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ESSENTIAL OIL FROM THE FRESH GREEN STALK OF *ALLIUM CEPA L.* AND THEIR DPPH ASSAY

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ABSTRACT

Fresh green stalk of the *allium cepa* were collected from the local field area near Lahore district. These bulbs were cut into small pieces and dried under shade. Essential oil extraction was carried out through hydro-distillation using Linkersson apparatus, followed by extraction with petroleum ether and drying with sodium sulfate anhydrous. GC-MS studies were performed to determine the components of the essential oil. Eleven components were identified and the major components were 1-allyl-3-methyl trisulfide (17.69%), 1-methyl-2-(prop-1-enyl) disulfide (15.90%), 1-methyl-3-(prop-1-enyl) trisulfide (15.64%) and 1-methyl-3-propyl trisulfide (12.24%). 2,2-Diphenyl picrylhydrazyl radicalwas (DPPH) was used for the determination of antioxidant activity of the essential oil from the stalk of *allium cepa*. Maximum antioxidant activity (36.55%) was observed at concentration 100 mg/ml while at the same concentration ascorbic acid gave 99.67%. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Sulfur containing compounds are responsible for its antioxidant activity.

KEYWORDS: Antioxidant, Fresh stalk, *Alium Cepa L*, Hydro-distilllation, Essential oil.

INTRODUCTION

Allium cepa is one of the edible species of a large genus (Allium), family *amaryllidaceae* consisting of more than 700 species.[1] Among the edible *allium*, the onion (*Allium cepa L.*)

stands in the first rank[2] and it is cultivated in all parts of the world.[3] Total volume of crop produced is 86.34 million tons from the 1.64 million hectares. The leading world producers of dry onions based on production figures are China 22,600,000 tonnes, India 16,308,990 tonnes, the United states 3,277,460 tonnes, Iran 2,260,000 tonnes, Russian federation 2,090,814 tonnes, Egypt 2,024,881 tonnes, Turkey 1,819,000 tonnes, Pakistan 1,692,300 tonnes, Nigeria 1,350,000 tonnes, Bangladesh 1,159.259 tonnes, Brazil 1,519,022 tonnes, Netherlands 1,353,000 tonnes, Mexico 1,238,602 tonnes, Myanmar 1,140,000 tonnes, Republic of Korea 1,195,737, Spain 1,187,1.[4]

From inception *Allium cepa* has been used as a food as well as medicine. Chemical compounds derived from the *allium cepa* have exerted anti-inflammatory,[5] antihistamine,[6] antibacterial, antiparasitic, and antifungal and antiparasitic activities.[7] Onion past was studied against the gram positive and gram negative bacteria by Kirilov et al, in 2014 and found that white variety gave highest inhibition.[8] it provides vitamins such as vitamin A and C, and a good amount of mineral elements to the human body.[9] In addition, onion is among the food plants to which moderate level of anticancer activities is associated with.[10]

Lachrymatory effect of allium cepa is well known from ancient times. Enzymatic reaction produces (Z)-propanethial-S-oxide which causes tear.[11] Aroma of the *allium cepa* is due to alkyl thiosulphonates which are released on freshly cut bulb, while propyl and propenyl sulfides are responsible for cooked onion. Dimethylthiophenes is found in fried *allium cepa* bulb. Precursor on the *allium cepa* are S-methyl and S-propyl-L-cysteine sulfoxide which are biosynthesized from valine and cysteine.[10] More than ninety percent soluble organic-bound sulfur in the *allium cepa* is found in γ-glutamilcysteine peptide not hydrolyzed by alliinase. γ-Glutamilcysteine peptide serve as storage reserve and are important for seed germination. Sulfur rich *allium cepa* compounds metabolized in the human liver microsomes are being substrates for FMO and CYPs.[12-15] Recent studies revealed that diallyl disulfide and dipropyl disulfide lowering the blood glucose and lipid levels in human and in animals.[16-19] The stalks are most often discarded or used only when the bulbs are not available. Nutrients of the stalk of allium cepa has been studied by Yahaya et al in 2010.[20] besides other nutrients vitamin A and C were also found in it.

Free radicals reactive oxygen species and reactive nitrogen species generated in our bodies can create oxidative stress. A balance between free radicals and antioxidants is necessary for proper physiological function. Free radicals thus adversely alter lipids, proteins, and DNA

and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping this oxidative stress. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be dangerous for human health.[21] Bulb and seeds of allium cepa have been studied and from the best of our knowledge this is the first report to study the essential oil of stalk and its antioxidant activity. Thus, the current search was designed to investigate the stalk of allium cepa for its chemical constituents and antioxidant activity.

