Determination of free radical scavenging activity of individual and combined extracts of *Zingiber officinale*, *Mentha spicata* and *Citrus limon*: most commonly used ingredients for preparation of a highly delicious and health boosting lemonade

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Abstract: Metabolic reactions, pollution and other environmental factors are cause of production of free radicals to human body. These free radicals may lead to chronic diseases including cancer, diabetes, obesity and others. Antioxidants present in our diet have the capacity to scavenge free radicals to reduce their damaging effects. Food components play vital role in controlling such diseases. Ginger (*Zingiberofficinale*), Mint (*Mentha spicata*) and lemon (*Citrus limon*) are important food ingredients used in treatment of numerous diseases. They are utilized in form of different formulations and teas; hence this study aimed to find out the antioxidant activity of all these ingredients individually and in combination. DPPH free radical scavenging assay was used for this purpose. Different concentrations of extracts (ranging from 10 to 100 micrograms per milliliters) of their samples were tested for free radical scavenging activity. Mixture of ginger, mint and lemon had superior scavenging activity showing significant difference followed by the Lemon. Altogether, our findings suggest that mixture of ginger, mint and lemon extracts possessed promising natural antioxidant activity for the reduction of oxidative stresses with potential applications in the pharmaceutical-food industry with reduce side effects.

Keywords: Antioxidant; diseases; Ginger; Mint; lemon; scavenging; oxidative stresses

Introduction

Many plants species are used for the treatment of human and animal abnormal functions from the ancient time history [1]. The evidence of natural plants and food business for medicinal purpose survives from Egyptian and Mesopotamian times. Some facts of use of plants as medicine have been information gathered about past times from Egypt, Africa, China, Indus Valley and worldwide of Pakistan as well [2,3].

Plant-based medicinal drugs have been commonly used in region of the worldwide [4,5]. The medicative plants are defined as the plants which have active ingredients of pharmaceutical importance in one or more than one part of the plant. This definition is published by the World

Health Organization. World Health Organization (WHO) has also confirmed that almost 80% world population use traditional medicines and the bulk of these medicines are based on medicinal plants [6].

It is reported that process of soaking a substance (usually in water) present in medicinal plants have important potential to fight diseases, due to their certain advantages, such as to their antibacterial, antioxidant, antifungal and antipyretic effects etc, they have been the first choice of treatment worldwide [7,8]. The advantages of medicinal plants are they are safe; easy availability; affordable; fulfill lack of advanced healthcare needs in underdeveloped countries [9,10] etc. Many plant-based medicines have been discovered based on traditional knowledge. Even nowadays, more people are fascinated towards herbal medicines applications. Nearly, about 40% drugs are originated from nature and have been used against infectious diseases. In developed countries, a significant percentage (25%) of medicines are derived from medicative plants [11,12].

Hence, it is a need of time to identify and acknowledge the use of plants for pharmaceutical gains [13]. The zest *Zingiber officinale* is obtained from the underground part of rhizomes of ginger. These plant species are utilized in home grown medicative purpose for the relieve illness of joint inflammation, rheumatological conditions and solid distress, atherosclerosis, headache migraines, rheumatoid joint inflammation, high cholesterol, ulcers, melancholy and ineptitude. a mild viral infection, influenza and regular difficult relating a menstruation etc [14,15], diarrhea caused by cancer, HIV treatment and vomiting after surgery. It is also used for pain relief from osteoarthritis, rheumatoid (RA), and menstrual pain. The fresh juice of ginger is used on their skin treat burns. It is used as a flavoring agent and for fragrance in cosmetics and soaps [16-18].

Mint (*Mentha spicata*) is an herbal plant with leafy stems. Mentha is a genus of plants in the family Lamiaceae [19,20]. It production is widespreads over Europe, Asia, Africa, Australia and North America [21,22] including Pakistan [23,24]. Mint is used as a medicinal herb to treat stomach ache and chest pains [25]. It is also used for the treatment of allergic reaction such as diarrhea [26], abdominal cramps[27], heart burn and headaches [28,29].

