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https://dx.doi.org/10.4314/nvj.v43i3.4 **ORIGINAL ARTICLE**

COMPARATIVE *IN-VITRO* ANTIOXIDANT STUDIES OF CRUDE METHANOL EXTRACT AND FOUR SOLVENT FRACTIONS OF RED CULTIVAR *ALLIUM CEPA* L. BULBS

Oyewusi, J. A[1*].; Oridupa, O.A[2].; Saba, A. B[2].; and Oyewusi, I. K[3].

[1]Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. [2]Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Ibadan, Nigeria. [3]Department of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. *Corresponding author: Email: oyewusija@funaab.edu.ng; Tel No: +234 803 667 6864

SUMMARY

Oxidative stress-induced disease conditions are on the increase and there is need for natural sources of antioxidant for relieving stress. The crude extract of *Allium cepa* L. has been well published as a potential natural source of antioxidant. But there is inadequate information on the antioxidant activities of fractionated extracts of the plant. The *in-vitro* antioxidant capacity of crude extract of red cultivar *A. cepa* bulbs and its four solvent fractions were studied and compared using Nitric oxide radical inhibitory assay (NORIA), FRAP and DPPH antioxidant models. The results of the three models revealed that the crude methanol extract exhibited very good antioxidant activities. However, with the aid of DPPH, the crude methanol extract, ethyl acetate and methanol fractions exhibited excellent antioxidant activities in comparison with ascorbic acid. Serial extraction did not make any of the fractionated extracts better than the crude extract of the red cultivar *A. cepa* bulbs. These results also showed that red cultivar *A. cepa* bulbs is a natural source of antioxidants and could serve as therapeutic agent in the prevention or slowing down of oxidative stress. Further studies are currently underway to identify the active component responsible for the observed antioxidant properties.

Key Words: Antioxidant, Red cultivar *Allium cepa* L., Fractionated extracts

Running title: Comparative antioxidant capacity of red Allium cepa extracts

INTRODUCTION

It is widely accepted that many diseases caused by oxidative stress associated with from an imbalance between formation of Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS) and their neutralization when the innate/endogenous antioxidant mechanisms fail to mop up the free radicals released into the circulation (Rao *et al.,* 2010). Excessive production of ROS is capable of inducing damages to the cellular biomolecules such as nucleic acids, carbohydrates, proteins, and enzymes, leading to development of several diseases (Prakash *et al.,* 2007). Medicinal plants have been widely reported as sources of antioxidant principles. Several thousands of people are becoming more conscious of their health and are aware of the importance of food nutritional values (Ola–Mudathir *et al*., 2018). This has reflected in the rate at which people use herbs and or their products in order to control the effects of stress factors produced in their systems. Antioxidants are popular among plant nutrients because of their potentials to prevent many pathophysiological diseases (González-Sarrías *et al.,* 2017). *Allium cepa* (Onions) is one of the most widely cultivated plant and consumed world-wide. It belongs to the plant Family, Liliaceae (Benito-Román *et al.,*2020; Milea *etal.,* 2021) which has about 250 genera and 3,700 species (Nasri *et al.,* 2012; Bisen and Emerald, 2016). *Allium cepa* is a medicinal plants reputed for exhibiting activities against several disease conditions (Nwaoguike, 2009). The *in-vitro* antioxidant activities of *Allium cepa* has been widely studied but most researchers (Benkeblia, 2005; Odukoya *et al.,* 2005; Suru, 2008; Skerget *et al.,* 2009; Obioha *et al.,* 2009; Alpsoy *et al.,* 2013; Ogunmodede *et al.,* 2012; Ashwini *et al.,* 2013) have concentrated their investigations on the crude extracts of the plants, except Roldan-Marin *et al.,* (2009) that investigated the *in-vivo* antioxidant defense ability using freeze-dried 'Recas' cultivar onion by-product from which two fractions were produced. From the available literature, Information on the antioxidant activity of red cultivar *A. cepa* beyond the crude extract level is apparently scanty in literature. Therefore, this work is designed to further investigate the *in-vitro* antioxidant activity of the crude methanol extract and four (gradient solvent fractions of the red cultivar *A. cepa* grown in Nigeria.