MATERIALS AND METHODS

Fresh green stalk of the *allium cepa* were collected from the local area nearby Lahore and essential oil was extracted through hydro-distillation by using Linkensson apparatus. Extracted oil was dried with sodium sulfate anhydrous and stored in the refrigerator at 4^0C and GC-MS analysis was performed by Agilent 5973-6890 gas chromatograph–mass spectrometry system, operating in EI mode at 70 ev equipped with a split-splitless injector. Radical scavenging activity of the essential oil against 2,2-diphenyl-1-picrylhydrazyle (DPPH) wad measured by Perkin Elmer Lambda 35, UV-Vis spectrophotometer. Petroleum ether was used for the separation of oil from water and methanol was used for the determination of antioxidant activity.

Extraction of essential oil

Fresh green stalk of *allium cepa* 5 Kg were collected from the local market and after that chopped into small pieces dried under the shade for ten days. Then it was further cut into small pieces and (2 Kg) subjected to hydro-distillation by using Linkersson apparatus for 10 hrs.[22] The steam distillate was extracted twice with petroleum ether (2×150 ml). The organic layer was dried over anhydrous sodium sulfate, which on removal of solvent afforded pale colored oil. Dried oil was stored in an air tight amber colored bottle ate 4^0C in refrigerator for further studies.

GC-MS studies of the essential oil of the mature bulb of *allium cepa*

Fresh green stalk of *allium cepa* were chopped, dried under shade and essential oil was extracted through hydro-distillation by using linkersson apparatus. Their chemical constituents were analyzed by GC-MS. Agilent 5973-6890 gas chromatograph–mass spectrometry system, operating in EI mode at 70 eV equipped with a split-splitless injector was used. Helium was used as a carrier gas at the flow rate of 1 ml/min, while HP-5MS (30 m, 0.25 mm, 0.25 um) capillary column was used. The initial temperature was programmed

at 50-100°C at the rate of 5°C/min and then 100-250°C at the rate of 3°C/min followed by a constant temperature at 260°C for a period of 20 minutes. Sample (2 µl) was injected to the column programmed at 200°C and resolution of components was attained. The identification of possible components was performed by matching their retention indices and mass spectra with those obtained the library of National Institute of Standard and Technology (NIST).

Antioxidant activity of essential oil of *allium cepa* mature bulb

Antioxidant activity of the essential oil of the fresh green stalk of *allium cepa* was evaluated by using 2,2-diphenyl-1-picrylhydrazyle (DPPH) radical. The DPPH assay was performed by following the method of Epsin et al., 2000.[23] Briefly, the samples (100 ul, each) of different concentration of 25 ul, 50 ul, 75 ul and 100 ul were mixed with 3 ml of methanol of DPPH solution. The absorbance of the resulting solution and the blank (with only DPPH) were recorded at λ 517 nm by UV-Vis spectrophotometer, after an incubation time of 30 minutes at ambient temperature against ascorbic acid as a positive control. For each samples three replicates were recorded. The percentage of radical scavenging activity was calculated using the following equation.

DPPH scavenging effect (%) = $(A_0-A_1)/A_0 \times 100$

Where A_0 is the absorption of the control at 30 minutes and A_1 is the absorbance of the sample at 30 minutes.

RESULTS AND DISCUSSION

The essential oil extracted from the green fresh stalk of *allium cepa* was pale in color and 0.001% yield was obtained. This yield of essential oil of fresh bulb was low as compared to fresh bulb and amature bulb.[24] It can be argued that the deposition of essential oil in the stalk is low and it may be due to immature collection of stalk[25,26] Eleven constituents were identified in the essential oil of *allium cepa* as shown in the table 1.

Table 1: Volatile Constituents of essential oil of mature bulb of *allium cepa*.

Peak #	Compound	Retention time (Min.)	Relative abundance (%age)
1	1-Methyl 3-propyle trisulfide	9.854	12.24
2	1-Allyl-3-methyl trisulfide	10.798	17.69
3	1,3 Dimethyl trisulfide	11.873	5.21
4	1-Methyl-2-(prop-1-enyl) disulfide	12.446	15.90

5	1,3-Dipropyle trisulfide	12.629	4.18
6	1-Methyl-3-(prop-1-enyl)trisulfide	13.12	15.64
7	1-Methyl-3-propyl trisulfide	14.322	7.44
8	3,4-Dimethyl-2-(methyl disulfury)thiophene	15.038	6.44
9	2-Methyl-5-(methyl thio)furan	17.647	4.17
10	1-(prop-1-enyl)-2-propyl disulfide	19.009	2.67
11	Methyl-5-methylfuryl sulfide	19.959	8.38

1-allyl-3-methyl trisulfide (17.69%), 1-methyl-2-(prop-1-enyl) disulfide (15.90%), 1-methyl-3-(prop-1-enyl) trisulfide (15.64%) and 1-methyl-3-propyl trisulfide (12.24%) were found major constituents in the essential oil of *allium cepa*. These results are similar to the findings of Corzmartine et al, 2007[27] and Dima et al, 2014.[28] Fresh green stalk of allium cepa has less oil than mature bulb, which may be due to extraction of oil at premature growing stage.