In Rutaceous family, *Citrus limon* is one of the most significant globe organic product harvest [30] and is expended generally as crisp produce or squeeze in light of its dietary benefits and uncommon enhance [31]. Lemons are a rich source of vitamin C, and contains various phytochemicals including terpenes, polyphenols such as flavanone glycosides hydroxycinnamic acid, and tannins and carotenoids [32,33]. For the treatment of various infections, lemons are very useful for it as a medicinal plant which is used for the antioxidant purpose. These plants are used for the medicinal field for the improvement of drugs and especially for diseases management of nephrotoxicity [34], protection of oral cavity [35] etc.

Antioxidant is a compound that suppresses the oxidative molecules of other substance. In oxidation process, free radicals are formed due to which chain reactions initiated. These reactions severely damage the cells of organisms. Therefore, antioxidants are used to stop oxidation reactions by neutralizing free radicals [36]. The natural antioxidants are available in all parts of plants like, fruits, vegetables seeds, bark, leaves, roots, nuts [37,38]. They are categorized into three groups i.e. carotenoids, vitamins and phenolic compounds. These antioxidants overcome the severe effects caused by free radicals [39,40]. Herbs have been

acknowledged broadly in certain countries because of the high antioxidant activity [41]and their benign properties [42]on individual well-being [41]. Herbs protect against oxidative stress and perform an essence job as anticancer, antitumor, anti-inflammation and cardiovascular diseases [43,44].

Previously broad range of research is being carried on different herbs with their oil. In this study, evaluation of antioxidant activity of ginger, mint, lemon extracts individually and also in combination was determined by free radical scavenging activity.

Materials and Methods

Chemicals used included ethanol and methanol from Thermo Fisher Scientific (Reagent Lane Fair Lawn, NJ, USA), DPPH (2,2-diphenyl-1-picrylhydrazyl) and DPPH (1,1-diphenyl-2-picrylhydrazyl), Corning® syringe filters 0.2 µm and L-*Ascorbic acid* 99% from Sigma-Aldrich (St. Louis, MO, USA). All reagents and chemicals utilized were of analytical grade.

Sample preparation and extractions

The following samples of plants were taken Ginger *(Zingiber officinale);* Mint *(Mentha spicata)* and Lemon *(Citrus limon)* from local market of Sialkot, Punjab, Pakistan. All samples were washed with distilled water and the samples of Ginger were peeled off, from which 4 grams of each sample (i.e., Ginger, Mint, and Lemon) were taken. Then all samples were crushed using pestle and mortar to produce a homogeneous paste. Approximately, 400 ml deionized water was added to every sample and mixed for 20 minutes and then filtered using filter syringe. The filtrate was evaporated, and the resultant extracts were used for preparation of different concentrations of extracts. Different concentrations of extracts of ginger, mint and lemon were prepared ranging from 10 to 100 µg/ml.

Determination of free radical scavenging activity of individual samples

Determination of free radical scavenging activity was done by using the method described by [45]. Approximately, 3ml of each concentration of sample, 1ml of 0.3mM DPPH was added (n=3), and incubated at 37 °C for 20 min. The optical density (O.D) was measured at 517nm spectrophotometrically by Shimadzu-1700 UV–VIS spectrophotometer (Japan). DPPH in methanol served as negative control and methanol were taken as blank, whereas ascorbic acid was used as standard.

Determination of antioxidant activity of mixture of ginger, mint and lemon

Approximately, 4grams of each of ginger, mint and lemon were taken and a homogenized by adding 400ml of water to this mixture. Sample was filtered and the solvent was evaporated at room temperature. Different concentrations of resultant extract were prepared ranging from 10 to 100 µg/ml.

Determination of antioxidant activity was done by using the method described as described previously [45] with following modifications. To Three milliliters of each concentration, 1ml of 0.3mM DPPH was added (n=3). These samples were incubated at 37 °C for 20 minutes. The

optical density (O.D) was measured at 517nm by spectrophotometer. DPPH in methanol served as negative control and methanol were taken as blank. Ascorbic acid was used as standard.

