EFFECT OF $Zn(NO_3)_2$ CONCENTRATION ON THE ANTIOXIDANT PROPERTY OF ALOE-VERA, CUMIN SEED AND GARLIC

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ABSTRACT

Antioxidants from organic compounds can reverse the adverse effect of skin aging by reacting with the free oxygen radical formed during the process of skin aging. This property of antioxidants has contributed in their popular use in many skin products. Aloe vera, cumin seed and garlic are some of such herbs which not only possess antioxidant property but also antibacterial, anti-inflammatory and many other properties proven beneficiary for human health and skin. As a result, these compounds are being used as a tool of skin care product mainly for their antioxidant property. To enhance the cleansing effect and give a proper absorbance of these antioxidants zinc salts are being used in commercial skin products along with these organic compounds. The aim of this study was to find out the effect of $Zn(NO_3)_2$concentration on the antioxidant property of Aloe vera, cumin seed and garlic with DPPH assay. The finding suggested that use of $Zn(NO_3)_2$ is beneficial for the antioxidant level of Aloe vera and cumin seed but it hinders the antioxidant level of garlic. Further study with topical application of gel or other skin product prepared with zinc nitrate and Aloe vera, cumin seed and garlic is needed to prove this with practical examples.

Keywords: Skin care, Oxidative stress, Antioxidant, Concentration, DPPH, Percentage inhibition

1. INTRODUCTION

1.1 Human skin and antioxidant:

Human skin works as a protective layer which keeps the internal environment of the body safe. Invasion by microorganisms, UV radiation and many mechanical damages is prevented by the defense mechanism present in our skin. Skin also has homeostatic ability along with immunological ability (Boelsma *et al.,* 2001). As a result, a healthy skin is an asset that helps with many biological processes in the body. Skin appearance is a scale for determining the condition of skin. It is determined by skin's surface texture, color, sebum and sweat production and elasticity (Boelsma *et al.,* 2001). Skin aging is a natural process which affects the appearance of skin with time. However, many genetic, hormonal and environmental factors play a vital role in skin aging. As an example, overexposure to solar radiation can accelerate the skin aging process (Schinitsky & Meisner, 1990). Similarly, formation of free radicals such as oxygen free radicals is considered as a critical mechanism for skin aging. Moreover, dietary products and drugs can produce toxicants which deteriorate the metabolism of skin.

Skin has a wide range of mechanism to overcome the effect of toxicants such as production of non-enzymatic and enzymatic molecules which work as antioxidants or oxidant-degrading systems. However, this defense mechanism of human skin has limitation which can be overridden by production of excessive toxicants due to many environmental factors (Bickers and Athar, 2006). In normal metabolic system, the production of toxicant or their metabolites are limited. These toxicants work directly as oxidants or indirectly produce a wide range of reactive oxygen species (ROS). ROS works as a free radical with a free electron in its orbital. It is highly reactive and unstable because of the presence of unpaired electron. It needs an electron to become stable which can be obtained from oxygen which is a biradical compound. However, this

reduction process has a great kinetic barrier for which often on single-electron reduction takes place which results in formation of more ROS (Benzie, 2003). Overproduction of ROS leads to an imbalance between the antioxidant defense and toxicant inhibition. This phenomenon is known as oxidative stress. Oxidative stress can lead to many skin diseases including cancer (Poljsak & Dhamane, 2012). It also impairs the body's antioxidant protective metabolism in various ways.Topical application of antioxidant or antioxidant reach products can help to reduce the effect of oxidative stress. Antioxidant is the substance that produces dioxygen while reacting with other substance such as ROS. It reverses the effect of free radicals and toxicants that produces ROS (Powell, 2000). As a result, the use of different fruits and herbs with antioxidant properties has been a centre of attraction to treat many skin diseases. Aloe vera, cumin seed and garlic are some such popular herbs for their acceptance in skin care products for topical supplementation of antioxidants. They do not have only antioxidant properties but also antibacterial and anti inflammatory properties. As a result, these herbs are being used in skin care products to treat the skin with their multiple properties.

1.2 Aloe vera as an antioxidant:

Aloe vera is being popularly used for its various affect on human and animal health. It has antibacterial, antiulcer, anticancer and anti inflammatory along with antioxidant properties (Joseph & Raj, 2010). Aloe vera has many components among which vitamin A, C and E and some minerals work as antioxidants. It has metallothionein protein which works as an active antioxidant against skin damage due to radiation (Surjushe, 2008). Both raw Aloe vera leaf and synthetic Aloe vera gel is being popularly used to heal skin from acne, scar and other inflammation.

