# Photoprotective potential of extract obtained from *Allium cepa (red onion)* against UVB-induced albino mice

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**Abstract:** Sunlight plays an important role in normal biological processes of the skin. However, too much exposure to ultraviolet (UV-B) rays without necessary protection can cause skin cell damage resulting to skin cancer and other skin complications. This study evaluated the photoprotective effect of *Allium cepa* (red onion) against the UV-B induced albino mice. Lipid peroxidation assay for Malondialdehyde (MDA) and histopathologic study were conducted in order to determine the photoprotective potential of *A. cepa*skin extract. Phytochemical analysis of the plant revealed the presence of flavonoids. Reduction in the MDA levels of the groups that received 2% (0.004), 3% (0.004) and 4% (0.007) topical *A. cepa*extract against 0.120 of the irradiated group was evident on day 15 which was also observed from days 3, 7 and 11. The results show the damaging effect of UV-B on skin of the albino mice and the protective effect of *A. cepa* skin since MDA level increases when UV-B dose was increased. Using ANOVA, the obtained p values were all greater than 0.05 level of significance showing that the treatments have similar photoprotective activity. Histopathologic results showed that the groups treated with 2%, 3% and 4% of the extract had very mild to moderate epidermal thickening, with cellular morphology almost similar to the unirradiated control group. Thus, *A. cepa*skin extracthas a photoprotective potential starting at 2% concentration as confirmed through histopathological findings. However, its photoprotective activity does not exhibit a concentration-dependent activity as suggested by the MDA levels of the irradiated and unirradiated controls and different experimental groups.

**Keywords:** *Allium cepa*, flavonoids, photoprotective, UV-B


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**INTRODUCTION**

The skin, as the outermost organ of the body, is frequently exposed to a pro-oxidative environment including ultraviolet (UV) radiation, drugs and air pollutants. Aside from these external factors, endogenous generation of reactive oxygen species (ROS) and other free radicals may cause oxidative stress (Thiele &Mudiyanselage, 2007). Being continuously exposed to potential hazards, the skin performs various tasks in order to maintain homeostasis. Among non-immune functions, the skin provides physical and biochemical barrier that ensures sufficient hydration and allows synthesis of vitamins and hormones. As an immune-protective organ, skin actively defends tissue especially deeper regions through the action of well-coordinated epithelial and immune cells (Meglio, Perera, & Nestle, 2011).

Skin pigmentation is the most important photoprotective factor of human skin. It is repeatedly exposed to UV that influences the function and survival of many cell types and is regarded as the main causative factor in the induction of skin cancer. The skin pigmentation is the most important photoprotective factor, since melanin, besides functioning as a broadband UV absorbent, has antioxidant and radical scavenging properties. Besides, many epidemiological studies have shown a lower incidence for skin cancer in individuals with darker skin compared to those with fair skin (Brenner & Hearing, 2008).

Skin cancer is the most common type of cancer in fair-skinned population. The incidence, morbidity and mortality rates of skin cancers are rising that pose a significant public health concern. The major etiologic agent in developing skin cancer is exposure to ultraviolet radiation.

DNA damage and genetic mutations are theconsequences of ultraviolet radiation exposure that may subsequently lead to skin cancer (Narayanan, Saladi, & Fox, 2010).

The main source of exposure to UV is the sunlight. It is composed of visible light (400-700nm), infrared (>700nm) and ultraviolet radiation. Ultraviolet radiation is further categorized as UVA (>315–400 nm), UVB (>280–315 nm) and UVC (>100–280 nm). The level of exposure to UV depends on latitude, altitude, time of day and other atmospheric components (Narayanan et al., 2010).

UVA rays enhances vitamin D production through the irradiation of 7-dihydrocholesterol and intensify the darkening of preformed melanin pigment favoring tanning. Adversely, it has been demonstrated that these rays are accountable for photosensitivity which result in several types of allergic reactions and actinic lesions including immunosuppression, photo aging, ocular damage, and skin cancer (Korać&Khambholja, 2011).


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As UVB is biologically active, it readily penetrates to the superficial level of the skin down to the basal layer of the epidermis. Harmful effects were generated and cause inflammation, sunburn and precipitating skin ageing. ROS are produced during energy release after light is absorbed by chromophores, such as melanin, in the skin. Also, when the high-energy photons of UVB is directly absorbed by the DNA bases of the cell, it can cause mutagenic lesion and to counter act such damage upon exposure to UVB, the skin synthesize new melanin that results to delayed tanning as the primary defense reducing sunburn. Unfortunately, its protective effect is not enough to protect the body against UV-induced cancer (Dupont, Gomez, &Bilodeau, 2012).

