



## FUNCTIONAL FOODS OF PAKISTAN AND THEIR ROLE IN SENESCENCE

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### ABSTRACT

Functional foods can be used as a tool for healthy & longer life span. The term “functional food” is tossed to emphasize the role of nutritive substances in the prevention and eradication of chronic disease development. The aim of this study is to identify functional foods of Pakistan for their anti-aging potential. In this regard, the ethanolic extract or fresh juices of fifty functional foods, mostly advised by nutritionists, were assessed for their ability to inhibit glycation and oxidative stress. Our analysis showed that out of 50 functional foods, 44 of them have significantly reduced the formation of advanced glycation end products. The Pomegranate peel extract, Strawberry juice, coffee powder, and areca nut showed more than 90% AGEs inhibition at the concentration of 1 mg/ml. Furthermore, antioxidant potential of all these 50 functional foods was also assessed for antioxidant activity using DPPH assay. In this assay, the most pronounced effect (90% free radical scavenging activity) was observed with Cumin seeds extract, Purple Onion extract, Grapefruit juice and Oregano extract. In conclusion, the present study highlights several easily available dietary substances of Pakistan, whom consumption can help prevent age related disabilities and may contribute to healthier and longer life span.

**Keywords:** Functional food, Glycation, Oxidative stress, Aging, Senescence

### INTRODUCTION

Aging or senescence is a physiological phenomenon of growing old that accompanies loss of bodily functions. One

of many theories that explained this process is known as “glycation theory”. It is a non-enzymatic reaction between protein and reducing sugars [1]. This type of reaction is



called Maillard reaction and leads to the formation of advanced glycation end products (AGEs). They are heterogeneous group of chemical compounds that are product of non-enzymatic reaction between sugars, amino acids, and other macromolecules [2]. Its most abundant concentration is found in old ages and strong scientific evidence supports that it has a role in decreasing life span. There is enough evidence that supports the theory that AGEs contribute to various degenerative disorders and diabetic complications [3]. Another theory that is readily accepted, explains “aging” as an outcome of oxidative stress to macromolecules. This theory is known as “Free radical theory”, according to which aging occurs because of excessive generation of free radicals and its accumulation inside the body [4]. Investigations in human and rodent models revealed that calorie restriction and low carbohydrate diet increases life span [5], most probably via reducing the phenomenon of glycation and free radical generation [6]. This leads to concept of combined approach for higher and specific molecular cause known as “network theory of ageing” [7], which elaborates that both processes (glycation and free radical generation) work in parallel.

For last 160 years, life expectancy has relentlessly expanded by a fourth of a year every year, an uncommon consistency of human accomplishment [8]. According to WHO, the world will have more individuals who live to see their 80s or 90s than any

time in recent memory. The quantity of individuals matured 80 years or more seasoned, for instance, will have nearly quadrupled to 395 million in the vicinity of 2000 and 2050 [9]. This is not the complete picture of reality as quality of life is equally important as quantity. This also means that there is the highest number of people living with morbidity of old age, which poses a challenge to health care providers. Food plays a vital role in determining quality of life [10]. Its importance was for the first time established in the ayurvedic concept of “we are what we eat” [11]. Nutritional neuroscience is an emerging field that deals with the role of diet on mental health. Prevention of disease by means of food is more readily accepted by the public than use of medication before onset of disease. This is one of the reasons due to which nutritional pharmacology is gaining importance now-a-days. All those food substances that helps in prevention of diseases or improving quality of life are considered as functional foods [12]. Purple colored food is highly recommended by nutritionist and dietitians. According to some, indigo colored food substances inhibit aging; one reason can be due to presence of anthocyanins [13]. Use of food as prevention of AGEs progression is well accepted and proven [14]. Using purple theory and functional food concepts, the present study was designed to investigate the anti-aging potential of fifty potential dietary substances available locally.

## **MATERIALS AND METHODS**

### ***Extract preparation***



Functional foods of Pakistan like Pomegranate, Areca nut, Indian Black berry (Jamun), Water Chest nut (Singhara), Fenugreek seeds and leaves, Spinach leaves, Rose petals, Black pepper, Turnip, Turmeric powder, Brinjal, Cumin seeds, Oregano, Ginger, Garlic, Purple cabbage, Lemon, Reddish, Cinnamon bark, Paprika, Oranges, Walnut, Falsa, Onion, Walnut, Almonds, Apple, Beet root, Tomato, Pineapple and Fig were obtained from local market. Ethanolic extract (70%) of these functional foods were obtained by maceration and subjected to rotary evaporation (Buchi rotavapor R-250). Afterwards, the extract was frozen and dried. Fresh juices of strawberries, Coconut water, Coconut milk, Lemon Juice, Oranges, Grapefruit and Pomegranate were also incorporated in this study after freeze drying.

