Groot Evstatieva LN (2002). Screening of plant extracts for antioxidant activity, comparative study on three testing methods. *Phytochem. Anal.*, 13(1): 8-17.
- Ma X, Hongxia W, Liqin L, Quansheng Y, Songbiao W, Rulin Z, Shanshan X and Yigang Z (2011). Polyphenolic compounds and antioxidant properties in mango fruits. *Scientia Horticulturea*, **129**(1): 102-107.
- Maisuthisakul P and Gordon MH (2009). Antioxidant and tyrosinase inhibitory Activity of mango seed kernel by product. *Food Chem.*, **117**(2): 332-341.
- Masibo M and He Q (2009). Antimicrobial activity and the major polyphenol in leaf extract of *Mangifera indica* L. *Malaysian J. Micrbiol.*, **5**: 73-80.
- Meijer L, Flajolet M and Greengard P (2004). Pharmacological inhibitors of glycogen synthase kinase-3. *Trends Pharmacol. Sci.*, **25**(9): 471-480.
- Nehme H, Reine N, Pierre L, Sylvain R and Philippe M (2013). Human protein kinase inhibitor screening by capillary electrophoresis using transverse diffusion of laminar flow profiles for reactant mixing. *J. Chromatography* A., **1314**: 298-305.

- Percival SS, Talcott ST, Chin ST, Mallak AC, Singleton AL and Moore JP (2006). Neoplastic Transformation of BALB/3T3 Cells and Cell Cycle of HL-60 Cells are Inhibited by Mango (*Mangifera indica* L.) juice and mango juice extracts. *J. Nut.*, **136**(5): 1300-1304.
- Prieto P, Pineda M and Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex, Specific application to the determination of vitamin E. *Annals of Bio Chem.*, **269**(2): 337-341.
- Ribeiro SMR, Barbosa LCA, Queiroz JH, Knodler M and Schieber A (2007). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food Chem.*, **110**(3): 620-626.
- Rice-Evans CA, Miller J and Paganga G (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Sci.*, **2**(4): 152-159.
- Rizvi ZF, Rabia M, Chaudhary MF and Zia M (2013). Antibacterial and antifungal activities of *Lawsonia inermis*, *Lantana camara* and *Swertia angustifolia*. *Pak. J. Bot.*, **45**(1): 275-278.
- Rodriguez J, Di-Pierro D, Gioia M, Monaco S, Delgado R, Coletta M and Marini S (2006). Effects of a natural extract from *Mangifera indica* L. and its active compound, mangiferin, on energy state and lipid peroxidation of red blood cells. *Biochem. Biophysics J.*, **1760**(9): 1333-1342.
- Scalzo J, Politi A, Pellegrini N, Mezzetti B and Battino M (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition*, **21**(2): 207-213.
- Schieber A, Berardini N and Carle R (2003). Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv. "Tommy Atkins"). peels by high-performance chromatographyelectrospray ionization mass spectrometry. *J. Agri. Food Chem.*, **51**(17): 5006-5011.
- Schieber A, Wieland U and Reinhold C (2000). Characterization of polyphenols in mango puree

- concentrate by HPLC with diode array and mass spectrometric detection. *Innovative Food Sci.* Em*erging Techno.*, **1**(2): 161-166.
- Soong Y and Barlow P (2006). Quantification of gallic acid and ellagic acid from longan (Dimocarpus longan Lour.). seed and mango (*Mangifera indica* L.). kernel and their effects on antioxidant activity. *J. Food Chem.*, **97**(3): 524-30.
- Soong YY and Barlow PJ (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chem.*, **88**(3): 411-417.
- Stoilova I, Gargova S, Stoyanova A and Ho L (2005). Antimicrobial and antioxidant activity of the polyphenol mangiferin. *Herb Polonica.*, **51**(1-2): 37-44.
- Stowe DJ, Atkinson T and Nicholas HM (1989). Protein kinase activities in cell-flee extracts of *Streptomyces coelicolor* A3(2). *Biochimie.*, **71**(9-10): 1101-1105.
- Tagashira M and Ohtake Y (1998). A new antioxidant 1, 3-benzodioxole from *Melissa officinalis*. *Planta Medica.*, **64**(6): 555-558.
- Vega-Vega V, Silva-Espinoza BA, Cruz-Valenzuela MR, Bernal-Mercado AT, Gonzalez-Aguilar GA, Ruiz-Cruz S, Moctezuma E, Siddiqui W and Ayala-Zavala JF (2013). Antimicrobial and antioxidant properties of byproduct extracts of mango fruit. J. Applied Bot. Food Quality, 86: 205-211.
- Von Gadow A, Joubert E and Hansmann CF (1997). Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chem.*, **60**(1): 73-77.
- Yen GC and Chen HY (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agri. Food Chem.*, **43**(1): 27-37.
- Zhang H, Andrew S, Emily S, Rose-Hellekant T and Chang LC (2008). Bioactive polybrominated diphenyl ethers from the marine sponge Dysidea sp. *J. Nat. Prod.*, **71**(2): 262-264.