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Research Article

QUANTIFICATION AND DETERMINATION OF ANTIOXIDANTS IN *SYZYGIUM CUMINI*: REVALANCE TO HUMAN HEALTH

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ABSTRACT

Introduction: As per the literature, *Syzygium cumini*, medicinal plant products, i.e., bark, leaves, seed, and fruits are used as an alternative medicine to treat various diseases. In spite of its medicinal uses, this work was further carried out to determine the protein content including antioxidant (i.e., vitamins C and E) content in aqueous leaves extract of *S. cumini* in lysed human whole blood.

Methods: For these studies, we analyzed various antioxidants especially vitamins (C and E) in lysed human whole blood using aqueous leaves extract of *S. cumini* (10 mg/ml; stock solution). In addition, estimation of protein content through NanoDrop method. For these studies, capsules of antioxidants (vitamin C [Celin] and vitamin E [Evion]) were used as standard.

Results: The results showed that *S. cumini* significantly increased in vitamin C production as compared to antioxidant (vitamin C capsule) control, but there is a little enhancement in vitamin E production**.** In addition, two bands of protein (45 and 60 kDa) are observed in aqueous leaves extract of *S. cumini.*

Conclusion: Aqueous leaves extract of *S. cumini* showed significant enhancement in vitamin C production. The study of this medicinal plant is very important and provides a scientific data of particular importance for the local people using these plant products for a variety of body disorders**.**

Keywords: Antioxidants, Protein, Vitamin, *Syzygium cumini.*

INTRODUCTION

Antioxidants are responsible for stopping or inhibiting the formation of free radicals including chain reactions otherwise, which ultimately leads to cell damage or even death [1]. In other words, antioxidants played an important role for our immune system, which enhances its immunity against various infectious diseases. Normally, these antioxidants are required both extracellularly and intracellularly, e.g. cytosol, nucleus, and mitochondria for maintaining hemostatis condition [2,3]. These antioxidants are generally derived from medicinal plant products especially leaves, seeds, and fruit, which includes vitamins (C and E), polyphenols, carotenoids, etc. Some of the antioxidant molecules are water soluble and some of them are lipid soluble [1-5].

As per the literature, numerous naturally occurring antioxidants, i.e., vitamin C and E are reported which is the more effective against various human infectious diseases [6]. In general, these antioxidants which played an important role in preventing peroxidation damage in the biological system. One of the antioxidants, vitamin C (L-ascorbate; hydrophilic molecule) is found mostly in bodily fluids and abundant in fruits and vegetables, and they serve as the main source for dietary food. However, new trends in food processing methods that generally leads to the loss vitamin C including other vitamins and nutrients as well. Vitamin C was firstly isolated in 1928 and recognized as the bioactive molecule that was missing in the diet of sailors, causing scurvy [7-9]. In general, vitamin C is known to take part in many physiological processes and showed some beneficial or therapeutic role in immune responses, cardiovascular disease, and cancer [8,9]. In addition, it is one of the most important antioxidants in extracellular fluids and can protect biomembranes against lipid peroxidation damage by inhibiting peroxyl radicals in the aqueous phase [7-10].

Vitamin E is a group of eight antioxidant lipophilic molecules (i.e., tocopherols and tocotrienols; each four in number) and is reported in green vegetables including oils, grains, and nuts as well as in eggs and milk [11,12]. Recently, vitamin E is commonly known for its antioxidant properties and possesses many immunobiological properties, e.g., antioxidant activity and the ability to modulate protein function and gene expression. As a lipophilic molecule, vitamin E is generally absorbed in the gut via micelles, and then incorporated into chylomicrons [11-14]. Finally, when vitamin E reached into the circulation, vitamin E is transferred to other lipoproteins by the action of phospholipid transfer protein (PLTP) and to cells by the action of PLTP and lipoprotein lipase. In contrast, the liver also secretes vitamin E through α-tocopherol-TP that is highly specific tor α-tocopherol and mediates its transfer to various lipoproteins [11-14].