#### **Glycation assay**

Inhibitory activity of extracts and juices on formation of AGES (advanced age glycation end products) was performed as described earlier by us [15]. AGEs reaction mixture was prepared using BSA (bovine serum albumin, 10 mg/ml) and fructose (100mM) prepared in 0.2M sodium Phosphate buffer (pH 7.34). This mixture was allowed to incubate for 24 hours. BSA (10 mg/ml) in 0.2M sodium phosphate buffer (7.34 pH) was used as blank while aminoguanidine (3mM) was used as positive control. For sample mixture, 1 mg/ml of test substance was used in AGEs mixture. Florescence intensity was measured at excitation emission wavelength of 340 – 435 nm. All

results were tested in triplicate. The percentage inhibition of AGEs was measured using the following formula:

$$\% \text{ Inhibition} = [1 - (\frac{\text{fluorescence Intensity of mixture with inhibitor}}{\text{Fluorescence Intensity of negative control}})] \times 100$$

(Fluorescence Intensity of negative control)

#### **DPPH Assay**

Free radical scavenging activity of functional foods were assessed by 1-1diphenyl-2-picryl-hydrazyl (DPPH) according to the method reported earlier. Briefly a 0.1mM solution of DPPH in methanol was prepared and 100ul of this solution was added to 300ul of extracts prepared in concentration of 1mg/ml. Mixtures were vigorously vortexed and kept in dark for 30 minutes at room temperature. The absorbance was measured at 517nm using UV spectrophotometer (Spectroscopy Synergy HTX BioTek, USA). Ascorbic acid (1mM) was used as standard positive control to compare results. All results were tested in triplicate. Percent free radical scavenging activity was calculated using the following formula [16]:

$$\text{DPPH Scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \times 100$$

Where,

Abs<sub>control</sub> = Absorption of control sample

Abs<sub>sample</sub> = Absorption of test substance sample

#### **RESULTS**

The edibles that are categorized earlier as functional foods due to their therapeutic potentials were screened for glycative stress reduction and their ability of free radical scavenging (FRS). In the extracts category,



most potent was found to be pomegranate peel extract which caused 95 % AGEs inhibition and 87% FRS activity Table 1. Although both pulp and seed of Indian black berry (Jamun) were almost equally effective as AGEs inhibitor (86% and 85% respectively), but seeds lack potential free radical scavenging ability. In case of fresh juices, most effective fresh juice was found to be that of strawberry, which exhibited 92% and 83% inhibition of AGEs and free radical, respectively Table 1. The results as percent inhibition are mentioned in Table 1. Aminoguanidine (AG) a known anti-aging is no longer used in clinical trials delaying senescence due to its reported adversities [17]. However, AG is among prototype anti-aging drug. Considering the perspective of a commoner instead of testing anti-aging potential of unknown drug nutritional substances are explored. To establish a simple efficacy of nutritional substances in terms of potency comparison with AG equivalence of nutritional items are calculated Table 1. Our equivalency data showed that the free radical scavenging ability (FRS) of certain functional foods like Cumin seeds (commonly known as zeera), Onion, Oregano powder and Grapefruits are comparable to that of standard ascorbic acid Graph 1. Similarly, 16 of the test substances like Pomegranate, Areca nut, Indian Black berry (Jamun), Water Chestnut (Singhara), Fenugreek seeds, Spinach leaves, Rose extract, Black pepper, Turnip and Turmeric powder were found to be more effective

than aminoguanidine (3mM) in reducing the extent of glycation Graph 2.

#### **STRENGTH AND LIMITATIONS**

This is an overly simplified explanation of potential ability of nutritional substances because of modification of Bovine serum Albumin in vitro however in physiological system their potential and efficacy may vary. We are currently screening their effects in various glycation models of rodents to establish the mechanism, but one cannot overlook the initial findings and power of nutritional substances.

#### **DISCUSSION**

Human beings are in search of “elixir of life” from centuries [18]. The search of anti-aging supernatural herb from the top of Himalayas has always been in the limelight [19]. Senescence is the natural, slow, and progressive phenomenon of growing old. On the other hand, humans always wish to maintain their quality of life for longer time. In this regard, dietary intervention holds tremendous potential and acceptability. There are many reasons for inclination towards nutritional solution for remedy majorly due to its affordability and easy excess in daily routine. All such nutritional substances are gaining popularity as “functional foods” that helps to prevent ailment through interfering in multiple pathological mechanisms [12] such as glycation and free radical generation. Keeping this in view, the present study was designed to identify the anti-aging functional foods of Pakistan, as little data is available.



