Potential significance of mango seed kernel in boosting the immune system

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Abstract
Consumption of junk food is increasing day by day leading to hazardous rise in lifestyle diseases and many other ailments related to various nutritional deficiencies. Rapidly growing interest in Nutraceuticals can possibly help in controlling them and also fulfill the health requirements. Use of antioxidant rich foods need to be incorporated in our daily diets to overcome these health problems. Fruits, being an excellent source of antioxidants can be used in form of fresh variety and also as preserved-dehydrated products. While processing of mango products, the peel and seed kernel are generated as waste; making them a source of environment pollution. Studies have reported that mango seeds are rich in antioxidants such as vitamin A, vitamin C and polyphenols; and can be used for edible purposes by humans. It is known that stimulation of the function and production of white blood cells assist in strengthening body’s immunity. So, developing products using mango seeds (in any form) may provide health-benefits beyond the traditional nutrients it contains; thereby promoting optimal health, longevity and quality of life. Therefore, it is resourcefully advantageous and environment friendly to procure and utilize valuable product - the mango seed kernel flour. Above all, this resource utilization can contribute towards food security.

Keywords: Immunity, Vitamin C, Vitamin A, antioxidants

1. Introduction
With the ever changing lifestyle of humans, the consumption of junk food / inappropriate nutrient intake is increasing. This has further negatively impacted the ability of our immune system leading to a number of lifestyle related diseases. It is known that immunity protects the host from pathogenic organism (bacteria, viruses, fungi, parasites) and the human immune system is always in an active state. This activity increases when an individual becomes infected and this activation further causes an increased rate of metabolism requiring energy sources, substances for biosynthesis and regulatory molecules; which are ultimately derived from our diet. A number of nutrients those enhance the ability of immune system are vitamins A, C, E (natural antioxidants), Vitamin B6, B12, folate and trace elements such as zinc, copper, selenium and iron (Calder, 2020)[10]. The recent corona virus i.e. severe acute respiratory syndrome corona virus 2 (SARS – Cov-2) was named COVID-19 by the World Health Organization in February 2020. The transmission of corona virus is human to human which causes various symptoms such as fever, dry cough, tiredness and breathing difficulty (WHO, 2020). During any infection, the body’s demand for energy and nutrition increases to support the functions of immune cells, so that they can respond effectively against virus. Despite the availability of a range of synthetic antibiotics and antioxidants (immunity enhancers), the infectious diseases continues to be a major health problem. Since the continued use of synthetic drugs is associated with side effects, human has generated knowledge and turned his attention towards use of natural alternatives. It has been documented that since ancient times, plant extracts have been used to heal diseases. So, a wide variety of compounds have been synthesized to improve their quality of life. Plants, vegetables and fruits have antimicrobial and antioxidant properties (Munguia et al., 2016) [12]. Antioxidants are compounds that can interact safely to neutralize or rather end the chain reaction with free radicals.
This resistance offered by the free radicals of endogenous mechanism of body protection plays a vital function (Vagg et al., 2021) [14]. The agriculture fruit crop plays an important role in maintaining health as well as national food security of people globally. Fruits are delicious in taste and are highly nutritious; majorly full of vitamins and minerals that can balance a cereal based diet (Chay et al, 2019) [3]. Among all fruits, Mango, the ‘King of Fruits’ continues to dominate the Indian fruit basket contributing 36 per cent to total fruit area. Mango grows almost in all states of India as a tropical and subtropical plant. Processing of the mangoes generates seeds and peel as wastes; and disposal of its seed is becoming a problem to the environment each day (Yatanatti and Vijayalakshmi, 2017). Mango seed is single, flat, oblong in shape and fibrous/hairy on the surface. The kernel is obtained by breaking the hard seed-coat of the mango seed. Studies show that mango seed contains crude protein, carbohydrate, fiber, ash and oil. Along with these nutrients, it also contains vitamin C, E and A (natural antioxidants). Presence of these antioxidant vitamins, suggests that it could be used as an alternative source of these vitamins (Kittiphoom, 2012) [6]. Besides the vitamins, its seed kernel is also good in polyphenols with potent antioxidant activity. A comprehensive review given by Masibo and He (2008) [9], quantified various polyphenolic compounds (gallic acid, tannin, coumarin, mangiferin and cinnamic acid) in mango seed kernel. The antioxidant activity of mango seed is higher than in jackfruit, longan, avocado and tamarind, due to its high polyphenolic content. Legesse and Emire (2012) [7] stated that 20 – 30 percent of mango seed kernel flour can be used in traditional food item, without adversely affecting their taste/acceptability. Therefore, it may be resourcefully advantageous and environmental friendly to produce valuable products from mango seed kernel.