Materials and methods

Ethical approval

This study was approved by the University of Ibadan, Animal Care Use and Research Ethic Committee (ACUREC) with approval number UI/ACUREC/AJO/16/0030.

Collection of plant and the preparation of crude methanol extract

Fresh bulbs of red cultivar *A. cepa* L were obtained from a popular onion market in Abeokuta, Ogun state, Nigeria. A specimen sample of the plant was already registered and deposited at the Herbarium of the Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria with voucher number: FUNAABH0029. The coverings of the bulbs were peeled off. The clean bulbs were weighed on a laboratory bench weighing scale. The onion bulbs were then chopped into small pieces. Two (2) kilograms of the chopped *A. cepa* bulbs was macerated in 3.1 L of 96 % methanol for 72 hours. The soaked bulbs were

filtered and the resultant extract solution was concentrated by evaporation using a rotary evaporator (BUCHI R0210, Switzerland). A dark brownish extract was obtained, stocked in glass bottles, labeled as crude methanol extract of red cultivar *Allium cepa* bulb (CME)) and kept in the refrigerator (4°C) until it was needed for the study.

Preparation of fractions

Another set of fresh red cultivar *A. cepa* L bulbs were purchased from the same source. The dry coverings of the bulbs were peeled off. The peeled onion bulbs were chopped into small pieces and oven dried at 30^0C. Two hundred grams of dried *A. cepa* was blended using a Waring Laboratory Blender (ThermoScientific, USA). The blended dried onion bulbs were used for the serial extraction using four solvents (*n*-hexane, ethyl acetate, chloroform and methanol) in increasing order of polarities. The dried and granulated onion was first macerated in n-hexane for 72 hours after which the filtrate was drained. The remains of the onion bulb granules were air dried for about 24 hours in order to remove the solvent. The subsequent dried onion bulb granules were again macerated in ethyl acetate for 72 hours, the filtrate was drained and remains of the granules were again air dried for about 24 hours in order to remove the solvent. The procedure was repeated for chloroform and methanol in that order using the same granulated onion bulbs. The extracts obtained from each these solvents were concentrated by evaporation using a rotary evaporator (BUCHI R0210, Switzerland) and properly labelled as: *n*-Hexane *Allium cepa* Fraction (nHACF); Ethyl acetate *Allium cepa* Fraction (EAACF); Chloroform *Allium cepa* Fraction (CACF) and Methanol *Allium cepa* Fraction (MACF).

***IN-VITRO* ANTIOXIDANT STUDIES**

1. Nitric Oxide Radical Inhibition Activity (NORIA)

The Nitric Oxide Radical Inhibition Activity of CME and all the four solvent fractions was evaluated according to method described by Garrat (1964). To 0.1 ml of varying concentration of each of the test sample, 0.9 ml of sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) was added. The mixture was then incubated in water bath at room temperature for 120 minutes under light. Sulphanilamide (1%; 0.5 ml) was then added to the solution. The reaction was allowed to stand for 10 minutes and protected from light. Naphthylethylene-diaminedihydrochloride (NED) solution (0.1%, 0.5 ml) was added and protected from light (Garrat, 1964). The absorbance of the chromophore formed was evaluated using spectrophotometer at 532 nm. Percentage inhibition of nitric oxide radical (%) by the extract was calculated using the formular [1-E/C] x100 (Marcocci *et al*., 1994). Where: E=absorbance in the presence of extracts; C=absorbance value of the fully oxidized control.

2. Ferric Reducing Antioxidant Power Assay (FRAP) Assay

Evaluation of the antioxidant capacity of CME and all the four solvent fractions of red cultivar *A. cepa* using FRAP assay was done as described by Benzie and Strain (1996). The FRAP assay used antioxidants in the redox linked colorimetric method with absorbance measured with a spectrophotometer. A 300 nmol/L acetate buffer of pH 3.6 (3.1 g of