1.3 Cumin as an antioxidant:

Cumin seed is readily found in our kitchen especially in south Asia. Cumin is very popular among the Asians for its cooling effect and giving remedy from overheating (Nag, 1994). It helps to reduce our salt intake by replacing salt with cumin. Moreover, cumin seed is also identified to have anticancer and antibacterial activity. Cumin consists of many chemical components such as coumarins, phthalides, acetylenes, and terpenoids(Craig, 1999). Among these components, coumarin is proven to have antioxidant property along with anti-inflammatory, antiallergic, hepatoprotective, antiviral and anticarcinogenic properties (Kostova, 2006). Cumin oil is being used in skin care products as a means of natural oil (Panasenko, 2010). Topical application of cumin paste is popular among young girls as a home remedy of acne and scar of skin.

1.4 Garlic as an antioxidant:

Garlic is well known for its various effects on health care which alone contributed in more than one thousand publications by last decade. Garlic has a wide range of biological attribution such as hypolipidemic, antiplateletand procirculatory effects. It is considered as the best disease preventive food, due to its effect on preventing flu, cold and enhancement of immunity and also working as an anticancer agent (Amagase, 2006). Garlic is proven to be effective for health problem caused by oxygen radical and lipid peroxidation such as cardiovascular diseases, liver damage and skin aging. Antioxidant components of garlic act against these diseases. Garlic has verities of chemical components among which only allin, allyl systeine, allyl disulfide and allicin has been proven to have antioxidant properties (Shenawy *et al*., 2008). However, different extraction process alters the chemical properties of garlic thus its

antioxidant potency. The most common extraction process of garlic is aqueous extraction which has been followed in this study.

1.5 Use of zinc Nitrate salt in skin product:

Use of natural herbs is very common from ages due to their affect in healing the skin. Among all other skin problems skin aging is one of the main concerns among people. There is a common tendency to look young as long as possible among most of us. As a result, product that helps to reverse the adverse effect of skin aging becomes very popular. Producers take this in count and invent many skin care products with antioxidant properties. However, there are some disadvantages of using natural herbs in skin care product as antioxidants. As example, stability of the product becomes a major concern as antioxidants are very unstable (Allen, 1990). Moreover, to get the expected result antioxidants should reach the target and remain there for enough time to show their activity. In normal condition the effectiveness of reaching target is very limited. Zinc salt such as zinc sulfate, zinc chloride, zinc nitrate can be a potential answer for the above mentioned problems. In this study, zinc nitrate salt has been used. Zinc nitrate is a hydratable inorganic anhydrous salt that produces heat while reacting with other substances (Giani, 1998). As a result, any product mixed with this salt will give a heating effect during use which will enhance the skins ability to absorb antioxidants (Scholz *et al.*, 2003). Moreover, zinc nitrate has been identified as a potent chemical to enhance the effectiveness of pharmacologically active agents such as antioxidants and reducing the systemic toxicity of the product (Allen, 1990). However, there is no study which shows a relation between the effect of zinc nitrate concentration and antioxidant level of various natural herbs. This finding will help to determine the suitable concentration of zinc nitrate to be used in skin product to get better cleansing effect without hindering the antioxidant property of natural herbs used in the product.

The goal of this experiment is to observe the effect of different concentration of zinc nitrate salt on the antioxidant level of garlic, cumin seed and Aloe vera which will help to determine which concentration of zinc nitrate salt should be used in skin care product in order to retain the antioxidant properties of Aloe vera, cumin seed and garlic.

2. MATERIALS AND METHODS

This research was conducted to identify the effect of different concentration of $Zn(NO_3)_2$on the antioxidant property of Aloe vera, cumin seed and garlic which were bought from a local grocery shop called “Shopno”. In each experiment fresh Aloe vera, cumin seed and garlic were used.

2.1. Sample Collection and Storage:

Fresh Aloe vera, cumin seed and garlic were bought one to two hours before the experiment. After extraction of each sample, it was stored in the refrigerator at 4^0 C for further experiment. A stock of 10M $Zn(NO_3)_2$ solution was prepared and stored at room temperature. This stock was used to make following final concentration of $Zn(NO_3)_2$ solution: 0.5M, 0.4 M, 0.3M, 0.2M, 0.1M, and 0.05M.

2.2. Extract Preparation:

2.2.1. Aloe vera extraction:

A fresh Aloe vera leaf was peeled with a sterile knife to take out the gel inside the leaf. It was cut into small pieces and weighed before homogenizing. Water was added into 1:1 ratio and then it was homogenized using a blender at high speed for 10 minutes. The mixed liquid was collected in an autoclaved beaker and was filtrated using cheesecloth for four times. Remaining extract was collected in another autoclaved beaker and stored in the refrigerator at 4^0C temperature.