The present study attempted to investigate on the following:

- To determine the natural antioxidant vitamins A, E & C in mango seed flour and find out the total antioxidant activity in the sample.

2. Methodology

2.1 Collection of sample

The fresh ripe mango was collected from the local market of Meerut District (U.P), India during the favorable season (month of June/July/August). The seed was removed.

2.2 Preparation and storage of seed kernel powder

Mango seed was washed thoroughly with water and then soaked for a minimum of 48 hrs to reduce the anti-nutritional components (tannins). Further, it was sun dried. After drying the sample, it was roasted and then finely ground in grinder. It was sieved, using a muslin cloth to obtain fine powder. This powder was then stored in refrigerator in air tight container until use for testing/extract preparation.

2.3 Laboratory analysis of seed kernel powder

Laboratory analysis of antioxidant activity and vitamins content were carried out in Multani Pharmaceuticals Ltd. (Analytical division), Bhagwanpur, District–Haridwar (Uttarakhand).

2.3.1 Estimation of vitamin A

In order to determine vitamin A content in mango seed kernel - 300 mg of sample, 50 mg of hydroquinone, and 30 ml of ethanol and 3 ml of 50 percent w/v solution of potassium hydroxide was taken. This mixture was boiled under a reflux condenser in stream of oxygen free nitrogen for 30 minutes. After that, it was rapidly cooled down and 30 ml of water was added to it. The solution was then transferred into a separator with the addition of three quantities of vitamin A extract (each 50 ml) and diethyl ether and shaken for 1 minute. After the completion of separation, the aqueous layer was discarded and extract was washed with four quantities (each 50 ml) of water. Then the separated extract was evaporated to nearly about 5 ml and the remaining solvent was removed in a stream of oxygen free nitrogen. The residue was dissolved in 2-propanol to produce a solution containing 9 to 15 units of vitamin A per ml. and then the absorbance was measured at about 310 nm. Further, the maximum absorption wavelength was determined (Indian Pharmacopeia, 2018).

2.3.2 Estimation of vitamin E

In order to determine the content of vitamin E in mango seed kernel flour - 0.3 g of sample was dissolved in 25 ml of ethanol (95%). Another 25 ml of 2.5 Methanolic sulphuric acid was added and kept for heating on a water bath under reflux condenser for 3 hours. It was then cooled and solution was transferred quantitatively to a 200 ml volumetric flask. The apparatus was rinsed with ethanol (95%), rinsing poured to the flask and the volume was made up with ethanol (95%) and mixed. 25 ml of resulting solution was taken in a flask and 25 ml of 0.25 methanolic sulphuric acid, 10 ml of water were added and titrated with 0.01 M ceric ammonium nitrate using 0.1 ml of diphenylamine as indicator, until a blue color persisting for at least 5 seconds was obtained (Indian Pharmacopeia, 2018).

2.3.3 Estimation of vitamin C

In order to determine the content of vitamin C in mango seed kernel flour - 0.1 g of sample was taken and dissolved in a mixture of 100 ml freshly boiled and cooled water. Then 25 ml of 1 M sulphuric acid was added to this mixture. Thereafter, this mixture was immediately titrated with 0.05 M iodine using starch solution as indicator. The titration continued until persistent blue-violet color was obtained (Indian Pharmacopeia, 2010).

2.3.4 Antioxidant (DPPH Radical Scavenging activity)

This assay was based on the measurement of scavenging ability of antioxidant towards stable radical DPPH (2, 2'-diphenyl - 1 - picrylhydrazyl). Firstly methonolic extraction of mango seed kernel was prepared. Then 3.9 ml aliquot of 0.0634 mM of DPPH solution was added in 0.1 ml of methanolic extraction. The prepared mixture was shaken in vortex and kept for 30 minutes in dark. The absorbance then was read in an UV visible spectrophotometer at wavelength of 515 nm. Analysis was performed in triplicate (Vega, 2013) [13]. Results were expressed in DPPH inhibition %.