Majority of the radiations absorbed in our atmosphere is filtered by the ozone layer, one which is very harmful on living things, the UVC. In recent time, the thickness of the layer has been reduced due to many reasons such as the use of chlorofluorocarbon (Yel & Türker, 2014).

Anti-oxidants are any substances that when present at low concentrations compared to those of an oxidizable substrate significantly delay or prevent oxidation of that substrate. They play an important role in the body's defense system by reducing the damaging effects of reactive oxygen species (ROS) within the body or free radicals generated during aerobic activity of cells. Increased intake of dietary antioxidant assists in maintaining the antioxidant status; thus, the body's normal physiological function (Ashwini, Balagnesh, Balamurugan, Murugan & Satiskumar, 2013).

Oxygen free radicals and reactive nitrogen species are renowned for their harmful effects. Free radicals cause possible biological damage known as oxidative stress and nitrosative stress. They occur in the biological system when there is an excessive ROS and a deficiency of enzymatic and non-enzymatic anti-oxidants. A variety of fruits and vegetables shows potential decreasing risk of having chronic diseases, such as cancer, coronary heart disease and many more. Vegetables, fruits and their seeds contain vitamins C, E, and β-carotene and compounds including bioflavonoids and proanthocyanidins, ellipticine and taxol, indole derivatives, dithiolthiones, phytoestrogens, which exhibit chemopreventive and/or anti-cancer properties. Polyphenols and flavonoids are predominant anti-cancer agents that are proven effective in treating ovarian, breast, pancreatic, cervical and prostate cancer. Flavonoids, which are relatively abundant in human diet usually present in food, have potential roles as anti-cancer agents. Frequently consumed fruits, wine and vegetables had anti-oxidants which prevent oxidative stress damage and are potentially anti-carcinogenic flavonoids which include quercetin, kaempferol, myricetin, apigenin and luteolin. There is a


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positive correlation between flavonoids-rich diet (from vegetables and fruits) and lower risk of acquiring cancer lead to a question that whether flavonoids mediate protective effects. Quercetin makes up majority of the flavonoid content obtained from the consumption of apples and onions (Ashwini et al., 2013).

Plant phenolics consist of phenolics acids, flavonoids, tannins and the less common stilbenes and lignans. Flavonoid is divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the oxidation state of the central C ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation (Dai & Mumper, 2010).

Flavonoids provide different physiologic functions for plants such as transport of plant hormone (auxin), defense, allelopathy, and modulating the levels of reactive oxygen species. Flavonoids essentially present immense medicinal value which includes anti-oxidant, anti-proliferative, antitumor, anti-inflammatory, and pro-apoptotic activities (Buer, Imin & Djordjevic, 2010). Cancers, cardiovascular disease and neurodegeneration are possibly prevented by flavonoid, especially flavanols, anthocyanins and flavonols.

*Allium cepa*has various other common names throughout the world. This name includes scallion, spring onion, gibbon, syboe and sibuyas. It is widely cultivated among Asian countries due to tropical season. *A. cepa* contains quercetin, a flavonoid that protects against cardiovascular disease, cataracts, and cancers and reduces swelling. It has been historically used for its medicinal value and has long been cultivated for food worldwide. With varying characteristics which include bulb colors such as white, yellow or red and flower colors such as white or purple, onions have green stems, hollow leaves and can reach three feet (1m) in height and which the bulb grows below the grounds which are edible and can be used medicinally. Al Masaudi and AlBureikan's study (2012) found that it is abundant in flavonoids, polyphenols, organic sulfur, saponins and many other secondary metabolites, which are liable for its medicinal potential.

***Figure 1. Allium cepa vegetable (red onion)***


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*A. cepa* is proven to have antioxidant, antihypertensive, antithrombotic, hypoglycemic, and antihyperlipidemic properties. Onion contains sulfur compounds and quercetin in traces of essential oils which are antioxidants that aid in neutralizing the free radicals present in the human body. It also contains mineral components such as calcium, magnesium, sodium, potassium, selenium and phosphorus (Sampathkumar, Bhowmik, Chiranjib, Biswajit&Tiwari, 2010).

Medical properties of *A. cepa* include improvement of kidney function and nitrogen absorption. It stimulates digestion and regulates bowel movement and removes active microorganisms. The seeds relieve dental worms and urinary disease. The stalks are source of Vitamin A, thiamine and ascorbic acid (Sampathkumar et al., 2010).

*A. cepa* has anti-inflammatory effects are due to its Vitamin C and quercetin contents and other active components called isthiocyanates. The use of fresh onion extracts is recommended by World Health Organization (WHO) for treating coughs, colds, asthma, bronchitis and also relieving hoarseness. WHO also supports the use of onions for the treatment of appetite loss and preventing atherosclerosis. It also contains organosulfur compounds that have been linked to regulate blood pressure, and cholesterol levels that reduce formation of blood. It also offsets tendencies towards angina and heart attack. Regular consumption of onion lowers blood pressure and the serum levels of cholesterol and triglyceride, while increasing HDL levels (Sampathkumar et al., 2010).