2.2.2. Cumin seed extraction:

Cumin seed was first weighed and water was mixed with it at 1:10 ratio (1gm cumin: 10 mL water). Cumin extract had high turbidity for which different dilution series were prepared from which 1:10 ratio was selected. The selected ratio of cumin and water was then added in the blender and homogenized for 15-20 minutes. Mixed liquid was collected in an autoclaved beaker and the above mentioned filtration and storing process was followed for cumin as well.

2.2.3. Garlic extraction:

Aqueous garlic extraction was followed for preparing garlic extraction mentioned by Maria Joao Fonseca and Fernando Tavares (2011). Garlic cloves were peeled and weighed. Cloves were cut into small pieces and homogenized in a blender by adding water into 1:1 ratio for 12-15 minutes. The homogenized mixture was filtered four times through cheese cloth and stored at 4^0C temperature.

2.3. Zinc Nitrate Solution Preparation:

2.3.1. Stock Preparation for Different Concentration of $Zn(NO_3)_2$Solution:

Zinc nitrate anhydrous used in this experiment had a molecular weight of 297.49g/mol. To make a stock solution of 10 M $Zn(NO_3)_2$ solution, 29.74g $Zn(NO_3)_2$ was weighed and dissolved in 10 mL distilled water. From this stock solution, the following concentration of $Zn(NO_3)_2$ solution was prepared: 8 M, 6 M, 4 M, 2 M and 1 M. For the reaction, 200 µL of $Zn(NO_3)_2$ was used with a volume of 200 µL of each sample along with 2 mL DPPH and 1.6 mL Tris-buffer which gave a final volume of 4 mL As a result, each concentration of $Zn(NO_3)_2$ solution had a 20 fold dilution during the experiment thus the final concentration was the following: 0.5M, 0.4 M, 0.3M, 0.2M, 0.1M, and 0.05M.

2.3.2. Absorbance Reading of Zinc Nitrate and Analytical Sample Reaction:

Before preparing DPPH solution, the absorbance reading for $Zn(NO_3)_2$ solution and analytical sample reaction was recorded. For this 2 mL of each sample was mixed separately with 2 mL $Zn(NO_3)_2$ solution of different concentration. Absorbance reading was recorded at 517 nm. This absorbance value had been denoted as A_R as it represents the absorbance value of the reaction between $Zn(NO_3)_2$ and the analytical samples only.

2.4. DPPH Solution Preparation:

The protocol described by Shimamura *et al*. was followed to make the DPPH (2,2-diphenyl-1-picrylhydrazyl) solution. 7.89 mg DPPH was weighed on a chemical balance which had a minimum limit of 10 µg. It was dissolved in 100ml of absolute ethanol which gave a solution of 0.2mM DPPH. As the absorbance of DPPH decreases with time approximately after 1 hour of preparation, it was kept at room temperature in dark for 2 hours to stabilize the absorbance. The solution was covered with aluminum foil paper and a black paper cup to avoid any contact with light as DPPH is photo reactive. After 2 hour, a test tube was filled with following reagents: 2 mL of DPPH solution, 400 µL of absolute ethanol and 1.6 mL of 0.1 M Tris HCl buffer (pH 7.4). This reaction mixer was then used to take absorbance reading at 517 nm. If the absorbance was within 1.00±0.05 range then it was directly used for further measurements. If it exceeded the range then ethanol was added and the solution was diluted to bring the absorbance value within the above mentioned range. This solution was used for further measurement and stored in dark at room temperature. All of the solution was used up during the day of preparation as the absorbance value of DPPH changes with time.

2.5. DPPH Assay Preparation:

After preparing the DPPH solution, sampling test tubes were prepared by the following

DPPH solution	Tris-HCl(pH 7.4)	Zinc Nitrate (with varying conc.)	Extracted sample
2mL	1.6 mL	200µL	200 µL

All of the samples were mixed for 10 seconds right after preparing them and kept at room temperature in the dark for 30 minutes after adding DPPH with it. After 30 minutes, the absorbance of the solution was measured at 517 nm wavelength using a blank prepared with 2.4 mL ethanol and 800 µL of Tris-HCl buffer. The absorbance after the addition of analytical sample with DPPH was denoted as A_D and the absorbance with only ethanol instead of sample was denoted as A_C. Following equation was used to calculate the inhibition ratio (%):

Inhibition ratio (%) = $\{(A_C - A_S)/A_C\} \times 100$

Where, A_C= Absorbance with only ethanol instead of sample

$A_S = A_D - A_R$ = Absorbance of the analytical sample

A_D= Absorbance after the addition of analytical sample with DPPH

A_R = Absorbance value of the reaction between $Zn(NO_3)_2$ and analytical samples

2.5.1. Statistical Analysis

All the absorbance values had been taken for three times and mean of the values were calculated differently for the three analytical samples. The standard deviation was calculated in Microsoft Excel 2007.