Statistical Analysis

The results of antioxidant activity were expressed as as means ± SD having triplicate analysis (n = 3). The ANOVA (Analysis of variance) was done to analyze the results at a significance level of $P < 0.05$, using MS Excel. The percent scavenging activity was determined by using the formula given below;

S.A. (%) = {(As-Ac)/Ac} x 100
Here, SA stands for Scavenging Activity; As for absorbance of sample; Ac for absorbance of control

Results and Discussion

Lemon, mint, and ginger are quite popular plant species which are commonly used in worldwide specially in South Asia either in form of 'Qehwa' or 'formulation'. Lemonade is one of the drinks/formulations used in Pakistan preferably in summer season. This drink is known as "Sikanjbeen" while 'Qehwa' of ginger/mint and/or lemon is used in cough and stomach related disorders, healthy and neat and clean skin. These ingredients are used throughout the year to tackle with different human health issues.

In the present research, the antioxidant activity of these three selected plant species was determined by using DPPH scavenging assay. In the first phase, the antioxidant activity of individual plant species was determined by using DPPH scavenging assay. In the next phase, activity of mixture of these three species was determined by the similar method. Ginger, mint and Lemon is a well-known herb used in everyday cooking worldwide. It is a compulsory part of Asian food dishes. It is well-known for its miraculous curing properties and health benefits.

The extracts of ginger were prepared and examined for their antioxidant potential. Figure 1 shows the values of percent scavenging activity of different concentrations of extracts of the samples of Ginger. It is evident that the activity did not increase in concentration dependent manner. The significant ($p < 0.05$) increase activity was shown by ginger extracts at a concentration of 70µg/ml while the lowest activity was observed at 10µg/ml. After 70µg/ml, there was not any significant increase for antioxidant activity (Figure:1).

These results are very close to values in literature [46] with water and ethanolic extracts. It's very well known that the development of free radicals and reactive oxygen species (ROS) are major cause for the formation of degenerative illnesses, including inflammation etc [47]. Use of Ginger in an amount could be very beneficial to combat effective diseases.

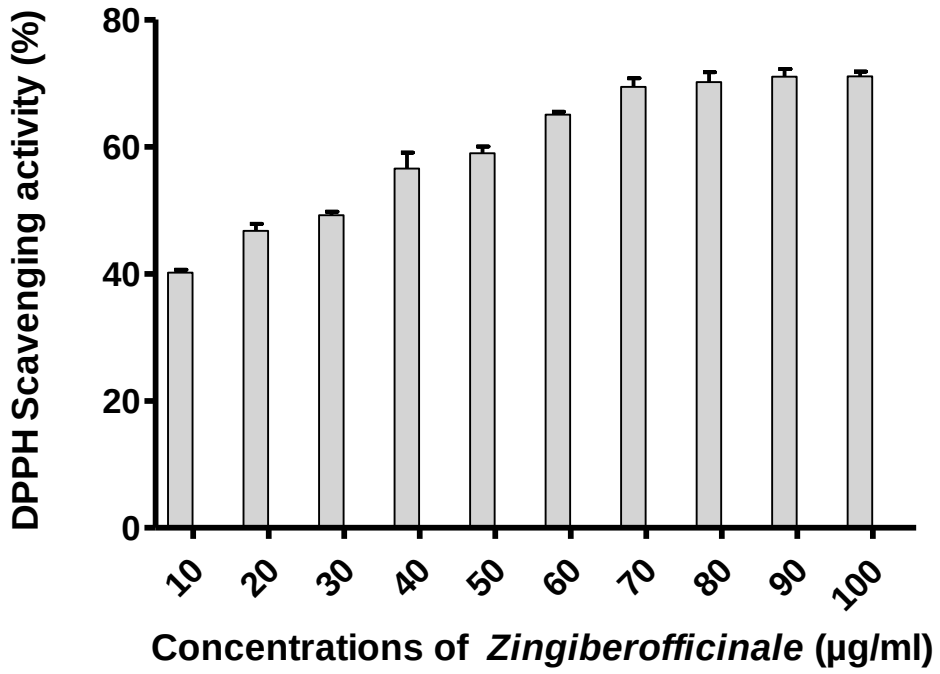

Fig. 1. Antioxidant activity of *Zingiberofficinale*. Each bar in graph are the mean values (*n*=3) that are significantly different (*p*<0.05).