Vitamin B6 contained in onions lowers homocysteine levels, a risk factor for heart attacks and strokes. Sulfur contents are responsible for its natural anti-clotting. Sulfides in onion provide protection against tumor growth especially stomach and colon cancer. Onions have active compounds that effectively inhibit the development of cancerous cells. It prevents prostatic hypertrophy, stomach and breast cancer (Sampathkumar et al., 2010).

*A. cepa* contains thiosulphate, a bactericidal compound which kills *Salmonella typhi, Pseudomonas aeruginosa*and *Escherichia coli* (Adeshina, Jibo, Agu, &Ehinmidu, 2011).

Sahin, Tuzcu, Akdemir, Ozercan, and Bayraktar's study (2011) found that concentration of flavonoid compound in foods may be affected by a variety of factors such as species, variety, degree of ripeness and storage after harvesting. According to Batra and Sharma (2013), because of lack standardized analytical method, estimation of daily human intake of flavonoids is difficult. Conjugated flavonoids half-lives are rather long which causes (23–28 h) flavonoids taken into the body regularly to readily accumulate which produces adequate active flavonoid concentrations.


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The overall objective of our study is to determine the ability of *A. cepa* for its photoprotective activity against UV radiation- induced damage to an artificial skin mimic. This study would provide additional knowledge in the medical field; thus, serves as a reference, foundation and support to the pre-existing, new and previous studies. As one of the endemic foods cultivated worldwide, onion being cost-effective and premeditated is prospectively preferred. The scope of the study includes determination of the potential photoprotective activities of *A. cepa*. Furthermore, the study includes the observational facets regarding different concentration of *A. cepa* extract and its relative effects. The study is delimited to the identification of phenolic extract from *A. cepa*. The study was conducted to create an alternative option that exhibit photoprotective activity from the phenolic extract of *A. cepa*that can be locally found, cost-effective and economical.

## MATERIALS AND METHOD

### Collection and Identification

Seven kilograms of ripe, firm vegetable of *A. Cepa* characterized by dry, unbroken outer skin that clings tightly to the bulb itself and bulb with no soft spots were purchased from a local market in Julian Pastor Memorial Market, Batangas City and was authenticated at the University of Philippines – Los Baños.

### Preparation of Extract (Yadav& Kumar, 2012)

The plants collected were washed in running water to remove dust. The bulb and skin parts of collected plants were separated, shade dried, powdered, weighed, and stored separately for extraction. Each of the dried powdered and weighed samples underwent Soxhlet extraction in 80 percent methanol for 24 hours and was filtered. The filtrate obtained from each sample was subsequently extracted in petroleum ether, diethyl ether, and ethyl acetate. Petroleum ether fraction was discarded due to its being rich in fatty substances. Ether fraction was used for free flavonoids whereas ethyl acetate fraction was used for bound flavonoids. Ethyl acetate fraction of each sample was hydrolysed further with seven percent $H_2SO_4$ for 24 hours and then re-extracted with ethyl acetate.

### Phytochemical Screening for Flavonoids

A stock solution of one ml was taken from a test tube and few drops of diluted NaOH solution were added. Appearance of intense yellow color on the test tube which will become colorless upon the addition of few drops of diluted acid indicated the presence of flavonoids (Hossain et al., 2013).


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**Preparation of Laboratory Animals**

Thirty Albino mice weighing 15-25 grams were purchased from University of the Philippines – Manila. Identification and ethical approval for all procedures required for the use of laboratory animals were secured from the Animal Care and Use Committee of Lyceum of the Philippines University. The laboratory animals were kept in plastic bottom cages and were provided with pellet-based feeds and adequate water throughout the experimental procedure. They were grouped as follows:

Group 1: Unirradiated Control Group
Group 2: Group treated with 2% *A. cepa*extract
Group 3: Group treated with 3% *A. cepa*extract
Group 4: Group treated with 4% *A. cepa*extract
Group 5: Irradiated Control Group

The first group was not exposed to radiation which served as the control. Group treated with 2%, 3%, 4% and the irradiated group was exposed to UV-B for 5 minutes, twice daily.

Trauma from shaving the dorsal part of the mice causes the formation of free radicals, thus, VeetCreme (Kosmetik E. Ritter, Switzerland) was applied on the defined area of approximately 4 $cm^2$ prior to hair removal. The mice were observed for 48 hours and those that showed abnormal hair growth and any reaction to the creme were excluded (Sachdeva, Kharya, Aljarbou, &Katyal, 2011).