3. Results:

Both Aloe vera and cumin seed showed an increase in percentage inhibition with the increase of $Zn(NO_3)_2$ concentration (Figure 1 &2). The absorbance value of aloe vera and cumin seed had been tabulated along with calculation of percentage inhibition and standard deviation value (Table 1-6). However, garlic showed a completely reverse trend where the inhibition percentage decreased with increasing concentration of $Zn(NO_3)_2$ (Figure 3). Absorbance value of garlic and its inhibition percentage had been tabulated along with the value of standard deviation (Table 7-9).

3.1. Results for Aloe vera:

Table 1: The absorbance value of Aloe vera decreases while reacting with increasing concentration of $Zn(NO_3)_2$ before adding DPPH

Zinc Nitrate Concentration (M)	Absorbance value of Aloe vera + $Zn(NO_3)_2$(A_R)
0.05	0.588
0.1	0.586
0.2	0.542
0.3	0.495
0.4	0.487
0.5	0.477

Table 2: The mean absorbance value of Aloe vera decreases with increasing concentration of $Zn(NO_3)_2$ with addition of DPPH

Zinc Nitrate Concentration	**Experiment 1**	**Experiment 2**	**Experiment 3**	**Mean (A_D)**
0.05	1.57	1.582	1.578	1.577
0.1	1.545	1.54	1.538	1.541
0.2	1.47	1.462	1.469	1.467
0.3	1.389	1.372	1.385	1.382
0.4	1.331	1.227	1.359	1.306
0.5	1.132	1.125	1.13	1.129

Table 3: The percentage inhibition of Aloe vera increases with increasing concentration of $Zn(NO_3)_2$

Zinc Nitrate Concentration	A_C (Absorbance of blank ethanol)	A_D (Absorbance of $Zn(NO_3)_2$ + Aloe vera+ DPPH)	A_R Absorbance of $Zn(NO_3)_2$ + Aloe vera)	A_S (Absorbance of Aloe vera due to reaction with DPPH)	Average Inhibition ratio (%)	Standard deviation Value of I (%)
0.05	1.05	1.577	0.588	0.989	5.841	0.581
0.1	1.05	1.541	0.586	0.955	9.048	0.343
0.2	1.05	1.467	0.542	0.925	11.905	0.415
0.3	1.05	1.382	0.495	0.887	15.524	0.846
0.4	1.05	1.306	0.487	0.819	22	6.624
0.5	1.05	1.129	0.477	0.652	37.905	0.343