Mint is a well-known and popular culinary herb used as an important food ingredient in worldwide. The extracts of mint were prepared and examined for their antioxidant potential. The Figure 2 shows the values of percent scavenging activity of different concentrations of extracts of the samples of mint. Figure 2, represents antioxidant activity increase and decrease, and till 80 µg/ml there was increase in concentration dependent manner [48]. The highest activity was shown by mint extracts at a concentration of 80µg/ml while the lowest activity was observed at 10 µg/ml.

There is high demand of Polyphenols for their qualitative and antioxidant property (Shahidi & Wanasundara, 1992). Phenolic compounds are good hydrogen donors, to act as antioxidants. Mentha species have been known to contain phenolic compounds with antioxidant properties in alcoholic and aqueous plant extracts [49]. Mint has been reported to work as shield to stop cell and individual from oxidative DNA damage linked with aging, cancer, and degenerative diseases etc. Its extract is a source to protect against DNA damage, free radicals, and lipid peroxidation. Hence, it is a cost-effective, easily available compound for daily use and biotechnological industry [48].

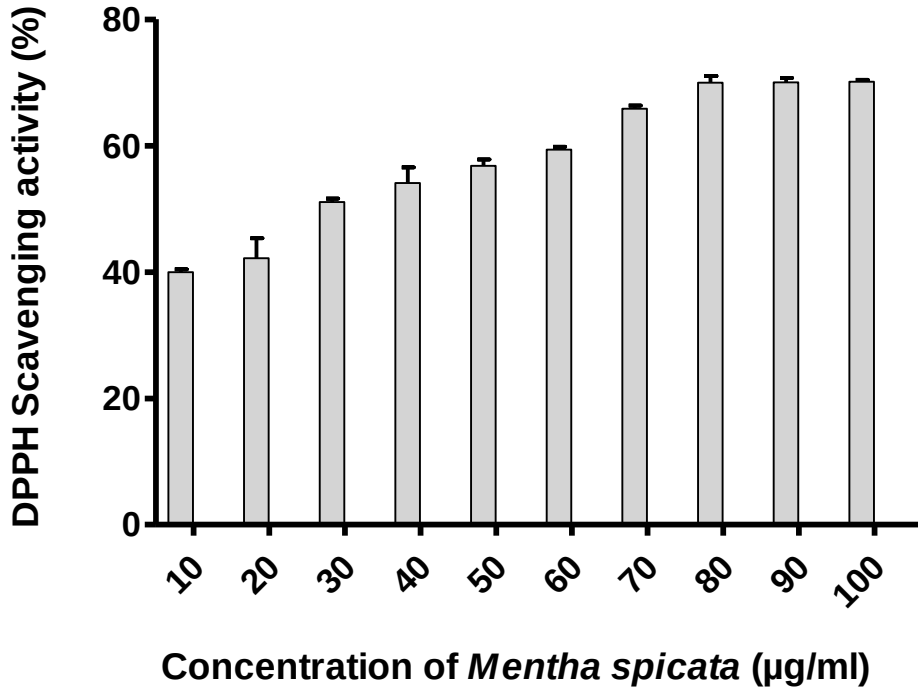

Fig. 2. Antioxidant activity of *Mentha spicata.* Each bar in graph are the mean values (*n*=3) that are significantly different (*p*<0.05).

Figure 3 shows the values of percent scavenging activity of different concentrations of extracts of the samples of lemon. It is evident that the activity did not increase in concentration dependent manner. The highest activity was shown by mint extracts at a concentration of 100µg/ml while the lowest activity was observed at 10µg/ml. Most of literature is carried out on lemon oil and peel, but rarely research is carried out on Lemon juice only. Lemon is known for high amount of phenolic and antioxidant properties [50], which could be used for energy, nutraceutical and health industry[51]. At 10µg/ml, 50% DPPH scavenging activity is represented, which demonstrate its higher and effective potential to be used for various health condition as a potent fruit having more antioxidant activity.

