WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 6, 1959-1965. Research Article ISSN 2277– 7105

ANTIOXIDANT ACTIVITY OF ESSNTIAL OIL OF MATURE BULB OF ALLIUM CEPA L. FROM PAKISTAN

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Article Received on
17 April 2016,
Revised on 08 May 2016,
Accepted on 29 May 2016
DOI: 10.20959/wjpr20166-6378

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ABSTRACT

Essential oil of allium cepa mature bulb was extracted through hydro-distillation followed by extraction with n-hexane and drying with sodium sulfate anhydrous. Dried clear oil was stored in a well tight amber color container at 4^0C in the refrigerator. Gas chromatography-mass spectrometry study of the essential oil was performed to determine the chemical constituents. Eleven constituents were identified and major constituents were methyl 5-methylfuryl sulfide (23.93%), dipropyl trisulfide (17.06%), diehyl sulfide (8.81%), allyl(but-3-enyl) sulfide (8.16%), 1-allyl-3-propyl trisulfide (8.07%). The antioxidant activity of the essential oil was measured by using DPPH and ascorbic acid as standard. The maximum antioxidant activity of the essential oil was 51.79% with 100 mg/ml while the antioxidant activity of ascorbic acid with similar concentration was 99.67%. The essential oil has specified strong pungent aroma containing mercaptanes. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Synthetic antioxidants are recently reported to be dangerous to human health. Antioxidants obtained from natural resources are better for human health as they are less harmful.

KEYWORDS: Alium Cepa L, Essential oil, Chemical Constituents, Methyl 5-methyl furyl sulfide, Antioxidant.

INTRODUCTION

Onion (allium cepa L.) belongs to the family amaryllidaceae which is one of the most important mono-cotyledonous crops in the world. The genus allium comprises over 700 species which can be found throughout the tropical, temperate and sub-temperate regions of the world.[1] Onion is the oldest known vegetable, indispensable and important vegetable item which is used throughout the year.[2,3] Onion is one of the most important commercial vegetable crop grown in Pakistan and believed to be native to south west asia and Mediterranean.[4] Total area of world under onion cultivation is 1.64 million hectares while the total production of onion is 86.34 million tones. In Pakistan its area under cultivation is 143.7 thousand hectares with a production of 1892 thousand tons during 2010-11.[5]

Folk healers traditionally used onions to prevent infections is among the oldest cultivated plants used both as a food and for medicinal applications.[6] Compounds derived from onion have exerted anti-inflammatory and antihistamine effects in vitro and in animal models.[2] In vitro studies have shown onion to possess antibacterial (including H. pylori), antiparasitic, and antifungal activity.[7,8] Antiparasitic, antifungal, anti-inflammatory and antihistamine activities of allium cepa have been studied.[9]

Free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensures. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases.[10] 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.[11] Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. Keeping in view the effective role of allium cepa in our daily life, the present study was conducted to investigate the essential oil for its antioxidant activity.

MATERIALS AND METHODS

The red onion (Allium cepa) was collected from the local market in Lahore and essential oil was extracted through hydro-distillation by using Dean Stark apparatus. Extracted oil was dried and GC-MS analysis was performed by Agilant 5973-6890 gas chromatograph–mass

spectrometry system. Antioxidant activity was measured by using DPPH on Perkin Elmer Lamda 35, UV-Vis spectrophotometer.

Extraction of essential oil

Red onion (allium cepa) dry mature bulb 500 g were collected from the local market and after peeling off, bulb were cut into small pieces with the help of mechanical chopper. Then 400 g were subjected to hydro-distillation by using Linkersson apparatus for 16 hrs.[12] The steam distillate was extracted twice with n-hexane (2×150 ml). The organic layer was dried over anhydrous sodium sulfate, which on removal of solvent afforded slightly pale colored oil. Dried oil was stored in well 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

Essential oil extracted from the mature bulb of allium cepa was analyezed for its chemical constituents by GC-MS. Agilant 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. Identification of components was performed by matching their retention indices and mass spectra with those obtained the NIST library.

Antioxidant activity of essential oil of allium cepa mature bulb

Antioxidant activity of the essential oil of the mature bulb of allium cepa was evaluated by the scavenging activity of 2,2-diphenyl-1-picrylhydrazyle (DPPH) radical. The DPPH assay was performed by following the method of Epsin et al., 2000.[13] The samples (100 ul, each) of different concentration of 25 ul, 50 ul, 75 ul and 100 ul were mixed with 3ml methanol of DPPH solution. The absorbance of the resulting solution and the blank (with only DPPH) were recorded 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 disappearance of the DPPH was measured spectro-photometrically at 517 nm. 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 dry mature bulb of allium cepa was pale in color and 0.03% yield was obtained. This yield was low as compared to Emad et al, 2011, they have reported 0.05% essential oil in allium cepa.[14] This yield was greater than foe et al, 2016,[15] they have reported 0.02% essential oil in allium cepa from Cameron. It can be argued that the variability of the yield of entire essential oil can be assigned to many factors including soil, age of the plants, climate and time of harvesting as reported previously,[16] 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	1,3 Divinyl trisulfide	7.38	4.55
2	1,3 Diethyl trisulfide	8.61	2.21
3	1,3 Dipropyl trisulfide	10.96	17.06
4	1-Allyl 3-propyl trisulfide	11.57	8.07
5	Diethyl sulfide	12.32	8.81
6	1-Allyl-3(but-3enyl) trisulfide	13.00	7.95
7	Dipropyl sulfide	13.95	6.44
8	Diallyl sulfide	14.31	7.24
9	Allyl(but-3-enyl)sulfide	15.76	8.16
10	Dipropyl disulfide	16.76	5.53
11	Methyl 5-methylfuryl sulfide	17.33	23.93

Methyl 5-methylfuryl sulfide, 1,3 dipropyl trisulfide were found major constituents in the essential oil of allium cepa. Chun et al, 2013[17] have also found similar results in the essential oil of allium cepa but these results are variable from the major constituents reported by Corzmartine et al, 2007[18] and Dima et al, 2014.[19] This variation may be due to variation in growing environment, seeds quality, mode of cultivation and geo-location.

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	% Inhibition
1	25	0.804	9.86
2	50	0.670	24.88
4	75	0.562	36.99
5	100	0.430	51.79

Antioxidant activity of the essential oil was measured by using DPPH, at 100 mg/ml was 51.79 against the ascorbic acid (Table 2) as standard which gave 99.67% inhibition. The lower value of inhibition of the essential oil of allium cepa is due to lower contents of phenolic compounds. This is clear from the chemical constituents that it contains aliphatic and aromatic molecule having sulfur element in their chemical architecture. The reducing power of the essential oil was dose dependent, and the reducing capacity of the oil was inferior to ascorbic acid, which may be due to the variability of the composition and synergetic effect of minor compounds.

CONCLUSION

The essential oil from the mature bulb of allium cepa was extracted through hydro-distillation and its GC-MS and antioxidant studies were carried out. Eleven components were identified and major components were methyl 5-methylfuryl sulfide (23.93%), dipropyl trisulfide (17.06%), diehyl sulfide (8.81%), allyl (but-3-enyl) sulfide (8.16%), 1-allyl-3-propyl trisulfide (8.07%). Antioxidant activity of the essential oil was determined by using DPPH and ascorbic acid as standard. Its activity at 100 mg/ml concentration was 51.79%. Lower antioxidant may be due to lower contents of phenolic compounds in the essential oil.

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