Syzygium cumini (family Myrtaceae), medicinal plant is distributed in tropical and subtropical regions. Most of these medicinal plants that belong to this family are generally known to be rich in volatile oils, which are reported and used as medicine [15,16]. In addition, most of the fruits belonging to this family also showed rich history of uses both as edibles and as traditional medicines in divergent ethnobotanical practices throughout the tropical and subtropical world [17-19]. In addition, this medicinal plant products - especially leaves, stem bark, leaves, seed, etc. - have shown various medicinal properties or uses including various immunopharmacological properties as well [15-19]. In this study, our group focused on this medicinal plant pertaining to determine its antioxidants (i.e., vitamin C and E).

METHODS

Collection of plant material and extraction

Leaves of *S. cumini* was collected from the Nakshatra Garden of Vidya Pratishthan in the month of October 2016 and identified by Dr. Bharat Shinde, Botanist at Vidya Pratishthan's Arts, Science and Commerce College, Baramati. Leaves of this medicinal plant were cut into small pieces and dried in shady area. Shade dried leaves of this medicinal plant were prepared using liquid nitrogen and then dissolved in phosphate buffered saline (PBS, pH 7.2). The powder was finally macerated in

mortar and pestle. Finally, collect the supernatant after centrifuging at high speed (15,000 rpm) for determining its antioxidant properties.

Preliminary phytochemical screening

Aqueous leaves extract of *S. cumini*, medicinal plant was subjected for phytochemical analysis [16] to identify the presence of various phytochemicals such as alkaloids, glycosides, saponins, flavonoids, tannins, sterols, phenols, etc. In addition, leaves are rich in acylated flavonol glycosides, quercetin, myricetin, myricitin, myricetin 3-O-4-acetyl-L-rhamnopyranoside, triterpenoids, esterase, galloyl carboxylase, and tannin.

Cell culture

Noninfected lysed human whole blood samples were used for antioxidant studies. All these cells were maintained in PBS supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C

Estimation of protein content

In this study, leaves dried powder (1 g) of *S. cumini* were taken and then add extraction buffer (i.e., 20 mM Tris-HCl). Incubate the sample for 5-8 minutes at room temperature and then centrifuged (6000 rpm; 10 minutes at 4°C). Afterward, supernatant was collected after centrifuging and then add similar volume of ice-cold acetone solution. Incubate for 10-15 minutes at room temperature and then centrifuged. Collect the pellet and then properly washed with acetone (ice cold) to remove the pigments including lipids as well. Finally, protein concentration was determined using NanoDrop method [20].

Estimation of vitamin C and E including total antioxidants using human whole blood

In this study, ethylenediaminetetraacetic acid non-infected human whole blood (n=10; 2 ml) samples were collected from Mangal Pathology Laboratory, Baramati. For these studies, serially diluted samples of antioxidants (vitamin C, E; 6.25-500 µg/ml; 1 ml) were taken and then add exact concentration of aqueous leaves extract, i.e., *S. cumini* (10 mg/ml; 100 µl) along with lysed human whole blood (100 µl). Incubate the samples for 2 hrs at 4°C. After incubation, centrifuging these samples at high speed (10,000 rpm, 4°C) and collect the supernatant for estimation of antioxidants and determined through NanoDrop method [21]. Vitamin C and E capsules are used as standard for these studies and diluted serially in PBS and used as negative control. On the basis of these studies, readings of aqueous leaves extract of *S. cumini* treated with various antioxidants of different concentration and compared with various antioxidants (vitamin C and E) diluted serially which is dissolved in PBS. Hence, calculations were done on the basis of slope intercept equation (y=mx+c) where m and c represent slope and intercept; y is optical density). All these readings or calculations were determined through NanoDrop method.

Statistical analysis

All values were mentioned as mean±standard error. Data were represented by one-way ANOVA test (Bonferroni multiple comparison test).

RESULTS

Estimation of protein content

In this study, aqueous leaves extract showed the presence of protein content which is determined through NanoDrop method. The quantity of protein in *S. cumini* was found to be 24.443 mg/ml (Fig. 1).

