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Nutraceuticals of Kinnow: A Review

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ABSTRACT: Drinking 100ml of fresh kinnow juice can give you 20 mg of vitamin C. This fruit is also loaded with vitamin B complex and minerals like sodium, potassium, calcium and copper. Kinnow contains about 2.5 times more calcium than other citrus fruits. Kinnow is a fruit crop which is famous among consumers due to its tasty and delicious juice. It is a multipurpose crop which could be utilized as an important source of nutrients for humans as well as animals. Depending on the environmental conditions and age of kinnow fruit the amount of juice may vary from 45 to 60%. Total soluble solids present in juice reside in range from 9.5 to 16%. Kinnow fruit is a rich source of bioactive constituents, along with specific minerals (sodium 0.01–0.03 mg/g; potassium 1.6–2.5 mg/g; calcium 0.14–0.47 mg/g, and copper 6–8 mg/100 ml), vitamins and volatile compounds. Kinnow fruit could be used for a variety of purposes ranging from fresh juice to candy, jellies and wine. The remaining waste of kinnow fruit after juice extraction could also be useful as animal feed. Addition of kinnow in diet chart could provide many health benefits. The present review paper focused on detail description of nutritional profile of kinnow fruits, pre- and postharvest factors affecting the bioactive profile, volatile compounds, and usefulness of specific compounds present in kinnow for health benefiting properties. Mandarins are of great interest because of their agro-industrial value, high nutrients, and bioactive constituents.

KEYWORDS-nutraceuticals, Kinnow, animal feed, health benefit, bioactive

I. INTRODUCTION

Kinnow is a member of Rutaceae family that belongs to class Magnoliopsida and order Sapindales. Kinnow has own importance at 3rd rank after banana and mango. Indian states like Haryana, Punjab, Himachal Pradesh, Rajasthan, U.P., and Jammu are major kinnow growing regions. It is a delicious fruit that have origin in South East Asia. Worldwide, it is well known for its nutrients rich juice and medicinal properties (Chopra et al., 2004; Hayat et al., 2010; Kelebek et al., 2008). Kinnow juice is a rich source of vitamin C and various antioxidant compounds that are required to sustain healthy life. It could be consumed in raw as well as processed form (Safdar et al., 2017a). Pharmacological importance of kinnow is not only limited to its edible parts, but also to non edible seeds and peel that are also a rich source of bioactive constituents with health benefiting properties (Magda et al., 2008). Peel is an outer part of kinnow fruit which comprises 15–20% of fruit weight. At present during processing of fruits the peel is discarded as a byproduct after extraction of juice. Researchers and food scientists are now focusing on utilization of waste/byproducts of fruits to use them as a substrate in food processing industries, cosmetic as well as pharmaceutical industries (Amir et al., 2019; Kelly et al., 2019; Lima et al., 2019; Majerska et al., 2019; Pereira and Maraschin, 2015). Kinnow juice, seeds and peel have received much attention particularly due to the presence of antioxidant, antimicrobial, and anticancerous properties. Kinnow is a cheap industrial fruit crop which is easily available in the market (Arruda et al., 2018; Barrales et al., 2018; Hayat et al., 2010; Nasir et al., 2016). Like other horticultural crops kinnow also require specific conditions for their optimal growth. Kinnow could be grown in soil ranged from clay loam, sandy loam to acidic soils with pH range 5.5–7.5. Small to medium sized, cadmium yellow-colored kinnow fruits are ready for harvesting during the month of December to January. The final yield depends on number of factors Figure 1. Fruits are particularly rich in bioactive compounds and are well known for their health benefiting properties such as prophylaxis of cardiovascular diseases [reduction of triglyceride levels, LDL level and increase HDL cholesterol], diabetes, allergies, early aging issues, and other chronic disorders (Ali et al., 2019; Andrade et al., 2019; Bvenura and Sivakumar, 2017; Chrczanowicz et al., 2008; Comert and Gokmen, 2018; Dar et al., 2019; Flammer et al., 2013; Kowalwska et al., 2017; Kyriacou and Rouphael, 2018; Majerska et al., 2019; Nowak et al., 2016a; Sarkar and Thirumurugan, 2019; Skoczyńska et al., 2007; Zheng et al., 2017). The present review paper is designed to provide depth knowledge on nutritional profile of kinnow fruit and the importance of specific compounds to maintain disease free life.[1,2]

Kinnow is available in the market with different coloration pattern, thickness of peel, amount of juice, and varying organoleptic properties (Goldenberg et al., 2014; Lado et al., 2016). Inner juicy portion of kinnow fruit is protected by

outer skin known as flavedo/exocarp (colored skin with essential oil secreting glands). Another important part just next to flavedo is white spongy tissues (albedo) which vary in thickness with complex network of vascular bundles (Lado et al., 2016). Edible part of kinnow fruit consist small-sized densely packed sacs which contain juice and seeds. Worldwide kinnow is famous for taste and nutritional importance of its juice. Another reason for the popularity of kinnow fruit is consumer preference. As consumers requirement is to buy a fruit crop that is easy to eat, dust free, and low seed number with maximal juice content. Kinnow fruit could fulfill the rising demands of consumers. Characteristic flavor of kinnow is due to the presence of volatile compounds in them. For marketing of kinnow at commercial scale it is necessary to detect the maturity level before plucking fruits from tree. The inner part of kinnow fruit mature prior to the development of specific color in the outer skin (Lado et al., 2014). Further, the taste of the kinnow fruit depends on the sugar-acid ratio in the juicy sacs. During the ripening period increase in temperature leads to decrease in acid level and increase in sugar content (Benjamin et al., 2013; Lado et al., 2016; Marsh et al., 1999). Amount of total soluble solids (TSS) present in pulp of kinnow fruit varies from 9.5–16% (Goldenberg et al., 2014; Shen et al., 2013; Ladaniya, 2011; Xu et al., 2008). Environmental conditions especially temperature, light irradiance along with agricultural practice significantly affects the sugar content in juicy sacs of fruit.