In our study, a list of fifty claimed functional foods were selected and divided into three major categories i.e., ethanolic extract, fresh juice, or raw product. Keeping in view the network theory of aging, these test substances were evaluated for their anti-glycation and free radical scavenging (FRS) potentials. The Pomegranate fresh juice also showed remarkable potential with 85% anti-aging potential and 61% FRS. Interestingly the most claimed, Grapefruit juice was found to be an AGEs promoter in Millard type reaction but showed excellent free radical scavenging ability. Other highly recommended food substances like coffee beans are more effective as brain stimulants for many decades but this has an impact on memory and cognition which is not seen with caffeine alone [20]. In recent years it was found to be effective in Alzheimer's disease apart from being a good anti-inflammatory, antioxidant, and cardio protective [21]. Its potential as an anti-ageing principle is constantly under investigation. We analyzed the role of coffee beans in the process of glycation because it contains rich number of tannins along with polyphenols. Most promising results were seen with coffee, as it has both anti-glycation (92 %) and free radical scavenging ability (74 %). Rose extract, which is frequently incorporated in Asian traditional cuisines, especially with sweets has found to be effective both as AGEs inhibitor (83%) and 77% FRS. Olive oil failed to exhibit any inhibition for advanced glycation end products in Millard type reaction however it

has excellent free radical scavenging ability of 83%. Our equivalency data shows are the free radical scavenging ability (FRS) of certain functional foods like Cumin seeds (commonly known as zeera), Onion, Oregano powder and Grapefruits are comparable to that of standard ascorbic acid (Graph-1). Similarly, 16 of the test substances like Pomegranate, Indian Black berry (Jamun), Water Chestnut (Singhara), Fenugreek seeds, Spinach leaves, Rose extract, Black pepper, Turnip and Turmeric powder were found to be more effective than aminoguanidine (3mM) in reducing the extent of glycation (Graph-2). Areca nut is a common Indian and Pakistani addiction this can worsen many medical conditions but when used in limited amounts it may offer its benefits as anti-aging. Its potential as effective antidepressant activity is already well established [22]. Apart from being anti-inflammatory and antioxidant it has a potential for analgesic activity [23]. This nut abuse is also known for its potential to cause oral cancer [24]. However, this nut is still selected due to the high content of tannins present in it that showed promising results. Singhara or water chestnut is a common fruit available in Pakistan which is often taken for granted but it is a powerful nutrition. Its scientific name is *Trapa japonica*. It is least investigated, but it is rich in anthocyanins particularly the inner peel [25]. However, it is observed that in our initial screening peel of this fruit it is more effective than aminoguanidine (Graph 1). Piper nigrum (black pepper) is a common



table spice long known for its traditional use against cough and cold [26]. It has strong anti-inflammatory and antibacterial properties and part of daily household cooking [27, 28]. It is commonly referred as “King of spices” due to its use in folklore and traditional medicine along with cuisines which renders it a special place among functional foods [26]. It has been extensively used in menstrual disorders, common flu, as a potent antioxidant and a free radical scavenger and in recent studies it is also found to be anticancer exhibiting potent cytotoxic and apoptosis in vitro [26, 29]. This finding (Graph 1) of being effective anti-aging now places this nutrition in a special category.

In recent years it is also established that poor diet is a leading cause of death after tobacco and hypertension [30, 31]. For a decade long, fats were the only notorious macronutrient in the list of morbidity causative agents [32]. After establishment of aging theories and search of the culprit’s carbohydrates and particularly reducing sugars are now considered more devastating than fatty food alone would ever be, that is why there is a sudden need of search for elixir of life like never before [33]. Functional foods are reported to have high impact on prevention of deleterious phenomenon, as observed in our study [34]. Antioxidant ability has been endowed to the polyphenolic contents, while anti-glycation ability has been attributed to anthocyanin contents, which imparts purple color to substances [35].