Estimation of antioxidants

To determine the antioxidants (vitamin C and E) in aqueous leaves extract of *S. cumini* as shown in Figs. 2 and 3. The results of these studies showed that *S. cumini* increased antioxidants (vitamin C) activity but it will show very less enhancement in case of vitamin E in lysed human whole blood. Overall, these studies claimed that aqueous leaves extract of *S. cumini* showed potent antioxidant activity especially in case of vitamin C.

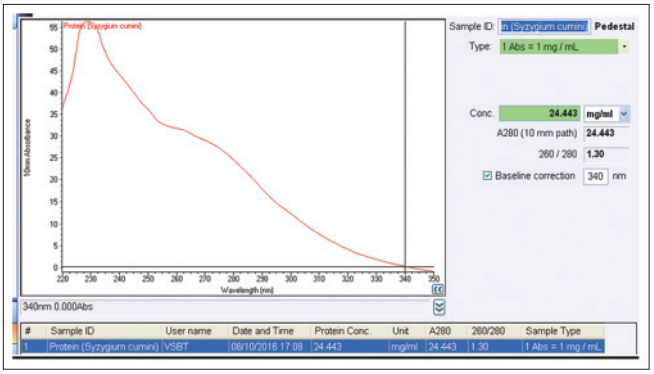

Fig. 1: Estimation of protein content. To determine the protein content from fresh mature plant leaves of *Syzygium cumini* using Tris-HCl and ice cold acetone which is determined through NanoDrop method. Results are expressed in mg/ml

DISCUSSION

As per the literature, human body produces natural antioxidants that protect against various infectious agents including free radicals that are present in our immune system. The most effective way is to increase the antioxidant activity, i.e. eating a diet which contained fresh fruits, vegetables, and grains including some food contained antioxidants as well. In other words, when these food materials are present in raw state and showed higher antioxidant properties, but heat will destroyed various antioxidants in many fruits and vegetables [2-7]. Hence, researchers start focusing on various medicinal plants for determining its antioxidant properties. Actually, these immunopharmacological activities (anti-inflammatory, antimicrobial, etc.) could be due to the presence of various phytochemicals (flavonoids, terpenoids, saponin, glycosides, alkaloids, phenolics, etc.) [22]. Among phytochemicals, phenolics is more important for antioxidant activity; literature revealed that medicinal plant products contained higher amount of phenolics content as compared to fruits and vegetables. In addition, these medicinal plants associated with anticancer agents that contained potent natural antioxidants and beneficial chemopreventive agents. In this study, we focused on medicinal plant products especially leaves of *S. cumini* pertaining to determine the vitamins (C and E) content in human whole blood samples.

In general, these antioxidants, i.e., vitamins (C and E) played an important role in protecting cells and neutralizing free radicals during exercise. Humans, unlike other animals, cannot be able to produce vitamin C with in the body [6-11] and consumed it in the form of the exogenous supplements. In human, absence of an enzyme, l-gulonolactone oxidase from the liver was reported and there is some defect or loss of gene that controlling the synthesis of this enzyme in humans and blocked the final phase in the series for converting glucose to ascorbic acid. In addition, a lot of benefits are reported related to vitamin E [8-19]. It may function as fat-soluble antioxidant and protect the cells from damage and might aid in lowering a variety of health problems, from heart disease to cancer, and possibly even dementia. In this regard, scientists start focusing on various medicinal plants especially leaves of *S. cumini* and tried to use as a supplementary source pertaining to enhance its immunity against various free radicals that are generated in our immune system.

Immunopharmacological finding of aqueous leaves extract was reported and showed a significant increase in vitamin C production but slightly enhancement in vitamin E production which is consistent with the normal physiological features as a result of normal metabolic processes in human. In this study, our results claimed that *S. cumini* showed the presence of primary metabolite, i.e., protein content and also showed enhancement in antioxidants especially vitamin C production in lysed human whole blood as compared to capsules of these antioxidants dissolved in PBS which is determined through NanoDrop method.