**UV Light Exposure Conditions and Development of UVB-induced Skin Damage in Mice (Sachdeva et al., 2011)**

The animals were divided into five groups with each group containing six members each. Group 1 served as the control. Groups 2, 3, and 4 were given UV radiation and different concentrations of extract treatment. Group 5 received a 5-minute exposure to UV light for twice daily and served as the irradiated control. The treatment was given four hours prior to the exposure to UV light as stated in the protocol. The mice were placed in a solar stimulator in a room fitted with UV lamp that is 40 cm away from the UV light. UV exposure was controlled by time. The exposure was given twice daily for five minutes per exposure. For the mice which received topical treatment, the extract was applied four hours prior to the exposure. The extract was delivered at a dose of 10 $\mu l/cm^2$ area of the skin by a micropipette. The animals were treated for three, seven, eleven, and fifteen days following skin excision that were then subjected to lipid peroxidation assay.


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**Biochemical Test**

Lipid peroxidation assay was assessed in all groups by estimating 2-thiobarbituric acid reacting substance, which is malondialdehyde (MDA). To 0.2 ml homogenized sample, 0.2 ml of 8.1 percent sodium lauryl sulfate and 1.5 ml of 20 percent acetic acid solution (pH adjusted to 3.5 with sodium hydroxide) were dispensed. Then 1.5 ml of 0.8 percent aqueous solution of thiobarbituric acid (TBA) was added. The mixture was made up to 40 ml with distilled water and heated in a water bath at 95˚C for 60 min. In cooling water, one ml of distilled water and 5.0 ml mixture of butanol and pyridine (15:1 v/v) were added. After centrifugation at 4000 rpm for 10 minutes, absorbance of the organic layer was read at 532 nm (Sachdeva et al., 2011).

**Histopathologic study**

The animals were treated for 15 days followed by the excision of the skin. Skin biopsies from dorsal portion of the unirradiated control group, irradiated control group and treated groups were fixed in 10 percent formalin, embedded in paraffin, and sectioned. The sections were stained with aqueous hematoxylin and alcoholic eosin stain for routine examination of the tissues (Sachdeva et al., 2011).

Histopathological analysis was performed at Lab Site Diagnosis Hub, D&C Bldg. D Canlapan Street, Batangas City.

**Statistical method**

The experimental results obtained were expressed as mean ± standard error of mean (SEM). All measurements were carried out in triplicate. Means were compared for statistically significant differences by one-way analysis of variance (ANOVA) with a 95 percent confidence interval. It is used to compare the differences between two samples labeled between groups and within groups which enable to determine the efficacy of a given treatment over a change period of time. All computations were done using PASW ver. 18.

## RESULTS AND DISCUSSION

**I. Plant Extract**

From 100 g of the blended and Soxhlet extracted sample, approximately 100 ml of extract were obtained. Subsequently filtered, separated and further hydrolyzed for 24 hours in which 40 ml was attained.

Figure 2 shows the phenolic extract of *A. cepa*after Soxhlet extraction which is red wine in color. The same appearance of extract


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was obtained in the research of Chandravanshi and Upadhyay in 2012 when they studied natural dye in *A. cepa*.

**Figure 2. *Allium cepa*extract**

## II. Phytochemical Screening for Flavonoids

For the identification of the presence of flavonoids in *A. cepa*skin, theappearance of intense yellow color in a test tube upon the addition of dilute NaOH which became colorless upon the addition of diluted HCl was obseved. This confirms the presence of flavonoids in the extract which is similar with the study conducted by Hossain, AL-Raqmi, AL-Mijizy, Weli& AL-Riyami Q (2013).

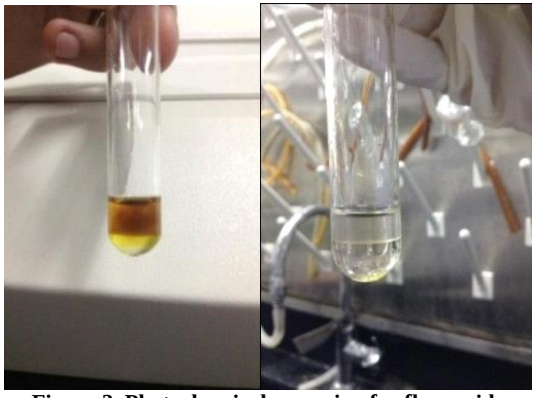

**Figure 3. Phytochemical screening for flavonoids**

## III. Lipid Peroxidation Assay

The skin acts as the first line of defense against environmental exposure like solar UV radiation in which exposure to such has shown dramatic effects on cell cohesion and mechanical integrity that are related to its effects on the intercellular components. Initiation of detrimental photochemical reactions causes both acute and chronic conditions. Such may include erythema, photo aging and different types of skin cancers. Alteration of the morphological composition of skin can lead to severe macroscopic skin damage such as chapping and cracking and associated inflammation, infection, scarring, and abnormal desquamation (Biniek, Levi, &Dauskardt, 2012).