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free radical-scavenging activity of antioxidant. In the DPPH test, the antioxidants were able to reduce the stable DPPH radical to the yellow-coloured diphenylpricryhydrazine. The effect of antioxidant on DPPH radical scavenging was conceived to their hydrogen-donating ability.

Table 2: Antioxidant activity of essential oil of mature dry bulb of *allium cepa.*

Serial no.	Concentration (mg/ml)	Absorbance (nm)	% Inhibition
1	25	0.830	4.70
2	50	0.737	17.37
4	75	0.650	27.13
5	100	0.566	36.55

Antioxidant activity of the essential oil was measured by using DPPH; at 100 mg/ml was 36.55% as compared to the ascorbic acid (Table 2) as standard which gave 99.67% inhibition. It is well known that flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donor and show radical scavenging activity.[29] Phenolic contents are good indicators of antioxidant activity, their high redox potential enable them to act as hydrogen donors or radical scavengers.[27] In our study, the main constituents are based on sulfur and less or negligible contents of phenolics.[30] So the efficiency is low, this antioxidant activity is partially due to sulfur compounds. A similar study has also been reported by Amagase et al, 2001.[31] They have reported that dially polysulfides contribute to

the antioxidant activity. Results indicate that essential oil was able to reduce stable radical DPPH to yellow colored DPPH-H independent of any enzymatic activity. Our results suggest that the essential oil of fresh green bulb of allium cepa have moderate antioxidant activity and it. These findings are consistent with previous reports.[32]

CONCLUSION

The essential oil from the fresh green stalk of *allium cepa* was extracted through hydro-distillation and its chemical constituents were determined by GC-MS while its antioxidant study was carried out with DPPH. Eleven components were identified and major components were dipropyl trisulfide (13.28%), methyl-5-methylfuryl sulfide (10.64%), 2-methyl-5-methylfuryl sulfide (10.64%) and diallyl sulfide (10.16%). Antioxidant activity of the essential oil was determined by using DPPH and ascorbic acid as standard. Its activity at 100 mg/ml concentration was 36.55%. Moderate antioxidant activity was observed in the essential oil. Sulfur containing compounds are responsible for its antioxidant activity. The essential oils from various sources have attained appreciable research interest in the scientific community.

REFERENCES

1. Burnie G, Forrester S, Greig D, Guest S, Harmony M, Hobley S, ckson G, Lavarack P,Melanie L, Donald RM, Macoboy S, Molynenx B, Moodie D, Moore J, North T, Newan D,80Pienaar K, Purdy G, Silk J, Ryan S and Schien G. Botanica: The illustrated A-Zof over 10,000 garden plants, (3rdEd.), RandomHouse Australia Pty Ltd, New South Wales, 1999; 74
2. Fritsch, K.M. and Friesen N. Evolution, domestication and Taxonomy. In: Allium Crop Science, Recent Advances (H.D. Rabinowitch and L. Currah. ed.). CAB1 Publishing, Oxon., 2002; 5-30.
3. Gautam SR, Neupane G,Baral BH, Rood PG and Pun L (1997)Prospects of onion cultivation in the warm-temperate hills of easternNepal and its research and development strategies for commercial production.ISHS Acta Horticultura., 1997; e433: 83-94.
4. FAO (2013) FAOSTAT. Food and Agricultural Organization of United Nations: Economic and Social Department; the Statistical Division. http://faostat.fao. org/site/567/DesktopDefault.aspx?PageID=567#ancor
5. Griffiths GL, Trueman, T, Crowther B, Thomas BS. Onions -A global benefit to health. Phytotherapy Res, 2002; 16: 603-615.