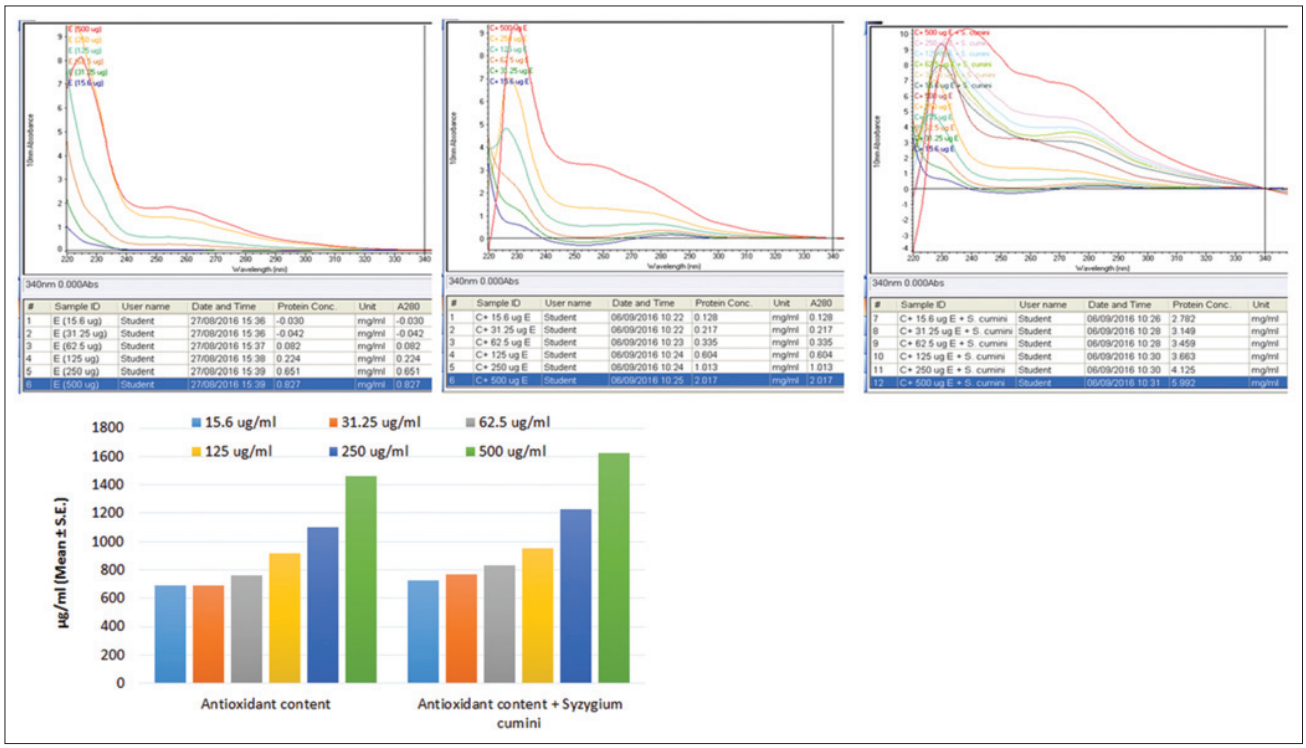

Fig. 2: Effect of variable doses of vitamin E along with or without aqueous leaves extract of *Syzygium cumini*. Lysed human whole blood (100 µl) were taken and incubate fixed concentration of aqueous leaves extract of *Syzygium cumini* (10 mg/ml 100 µl) including vitamin E of variable concentration (6.25-500 µl/ml, 1 ml; serially diluted). Incubate these blood samples with vitamin E, antioxidant along with or without aqueous leaves extract for 2 hrs at 4°C. After incubation, centrifuged these samples at high speed (10,000 rpm, 4°C) and collect the supernatant for estimation of vitamin E and determined through NanoDrop method. For these studies, standard curve were generated using vitamin E of different concentration dissolved in phosphate buffered saline, calculate slope and intercept. Readings will be expressed in jig/ml.