sodium acetate + 16 ml of glacial acetic acid made up to 1 litre with distilled water, 10 nmol/l12,4,6-tri 2-pyridyl 1,3,5-triazine, 98% (Sigma Aldrich), 3.1 mg/ml in 40 mmol/HCl) and 20 mmol/l of ferric chloride were mixed together in the ratio of 10:1:1 respectively to give the FRAP working reagent.
Each of 50 µl aliquot of each of the test sample or standard was added to 150 µl of FRAP reagent in a semi-micro plastic cuvette and was run in triplicate. Absorbance measurement was taken at 593 nm (A_{593}) exactly 10 minutes after mixing, using 50 µl of water as the control. To standardize 50 ml of the standard [iron (III) sulphate, 1 mM] was added to 1.5 ml of FRAP reagent. All measurement was taken at room temperature with extracts protected from direct sunlight. The FRAP values of the extracts were determined from a calibration curve of ferrous sulfate solutions at concentrations of 125, 250 and 500 M, expressed as M of ferric iron reduced per gram of dried sample. The antioxidant activity was measured by its ability to reduce the Fe^{3+}/ferricyanide complex by forming ferrous products. Fe^{2+} can be monitored by measuring the formation of Perlûs Prussian blue at 593 nm. Increased absorbance at 593 nm indicates a stronger reducing power.

3. DPPH Radical Scavenging Assay

The stable DPPH radical scavenging effect of test samples was carried out according to Viturro, *et al.* (1999). A methanol DPPH solution (0.15%) was mixed with serial dilutions of the extracts. A 20 min incubation period at room temperature was used before reading the absorbance at 515 nm. All the test and analyses were performed in triplicate and average. The inhibitory percentage of DPPH was calculated according to the following equation:

$$\% \text{ inhibition} = \frac{\text{absorbance (control) - absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

Statistical Analysis

Data generated from this study were presented as mean ± SEM. The difference between the means in the treated groups and in the untreated groups were compared by one-way analysis of variance (ANOVA) at 95% confidence interval using the Prism 5.0 Graphpad Statistic software.

RESULTS

Nitric Oxide Radical Inhibition Activity (NORIA)

There was no significant ($p>0.05$) difference between the percentage nitric oxide radical inhibitory action of ascorbic acid and that of the crude methanol extract of red cultivar *A. cepa* at all concentrations, except at 0.25 mg/ml at which that of the crude extract (51.44±10.42%) was significantly ($p<0.05$) lower than that of ascorbic acid. There was no significant ($p>0.05$) difference between the IC_{50} (inhibitory concentration) of ascorbic acid and that of crude methanol extract of red cultivar *A. cepa* (Table 1). There was no significant ($p>0.05$) difference between the percentage nitric oxide radical inhibitory action of ascorbic acid and that of any of the solvent fractions of *Allium cepa* at 0.5 mg/ml. However, at all other concentrations, the percentage values of all the *A. cepa* fractions were significantly lower ($p<0.01$) than those of ascorbic acid except for chloroform fraction (65.66±4.80% at 0.25mg/ml) which was not significantly ($p>0.05$) different. There was no significant ($p>0.05$) difference between the IC_{50} of

ascorbic acid and that of any of the A. cepa extracts (Table 1).

TABLE I: Percentage nitric oxide radical inhibitory action of crude methanol extract and solvents fractions of Allium cepa

	Crude Methanol A. cepa extract	n-Hexane fraction	Ethyl acetate fraction	Chloroform fraction	Methanol fraction	Ascorbic acid
Concentration		Percentage Inhibition (%)				
0.5 mg/ml	45.29±10.87	45.24±5.22	47.62±2.32	52.74±1.45	50.65±8.12	43.94±5.95
0.25 mg/ml	51.44±10.42*	54.98±4.99*	49.86±7.11*	65.66±4.80	52.45±2.15*	76.69±1.15
0.125 mg/ml	62.84±6.65	56.42±5.81*	47.47±5.60*	58.66±9.07*	40.76±11.79	78.35±2.78
0.0625 mg/ml	63.49±4.36	46.10±4.97*	39.83±6.26*	46.61±6.76*	52.74±6.84*	76.41±6.14
IC_{50} (mg/ml)	2.86mg/ml	2.97 mg/ml	3.16 mg/ml	2.62 mg/ml	3.09 mg/ml	2.36mg/ml

*Indicates significant ($p<0.05$) difference compared to the Ascorbic acid values at the same concentration.

