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Phytochemicals Screening, Antioxidant and Anticancer Activities of Garlic (*Allium sativum*) Extracts

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ABSTRACT

The objective of this study is to determine the phytochemicals of garlic, antioxidants and anticancer activities against cancer cell line (MCF-7) of hot water, cold water and ethanol extracts of garlic (*Allium Sativum*). The results showed that garlic contains more than 80% total carbohydrates and proteins. Also, qualitative phytochemical analysis of garlic extracts indicated the presence of flavonoids, alkaloids, tannins, phenols, saponins, terpenoids, steroids and phytosterols in ethanol and hot water extracts, whereas alkaloids and saponins are absent in cold water extract. Moreover, antioxidant activity of garlic extracts by DPPH method showed a strong effect on the activity scavenging of free radicals. In addition, ethanol extract had higher reducing power, total flavonoids and total phenolics contents in comparison with hot water and cold water extracts. Finally, garlic extracts also showed very high cytotoxic activity against human breast cancer cell line type (MCF-7) especially ethanol extract, which makes us recommend that garlic is useful as a rich antioxidant and has great strength against cancer cells.

Keywords: *Allium Sativum*, phytochemicals, total flavonoids, total phenolics, DPPH, MCF-7

INTRODUCTION

The common herbs such as garlic, thyme, ginger, onion, basil etc., offer large health benefits by means of strong phytochemical and antioxidant characteristic. Even though there is few literature on the health benefits of herbs and extracts of these, the number of studies research the potential health effects of phytochemicals emerging from herbs are great. Most of the products classified as herbal and plant medicines also depend on its richness with antioxidant or phytochemicals (Anjeza and Mandal, 2012). Garlic is one of the primeval plants used in medicine and as a flavoring in food, it ranks the highest of all the herbs remedies consumed for its large health benefits. Garlic belongs to the Alliaceae family and grows as an annual herb in cold climates and dry weather. There are a wide range of treatment effects for garlic as prevention and treatment of diseases such as cold and flu syndrome through immune increases and shows anti-microbial, anti-inflammatory, anti-parasitic, anti-diabetic, antioxidant, anti-cancer effects and immunomodulatory properties (Mnayer *et al*., 2014 and Lorigooinia *et al*., 2015). Besides nutritional and medicinal values, garlic has antioxidant potentials. Antioxidants are molecules that have great attraction for free radicals and are described by their ability to act powerful radical scavenging activity. Free radicals are unsettled and highly reactive molecules that attract electrons from bimolecular, oxidizing them and overlapping with their activity. They are produced as secondary products of natural biological processes such as oxidative phosphorylation and synthesis of prostaglandin (Halliwell, 1989). Also, free radicals are produced by exposure to environmental factors such as radiation, pollution and cigarette smoking (Hamid *et al*, 2010). Therefore, our body is exposed to many free radicals that constantly overlapping with the function of the cells. Garlic is a good source of natural antioxidants that can be used as scavenging of free radicals. It is expansively renowned that many of the present diseases are due to oxidative stress that caused by imbalances between formation and neutralization of prooxidants (Hazra *et al*., 2008). Garlic has phytochemicals, it has been proven to contain tannins, alkaloids, flavonoids and phenolic compounds (Olusanmi and Amadi, 2010). Phytochemicals are bio-active compounds found in plants, it is work with nutrients and dietary fiber to protect human from diseases. They are nonnutritive compounds that used to flavor and color. Phytochemicals have antioxidant activity and reduce the hazard of several diseases (Craig, 1999). Phenolic compounds are great groups of secondary metabolites which have the ability to neutralize the free radicals (Picchi *et al*., 2012). Flavonoids are the large group of polyphenols found in plants that have powerful antioxidant activities due to scavenging of reactive oxygen species (ROS) and inhibition of oxidative stress (Pourcel *et al*., 2006 and Hounsome *et al*., 2009). The reducing power of compound may serve as an indicator of its potential antioxidant activity. The activity of antioxidants and reducing power is believed to be related to high levels of total phenolic (Gavamukulya *et al*., 2014). Breast cancer (MCF-7) is malignant cancer cells that begin in the breast cells and from it to distant areas of the body and occurs almost in women and may occur in men (Abeloff *et al*., 2008). A great relationship between antioxidants efficiency in extracts of different plants and anticancer potency was reported by (Aboul-Enein *et al*., 2012)

Therefore, the aim of this study is to determine the phytochemicals of garlic, antioxidants and anticancer activities against cell line (MCF-7) of garlic hot water, cold water and ethanol extracts.

MATERIALS AND METHODS

Plant materials

Garlic plant (Balady variety) used in this study were collected from Beheira Governorate, Egypt.

Tumor cells

Human breast cancer cell line (MCF-7) was used in this study. The tumor line is maintained in the National Cancer Institute (NCI) Cairo University, Egypt.

The chemical composition of garlic

Moisture, Ash, protein, fat, fiber, and carbohydrates were determined in garlic powder by Near-InfraRed (NIR) Specrtoscopy apparatus, model DA1650, which manufactured by FOSS Corporation (Taha *et al*., 2016). according to (AOAC, 2010) Carbohydrates content was calculated by difference from the following equation : % carbohydrates content = 100- (% protein + % moisture + % ash + % lipids + % fibers)

Plant extraction and preparation

Garlic cloves were separated and peeled. Then dried in air and pulverized to powder to be ready for testing. The extracts were performed by 5 g of plant material with 50 ml of cold water, 70% ethanol (v/v) and hot water (80oC) which was used for a half hour and soaked overnight, then were filtered using Whatman no.1 filter paper. After filtration, the filtrate was centrifuged at 4000 rpm for 10 minutes using a centrifuge and kept at 4oC until used.