## CONCLUSIONS

The natural process of aging can be slowed with lesser devastation in terms of cognitive decline by dietary management with functional foods. In the light of our investigation, the edible test substances such as pomegranate, Indian black berry, coffee, and strawberries provide best natural intervention for treatment and prevention of oxidative and glycative stress. It is highly recommended to incorporate the use of this variety of functional foods on daily basis as they not only prevent free radical injuries but also help in delaying senescence and AGE-related morbidities. Additionally, people working in hazardous milieu can adopt prophylactic approach by taking these edibles to avoid biochemical stress. Hence one can safely say that these substances may serve as “elixir of life” in long term, if consumed on daily basis thus preserving the quality and quantity of life.

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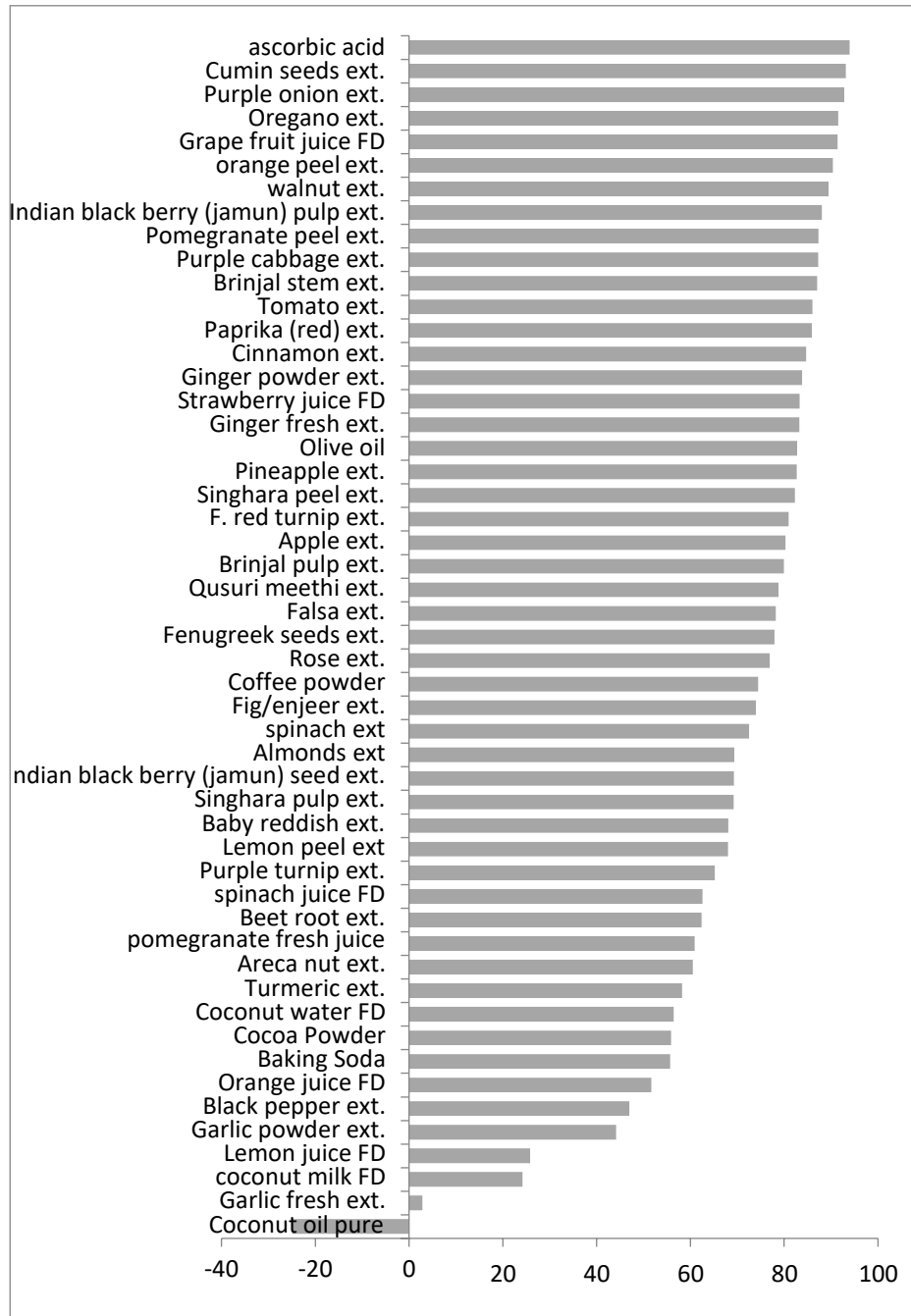