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The photoprotective activity of *A. cepa*extract was assessed by quantification of malondialdehyde (MDA) level present in the skin and evaluation of morphological appearance through histopathologic study. Assessments of the MDA level in the homogenized skin sample was performed through a Lipid Peroxidation assay.

Lipid peroxidation is a key chemical event in the oxidative stress. Lipid peroxidation is a complex molecular mechanism which involves formation of lipid radicals and destruction of cell membranes, through oxidative damage and toxicity to cell structures leading to cell death. It significantly promotes structural damage to membranes and formation of secondary products that cause enzymatic inactivation and produces toxic effects to cellular division and function. MDA was considered as the most important metabolite of lipid peroxidation but is practically not toxic. MDA as a metabolite of lipid peroxidation, it was used in Lipid peroxidation assay (Repetto, Semprine, &Boveris, 2012). Lipid Peroxidation assay was used to measure a biomarker for oxidative stress, the MDA levels which is the product of polyunsaturated lipids degradation facilitated by reactive oxygen species.

**Table 1**
**Levels of Skin Malondialdehyde in the unirradiated and irradiated controls and treated groups**

| | Level of Malondiadehyde | | | |
|---|---|---|---|---|
| | Day 3 | Day 7 | Day 11 | Day 15 |
| Group 1 Unirradiated Control | 0.002 | 0.003 | 0.002 | 0.002 |
| Group 2 Treated w/ 2% extract | 0.007 | 0.006 | 0.004 | 0.004 |
| Group 3 Treated w/ 3% extract | 0.006 | 0.005 | 0.004 | 0.004 |
| Group 4 Treated w/ 4% extract | 0.004 | 0.005 | 0.005 | 0.007 |
| Group 5 Irradiated Control | 0.015 | 0.090 | 0.100 | 0.120 |

The MDA levels are presented on Table 1. For the unirradiated control group, the MDA level on the third day was found to be 0.002 as opposed to the irradiated control group which showed the maximal MDA level of 0.015 that served as the baseline. With the values obtained, it is evident that the skin of the test animals remained intact for the unirradiated group while evidence of oxidative stress resulting to cellular damage on the skin was detected in irradiated control group. This implies the damaging effect of radiation on the skin. According to Sachdeva et al. (2011) UV-irradiated mice skin result to morphological changes in the form of deep lesions and


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ulcerations along with an overall erythema. The experimental Groups 2, 3 and 4 on the third day resulted to MDA levels of 0.007, 0.006 and 0.004, respectively. The attained values were close to that of the unirradiated control group and were lower than the values of the irradiated control group. This implies that there was no free radical development even though they were exposed to radiation. This is more likely due to the effect of flavonoids of *A. cepa* extract on the skin of the test animals. According to Buer, Imin&Djordjevic (2010), flavonoids present immense medicinal value like anti-oxidant. Moreover, the MDA levels on the third day of the treated groups showed no cellular damage in comparison with the unirradiated and rradiated control group, as exhibited in the study performed bySachdeva et al. (2011).

For day 7, the MDA level of the unirradiated control was 0.003 as compared to the 0.090 MDA levels from the irradiated control group which showed the minimal MDA level of 0.003. The MDA level of the groups consistently treated with 2%, 3% and 4% of the extract were 0.006, 0.005 and 0.005, respectively. The values of the groups treated with 2% and 3% of *A. cepa*extract decreased in contrast with the group treated with 4% of *A.cepa* extract. However, the obtained results were near that of the unirradiated control group suggesting no free radical development between the groups treated with *A. cepa* extract. This indicates that the effect of extract does not depend on its concentration. Treatment with three doses exhibited elevated and reduced MDA levels. However, the reduction of MDA levels was not dose-dependent as stated in the study presented by Sachdeva et al. (2011).

The MDA level of the unirradiated control group for day 11 was still noted with 0.002 and an MDA level of 0.100 was observed in the irradiated control group. The values 0.004, 0.004 and 0.005 were the attained MDA levels from the groups treated with 2%, 3% and 4%, respectively which was in agreement with the irradiated control group. This implies that the treatment seems to act as a sunscreen reducing the formation of free radicals brought about by the radiation. MDA levels being considered as an important metabolite of lipid peroxidation is used as a biomarker of oxidative stress. Oxidative stress is a well-known mechanism that indicates cellular injury that occurs with increased lipoperoxidation of cell phospholipids and that has been implicated on various cell dysfunctions (Repetto, Semprine, &Boveris, 2012).