Ferric reducing antioxidant potential (FRAP)

The FRAP values for all the concentration of crude methanol extract of red cultivar *A. cepa* were significantly ($p<0.01$) lower than that of the ascorbic acid except at 1mg/ml [0.62±0.12 mM Fe (II)/L], which was not significantly ($p>0.05$) different from that of the ascorbic acid [0.89±0.07 mM Fe (II)/L] (Table 2). The FRAP values of all the solvent fractions of *A. cepa* at all concentrations were significantly ($p<0.01$) lower than that of the ascorbic acid except for ethyl acetate fraction [0.72±0.26 mM Fe(II)/L] at 1mg/ml and N-hexane fraction [0.75±0.10mM Fe(II)/L] at 0.25 mg/ml which were not significantly ($p<0.05$) different from that of the ascorbic acid at the same concentration (Table 2).

TABLE II: Ferric reducing antioxidant power (FRAP) activity of crude methanol extract and four solvent fractions of *Allium cepa*

Concentration	Crude Methanol *A. cepa* extract	N-Hexane fraction	Ethyl acetate fraction	Chloroform fraction	Methanol fraction	Ascorbic acid
			(mM Fe(II)/L)			
1 mg/ml	0.62±0.12	0.24±0.12*	0.72±0.26	0.13±0.09*	0.20±0.04*	0.89±0.07
0.5 mg/ml	0.95±0.13	0.25±0.03*	0.20±0.09*	0.59±0.21*	0.23±0.11*	1.03±0.14
0.25 mg/ml	0.53±0.06*	0.75±0.10	0.12±0.09*	0.32±0.16*	0.41±0.08*	0.91±0.30

*Indicates significant ($p<0.05$) difference compared to the ascorbic acid values at the same concentration.

The DPPH radical scavenging activity

The extract-induced percentage inhibition of DPPH was significantly ($p<0.01$) lower than that of the ascorbic acid at all the applied concentrations. However, there was no significant ($p>0.05$) difference between the percentage inhibition of DPPH scavenging activity of crude methanol extract of red cultivar *A. cepa* (84.93 ± 1.73%) and that of ascorbic acid (85.96 ± 0.62%) at 10 mg/ml. The IC_{50} of crude methanol extract of red cultivar *A. cepa* (3.37 mg/ml) was also significantly ($p<0.01$) higher than ascorbic acid (0.78 mg/ml) (Table 3). At concentration of 0.01 – 10 mg/ml, the percentage DPPH scavenging activities of all the solvent fractions of *A. cepa* were significantly ($p<0.01$) lower than that of the ascorbic acid. At 10mg/ml concentration, the percentage DPPH scavenging activities of N-hexane (54.72±4.71%) and chloroform (61.96±1.33) fractions were significantly ($p<0.05$) lower than that of ascorbic acid while that of ethyl acetate (86.12±3.39%) and methanol fraction (87.19±0.19%) were insignificantly ($p>0.05$) higher than that of ascorbic acid (85.96±0.62%). The IC_{50} of all the fractions of *A. cepa* were extremely significantly ($p<0.001$) higher than that of the ascorbic acid (0.78 mg/ml) [Table 3].

TABLE III: Percentage DPPH scavenging activity of crude methanol extract and four solvent fractions of *A. cepa* extracts compared to ascorbic acid

Concentration	Crude Methanol A. cepa extract	N-Hexane fraction	Ethyl acetate fraction	Chloroform fraction	Methanol fraction	Ascorbic acid
Percentage inhibition (%)						
10 mg/ml	84.93±1.73	54.72±4.71*	86.12±3.39	61.96±1.33*	87.19±0.19	85.96±0.62
1 mg/ml	49.51±5.51*	26.61±1.25*	23.52±3.44*	34.50±10.32*	45.31±1.98*	81.11±0.75
0.1 mg/ml	45.82±3.13*	23.61±4.15*	21.83±2.47*	18.46±2.47*	37.76±1.75*	87.21±1.21
0.01 mg/ml	38.81±6.68*	29.65±6.05*	21.54±2.24*	18.71±1.84*	33.63±2.92*	85.13±0.43
0.001 mg/ml	46.63±2.67*	22.73±0.97*	22.77±0.68*	17.99±1.35*	40.12±3.53*	56.79±2.65
0.0001 mg/ml	39.60±7.37	22.40±4.08*	15.94±0.59*	17.13±2.06*	43.88±4.81	41.82±1.08
IC_{50} (mg/ml)	*3.37 mg/ml	*7.69 mg/ml	*4.58 mg/ml	*6.30 mg/ml	*3.80 g/ml	0.78 mg/ml

*Indicates significant ($p<0.05$) difference compared to the Ascorbic acid values at the same concentration.