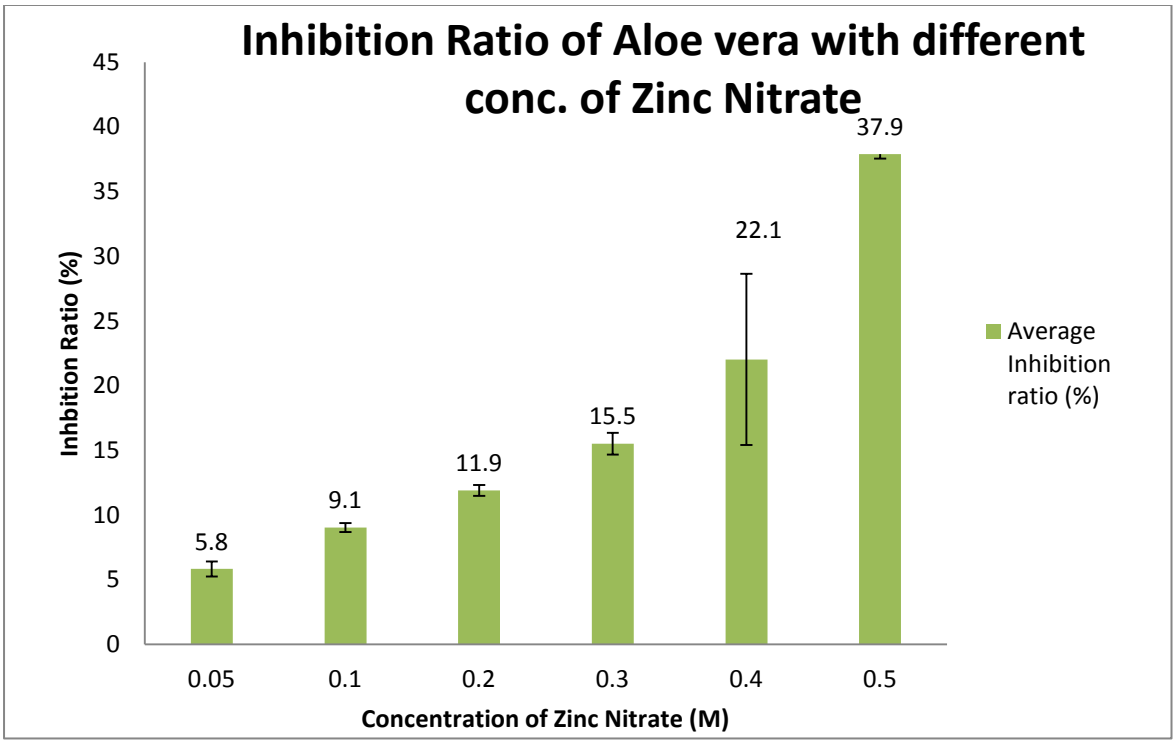

Figure 1: Inhibition ratio of Aloe vera increases with increasing concentration of Zinc nitrate with negligible deviation from the STDEV value with and exception at 0.4M

3.2. Results for Cumin Seed:

Table 4: The absorbance value of Cuminseed decreases while reacting with increasing concentration of $Zn(NO_3)_2$ before adding DPPH

Zinc Nitrate Concentration (M)	Absorbance value of Cumin + $Zn(NO_3)_2$ (AR)
0.05	1.125
0.1	0.988
0.2	0.864
0.3	0.787
0.4	0.696
0.5	0.509

Table 5: The mean absorbance value of Cumin seed decreases with increasing concentration of $Zn(NO_3)_2$with addition of DPPH

Zinc Nitrate Concentration	**Experiment 1**	**Experiment 2**	**Experiment 3**	**Mean (A_D)**
0.05	2.170	2.032	2.124	2.109
0.1	2.016	1.986	1.885	1.962
0.2	1.704	1.770	1.708	1.727
0.3	1.249	1.704	1.514	1.489
0.4	1.158	1.674	1.323	1.385
0.5	1.007	1.054	1.021	1.027

Table 6: The percentage inhibition of Cumin Seed increases with increasing concentration of $Zn(NO_3)_2$

Zinc Nitrate Concentration	A_C (Absorbance of blank ethanol)	A_D (Absorbance of $Zn(NO_3)_2$ + Cumin+ DPPH)	A_R Absorbance of $Zn(NO_3)_2$ + Cumin seed)	A_S (Absorbance of Cumin due to reaction with DPPH)	Average Inhibition ratio (%)	Standard deviation Value of I (%)
0.05	1.05	2.109	1.125	0.984	6.2856	6.692015
0.1	1.05	1.962	0.988	0.974	7.238	6.536368
0.2	1.05	1.727	0.864	0.863	17.809	3.524238
0.3	1.05	1.489	0.787	0.702	33.143	21.76456
0.4	1.05	1.385	0.696	0.689	34.381	25.0979
0.5	1.05	1.027	0.509	0.518	50.667	2.298246