For day 15, the MDA level of the irradiated control group was 0.120, which greatly increased compared to first day of exposure. Exposure to UVB shows significant increase in MDA level which means that the radiation is harmful to the skin as it correlates to the


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formation of free radicals producing oxidative damage as an outcome during which was measured as MDA. In comparison to MDA level of the unirradiated control group which is 0.002, normal DNA repair and cell regeneration was maintained given that it was not exposed to UV light. Moreover, the groups treated with 2%, 3% and 4% of extract have MDA levels of 0.004, 0.004, and 0.007, respectively, corresponding with the MDA level of unirradiated control group.

It can be implied that there was minimal MDA level within the unirradiated control and maximal level of MDA in the irradiated control group. Considering that the unirradiated control was not exposed to ultraviolet light, wherein no cellular damage was produced. In comparison to the irradiated control group which was exposed to the ultraviolet radiation that produced oxidative damage as an outcome which was measured as MDA. MDA levels of treated groups with 2%, 3% and 4% *A. cepa* extract slightly decreased as the concentration increased. The MDA levels were measured continuously from day 3, 7, 11 and 15.

It is also indicated in Table 1 that the shorter the exposure of the laboratory animals under UV light was, the lesser MDA level was observed. Longer length of exposure yielded larger UV damage to the laboratory animals. Hence, the MDA level is proportional to the length of exposure of the laboratory under UV light. However, the MDA value of unirradiated group and treated groups were adjacent. Longer UV wavelengths such as UV-B induced oxidative stress and protein denaturation whereas short wavelength UV radiation causes predominantly DNA damage to cells and cause apoptosis (Gaiba et al., 2012). Oxidative stress in determined by MDA levels through lipid peroxidation (Repetto, Semprine, &Boveris, 2012).

The highest concentration of MDA is found on the irradiated control group as an indication that free radicals are formed and oxidative stress on the cells of the irradiated control group, whereas MDA, being the product of polyunsaturated lipids degradation facilitated by reactive oxygen species was considered as a biomarker for oxidative stress as stated by Sachdeva et al. in 2011. MDA formation is usually used as a marker for oxidative stress (Ahmad, Tripathi, Tripathi, Singh, Singh & Singh, 2008). Oxidative stress results when there is imbalance in the steady state of prooxidants and antioxidants usually shifts to increase prooxidants initiating possible organ damage (Aguiar, Almeda, Araujo, de Abreu, & Chavez et al., 2012). MDA was formed during irradiation of UV-B and its level increased with increased dose of UV-B. Therefore, MDA levels with 2% 3% and 4% treatment of *A. cepa*extract that showed a


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decrease steady-state is indicative of reduced lipid peroxidation and oxidative stress in the cells.

A great significance was seen in the MDA levels of groups treated with 2%, 3% and 4% with MDA levels of 0.004, 0.002, and 0.007, respectively, corresponding with the MDA level of unirradiated control group; thus, indicative of reduced lipid peroxidation and oxidative stress in the cells. The treatment helped in blocking the UVB rays as the *A. cepa*extract absorbs the radiation before it gets to penetrate the skin and causes skin cell damage.

An organism should continuously control and regulate the redox potential, the balance of pro-oxidants and antioxidants which are important for maintaining vital cellular and biochemical functions. Increase in pro-oxidant cause oxidative damage while increase in antioxidant is termed reductive stress.(Repetto, Semprine, &Boveris, 2012).

Oxidative stress and damage have been implicated in numerous disease processes. Higher concentrations of lipid radicals correspond to pathological situations. Hydroxynonenal (HNE) and MDA, have been shown to modify DNA bases therefore, DNA damage caused by lipid peroxidation yields promutagenic lesions and carcinogenic effects associated with oxidative damage providing promising markers for risk prediction and targets for preventive measures (Repetto, Semprine, &Boveris, 2012).