DISCUSSION

Oxygen is essential for metabolic processes in biological systems as well as production of free radicals that are essential for the body's signaling mechanisms (Meo and Venditti, 2020). However excessive production of free radicals can lead to imbalance between oxidants and the innate antioxidants resulting into oxidative stress (Mattson, 2004; Pisoschi and Pop, 2015; Rani *et al.,* 2016). A lot of disease conditions such as cancer, diabetes mellitus, Alzheimer's disease, and cardiac and liver diseases have been associated with long-term oxidative stress that results in damages to body's cells, nucleic acids, proteins, and lipids, (Rao *et al,* 2010; Arka *et al.,* 2022). The *in-vitro* antioxidant capacity of crude extract of red cultivar *A. cepa* bulbs and its four solvents fractions were studied and compared using Nitric oxide radical inhibitory assay (NORIA), FRAP and DPPH antioxidant models. The results of the nitric oxide-scavenging activity test in this study revealed that the crude methanolic extract of red cultivar *A. cepa* demonstrated a moderate antioxidant effect in nitric oxide scavenging activity. Though, not as effective as ascorbic acid (reference drug) but, its effect was higher than those of other solvent fractions of the extracts. This nitric oxide scavenging action observed with the crude extract indicates its antioxidant capability and this might be due to synergistic effect of one or two secondary metabolites in the crude extract that may have been separated during solvent fractionation. Nitric oxide (NO) is produced from amino acid L-arginine by vascular endothelial cells, phagocytes, and certain cells

of the brain. Nitric oxide is considered to be a free radical because of its unpaired electron. It also exhibits important reactivity with certain types of proteins and other free radicals. The toxic effects of NO become deleterious when it reacts with superoxide radical, forming a highly reactive peroxy-nitrite anion ($ONOO^-$) [Nagmoti *et al.*, 2011]. Chronic exposure to NO radical is connected with series of diseases such as carcinomas, juvenile diabetes, multiple sclerosis and inflammatory conditions such as arthritis, and ulcerative colitis (Huie and Padmaja, 1993). Therefore, the need for removal of these free radicals is very essential for the prevention of disease occurrence and for amelioration of the effects of presence of disease conditions. The results of the FRAP test in this study showed that the crude methanol extract, the n-hexane and ethyl acetate fractions of red cultivar *A. cepa* were moderately effective as an antioxidant as observed in their ferric reducing activities. The crude extract had the highest ferric reducing capacity compared with the solvent fractions indicating that it has the strongest antioxidant activities. As observed with the nitric oxide scavenging activity test, the antioxidant activity of the crude extract may be due to the synergistic interaction of one or two secondary metabolites in it. The DPPH is a stable free radical and it has been widely employed for the estimation of free radical scavenging capabilities of extracts from medicinal plants (Murugan and Parimelazhagan, 2013). The results of the DPPH antioxidant test in this study revealed that the crude methanol extract, the ethyl acetate and methanol fractions exhibited very high scavenging activities for DPPH radical compounds; which indicate that they have very high hydrogen atom donor capacity compared to the other solvent fraction extracts (Contreras-Guzman and Srong, 1982; Kedare and Singh, 2011). The DPPH scavenging capacity of the crude methanol extract and that of the ethyl acetate and methanol fractions showed that they have very high antioxidant activities as observed with the standard antioxidant (ascorbic acid) used in this study. The results of the DPPH in this study showed that only the highest concentration (10 mg/ml) exhibited potent antioxidant activities at par with ascorbic acid. While other lower concentrations of ascorbic acid also showed high antioxidant activities, none of the lower concentration of any of the extracts showed up to 50% of the antioxidant activities observed with the standard antioxidant. This indicates that the antioxidant actions of the crude extract and the solvent fractions of red cultivar *A. cepa* was concentration/dose dependent. This suggests that if the concentration of the extracts were to be raised beyond 10 mg/ml, other solvent fraction extracts of red cultivar *A. cepa* would have exhibited significant level of antioxidant potential. The results of the three *in-vitro* antioxidant tests used in this study revealed that red cultivar *A. cepa* and its solvent fractions have strong antioxidant activities but they manifested higher antioxidant activities with DPPH than the two other antioxidant tests employed in this study. This may imply that *A. cepa* bulbs exhibits its antioxidant activities by hydrogen (H^+) donation to neutralize free radicals. This report is in agreement with the report of Lisanti *et al.* (2016) who reported high antioxidant activities of three *A. cepa* cultivars of Italian origin. All the cultivars exhibited high antioxidant activities with the aid of DPPH antioxidant assay with *Rossa di Toscana* (which is a red onion) presenting the highest antioxidant activities. The results of our study also agree with the report of Lee *et al.* (2014) who compared the antioxidant activities of orange-