Kinnow juice is a rich source of primary metabolites such as amino acid, vitamins (ascorbic acid, provitamin-A, and folate) as well as secondary metabolites [bioactive compounds (limonoids, flavones, phenolics, and flavonoids) and carotenoids]. The amount of amino acids present in kinnow juice depends on the storage conditions (temperature and humidity of storage chamber). Major amino acid present in kinnow juice are asparagine (3.5 $\mu\text{mol/g}$) followed by arginine (2.4 $\mu\text{mol/g}$), aspartic acid (2.0 $\mu\text{mol/g}$), proline (1.5 $\mu\text{mol/g}$), and glutamine (1.0 $\mu\text{mol/g}$) (Matsumoto and Ikoma, 2012). Kinnow juice is an important source of osmotic stabilizer and blood pressure regulating minerals (sodium and potassium). Sodium concentration in kinnow juice may vary from 0.01 to 0.03 mg/g whereas potassium ranged from 1.6 to 2.5 mg/g (Barros et al., 2012; Codoñer-Franch and Valls-Bellés, 2010). Mineral profile of kinnow juice indicates the presence of calcium in range 0.14–0.47 mg/g followed by magnesium (0.11–0.12 mg/g), phosphorus (0.10–0.30 mg/g), iron (0.0015–0.0060 mg/g), zinc (0.0007–0.005 mg/g), copper (6–8 mg/100 ml), and manganese (0.0005 mg/g) (Barros et al., 2012; Lado et al., 2016). Minerals are required to maintain healthy bones and activity of mitochondrial enzymes.

Bioactive compounds are present in fruits/vegetables/natural resources as a specific class of phytochemicals with antioxidant/pharmacological properties (Arruda et al., 2018; Dhull et al., 2016; Kaur et al., 2019a; Purewal et al., 2019; Salar et al., 2017a; Singh et al., 2019). These compounds of natural origin contribute a major portion of our daily routine diet (Hayat et al., 2010). Zhang et al. (2014) reported another important derivatives of flavonoids, i.e., narirutin and eriocitrin which is present in 10X amount as compared to hesperidin. The type and amount of bioactive compounds present in natural resources may vary depending on cultivars, conditions under which they are growing (Kaur et al., 2019b; Salar and Purewal, 2017). As compared to fruit juice peels contains highest amount of bioactive compounds (Lafuente et al., 2011).[3,4,5]

Hayat et al. (2011) reported the presence of phenolic compounds and flavonoids in extracts prepared from Kinnow, Feutrell's Early, and Ponkan. Phenolic compounds were present in range 32.9– 63.2 mg GAE/g. The highest phenolic compounds were present in Ponkan whereas lower amount was observed for Kinnow. Comparative analysis of flavonoids indicates the presence of their maximum amount in Ponkan peel extract (35.6 mg REQ/g) and minimum amount in Kinnow peel extract (11.7 mg REQ/g). Sharma et al. (2016) studied pattern of phenolic compounds with antioxidant properties in granulated and nongranulated juicy sacs of Kinnow mandarin. The resulting output of their study indicate that granulated kinnow possess low amount of phenolic compounds (4.3 mg GAE/100 ml); ascorbic acid (16.2 mg/100 ml) with antioxidant capacity (1.78 $\mu\text{mol Trolox/g}$). Whereas, extracts prepared from nongranulated kinnow showed higher phenolic compounds (11.3 mg GAE/100 ml); ascorbic acid (28.6 mg/100 ml) with significantly higher antioxidant capacity (9.51 $\mu\text{mol Trolox/g}$).[3,4]

Zahoor et al. (2016) reported significant variations in antioxidant properties, phenolic compounds, flavonoids and phenolic profile of five citrus fruits. The selected species was *C. reticulata* (Kinnow); *C. sinensis* 'Valancia' (Mausami); *C. paradisi* 'Macfadyen' (Grapefruit); *C. reticulata* Blanco 'Merisol' (Fruiter); and *C. sinensis* 'Maltaise Sanguine' (Red blood). Four different concentration of extraction phase was used for the release of phenolic compounds from selected fruit samples. The selected extraction phases were methanol (100%); ethanol (100%); methanol (80%); and ethanol (80%). Each extraction phase showed their synergistic effect on phenolics as well as flavonoids of selected fruits. Phenolic compounds in different extracts of Kinnow peel were 66.1 mg GAE/g (methanol 100%); 37 mg GAE/g (methanol 80%); 35.9 mg GAE/g (ethanol 100%); and 28.2 mg GAE/g (ethanol 80%). Whereas

flavonoids in different kinnow peel extracts were 25.5 mg CE/g (methanol 100%); 17.3 mg CE/g (methanol 80%); 29.4 mg CE/g (ethanol 100%); and 14.2 mg CE/g (ethanol 80%). Specific phenolic acids in kinnow peel extract (methanol 100%) were gallic acid (45.6 mg/Kg); proto catechuic acid (111.7 mg/Kg); and 3-hydroxybenzoic acid (126.4 mg/Kg). Phenolic compounds in different extracts of mausami peel were 60.5 mg GAE/g (methanol 100%); 30.1 mg GAE/g (methanol 80%); 23.4 mg GAE/g (ethanol 100%); and 21.1 mg GAE/g (ethanol 80%). Whereas flavonoids in different mausami peel extracts were 25.6 mg CE/g (methanol 100%); 11.1 mg CE/g (methanol 80%); 9.3 mg CE/g (ethanol 100%); and 10.4 mg CE/g (ethanol 80%). Phenolic compounds in different extracts of grapefruit peel were 58.2 mg GAE/g (methanol 100%); 36.8 mg GAE/g (methanol 80%); 39.4 mg GAE/g (ethanol 100%); and 22.8 mg GAE/g (ethanol 80%). Whereas flavonoids in different grapefruit peel extracts were 28.8 mg CE/g (methanol 100%); 27 mg CE/g (methanol 80%); 19.4 mg CE/g (ethanol 100%); and 19.2 mg CE/g (ethanol 80%). Phenolic compounds in different extracts of Fruiter (*C. reticulata* Blanco 'Merisol') peel were 55.9 mg GAE/g (methanol 100%); 25.6 mg GAE/g (methanol 80%); 15.7 mg GAE/g (ethanol 100%); and 22.9 mg GAE/g (ethanol 80%). Whereas flavonoids in different Fruiter (*C. reticulata* Blanco 'Merisol') peel extracts were 13.1 mg CE/g (methanol 100%); 15.3 mg CE/g (methanol 80%); 12.5 mg CE/g (ethanol 100%); and 10.1 mg CE/g (ethanol 80%). Phenolic compounds in different extracts of Red blood (*C. sinensis* 'Maltaise Sanguine) peel were 62.3 mg GAE/g (methanol 100%); 20.8 mg GAE/g (methanol 80%); 13.6 mg GAE/g (ethanol 100%); and 26.7 mg GAE/g (ethanol 80%). Whereas flavonoids in different Red blood (*C. sinensis* 'Maltaise Sanguine) peel extracts were 28.9 mg CE/g (methanol 100%); 17.6 mg CE/g (methanol 80%); 21.6 mg CE/g (ethanol 100%); and 19.3 mg CE/g (ethanol 80%). Methanol (100%) seems to be best extraction phase for the recovery of phenolic compounds from selected fruits. Whereas the best extraction phase for flavonoids was vary with the fruit type such as Mausami (*C. sinensis* 'Valancia') (25.6 mg CE/g; methanol 100%); Fruiter (*C. reticulata* Blanco 'Merisol') (15.3 mg CE/g methanol 80%); Red blood (*C. sinensis* 'Maltaise Sanguine); (28.9 mg CE/g methanol 100%); Kinnow (*C. reticulata*) (29.4 mg CE/g ethanol 100%); and Grapefruit (*C. paradise* 'Macfadyen') (28.8 mg CE/g methanol 100%). Comparative analysis results indicate the presence of phenolic compounds in trend Kinnow (*C. reticulata*)>Red blood (*C. sinensis* 'Maltaise Sanguine)>Mausami (*C. sinensis* 'Valancia')>Grapefruit (*C. paradise* 'Macfadyen')>Fruiter (*C. reticulata* Blanco 'Merisol'). Safdar et al. (2017a) compare the efficiency of ultrasound and maceration assisted extraction of phenolic compounds from kinnow peel. Their results indicate that ultrasound assisted extraction is more efficient than maceration. Recovery of phenolic compounds during ultrasound extraction process was 32.4 mg GAE/g whereas during maceration the amount was 8.6 mg GAE/g. In addition to mandarins and other citrus, berry fruit is a wealth of bioactive compounds (Nowak et al., 2016b; Mikulic-Petkovsek et al., 2012; Bermudez-Soto and Tomas-Barberan, 2004).[5,6]