6. Rose PM, Whiteman PK, Moore and Zhu YZ. Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus *allium*: the chemistry of potential therapeutic agents. Natural Product Rep, 2005; 22: 351-368
7. Wani AH, Nisa TU.. Management of black mold rot of onion. Mycopath., 2011; 9: 43-49.
8. Kirilov A, Doycheva A and Satchanska G. Antibacterial activity of mature and green allium cepa. Ecol Engin Envi Prot, 2014; 12-17.
9. Paul, S. Mineral and Trace Elements. Human Nutrition. Eleventh Edition, Elsevier Churchill Livingstone. Netherlands., 2006; 231-249.
10. Michael, H. G. Food and Nutrient Structure. Human Nutrition. Eleventh Edition, Elsevier Churchill Livingstone. Netherlands., 2006; 44.
11. Lanzotti V, The analysis of onion and garlic. J Chromatogr A, 2006; 1112: 3-22.
12. Elnima EI, Ahmed SA, Mekkawi AG, and Mossa JS. The antimicrobial activity of garlic and onion extracts. Pharmazie, 1983; 38: 747-748.
13. Zohri AN, Gwad KA and Saber S. Antibacterial, antidermatophytic and antioxigenic activities of onion (*Allium cepa*) oil. Microbiol Res, 1995; 150: 167-172.
14. Bakhat J, Shehla K, Shafi M. Antimicrobial potential of fresh *allium cepa* agains gram positive and gram negative bacteria and fungi. Pak J Bot., 2013; 45(S1): 1-6.
15. Belitz HD, Grosch W, Schieberle P. Food Chemistry, 4th Edition, Springer-Verlag Berlin-Heidelberg, 2009; 790-791.
16. Testa B, Kraemer S. The biochemistry of drug metabolism: principles, redox reactions, hydrolyses. Willey-VCH. Verlage Helvetica Chimica Acta, Zurich, Switzerland, 2008; 124.
17. Yamaguchi M. Allium: Onion, garlic and others. World Veg, 1983; 184-206.
18. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidant and functional foods: impact on human health. Pharmacognosy Rev., 2010; 4(8): 118-126.
19. Ramy M, Sayed R, Mahmoud G. Chemical composition , antiviral and antioxidant activities of seven essential oils. J Appl Sci Res, 2010; 6(1): 50-62.
20. Y.Yahaya1, U. A. Birnin Yauri and B. U. Bagudo. Study of Nutrient Content Variation in Bulb And Stalk of Onions (Allium Sepa) Cultivated in Aliero, Aliero, Kebbi State, Nigeria. Nigerian Journal of Basic and Applied Science., 2010; 18(1): 83-89.
21. Rao AL, Bharani M, Pallavi V. Role of antioxidants and free radicals in health and disease. Adv Pharmacol Toxicol, 2006; 7: 29–38.

22. Shahzad K, Shaista N, Ahmad R, Zafar I, Khalid S. Evaluation of antioxidant and antimicrobial activity of essential oil of tangerine fruit peel. Pak J Biochem Mol Biol, 2009; 42(1): 4-7.
23. Epsin JC, Soler-Rivas C, Wichers HJ. Charactrization of the total free radical scavenging capacity of vegetable oil and oil fractions using 2,2diphenyl -1-picrylhydrazyl radical. J. Agric. Food Chem, 2000; 48: 648-56.
24. Chun LY, Dai DH, Wei LH. Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa L*.) Food Control, 2013; 30: 48-53
25. Emad A, Shalaby N, Nasr F and Samia ME. In vitro study of the antimicrobial and antioxidant efficacy of some nature essential oils. J Med Plants Res, 2011; 5(6): 922-931.
26. Foe FMC, Tatiana FKT, Ascencion MN, Jean A, Abel JGY, Alembert TT, Jean LOE, François XE. Chemical composition, in vitro antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon. Compl Alterna Med, 2016; 16(117): 2-12.
27. Corozmatine N, Villamiel M. Biological properties of onion and garlic. Trends Food Sci Tech, 2007; 18: 609-625.
28. Dima M, Annes F, Emmanuel P, Tayysir H, Nancy N, Christine F, Xavier F, Farid C. Chemical composition, antimicrobial and antioxidant activities of six essential oil from the *alliaceae* family. Molecule, 2014; 19: 20034-20053.
29. Cosentino S, Tuberoso CI, Pisano, B.; Satta, M.; Mascia, V.; Arzedi, E.; Palmas, F. In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Lett. Appl. Microbiol., 1999; 29: 130–135
30. Eman AEW, Mortada MES, Ezzat ESA. GC-MS Investigation of essential oil and antioxidant activity of Egyptian white onion (*allim cepa*). Int. J Pharma Sci Res, 2015; 6(3): 537-543.
31. Amagase, H, Petesch B, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive compounds. J Nutr, 2001; 131: 955S–962S.
32. Takahashi M, Shibamoto T. Chemical composition and antioxidant /anti-inflammatory activities of steam distillate from freeze-dried onion (allium cepa L) spout. J Agri Food Chem, 2008; 56: 10462-10467.