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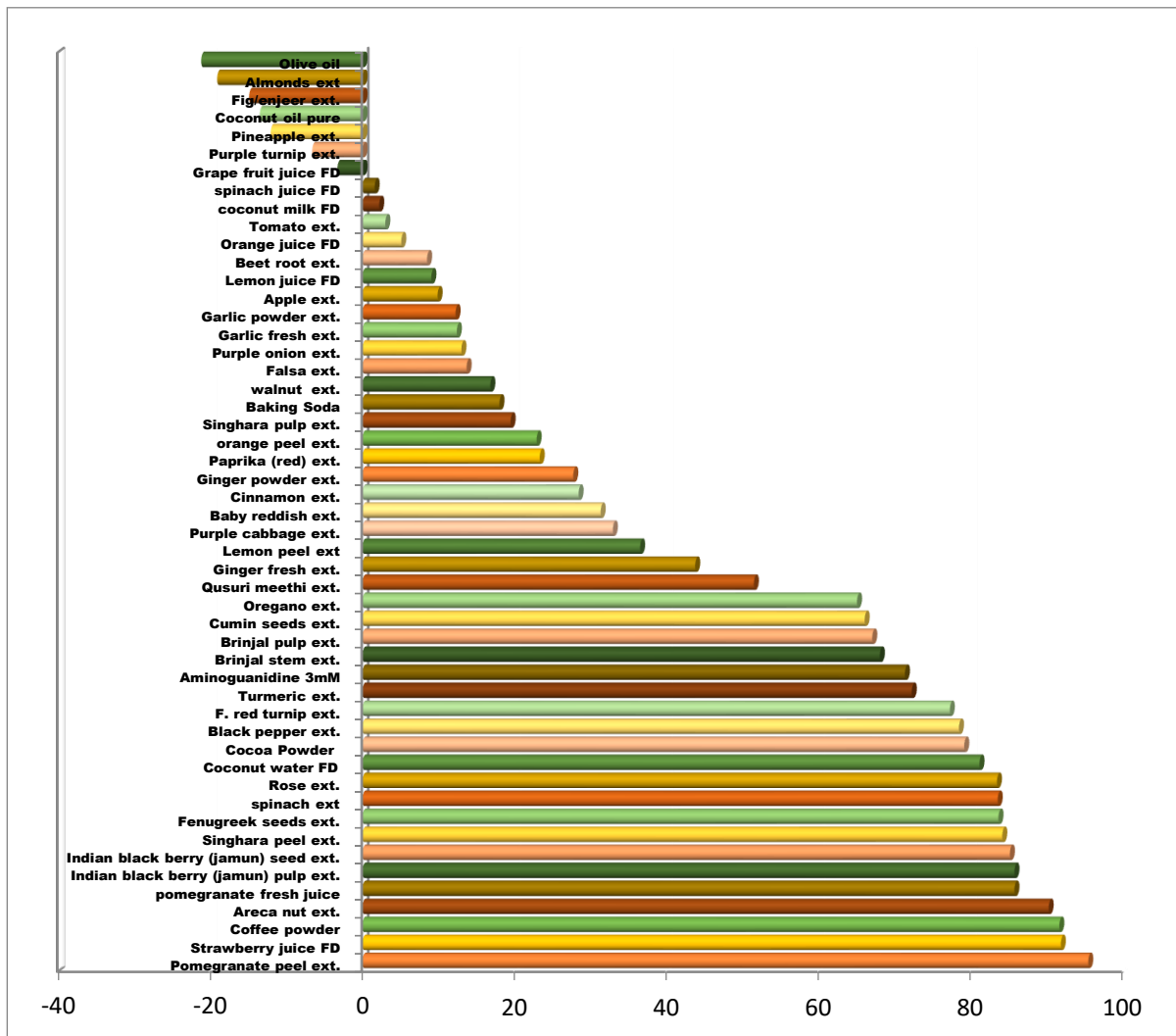


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**Graph 1: Equivalency graph of Antioxidant functional foods and Ascorbic acid.**

The figure depicts the equivalency of antioxidant potential of functional foods with Ascorbic acid. The data shows that FRS certain functional foods like Cumin seeds (commonly known as zeera), Purple Onion, Oregano powder and Grapefruits are comparable to that of standard ascorbic acid



**Graph 2: Equivalency graph of Anti-glycation functional foods and Aminoguanidine.**

The figure depicts the equivalency of anti-glycation potential of functional foods with standard Aminoguanidine. Our data shows that 16 of the test substances like Pomegranate, Areca nut, Indian Black berry (Jamun), Water Chestnut (Singhara), Fenugreek seeds, Spinach leaves, Rose extract, Black pepper, Turnip and Turmeric powder were more effective than aminoguanidine (3mM) in reducing the extent of glycation.