coloured onion peels extracts obtained with water, ethanol and subcritical water separately. Their report revealed that all the extracts of the South Korean onion peel exhibited their highest antioxidant activity with DPPH antioxidant test. *Allium cepa* is known to possess organosulfur compounds, polyphenols and flavonoids (Oyewusi *et al.,* 2015); all of which are known to be associated to antioxidant activities of medicinal plants (Xu *et al*., 2017; Chinaka and Edwin, 2019; Cherubim *et al.,* 2020; González-de-Peredo *et al.*, 2021). Soto *et al.* (2016) attributed the phenolic content of three Spanish onion cultivar and one from USA to their antioxidant activities. Research results of Prakash *et al.* (2007) revealed that an Indian red onion had the highest phenolic content when compared with three other cultivars and it is highly associated with its antioxidant activities. This suggests that the antioxidant activities of red cultivar *A.cepa* bulbs in this study may be due to any of its flavonoid or phenolic contents. The results of the antioxidant evaluation of the red cultivar *A. cepa* in this study corroborate the reports of previous research reports that *A. cepa* possess strong antioxidant activities. However, within the available literature, this is the first antioxidant evaluation of Nigerian red cultivar *A. cepa* bulbs that compares the crude methanolic extract with four other solvent fractions of the same. The results of the three *in-vitro* antioxidant tests showed that the crude methanol extract exhibited the best antioxidant activities in all the three evaluation methods. However, with the aid of DPPH, the crude methanol extract, ethyl acetate and methanol fractions exhibited excellent antioxidant activities in comparison with ascorbic acid.

CONCLUSION

These results indicate that serial extraction of the extracts did not make any of the fractionated extracts better than the crude extract of the red cultivar *A. cepa* bulbs. These results also showed that red cultivar *A. cepa* bulbs is a natural source of antioxidants and could serve as therapeutic agent in the prevention or the slowing down of oxidative stress.

REFERENCES

LISANTI, A., FORMICA, V., IANNI, F., ALBERTINI, B., MARINOZZI, M., SARDELLA, R., and NATALINI, B. (2015). Antioxidant activity of phenolic extracts from different cultivars of Italian onion (*Allium cepa*) and relative human immune cell proliferative induction. *Pharmaceutical Biology*, 54(5): 799–806. doi:10.3109/13880209.2015.1080733.

ALPSOY, S., CEVAT, A., RAMAZAN, U., BIROL, T., MEHMET K., MUSTAFA, E., OSMAN, K.. and ASUMAN, G. (2013). Antioxidant and anti-apoptotic effects of *A. cepa* (*Allium cepa*) extract on doxorubicin- induced cardiotoxicity in rats. *Journal of Applied Toxicology*, 2013(33): 202–208.

ARKA, J.C., TANVIR, M.U., BM- REDWAN, M. Z., SAIKAT, M., RAJIB, D.A,S., FIRZAN, N., KULDEEP, D., ARPITA, R., MD-JAMAL, H., AMEER, K. and TALHA, B.E. (2022). Allium cepa: A Treasure of Bioactive Phytochemicals with Prospective Health Benefits.

Evidence-Based Complementary and Alternative Medicine, 2022., Article ID 4586318, 27 pages. https://doi.org/10.1155/2022/4586318.