Volatile compounds

Prior to consumption, consumers judge the quality of fruits as well as vegetables by its aroma. Aroma is related to the freshness of the fruits, taste, and maturity. Flavor of fruits could be determined using various sensory features like sweetness of pulp/juice, acidic level and nature of bitterness causing compounds (Maffei, 2010). Aroma in fruits and vegetable is due to the presence of certain specific volatile compounds like aldehydes, ketones, carotenoids derivatives, terpenoids, and esters. The amount and type of volatile compounds in certain fruits and vegetables depends on the genetic makeup, conditions (temperature, pH, and moisture) under which plants grown, postharvest practices, and storage conditions (El-Hadi et al., 2013).

Genetic factors are the main reasons for the presence or absence of any specific features in the fruits. The type of genes and their location in genetic material significantly affects the fruits characteristics. Evaluation of genetic makeup at initial stage could help the breeders to select productive cultivars for farmers. Baldwin et al. (2000) reported that fruits with antisense pectinmethylesterase possess comparatively lowest amount of methanol than other fruits. Shan et al. (2012) observed that during ripening matured melon plants consist only 78% of the volatile esters. Age of fruits is an important determinant factor of aroma as it determines the increase or decrease in volatile compounds. Maturity of fruits could be evaluated by the presence of specific aroma in them. Age not only affects the softness of fruit but at the same it also affects the volatile compounds type and their composition within the fruit. It is recommended that fruits should be harvested in the appropriate stage of maturity. Amount of volatile compounds in fruits plucked at earlier stage is lesser as compared to mature one (Fellman et al., 2003). However, for the purpose to improve the shelf life, they are often harvested at earlier stage to ease their transportation. The amount of volatile compounds also affects the taste of fruit. During storage conditions fruits may lose the capability to synthesize volatile compounds. Menager et al. (2004) observed that fruits at ripening stage have maximum furanone acid lactone and esters. In contrast to above said compounds fruit before ripening possess high level of C₆ aldehydes. Pereira (2010) observed sesquiterpenes as the major volatile compound in immature avocado and the compounds start decreasing with the increase in age of fruit.

Obenland et al. (2012) reported that after attaining maturity, fruits possess maximum methyl acetate, β -myrcene, and acetaldehyde.[7,8]

Application of Chemicals/Growth Regulators

Chemicals/growth regulators and gaseous treatment for fruit ripening significantly affects the concentration of volatile compounds (Brasil and Siddiqui, 2018; Pech et al., 2008; Vendrell, 1985; Wang et al., 2016). Bitterness is the common problem of fruits which is being eradicated by using the calcium treatment which results in enhanced production of compounds related to aroma/flavor (Hocking et al., 2016; Moor et al., 2006). Biochemical pathway of volatile compounds could be altered using methyl jasmonate (He et al., 2018; Liu et al., 2018). Scientific reports suggest that methyl jasmonate has a significant effect of volatile compounds of some specific fruits only. As its application on strawberries results in enhanced production of volatile compounds whereas in blackberries there is no significant change observed. Similar response from fruits in relation to methyl jasmonate could help to solve the mystery of volatile compounds production/alteration in horticultural crops (Moreno et al., 2010b, 2010a; Schwieterman et al., 2014).

Agricultural practices and environmental factors have significant effect on physical and anatomical features of fruits. Cultivation season, timing, use of synthetic fertilizers, and conditions during the growth of fruits, influence the quality of fruits (Juan and Jiezhong, 2017; Madakadze and Kwaramba, 2004). In contrast to growing conditions, harvesting, transportation to market, and storage conditions, other factor that are contributing in changing fruits qualities are insect pests (Adewoyin, 2017; Babatola et al., 2008; Baloch and Bibi, 2012). They are one of the challenging tasks for the farmers for better agricultural practices. The external as well as the internal features of the fruits are generally affected by the application of fertilizer in the field. The type and doses of the chemicals applied in the field affect the fruits quality such as amount of nutrients, color, maturation time, and aroma (Heeb et al., 2006; Liu and Liu, 2012). Use of growth regulators while fruits are at growing stage/harvested has their own significant effects on quality as well as their taste. Farmers use synthetic growth regulator for fruit crops to achieve desirable fruit size, pigmentation, aroma, flowering, abscission of fruitlets, and fruits maturity (early/late).