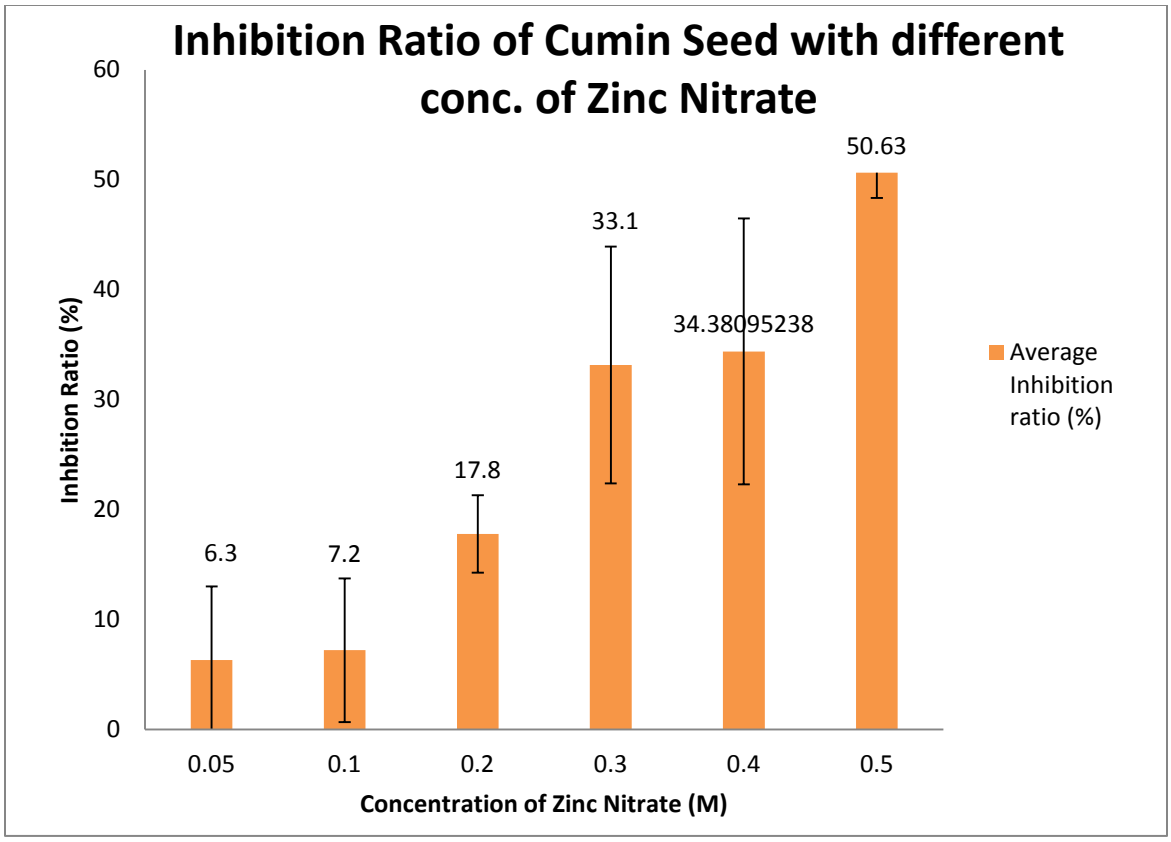

Figure 2: Inhibition ratio of Cumin Seed increases with increasing concentration of Zinc nitrate with significant deviation from the STDEV value 0.3 & 0.4 M.

3.3. Results for Garlic:

Table 7: The absorbance value of Garlic increases while reacting with increasing concentration of $Zn(NO_3)_2$ before adding DPPH

Zinc Nitrate Concentration (M)	Absorbance value of Garlic+ $Zn(NO_3)_2$(A_R)
0.05	0.484
0.1	0.502
0.2	0.521
0.3	0.532
0.4	0.547
0.5	0.552

Table 8: The mean absorbance value of Garlic increases with increasing concentration of $Zn(NO_3)_2$ with addition of DPPH

Zinc Nitrate Concentration	**Experiment 1**	**Experiment 2**	**Experiment 3**	**Mean (A_D)**
0.05	0.725	0.728	0.727	0.727
0.1	0.752	0.751	0.752	0.752
0.2	0.792	0.787	0.790	0.789
0.3	0.802	0.800	0.801	0.801
0.4	0.826	0.827	0.826	0.826
0.5	0.836	0.829	0.832	0.832

Table 9: The percentage inhibition of Garlic decreases with increasing concentration of $Zn(NO_3)_2$

Zinc Nitrate Concentration	A_C (Absorbance of blank ethanol)	A_D (Absorbance of $Zn(NO_3)_2$ + Garlic+ DPPH)	A_R Absorbance of $Zn(NO_3)_2$ + Garlic)	A_S (Absorbance of Garlic due to reaction with DPPH)	Average Inhibition ratio (%)	Standard deviation Value of I (%)
0.05	1.05	0.727	0.484	0.243	76.857	0.145
0.1	1.05	0.752	0.502	0.25	76.190	0.055
0.2	1.05	0.789	0.521	0.268	74.476	0.239
0.3	1.05	0.801	0.532	0.269	74.381	0.095
0.4	1.05	0.826	0.547	0.279	73.428	0.055
0.5	1.05	0.832	0.552	0.28	73.333	0.334