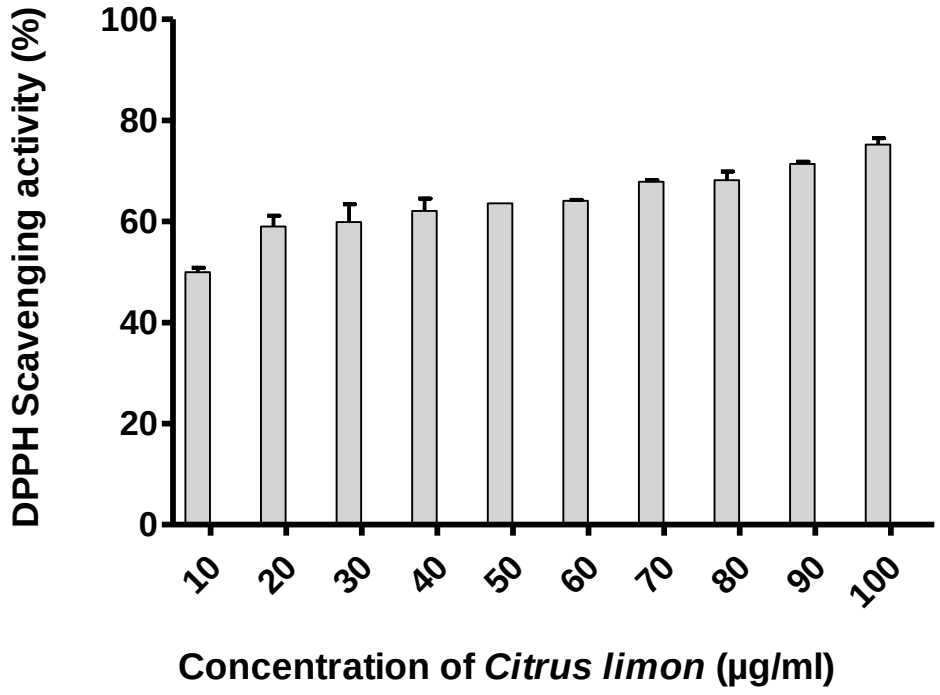

Fig. 3. Antioxidant activity of of *Citrus limon*. Each bar in graph are the mean values (n=3) that are significantly different (p<0.05).

This kind of formulations is routinely used in South Asia. For example, Tamarind, Plum, Grapefruit formulation e.g. Ruhafza and Jam-e-shireen are quite famous among masses. But it is interesting to note that such formulations have not been investigated for their antioxidant activities from any part of the world especially in Asia specially in Pakistan, China and India. Such formulations are healthy and nourishing as well and need to be investigated for their phytochemicals and antioxidant potentials. Ginger, mint and lemon being the most commonly used constituents of such formulations need to be studied for their bioactivities and phytochemicals. The aim of the present study was to assess the antioxidant potential of the mixture of these three ingredients of formulations.

The DPPH scavenging assay was conducted to assess the antioxidant activity of above-mentioned ingredients. The percent scavenging activity of different concentrations of mixture of ginger, mint and lemon is shown in the Figure 4. It is evident that the activity decreased in concentration dependent manner. The highest activity was shown by mixture of ginger, mint and lemon extracts at a concentration of 100µg/ml while the lowest activity was observed at 10µg/ml (Figure 4). This is because lemon is rich in ascorbic acid which is well known for its strong antioxidant activity the other two ingredients in the mixture have low antioxidant activity as compared with lemon. Hence the overall activity of mixture is higher than individual extracts. It is interesting to note that lemon alone cannot be consumed at higher concentrations due to its sour and slightly bitter taste so, if it is utilized in combination with ginger and mint than its taste in comparatively less bitter[52].