ASHWINI, M., JANAKIRAMAN, B., SRINIVASAN, B., SHANMUGARAJ, B.M. and RAMALINGAM, S. (2013). Antioxidant Activity in *in Vivo* and *in Vitro* Cultures of *A. cepa* Varieties (Bellary and CO_3). *Food and Nutrition Sciences*. 2013(4): 918-923.

Benkeblia, N. (2005). Free-Radical Scavenging Capacity and Antioxidant Properties of Some Selected *A. cepa* (*Allium cepa* L.) and Garlic (*Allium sativum* L.) Extracts. *Brazilian Archives of Biology and Technology*. 48 (5): 753-759.

CHERUBIM, D.J., MARTINS, C.V., FARIÑA, L. and LUCCA, R. A. (2020). Polyphenols as natural antioxidants in cosmetics applications. *Journal of Cosmetic Dermatology*. 19(1): 33-37. doi:10.1111/jocd.13093

XU, D.-P., LI, Y., MENG, X., ZHOU, T., ZHOU, Y., ZHENG, J., Zhang, J.-J. and LI, H.-B. (2017). Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *International Journal of Molecular Sciences*, 18(1): 96. doi:10.3390/ijms18010096

PRAKASH, D., SINGH, B.N. and UPADHYAY, G. (2007). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chemistry*, 102(4): 1389–1393.

OLA-MUDATHIR, F.K., ABDUL-WAHAB, A., MOSHOOD, A.I., and OBUOTOR, E.M. (2018). Comparative evaluation of antioxidant properties of methanol extracts of *Allium cepa* bulb, *Allium cepa* bulb peels and *Allium fistulosum*. *Kragujevac Journal of Science*, 40 (2018): 131-141.

GARRATT, D.C. (1964). The Quantitative Analysis of Drugs. In: The Quantitative Analysis of Drugs. 3rd Edition, Springer, Boston, MA. Chapman and Hall Ltd., London, 456-458. https://doi.org/10.1007/978-1-4613-3380-7_1.

GONZÁLEZ-DE-PEREDO, A.V., VÁZQUEZ-ESPINOSA, M., ESPADA-BELLIDO, E., CARRERA, C., FERREIRO-GONZÁLEZ, M., BARBERO, G.F., and PALMA, M. (2021). Flavanol Composition and Antioxidant Activity of Onions (*Allium cepa* L.) Based on the Development of New Analytical Ultrasound-Assisted Extraction Methods. *Antioxidants*, 10(2): 273. https://doi.org/10.3390/antiox 10020273

GONZÁLEZ-SARRÍAS, A., NÚÑEZ-SÁNCHEZ, M., TOMÁS-BARBERÁN, F. and ESPÍN, J. (2017): Neuroprotective effects of bioavailable polyphenol-derived metabolites against oxidative stress-induced cytotoxicity in human neuroblastoma SH-SY5Y cells. *Journal of Agricultural and Food Chemistry*, 65 (4): 752–758. doi: 10.1021/acs.jafc.6b04538

KEDARE, S.B. and SINGH, R.P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4): 412–422. doi:10.1007/s13197-011-0251-1

LEE, K.A., KIM, K.-T., KIM, H.J., CHUNG, M.-S., CHANG, P.-S., PARK, H., and PAI, H.-D. (2014). Antioxidant activities of onion (*Allium cepa* L.) peel extracts produced by ethanol, hot water, and subcritical water extraction. *Food Science and Biotechnology*, 23(2): 615–621. doi:10.1007/s10068-014-0084-6

MARCOCCI, L., MAGUIRE, J.J., DROY-LEFAIX M.T. and PACKER, L. (1994). The nitric oxide scavenging properties of *Ginkgo biloba* extract EGb761. *Biochemical and Biophysical Research Communication*, 201(2): 748-55. doi: 10.1006/bbrc.1994.1764.

MATTSON, M.P. (2004). Pathways towards and away from Alzheimer's disease. *Nature*, 430,631–639. https://doi.org/10.1038/nature02621

MILEA, S,.A, APRODU, I., ENACHI, E., BARBU, V., RÂPEANU, G., BAHRIM, G.E., STĂNCIUC, N. (2021). Whey Protein Isolate-Xylose Maillard-Based Conjugates with Tailored Microencapsulation Capacity of Flavonoids from Yellow Onions Skins. *Antioxidants*, 10(11): 1708.