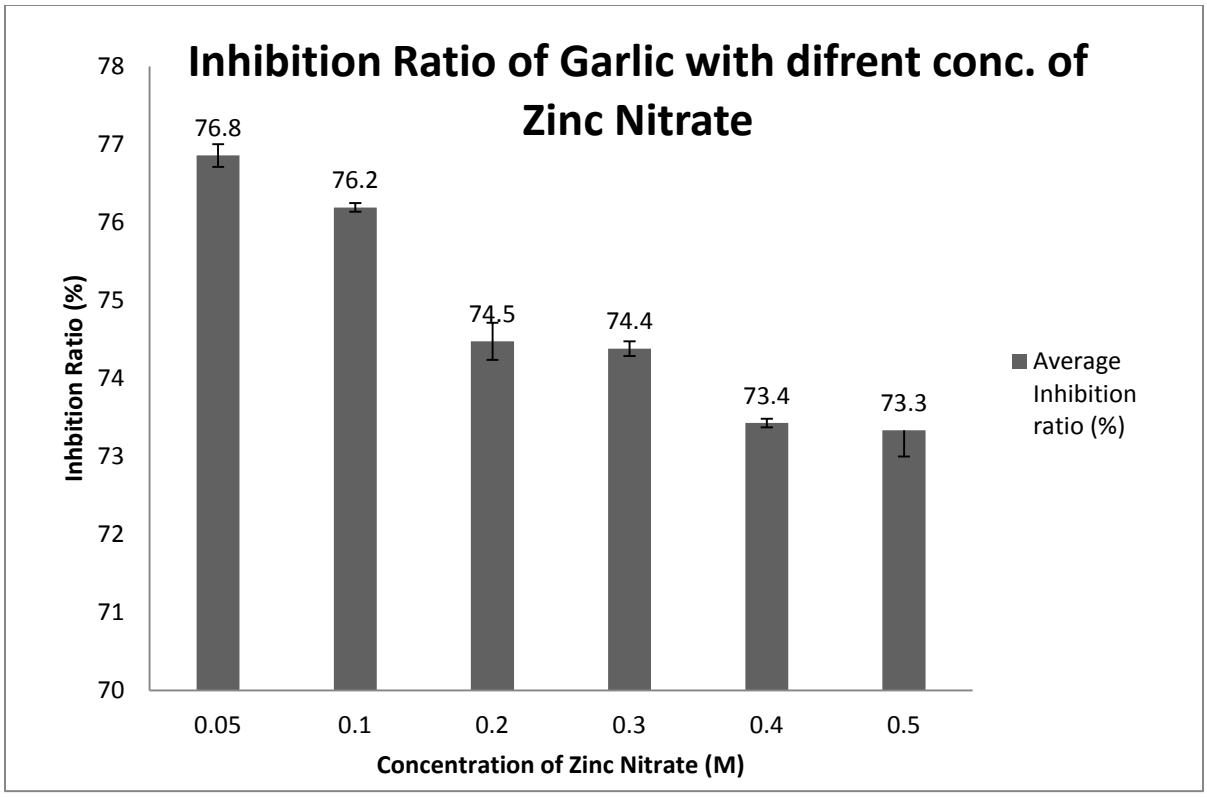

Figure 3: Inhibition ratio of Garlic decreases with increasing concentration of Zinc nitrate with negligible deviation from the STDEV value.

4. Discussion

Use of DPPH assay to calculate antioxidant level is a common method. In this assay, DPPH is reduced by the antioxidant which decreases the absorbance at 515-528 nm wavelengths (Magalhaes *et al.,* 2008). It means organic compounds with high antioxidant level will decrease the absorbance of DPPH .Thus any organic compound with high antioxidant level will give a low A_S value after reacting with DPPH which will in return increase the percentage inhibition value.Calculating the percentage inhibition will help to determine the strength of antioxidant property of organic compounds.

From figure 1 and 2, it can be observed that the initial percentage inhibition at 0.05M $Zn(NO_3)_2$ concentration was low. However, with increasing concentration of $Zn(NO_3)_2$ the percentage inhibition increased. It means $Zn(NO_3)_2$ increases the reducing strength of Aloe vera and cumin seed. Reaction with increasing concentration of $Zn(NO_3)_2$ will help both Aloe vera and cumin seed to show their antioxidant potency. On the other hand, figure 3 shows that the percentage inhibition of garlic decreases with increasing concentration of $Zn(NO_3)_2$. It means the reaction with increasing $Zn(NO_3)_2$ concentration hinders the antioxidant property of garlic. The mode of action has been discussed below.

4.1. Mode of action of DPPH:

DPPH assay method is electron transfer based where the DPPH acts as a stable free radical and the antioxidant works as electron donor. The free radical in DPPH is nitrogen centered free radical. It is quite stable and easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Patel & Patel, 2011). As a result, the free radical from DPPH pairs off with antioxidant and reduces to DPPH which in return decreases the absorbance of the DPPH. The more the decolorization is the more is the reducing ability of the antioxidant. This is called

free radical scavenging of antioxidants. The reaction rate is influenced by the solvent and pH of the solution. As example, if water is used instead of ethanol the absorbance of the DPPH decreases as it coagulates and decreases the amount of free radical accessible for the reaction with antioxidant. Moreover, the size of the reactant molecule also influences the rate of reaction. Smaller molecule has better access to reaction site. As a result, antioxidant with large reactant molecule will react slowly or may even act as inert in this assay (Magalhaes*et al.,* 2008). Addition of $Zn(NO_3)_2$ in this assay will either help the antioxidant by increasing the strength of their free radical scavenging ability by adding nitrogen free radical in the reaction or increasing the availability of the reactant molecules from the antioxidants. It can inhibit the antioxidant property by reacting with the antioxidant reactant molecule thus making them unavailable for the reaction.