**Table 2**
**Comparison of the Mean of the Groups Exposed to UV Light**

| Group | p value | Interpretation |
|---|---|---|
| Group 1 Unirradiated Control | 0.079 | Not Significant |
| Group 2 Treated w/2% extract | 0.076 | Not Significant |
| Group 3 Treated w/ 3% extract | 0.077 | Not Significant |
| Group 4 Treated w/ 4% extract | 0.0762 | Not Significant |
| Group 5 Irradiated Control | -0.0770 | Significant |

**Significant p value = < 0.05*

Presented in Table 2 is the mean comparison of p values of UV light exposed groups. It is documented that the obtained p values for Groups 1, 2, 3, 4, and 5 were 0.079, 0.076, 0.077, 0.0762 and -0.077, respectively. Groups 1, 2, 3 and 4 had a value greater than that of the confidence value of 0.05 ($p>.05$) which indicates non-significance. On the contrary, Group 5 yielded less than that of the confidence value of 0.05 ($p<0.05$), which were considered significant. This indicates that upon radiation, development of cellular damage due


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to oxidative stress was induced among albino mice belonging to irradiated group. This clearly shows that the *A. cepa*extract was able to protect the skin of the test animals despite the exposure to radiation which is more likely due to its phenolic compounds such as flavonoids. UVB irradiation damages skin cells by the formation of ROS (Reactive Oxygen Species) resulting in oxidative stress, an important mediator of damage to cell structures, including lipids, membranes, proteins and DNA (Gaiba et al., 2012).

Photoprotective effects of the extract of *A. cepa*prepared in different concentrations were presented in Table 3.

**Table 3**
**Multiple Comparisons of the Photoprotective Effect of the Crude Extract of *Allium cepa***

| Group | Concentration of *A. cepa* | p value | Interpretation |
|---|---|---|---|
| Group 1 | 2% | 1.000 | Not Significant |
| Unirradiated | 3% | 1.000 | Not Significant |
| Control | 4% | 1.000 | Not Significant |
| | Irradiated | 0.002 | Significant |
| Group 2 | Unirradiated | 1.000 | Not Significant |
| Treated w/ | 3% | 1.000 | Not Significant |
| 2% extract | 4% | 1.000 | Not Significant |
| | Irradiated | 0.002 | Significant |
| Group 3 | Unirradiated | 1.000 | Not Significant |
| Treated w/ | 2% | 1.000 | Not Significant |
| 3% extract | 4% | 1.000 | Not Significant |
| | Irradiated | 0.002 | Significant |
| Group 4 | Unirradiated | 1.000 | Not Significant |
| Treated w/ | 2% | 1.000 | Not Significant |
| 4% extract | 3% | 1.000 | Not Significant |
| | Irradiated | 0.002 | Significant |
| Group 5 | Unirradiated | 0.002 | Significant |
| Irradiated | 2% | 0.002 | Significant |
| Control | 3% | 0.002 | Significant |
| | 4% | 0.002 | Significant |

A significant difference was observed upon the comparison of the unirradiated control group from the irradiated control group, while no significance was observed on the groups treated with 2% 3% and 4% of the extract in comparison to the unirradiated control using multiple comparisons test (Scheffe's test). The group treated with 2% of


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the extract was compared with the irradiated control wherein a significant difference was noted. However, with the comparison of that same group with the unirradiated control group and the groups treated with 3% and 4%, no significant difference was observed. On the other hand, a significant difference was observed when the group treated with 3% was compared with the irradiated control. In comparison with the group treated with 3% of extract from the unirradiated control, 2% and 4% of the extract, no significant difference was observed. The group treated with 4% of the extract when compared with the irradiated control exhibited a significant difference. Furthermore, when the same group was compared with the unirradiated, 2% and 3%, no significant difference was observed. Lastly, upon the comparison of irradiated control with the unirradiated control group and the groups treated with 2%, 3% and 4% extract, significant differences were seen.

The MDA levels of treated groups markedly decreased as compared to irradiated group which MDA level continued to increase upon exposure. Moreover, in accordance with the study conducted by Sachdeva et al. in 2011, there was no significant difference observed when the treated groups were compared with each other. This suggests that the photoprotective effect of the *A. cepa* extract does not exhibit a concentration-dependent manner. According to Procházková, Boušová and Wilhelmová (2011), antioxidant properties of a particular flavonoid depend most of all on its concentration. Their prooxidant properties could be associated with a cell signaling by which flavonoids contribute to the coordination of cell functions.

**IV. Histopathologic Result**

Histopathologic examination was performed in order to further confirm the photoprotective of *A. cepa*extract as shown in Figure 4.

In Figure 4A, localized and segmental sloughing off of epithelium with no associated inflammatory cells were observed. The adnexal structures and dermis are within normal limits. This confirms that the skin of the albino mice remained normal during the duration of the study, the fact that it has not been exposed to radiation. According to the study of Okhumo et al. in 2006, there is no remarkable change for unirradiated mice since it did not undergo UVB exposure.

The group that received 2% of the *A. cepa*extract demonstrated a mild hyperkeratoticacanthotic epidermis with normal adnexal structures as seen in Figure B. Developing hair follicles effacing the dermis were also found in the group treated with 2% *A.cepa*extract. UV-B radiation is responsible for hair protein loss; thus, development of hair follicles indicates repair of cellular injury. Although damage was induced on the skin of albino mice upon radiation, results presented minor damage compared to the irradiated


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group more likely due to the effect of *A. cepa*extract. According to Joiner and van der Kogel (2009), recovery is faster after lower initial doses that which induces clinical desquamation of epidermidis and complete compensation can be observed.