Transportation and Storage Conditions

Transportation of fruits within state/outside states/countries could be carried out in two different ways. In the first stage farmers sell their fruits directly to consumers either directly from farmland or through local marketing. In the second way farmers send horticultural output for suitable packing followed by cooling in specific chambers and transportation to distant place through suitable vehicles. However, due to road conditions and transportation time some undesirable changes may occur that will affect the aroma of fruits. Other factors that contribute in changing fruit quality is (a) type and conditions of fruit storage chambers, (b) effect of sunlight and moisture loss during transportation, (c) fruit softening/damage, and (d) distribution/circulation of air within the storage chamber.[7,8]

It is important to understand the effect of atmospheric conditions on fruits. Fruits could be stored for a long period by optimizing the conditions they require during storage. Controlled atmospheric conditions could help to maintain the freshness of fruits as well as their supply even during off seasons. For long-term storage, maintenance of gaseous concentration within the chamber is necessary as it could help the fruits to combat ethylene production. Lower concentration of oxygen and increased concentration of carbon dioxide is desirable to maintain freshness in fruits. However, the requirement of O_2 and CO_2 may vary with the type of fruit and their metabolic reactions rate. Without the maintenance of O_2 concentration in storage chamber the metabolic reactions within fruits may shift toward anaerobic pathways which ultimately results in development of off flavors in fruits. Waxing of fruits and use of edible films/packing material is an alternate strategy that is currently being used for the enhancement of shelf life of fruits (Prasad et al., 2018; Li et al., 2018; Singh et al., 2016b; Lin and Zhao, 2007; Saftner, 1999). However, their use in excess amount may affect the volatile compounds of fruits and results in formation of acetaldehyde. Coating improves the external appearance of fruits and decreases the rate of weight loss and helps to retain fruit firmer (Farooqi and Ahmad, 1988). Kaur et al. (2004) reported the effectiveness of wax combination with fungicides toward storage behavior of packaged kinnow fruit. They observed that treatment of kinnow fruits with Imazalil [(1000 ppm with high density polyethylene (HDPE) film] results in better fruit appearance, quality, and low rate of pathological rotting. Mahajan et al. (2015) studied the effect of packing material on TSS, acidity, ascorbic acid, and carotene content. Packing material used in their study was cryovac heat shrinkable RD-106 film (15 μ), low density polyethylene film (LDPE, 25 μ), and high density polyethylene film (HDPE, 10 μ). TSS content of kinnow fruit during the storage period

(5–25 days) in different packing was 9.6–12.6% (Shrink film); 9.1–11.2% (LDPE); 9.3–12% (HDPE). TSS (%) was observed highest in packing materials as compared to control. Maximum acidity (0.54%) was observed in the fruit wrapped in shrink films whereas; the minimal acidity (0.44%) was recorded in control fruits. The changes in acidity during storage period might be due to the increased organic acids catabolism through respiration process. A continuous decline in ascorbic acid content in Kinnow fruits was noticed with the advancement of storage period. The film packed Kinnow fruits in packing materials showed a gradual and steady increase in carotene content up to 15 days, thereafter, a decline was observed; whereas in control fruits, the carotene content increased only up to 10 days of storage and thereafter a decline was observed.

Health Benefits of Kinnow and its Bioactive Compounds

Since long time citrus fruits are being used as traditional medicine for the welfare of human beings. The medicinal properties in them are gaining more interest from scientists/researchers/medical professionals throughout the world (Safdar et al., 2017b; Shetty et al., 2016). Kumar and Sharma (2016) studied the therapeutic potential of kinnow juice on scopolamine induced amnesic rats. Experimental organisms (Albino wistar rats) were divided in to seven different groups and have been given specific doses of kinnow juice (2.5%, 5%, 7.5%, and 10%). The standard group was given treatment with Piracetam (400 mg/kg) for a period of 30 days. For the purpose to induce amnesia, selected rats group was treated with Scopolamine (1 mg/kg body weight; single intraperitoneal injection). Locomotor activities were evaluated using digital photoactometer and the same was expressed as counts/5 min. Kinnow juice was fed to rats and effect on level of cholinesterase was assessed in brain tissue of albino wistar rats. Scopolamine treated albino wistar rats showed significant impairment in acquisition and retention as compare to normal control group. Pre treatments of rats with Kinnow juice for a period of 30 days had shown significant decrease in transfer latency in elevated plus maze. Significant decrease in brain cholinesterase level was also observed. The results of their study clearly indicate that kinnow juice have potential role in the management of cognitive dysfunctions. Higher dose of Kinnow fruit juice (10% v/v, p.o.) for 30 days was comparable to the standard treatment of Piracetam. Safdar et al. (2017b) administered different concentrations of kinnow peel extracts (75 mg, 150 mg, and 300 mg/Kg) in rats for a period of 60 days and myocardial infarction was induced by administering doxorubicin injection (2.5 mg/Kg body weight). Serum and heart tissues were evaluated for the presence of specific cardiac biomarkers and functional activities such as aspartate transaminase, creatine phosphokinase, lactate dehydrogenase, creatine kinase-MB fraction, lipid profile (triglycerides, cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL), renal function activities (creatinine, uric acid, and blood urea nitrogen) and antioxidant enzymes (glutathione peroxidase, superoxide dismutase, and catalase). Histopathological studies were carried out for cardiac tissues of the studied rat groups. Significant protection against doxorubicin-induced myocardial infarction was observed. Administration of doxorubicin resulted in significant elevation of serum lactate dehydrogenase (342.3 IU/L) as compared to untreated counterparts (182.8 IU/L). Pretreatment with peel extracts significantly minimize the effect of doxorubicin administration on serum cardiac biomarkers level in different treatment groups. However, the changes were more pronounced at low dose of peel extracts especially kinnow peel extracts (304.6 IU/L). For pretreatment groups, highest and lowest elevation in creatine kinase myocardial band level in serum was observed in kinnow peel extract low dose (46.5 IU/L). Use of kinnow peel extracts shown significant reduction in LDL. Presence of significant activity in fruits is an indication of good amount of bioactive compounds (Ashraf et al., 2017). Apraj and Pandita (2016) demonstrated that orange peel extract had significant activity for inhibition of elastase (80%) and collagenase (76%). The potential of orange peel could be helpful for the preparation of antiaging products. Shetty et al. (2016) reported that orange peel extracts have antimicrobial potential especially against *Lactobacillus acidophilus* and *Streptococcus mutans* and hence the extracts could be used in treatment dental caries. Ferreira et al. (2018) observed that mandarin peel (ethanolic extract; 500 µg/ml) was capable to reduce the viability of human breast carcinoma cells by 60%. [9,10]