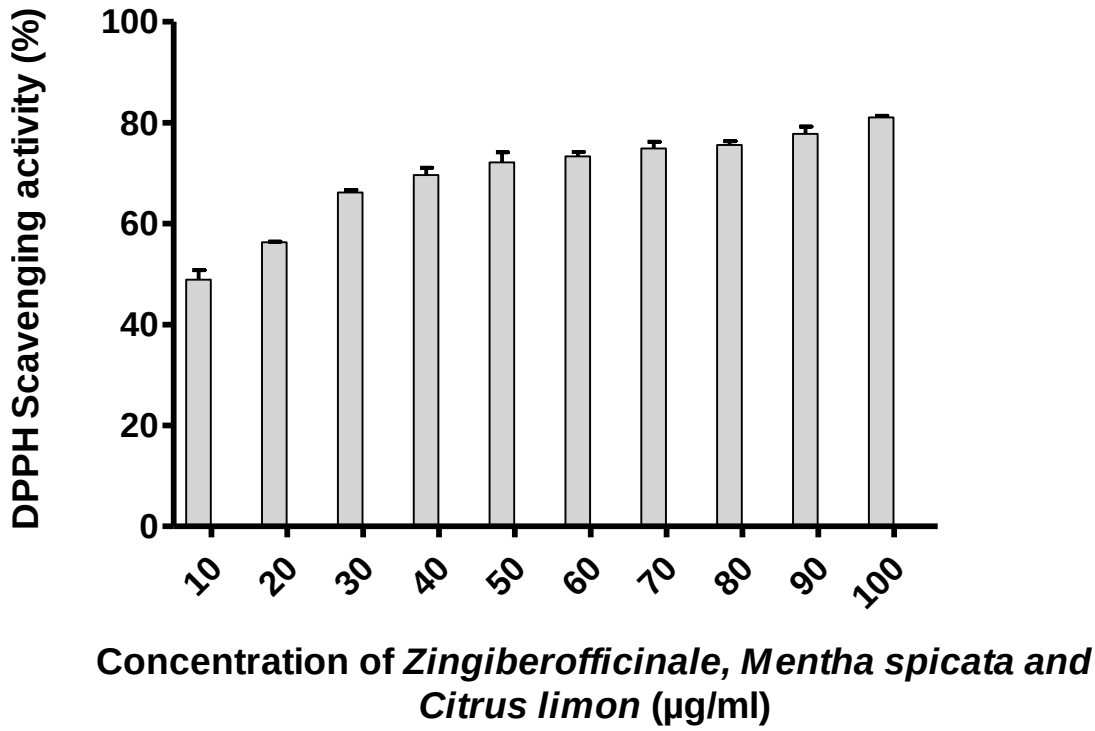

Fig. 4. Antioxidant activity of of Zingiberofficinale, *Mentha spicata* and *Citrus limon.* Each bar in graph are the mean values (n=3) that are significantly different (p<0.05).

Consequently, for Ginger, mint and lemon antioxidant activity is concentration dependent and also depend on the structure and interactions in the extracts, representing that samples with same concentrations of phenolic compounds can fluctuate in their antioxidant activities [53]. The stomach related disorders and skin problems can be cured by using mixture of these three plant species This kind of research has not been performed earlier. In future, these species are required to be characterized and investigated further for their potential bioactivities including different biological and antimicrobial, anti-andipogenic [54] and antidiabetic activities [55]. These ingredients act slowly but prove effective in dealing with health problems. The free radical scavenging compounds found in plants could prove effective in controlling health issues generated by excess free radicals in the body [56].

Conclusion:

Generally, herbal products are considered safer to use. Instead of taking synthetic medications, majority of population prefer to use food for healthy life and to protect against diseases. Ginger, Mint and Lemon are cost effective and easily available plant species. The understanding of chemical nature of plant species is also important and needs to be understood. Our result indicates that the activity of individual components in pure form higher for lemon but in mixture

form, the activity is most effective. Hence, it is recommended that regular use of these ingredients as mixture have potential to strengthen body and mind and to detoxify our body of toxic substances. These components are compatible with the environment of human body and are not known yet for any side effects.

Author Contributions: All authors contributed to the study, conception and design. The research idea was perceived by Dr. Mehmooda Munazir, the practical work was performed by Hina Aslam with the help of Zulnoreen Sarfaraz and Ayesha Tahir, under supervision of Dr. Mehmooda Munazir. Saira Hussain, Mehmooda Munazir and Hina Aslam wrote the first draft of article which was reviewed and edited by Dr. Asma Ahmed, Rehana Badar and Dr. Wahid Hussain. The final version of paper was prepared by Dr. Mehmooda Munazir. All authors read and approved the final manuscript. The consent of all authors was taken before submitting the paper to the journal.

Conflicts of Interest: The authors declare no conflict of interest.

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