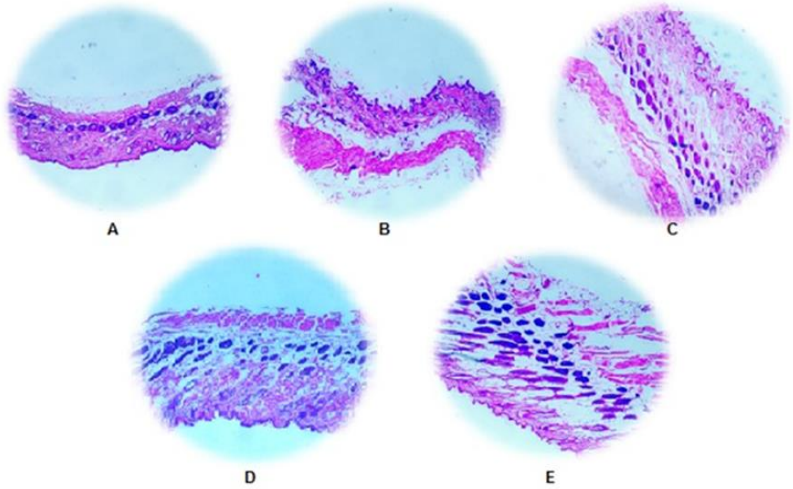


**Figure 4**.Histopathologic evaluation of A. Unirradiated Control, Group B. Group treated with 2%, C. Group treated with 3%, D. Group treated with 4%, & E. Irradiated Control Group

The group treated with 3% *A.cepa*extract showed the same results with the group treated with the 2% extract. Both showed increase in pigmentation and epidermal thickness.This suggests that the extract contributed to the elevated pigmentation and epidermal thickness in addition to the natural mechanism of the skin. This shows that there was less cellular injury in the treated groups as compared with the irradiated control group. Excessive UV exposure can lead to cell death. However, cellular injury can be repaired by the increase keratinocyte cell division that results to the accumulation in the epidermis causing epidermal thickness which protects the skin better against UV penetration. Production and accumulation of melanin pigment in the skin is an important physiologic response protecting the skin against subsequent damage. This is a response to ultraviolet exposure to protect against UV-associated to carcinogenesis (Korać&Khambholja, 2011).

It can be seen in Figure 4D that the group treated with 4% *A. cepa* extract exhibited a moderate hyperkeratoticacanthotic epidermis with very mild dermal edema. The adnexal structures were within normal limits and there was a random increase in numbers of developing hair follicles. This clearly shows the protective effect of *A.*


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*cepa*on the test animals' skin when exposed to radiation. This is more likely due to the phenolics such as flavonoids present in the extract that reduced the cellular damage after exposure to UV-B. The thickened epidermis is caused by constant exposure to the ultraviolet light and considered a reaction due to ROS formation. This is supported by level of MDA in the lipid peroxidase assay. In the study conducted by Joiner and van der Kogel (2009), high initial doses cause acute severe damage that may results in residual damage and reduced tolerance.

Histopathologic assessment of irradiated group that can be seen in Figure 4E reveals mild hyperkeratoticacanthothic epidermis with moderate numbers of inflammatory cells predominantly neutrophils, some lymphocyte and plasma cells infiltrating the submucosal (fat) layer. This confirms that the skin has been damaged by the radiation. This is more likely due to chronic effect of subsequent exposure to radiation. The induced epidermal dysplastic changes in form of focal acanthosis, loss of polarity, variation in size and shape of nuclei, nuclear aggregation, and presence of prominent nucleoli, mitosis, cytoplasmic basophilia and loss of keratohyaline granules that causes chronic inflammation are evident in skin damage (El-Sherry, Zaher, Youssef & El-Amir, 2007).

## CONCLUSION

It is therefore concluded that the extract of *A. cepa* has a protective effect against UV radiation at an optimum concentration of 2% as supported by lipid peroxidation assay and histopathologic assessment. However, the photoprotective effect of *A. cepa*extractdoes not exhibit a concentration-dependent activity. The findings suggest that *A. cepa* extract can be a potential alternative agent in treating skin damage caused by radiation.

## RECOMMENDATIONS

Isolation of specific active phytochemical components of *A. cepa* must be done in order to investigate which exhibits the most effective photoprotective activity. Other extraction procedures can be carried out in order produce larger amount of extract. Additional studies and researches must be conducted to investigate the photoprotective property of other plants with the same phytochemical components.

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