Naim et al. (2012) demonstrated the presence of blood glucose lowering capability in lemon peel (hexane fractions). Kabra et al. (2012) studied the role of *Citrus medica* extract in management of blood glucose level. Significant activity was observed in lemon peel extract as they stimulate β -cell (islets of Langerhans) to secrete glucose lowering insulin hormone. Treatment of streptozotocin-induced diabetic rats with suitable dose of ethanolic extract (400 mg/kg) for a period of 8 weeks resulted in decreased blood glucose, glycosylated hemoglobin and thiobarbituric acid by inhibiting the lipid peroxidation. Sridharan et al. (2016) reported that extracts prepared from lemon peel have potential to prevent calcification in urinary system as they play an important role by inhibiting the calcium oxalate formation (stony concretions). Lemon peel could manage stone induced damages in urinary tract. Kim et al. (2013) studied the medicinal properties in Yuzu (*C. junos*) peel extract prepared in ethanol (5%). They observed the presence of cholesterol lowering properties, weight management potential. Tangerine is an important citrus fruit which could be used in preparation of

dietary supplements for the treatment of neurodegenerative disorders. The anti-neuroinflammatory potential was mainly due to the specific bioactive constituents (hesperidin, nobiletin, and tangeretin) that play an important role during inhibition of LPS-induced proinflammatory cytokine expression (Ho and Kuo, 2014).

Role of Bioactive Compounds in Health

Bioactive phytochemicals present in kinnow fruit have many health benefiting properties.

Scientific reports suggest that ferulic acid has its own importance as a strong antioxidant among other bioactive compounds (Graf, 1992; Itagaki et al., 2009; Shahidi and Chandrasekara, 2010). Edeas et al. (1995) observed the role of ferulic acid in tumor necrosis factors induced activation of HIV infected cell lines. Ferulic acid is active against a broad categories of microbial entity including yeast, gram (+ve) as well as gram (-ve) microbial strains (Borges et al., 2013; Daglia, 2012; Lo and Chung, 1999). Saavedra et al. (2010) studied the combination of ferulic acid with synthetic drug streptomycin and they observed that the combination resulted in more antimicrobial potential in comparison with single forms. Borges et al. (2013) reported that application of ferulic acid as an antimicrobial drug resulted in pore formation in membranes, imbalance in membrane ionic system through hydrophobic changes and finally rupturing of membrane which leads to death of entity. Diabetes is one of most increasing pancreatic disorder in developing/developed nations. Balasubashini et al. (2004) observed a decrease in blood glucose level in diabetic rats upon use of ferulic acid as a remedy. Use of ferulic acid in drug formulation also results in decreased peroxidation of lipids, increased activity of catalase, superoxide dismutase and expanded pancreatic islets (Choi et al., 2011; Ohnishi et al., 2004). Several authors of scientific world suggest that regular consumption of natural resources especially fruits and vegetables that are rich in ferulic acid content results in significant decrease in many type of cancers (Anson et al., 2009; Bourne and Rice-Evans, 1998; Kampa et al., 2007). Being a multifunctional bioactive compounds ferulic acid also has uses in cosmetic industry also. Ferulic acid is used in the preparation of skin lotions, pharmaceutically important formulations and sunscreens creams (Compton and Laszlo, 2002; Graf, 1992; Lin et al., 2005; Oresajo et al., 2008).

p-Coumaric is an another important bioactive constituent which is present in fruits (kinnow, pear and apples) and vegetables/plant parts/cereals. p-Coumaric acid is formed in natural resources as an intermediate complex of phenylpropanoid pathway (Zang et al., 2000). p-Coumaric acid is identified by researchers as an important nutraceutical/bioactive phyto-constituent which exist naturally in three isomeric forms including ortho form; meta form and para form. p-Coumaric acid is an antimicrobial compounds which is active against broad category of microbiota (*B. subtilis*, *B. cereus*, *S. typhimurium*, and *S. dysenteriae*) (Lou et al., 2012). The antimicrobial activity of p-Coumaric acid relies on pH as slight decrease in pH leads to increased activity of compounds against microbial strains. Basically the action of p-Coumaric acid includes membranal pores formation and change in membrane permeability. Antimicrobial activity of p-Coumaric is dependent on number of hydroxyl group present within the structure (Sanchez-Maldonado et al., 2011). Caffeic acid is a bioactive constituent which is present in a wide variety of natural resources. Being bioactive in nature it performs various biological and pharmacologically functions including antioxidant, lipooxygenase inhibitor, oxidative stress retardant, anticancerous properties, and anti-inflammatory activities (Dhull et al., 2016; Karthikesan and Pari, 2007; Natarajan et al., 1996; Pascual et al., 1994; Salar et al., 2017b; Song et al., 2008). Current research related to antioxidant and anti-inflammatory studies has more focus to elaborate the capability of natural extracts to protect DNA from oxidative stress mediated responses (Kaur et al., 2018; Salar et al., 2017c). However, the underlying mechanism related to protection at molecular level is still unclear. In addition to above discussed biological functions caffeic acid is also involved in wound healing process (Bodeker and Hughes, 1998). Sudina et al. (1993) observed the Inhibitory effect of caffeic acid against superoxide generation. Due to the antimicrobial nature of caffeic acid it is also used in the preparation/formulation of various beauty products/skin care formulations (Paulo and Santos, 2018).

Catechins are well known bioactive compounds for their health benefitting properties. Diversity in types of catechin may occur due to their auto-oxidation which results in structural changes and polymerization degree (Singh et al., 2019; Matsui, 2015). One of the monomeric form of catechin, i.e., epigallocatechin could be observed direct in an intact form through specific transporter (Konishi et al., 2003). The specific functionality of catechins within the body may depends on their bioavailability and may vary from source to source (Salar and Purewal, 2016; Scalabert and Williamson, 2000). Catechin and their derivative may affect ionic absorption within the body thus affecting various biological pathways (Nelson and Poulter, 2004; Samman et al., 2001; Zeyuan et al., 1998). The effectiveness of

catechin within digestive tract is dose dependent and the effect on target organs/tissues may be evaluated accordingly (Cabrera et al., 2006; Manach et al., 2004).

Hesperidin an important derivative of flavonoids present in citrus family fruits. Hesperidin is an important bioactive plant pigment with antioxidant properties, metal chelator, required for healthy blood vessels, blood pressure controller, and anti-inflammatory actions. Regular consumption of fruits containing hesperidin may serve as medicinal treatment for hay fever, control changes during menopausal phase and improved blood circulation (Bonina et al., 1996; Deng et al., 1997; Jovanovic et al., 1994; Nandakumar and Balasubramanian, 2012; Suarez et al., 1998). Various in-vitro as well as in-vivo scientific studies reported that hesperidin is involved in diuretic activities, antihypersensitive reactions and control of colon cancer with its antiapoptotic activities in keratinocyte cell line system of humans (Banjerdpongchai et al., 2016; Hewage et al., 2016; Li and Schluesener, 2017; Liu et al., 2017; Natarajan et al., 2011; Saiprasad et al., 2014; Tamilselvam et al., 2013).