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\% \text{ inhibition} = \left(\frac{A0 - A1}{A0}\right) \times 100
\]

Where,

- A0 = Absorbance of the control sample
- A1 = Absorbance of the test sample

3. Results and Discussion

The results obtained by the chemical testing of mango seed kernel flour are summarized below-
Table 1: Vitamin content and scavenging activity of Mango Seed Kernel Flour

| Parameters                  | Mango seed kernel flour |
|-----------------------------|-------------------------|
| Vitamin A (µg/g)            | 80                      |
| Vitamin C (mg/g)            | 1.75                    |
| Vitamin E (mg/g)            | Nil                     |
| (DPPH) Scavenging activity (%) | 78.79                  |

The results of antioxidant potential vitamin A, E and C and DPPH radical scavenging activity are given in Table 1. The vitamin A content of the given sample is shown in table is 80µg/g, which is higher than the value of 13 µg/g given by Patel and Kheni (2018) [10]. This variation may be due to the different variety of mango, region or possibly the method of production. The estimation shows good presence of vitamin A in mango seed kernel, which is essential for normal cell functions, differentiation of epithelial tissue and for immune cell maturation and function (Calder, 2020) [1].

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Table 2: RDA percentage contribution of Vitamin A and Vitamin C per given sample

| Nutrient | Mango Seed Kernel Powder/1g | Mango Seed Kernel Powder/5g | %RDA/Day | Percent/day |
|----------|-----------------------------|-----------------------------|----------|-------------|
| Vitamin C | 1.75 mg                     | 8.75 mg                     | 80 mg    | 65 mg       | 10.9%       | 13.5%       |
| Vitamin A | 80 µg                       | 400 µg                      | 1000 µg  | 840 µg      | 40%       | 47.6%       |

*Source: Nutrient requirements for Indians, 2020

Table 2 clearly shows that Mango Seed Kernel flour’s 5gm (1 tsp) can contribute to 10.9 - 13.5 percent of Vitamin C and 40 - 47.6 percent of Vitamin A of our daily recommended requirements, if added in any appropriate acceptable form. These may be considered a good contributory percentage being provided by a small quantity of Mango Seed flour. It may be consumed as a flour (added to wheat flour for regular consumption) and as well as by incorporating in any recipe as an ingredient. Mango being one of the most economical seasonal fruits; may thus be optimally utilized to provide benefits.

4. Conclusion
The results obtained above are an indicator for the possibility of favorable advantages of mango seed kernel. Considering these positive health benefits in addition to it being a cost effective and eco-friendly measure, the consumption of mango seed flour (by supplementing wheat flour with it or by adding it to a recipe as an ingredient) can be best utilized to support the immune system. The host can be in a better capacity to fight against respiratory infections such as bronchitis, pneumonia (due to recent coronavirus). The presence of high antioxidant activity also indicates that mango seed flour is able to fight against diseases those are caused due to immense impact of oxidative stress, such as Alzheimer’s disease, cardiovascular disease etc. It is also suggested that a variety of nutritional dishes starting from curry, bread, milk, snacks, desserts/sweets and many specialty dishes can be prepared at home level by incorporating a small quantity of mango seed flour.

The vitamin C content in the given sample is 1.75 mg/g and is comparatively higher than the value of 0.56 mg/100g given by Patel and Kheni (2018) [10]. Vitamin C acts as an antioxidant and is also known as the immunity enhancer vitamin. It has ability to regulate the immune functions of body by modulating redox sensitive cell signaling pathway or by instantly safeguarding essential structure components of the cell. It has been stated that the administration of vitamin C in the respiratory infected patients, restores their normal vitamin C plasma levels and also improves the rigidity of the breathing problems (Vagga et al., 2021) [14]. Vitamin A and C are also the source of natural antioxidants. Results of mango seed kernel in Table 1 indicate that DPPH radical scavenging activity value is 78.79%, which is comparable with the value of 73% given by Kaur (2015) [3]. Reviews state that antioxidant activity of mango seed kernel flour is higher than the seeds of jackfruit, tamarind and avocado due its high poly-phenolic content.