BENITO-ROMÁN, Ó., BLANCO, B., SANZ, M.T. and BELTRÁN, S. (2020). Subcritical Water Extraction of Phenolic Compounds from Onion Skin Wastes (*Allium cepa* cv. Horcal): Effect of Temperature and Solvent Properties. *Antioxidants*, 9(12): 1233. doi: 10.3390/antiox9121233.

BISEN, P.S. and EMERALD, M. (2016). Nutritional and therapeutic potential of garlic and onion (*Allium sp*). *CNF*. 12(3): 190–199.

NASRI, S., ANOUSH, M. and KHATAMI, N. (2012). Evaluation of analgesic and anti-inflammatory effects of fresh onion juice in experimental animals. *African Journal of Traditional and Complement Alternative Medicine*, 6: 1679–1684.

NAGMOTI, D.M., D.K. KHATRI., P.R. JUVEKAR., and A.R. JUVEKAR. (2011). "Antioxidant activity and free radical-scavenging potential of *Pithecellobium dulce* Benth seed extracts," *Free Radical and Antioxidants*, 2(2): 37–43.

OBIOHA, E.U., STEPHEN, M.S., KIKELOMO F.O. and TOYIN Y.F. (2009). Hepatoprotective Potentials of *A. cepa* and Garlic Extracts on Cadmium-Induced Oxidative Damage in Rats. *Biological Trace Element Research*. 129(1-3) :143.

ODUKOYA, A.O., ILORI, O.O., SOFIDIYA, M.O., ANIUNOH, O.A., LAWAL, B.M. and TADE, I.O. (2005). Antioxidant activity of Nigerian dietary spices. *Electronic Journal of Environmental, Agriculture and Food Chemistry*, 4(6): 1086-1093.

OGUNMODEDE, S.O., SAALU L.C.,

OGUNLADE, B., AKUNNA, G.G and OYEWOPO, A.O. (2012). An Evaluation of the Hypoglycemic, Antioxidant and Hepatoprotective Potentials of *A. cepa* (*Allium cepa* L.) on Alloxan-induced Diabetic Rabbits. *International Journal of Pharmacology*, 8 (1): 21-29.

PISOSCHI A. M. and POP A. (2015). The role of antioxidants in the chemistry of oxidative stress: a review. *European Journal of Medicinal Chemistry,* 97:55–74. DOI: 10.1016/j.ejmech.2015.04.040

RAO, A.S.V., REDDY, S.G., BABU, P.P, and REDDY, A.R. (2010). The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. *BMC* Complementary and Alternative Medicine, 10(1): 1-9. doi:10.1186/1472-6882-10-4

ROLDAN-MARIN, E., BRITTA, N.K., MORTEN, P., MONA-LISE, B., TOM H.N., MAX, H., THAER, B., SOREN, L., PILAR, C.M., CONCEPCION S. and LARS O. D. (2009). Effects of an *A. cepa* by-product on bioactivity and safety markers in healthy rats. *British Journal of Nutrition,* 102(11): 1574–1582. DOI: https://doi.org/10.1017/S0007114509990870

DI-MEO, S. and VENDITTI, P. (2020). Evolution of the Knowledge of Free Radicals and Other Oxidants. *Oxidative Medicine and Cellular Longevity,* 2020(3): 1–32. doi:10.1155/2020/9829176

ŠKERGET, M., MAJHENIÈ, L., BEZJAK, M. and KNEZ, Z. (2009). Antioxidant, Radical Scavenging and Antimicrobial Activities of Red *A. cepa* (*Allium cepa* L) Skin and Edible Part Extracts. *Chemical and Biochemical Engineering*, 23 (4): 435–444.

SURU, S.M. (2008). *A. cepa* and garlic extracts lessen cadmium-induced nephrotoxicity in rats. *BioMetals*. 21(6): 623–633.

RANI, V., DEEP, G., SINGH, R.K., PALLE, K., and YADAV, U. C. S. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sciences,* 148, 183–193. doi:10.1016/j.lfs.2016.02.002