Fruits of citrus family are rich sources of specific flavonoids (narirutin, naringenin, nobelitin, and Naringin) (Alam et al., 2014; Tripoli et al., 2007). Naringenin and Naringin present in kinnow fruit are well known for their strong antioxidant properties (Jung et al., 2003; Renugadevi and Prabu, 2009). Horiba et al. (2010) reported the involvement of naringenin in promotion of gene expression and secretion of adiponectin protein from adipocytes. Saponara et al. (2006) observed the regulatory conductance of Ca^{2+} and K^{+} in artery myocytes of rat. Flavonoids along with other bioactive constituents are involved in inhibition of TNF- α induced smooth muscle cells proliferation (Chen et al., 2012). Reddy et al. (2008) evaluated the dose dependent effect of naringin on antioxidant properties and concentration of 8-hydroxy-29-deoxyguanosine and polymerase activity in mice heart and liver. Scientific reports suggest that supplementation of naringin in specific dose results in decreased plasma transaminase activity during Nickel-Cadmium induced liver toxicity in rats (Pari and Amudha, 2011; Renugadevi and Prabu, 2010). Lee et al. (2004) reported the role of naringenin in restoration of protein concentration and serum albumin level in dimethylnitrosamine-induced hepatotoxicity.

Functional properties of bioactive constituents with in living organisms are dependent on dose consumed/type of natural resources consumed in daily diet menu. Quercetin is a biologically active phytochemical which has high molecular weight and sparingly soluble in aqueous phase. Bioactive constituents present in natural resources neutralize the effect of free radicals due to the presence of antioxidant properties in them. Various scientific reports the neuro-protective and neuro-toxic nature of quercetin. Joseph and Muralidhara (2013) evaluated the neuro-protection activity of quercetin in combination with fish oil against Alzheimer disease in rat brain. Quercetin is capable to alter the enzymatic activity of cyclooxygenase (COX) and lipooxygenase (16–17). Consumption of quercetin rich natural sources results in inhibition of platelets aggregation thus improves endothelium health. Quercetin is well known for decrease in mortality rate in low density lipoprotein-related chronic disorders. Dong et al. (2014) reported the dose dependent response of quercetin toward leucocyte suppression, decreased chemokine, and enhanced antioxidant enzyme activities.[11,12]

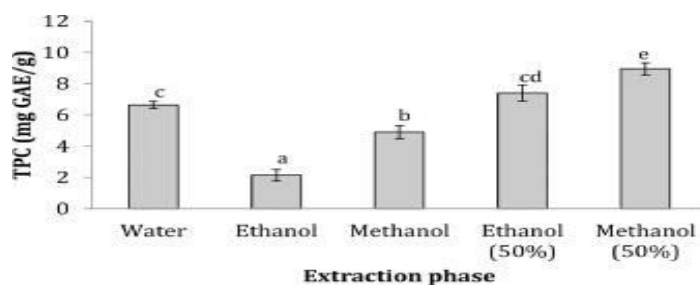
Kaempferol is an important polyphenolic compound which is present in natural resources like vegetable and fruits (Somerset and Johannot, 2008). Scientific reports demonstrate the role of kaempferol in reducing the risk of chronic disorders. Kim et al. (2008) reported the role of kaempferol in signaling pathway which is linked to cancer cells. The compound kaempferol have capability to bind with RSK2 protein especially at the specific positions Val⁸² and the Lys¹⁰⁰ that have critical role in RSK2 functioning (Choi and Kim, 2009). Action of kaempferol on p53 results in phosphorylation at serine¹⁵ which is associated in apoptosis phenomenon (Chen and Chen, 2013; Ito et al., 2004). The activity of extracts containing kaempferol involves the inhibition of VEGF expression and angiogenesis (Luo et al., 2012). Limonin present in fruits of citrus family is a multifunctional bioactive compound which posses antiobesity (Ono et al., 2011); anticancerous (Tian et al., 2001); and many more activities of pharmacological importance (Mandadi et al., 2007; Qin et al., 2018; Ribeiro et al., 2008).

Throughout the world, demand for fruits rich in specific nutrients such as minerals/vitamins and antioxidants is increasing day by day. Kinnow is known as dual purpose horticultural crop which could be used as both food and feed. It is a promising natural source of specific minerals (potassium, magnesium, sodium, calcium, copper, zinc and manganese) and bioactive compounds such as gallic acid, p-hydroxybenzoic acid, vanillic acid, p-Coumaric acid, ferulic acid, chlorogenic acid, caffeic acid, catechin, hesperidin, naringenin, quercetin, and kaempferol which makes it as important as medicine. Therefore kinnow could be recommended as important fruit which could be used in natural

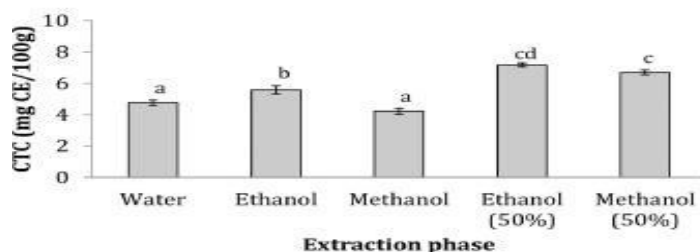
form to combat diseased conditions (diabetes, cancer, blood pressure, and oxidative stress-related disorders). Regular consumption of kinnow based products could be helpful to sustain healthy lifestyle.