4.2. Antioxidant property of Aloe vera with $Zn(NO_3)_2$:

Aloe vera has vitamin A, C, E and protein metallothionein which gives Aloe vera its antioxidant properties. Vitamins work as a source for tocopherol or tocotrienol. It can produce singlet oxygen which is highly reactive oxygen molecule. Singlet oxygen works as a resonant absorbers and scavenges the free radical here the DPPH free radical. On the other hand, metallothionein can bind with metal ions here Zn ion from $Zn(NO_3)_2$ solution(Benzie, 20030. Reaction of Aloe vera and $Zn(NO_3)_2$ releases more tocopherol and tocotrienol to react with DPPH mean while metallothionein binds with zinc ions. As a result, the single oxygen from vitamins reduces DPPH by scavenging the free radicals at a high reaction rate. This is why the inhibition ratio of Aloe vera increases with increasing concentration of $Zn(NO_3)_2$ as shown in figure 1.

4.2. Antioxidant property of Cumin with $Zn(NO_3)_2$:

Cumin has coumarin which has been proven to have antioxidant property. Coumarin is concentration dependent. It shows better reactivity at higher concentration. In DPPH assay coumarin acts as an electron donor and this is attributed by substituent like –OH, -CH3 and –Cl which reduces the DPPH (Patel & Patel, 2011). As a result, it can be said that reaction with $Zn(NO_3)_2$ and cumin seed produced more free coumarin compounds. Since the coumarin is concentration dependent, the availability of more coumarin in the reaction mixer will increase the strength of DPPH scavenging of the cumin seed. As a result, the inhibition ratio of the cumin seed increases with increasing concentration of $Zn(NO_3)_2$ (figure 2). Another possibility can be the increasing strength of DPPH by the addition of $Zn(NO_3)_2$ solution along with cumin. While cumin reacts with $Zn(NO_3)_2$ the reaction might facilitate the formation of free nitrate radical from DPPH which will increase the DPPH scavenging thus increases the inhibition ratio.

4.2. Antioxidant property of Garlic with $Zn(NO_3)_2$:

Garlic has many chemical compounds among them allin, allyl systeine, allyl disulfide and allicin components of garlic possess antioxidant activity. However, among all of them allyl systeine has been reported to have highest scavenging capacity (Londhe*et. al,* 2011). It means the presence of allyl systeine in the reaction will increase the antioxidant property of garlic. In this experiment, the inhibition ratio of garlic decreases with increasing concentration of $Zn(NO_3)_2$ (figure 3). It can be deduced that the reaction of $Zn(NO_3)_2$ and garlic inhibits the reaction of allyl systeine which decreases the antioxidant strength of garlic. Moreover, the size of the chemical compounds produced from garlic can also affect the rate of reaction. If the size of

allyl systein or other chemical compounds inhibits the reaction site of DPPH it will decrease the inhibition ratio. In return, the overall antioxidant property will decrease.

This study has some limitations. As an example, DPPH used in this experiment is photo reactive and time sensitive. The absorbance of DPPH changes with its reaction with light and also with time. To minimize its reaction with light, the DPPH mixer was covered with foil paper and then covered with a black cup. However, the number of analytical sample for this experiment was 36 but the spectrophotometer used for the experiment had only two cuvettes. As a result, the absorbance reading of all samples was not taken within a same time. Some of the samples waited for extra couple of minutes. Moreover, the analytical sample of Aloe vera. Cumin seed and garlic varied each time as all of them were bought freshly right before the experiment and the species of the sample was unknown. This can also have contributed in the variation of inhibition ratio. However, as the antioxidant property of all these herbs is attributed by the chemical components present in them which are not much influenced by their variation in physical characteristic or their species, the results obtained from the experiment can be generalized for Aloe vera, cumin seed and garlic regardless of their variation is species.

5. Conclusion:

The aim of this study was to find out the effect of $Zn(NO_3)_2$ concentrations on the antioxidant property of Aloe vera, cumin seed and garlic. From the results obtained throughout this study, it was found out that $Zn(NO_3)_2$ enhances the antioxidant property of Aloe vera and cumin seed but it hinders the antioxidant level of garlic. Further study is needed to find out its real effect on human skin by making topically applicable gel and observing its effect on the skin.

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