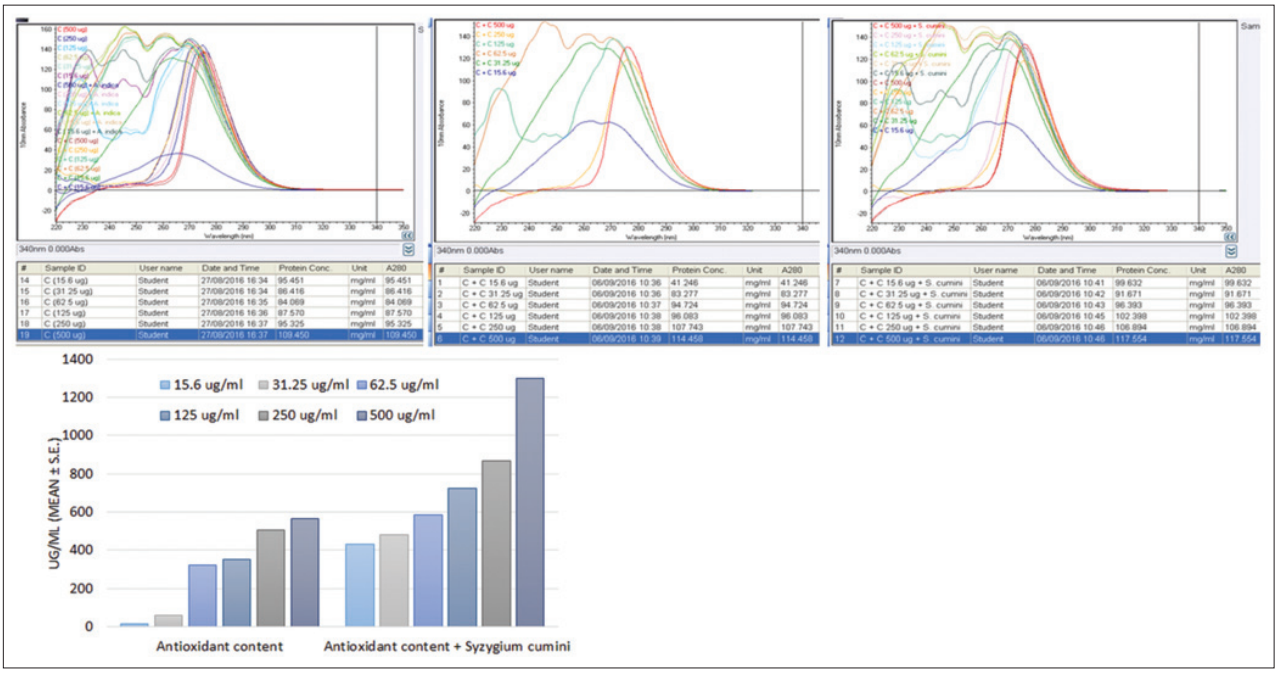

Fig.3: Effect of variable doses of vitamin C along with or without aqueous leaves extract of *Syzygium cumini*. Lysed human whole blood (100 µl) were taken and incubate fixed concentration of aqueous leaves extract of *Syzygium cumini* (10 mg/ml 100 µl) including vitamin C of variable concentration (6.25-500 µl/ml, 1 ml; serially diluted). Incubate these blood samples with vitamin C, antioxidant along with or without aqueous leaves extract for 2 hrs at 4°C. After incubation, centrifuged these samples at high speed (10,000 rpm, 4°C) and collect the supernatant for estimation of vitamin C and determined through NanoDrop method. For these studies, standard curve were generated using vitamin C of different concentration dissolved in phosphate buffered saline, calculate slope and intercept. Readings will be expressed in µg/ml.

Due to the enhancement of these antioxidants especially (vitamin C) containing aqueous leaves extract of *S. cumini* may help to prevent damage that is associated with cancer, heart disease, and other related human diseases.

CONCLUSION

In this study, aqueous leaves extract of *S. cumini* was and found to be more potent because of higher amount of antioxidants especially vitamins C production. All these studies were conducted in lysed human whole blood, and these studies claimed that leaves can be used in various immunopharmacological applications as a valuable antioxidant natural source and medicine.

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