II. DISCUSSION

Kinnow seeds were studied in terms of their bioactive profile and antioxidant properties. Seeds extracts prepared in water, ethanol, ethanol (50%), methanol and methanol (50%) were screened for bioactive compounds (condensed tannin and total phenolic content) and antioxidant properties. For the estimation of antioxidant properties in Kinnow seed extracts five antioxidant assays [DPPH (2, 2 diphenyl-1-picryl hydrazyl), FRAP (ferric reducing antioxidant power), ABTS, RPA (reducing power activity) and TAC (total antioxidant capacity)] was performed. Among studied seed extracts those which were prepared in methanol (50%) showed significantly higher TPC value (8.95 mg GAE/g) and antioxidant properties. The correlation observed in TPC and antioxidant properties was (DPPH, $r = .996$, $p < 0.01$; ABTS, $r = .997$, $p < 0.01$; TAC, $r = .990$, $p < 0.01$; RPA, $r = .993$, $p < 0.01$; FRAP, $r = .975$, $p < 0.01$). Observation of study demonstrated that Kinnow seeds could be used in the preparation of antioxidant rich low cost cosmetic, dietary and other supplements of pharmaceutical importance.



a Effect of extraction phase on TPC value of Kinnow seeds



(b) Effect of extraction phase on CTC values of Kinnow seeds

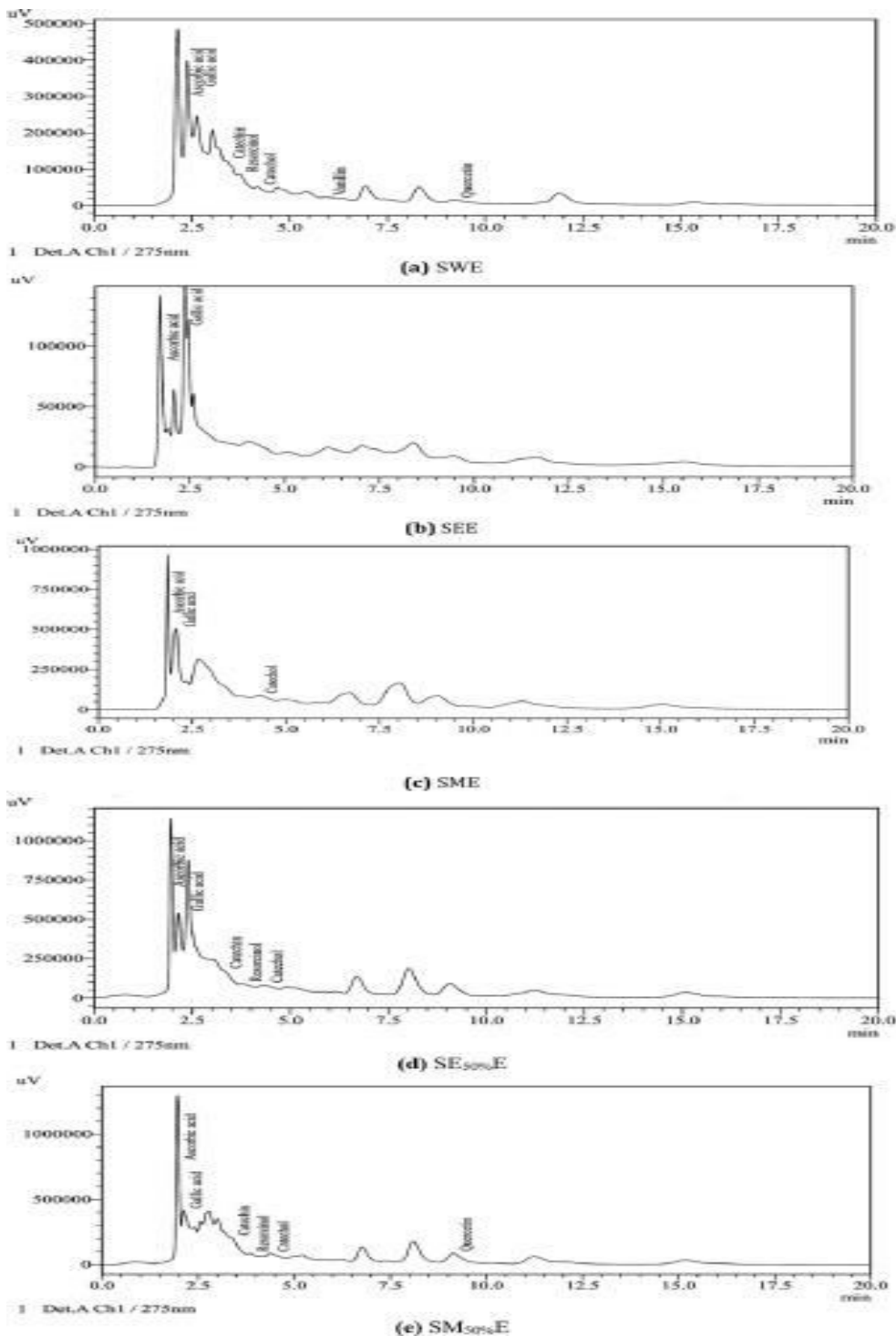
TPC in seed extracts (SWE, SEE, SME, SE_{50%}E, SM_{50%}E) was determined according to the method as described by Yu et al. (2004). Aliquot of seed extracts (100 μ L) were added in reaction vial (sample tube) to react with Folin & Ciocalteu's phenol reagent (500 μ L), thereafter, aqueous sodium carbonate solution (1500 μ L, 20%) was added. Seed extracts-reagents mixture was mixed well using vortex mixer and left at ambient room temperature for 15 min. Thereafter, using distilled water (7.9 mL), the final volume (10 mL) was prepared. Gallic acid standard stock (mg/ml) was prepared which was further diluted (100 μ g/ml, 200 μ g/ml, 300 μ g/ml, 400 μ g/ml, 500 μ g/ml, 600 μ g/ml and 700 μ g/ml), for the purpose to prepare standard curve. TPC of seed extracts (SWE, SEE, SME, SE_{50%}E, SM_{50%}E) was quantified using gallic acid standard curve equation and results were expressed as mg GAE (Gallic acid equivalent)/g.

Condensed tannin content (CTC)

CTC in seed extracts (SWE, SEE, SME, SE_{50%}E, SM_{50%}E) was determined by the C₈H₈O₃-HCl method (Julkunen-Titto, 1985). Briefly, 100 μ L of each extract was added separately in each sample vial (10 mL) followed by addition of Vanillin (3 mL, 4%, w/v) and conc. HCl (1.5 mL). The extract-reagent mixture was then incubated at room temperature (20 min) for the purpose to develop specific color in the reaction mixture. The absorbance of colored reaction mixture (seed extracts and C₈H₈O₃:HCl) against a blank was recorded (500 nm). Catechin standard stock (mg/ml) was prepared which was further diluted (100 μ g/ml, 200 μ g/ml, 300 μ g/ml, 400 μ g/ml, 500 μ g/ml, 600 μ g/ml and 700 μ g/ml), for the purpose to prepare standard curve. CTC in extracts (SWE, SEE, SME, SE_{50%}E, SM_{50%}E) were calculated using



Catechin as standard and CTC value was expressed as mg CE (Catechin equivalent)/g. Seed extracts (SWE, SEE, SME, SE_{50%}E, SM_{50%}E) were evaluated for % DPPH inhibition using the DPPH assay as described by Yen and Chen (1995).



Aliquot of each seed extract (SWE, SEE, SME, SE_{50%}E, SM_{50%}E) (100 μ L) was added in reaction vial (sample tube), thereafter, DPPH reagent (3 mL, 100 μ M) was added and the resulting mixture was then incubated at room temperature under dark conditions (30 min). Absorbance (A) of the colored extracts-reagent mixture was noted (517 nm) at 0 and 30 min against blank. DPPH inhibition activity (%) was calculated. Experimental data generated from present investigation suggests that seed extracts of Kinnow is a source of bioactive compounds with antioxidant properties.



Aqueous methanol (50%) was observed as best extraction medium as its use results in higher recovery of bioactive compounds from seed flour. [12,13]

Specific bioactive compounds detected in seed extracts.

Extraction phase	Ascorbic acid (µg/mL)	Gallic acid (µg/mL)	Catechin (µg/mL)	Resorcinol (µg/mL)	Catechol (µg/mL)	Vanillin (µg/mL)	Quercetin (µg/mL)
SWE	8.90	11.3	9.05	10.25	2.97	0.94	9.86
SEE	2.25	6.1	ND	ND	ND	ND	ND
SME	13.66	17.89	ND	ND	12.41	ND	ND
SE _{50%} E	18.22	15.8	2.74	8.96	5.36	ND	ND
SM _{50%} E	19.91	16.9	14.27	8.51	8.72	ND	23.80

ND: Not detected SWE: Seed water extract; SEE: Seed ethanol extract; SME: Seed Methanol extract; SE_{50%}E: Seed aqueous ethanol (50%) extract; SM_{50%}E: Seed aqueous methanol (50%) extract

Comparatively aqueous methanol (50%) and ethanol (50%) was good in fair amount of phenolics as compared to pure extraction phase (water, ethanol and methanol). Strong positive relationship was observed between antioxidant properties and bioactive compounds. Antioxidant potential in Kinnow seeds help to extend its use in preparation of low cost antioxidant rich supplements/cosmetic products. HPLC results indicate the presence of seven health benefiting compounds (quercetin, vanillin, catechol, catechin, resorcinol, gallic acid and ascorbic acid) in studied samples. Further, experimental parameters studied in the present study might be further explored to optimize the concentration of specific compounds in extracts.

Antioxidant properties in Kinnow seed extracts

Extraction phase	DPPH (% inhibition)	ABTS (% inhibition)	TAC (AAE/100g)	RPA (mg QE/g)	FRAP (mM/100g)
SWE	41.26±1.05	59.44±0.05	8.25±0.19	5.11±0.08	12.46±0.91
SEE	31.70±0.94	54.72±0.13	5.12±0.11	1.80±0.16	5.87±1.05
SME	37.05±0.86	58.81±0.19	7.18±0.05	3.72±0.23	8.23±0.85
SE _{50%} E	49.25±0.92	68.17±0.31	10.34±0.08	12.10±0.29	14.33±0.78
SM _{50%} E	60.78±0.66	79.61±0.22	17.81±0.07	18.46±0.22	20.23±0.82

SWE: Seed water extract; SEE: Seed ethanol extract; SME: Seed Methanol extract; SE_{50%}E: Seed aqueous ethanol (50%) extract; SM_{50%}E: Seed aqueous methanol (50%) extract[12,13]

III. RESULTS

Though kinnow does not seem nearly as popular as mandarins and oranges, there are many reasons to work on trying to include more of it in your diet. Kinnow contain 2.5 times the calcium of other citrus fruits, and thus regular consumption will help to make the bones stronger. Additionally, the vitamin A content is as much as three times more than it is in other citrus fruits. The high fibre content helps to relieve chronic constipation. Kinnow juice helps strengthen the digestive system and naturally gives relief from acidity, thus being an excellent replacement for milk for vegans. Kinnow also reduces the sluggishness of the intestines.

Those recovering from typhoid will benefit greatly from consuming the fruit. For those suffering from recurrent fever, kinnow is a boon as it helps quench thirst, bring down fever and eliminate toxins from the body.



The Punjab Horticultural Postharvest Technology Centre has noted the antioxidants in kinnows could potentially help lower both the risk of cancer and contracting HIV. PubMed with the National Library of Medicine writes that "deficiencies in antioxidant nutrients lead to more rapid HIV-associated disease progression" but noted that "correlating antioxidant levels with disease progression is not the same as determining that taking extra vitamins slows HIV progression." Whatever the case may be, it seems as though the fruit is healthy and good to consume.

Fresh juice from the kinnow fruit helps perk up one's appetite and fires up pancreatic function. It acts as an effective anti-poison medication. Regular consumption of kinnow will help give you a clear and radiant complexion. On top of all that, the dried peel is used in various cosmetic products and is also used as an effective home remedy to treat blackheads.

Kinnow works to lower bad cholesterol and raises good cholesterol levels, while the potassium and magnesium in it help regulate one's blood pressure. Because of the fruit's large carbohydrate content, consumption gives the body a lot of energy. This is especially nice during the winter season when the body craves larger amounts of it.[12]

Nutrients in Kinnow per 100 grams

Nutrient	Quantity	% Daily Value
Calories	50 K.cal	2
Potassium	200 mg	
Carbohydrates	11 grams	5
Dietary Fiber	2 grams	8
Sugar	10 grams	13
Protein	1 gram	2
Vitamin A		2
Vitamin C		110
Calcium		4
Iron		2

IV. CONCLUSION

A dietary fiber enriched vermicelli from wheat flour supplemented with debittered kinnow industry by-products (pulp residue and pomace) has been developed. Functional, cooking and textural properties of both supplemented and unsupplemented vermicelli were evaluated. Vermicelli containing 15% debittered kinnow pulp residue and pomace showed minimum cooking loss (18.5, 20.0%) but higher swelling index (2.06, 1.87), water absorption capacity (153, 202 g/100 g) and optimal cooking time (9.34, 9.02 min). Firmness and fracturability of vermicelli supplemented debittered pulp residue (10.0 and 21.5) and pomace (16.7 and 16.1) was higher as compared to control sample (6.1 and 2.1) respectively. Further, redness, firmness, TPC, DPPH activity and water absorption capacity of vermicelli got



increased with addition of debittered kinnow pulp and pomace. The utilization of debittered kinnow pulp and pomace in vermicelli can provide dual benefit like production of healthy food products along with solving the problem of solid waste disposal of kinnow industry byproducts.[13]

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