Detection of Phytochemicals

The following phytochemicals were qualitatively determined in garlic extracts: Alkaloids, tannins and terpenoids were detected by Wagner's, Braemer's and Salkowski test, respectively according to Sasidharan *et al.* (2011). Flavonoids and phytosterols were detected by Alkaline reagent and Salkowski test, respectively according to Tiwari *et al.* (2011). Phenolic was detected by ferric chloride test according to Cai *et al.* (2011). Saponins and steroids were detections by the froth and Salkowski test, respectively according to Savithramma *et al.* (2011).

DPPH free-radical Scavenging Activity

DPPH radical scavenging activity of garlic extracts were evaluated according to Burtis and Bucar(2000). Inhibition of DPPH free radical was calculated by the following equation; Inhibition (I %) = (A blank – A sample)/ (A blank) × 100 Where A blank= absorbance of the control reaction. A sample= the absorbance of the test extract.

Determination of reducing power

The reducing power of garlic extracts by hot water, cold water and ethanol was determined according to (Dorman *et al*.,2003) who used gallic acid as standard.

Determination of total flavonoids

Flavonoids contents were a determination in garlic extracts according to the aluminum chloride colorimetric method described by Matyuschenko and Stepanova (2003). The data were expressed as: µg rutin equivalents per g dry weight.

Determination of total phenolics

Total phenolics were determined in garlic extracts using the method of Folin-Ciocalteu described by Singleton and Rossi (1965). Gallic acid was used as a standard, the data were expressed as; mg of gallic acid equivalents (GAE) per gram dry weight.

Anticancer activity of garlic extracts against MCF-7 cell line

The cytotoxicity on MCF-7 cell line (Breast cancer) was evaluated according to Neutral uptake red assay (Repetto *et al*., 2008).The concentration of a test chemical reflecting a 50% inhibition of the uptake (IC_{50}) was calculated and the confidence interval using a mathematical model.

Data Analysis

Complete Randomized Design analysis for all data obtained was carried out with three replications and differences between means were calculated using L.S.D test according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The chemical composition of garlic

The chemical composition of garlic is presented in table (1), from which it can be observed that garlic contents were 2.0% fat, 3.39% crude fiber, 5.18% moisture, 9.31% ash, 21.04% protein and 59.08% total carbohydrates. These results in agreement with the results of Otunola *et al*. (2010) found that 0.72% fat, 2.1% fiber, 4.55% moisture, 4.08% ash, 15.33% protein and 73.22% total carbohydrates. From that results, garlic contains more than 80% total carbohydrates and proteins.

Detection of phytochemicals

Qualitative phytochemical analysis of garlic extracts indicated the presence of flavonoids, alkaloids, tannins, phenols, saponins, terpenoids, steroids and phytosterols in ethanol and hot water extracts, whereas alkaloids and saponins are absent in cold water extract (Table 2). These results are consistent with Huzaifa *et al*. (2014) who found that the presence of flavonoids, alkaloids, saponins and tannins in aqueous extract of garlic.

Table 1. Chemical composition of garlic

Parameters	Quantity (%)
Fat	2.00
Crude fiber	3.39
Moisture	5.18
Ash	9.31
Protein	21.04
*Total carbohydrates	59.08

***Carbohydrates were calculated by difference.**

Table 2. Qualitative phytochemical screening of garlic extracts

Phytochemical	Ethanol	Cold water	Hot water
Alkaloids	+	-	+
Flavonoids	+	+	+
Tannins	+	+	+
Phenolics	+	+	+
Saponins	+	-	+
Terpenoids	+	+	+
Steroids	+	+	+
Phytosterols	+	+	+

(+) present; (-)absent

DPPH free-radical Scavenging Activity

DPPH is stable free radical and is mostly used to estimate the ability of antioxidant activity of plant extract or natural compounds to work as scavenging of free radical or donating hydrogen atom (Nurliyana *et al*., 2010). Antioxidant activity of garlic extracts by DPPH method is shown in (Table 3). Garlic extracts of hot water, cold water and ethanol showed scavenging activity by inhibition of free radicals. The scavenging activity of garlic extracts was very effectual and the power of extracts non-significantly decreased with increasing of concentrations. Hot water extract showed anti-radical activity (66.3 % at 50 µg/ml and 61.6 % at 100 µg/ml) followed by ethanol extract (60% at 50 µg/ml and 56.6 % at 100 µg/ml) at the same concentration while cold water showed anti-radical activity (59.1 % at 50 µg/ml and 57.3 at 100 µg/ml) (Table 3). The results showed a strong effect of garlic extracts on scavenging activity of DPPH free radicals with non-significant difference between the effect of garlic extracts.

Table 3. DPPH radical-scavenging activities, reducing power, total Flavonoids and total phenolics of garlic extracts

Garlic extracts	Concentration extracts: Antioxidant activity by DPPH (%) 50µg/ml	100µg/ml	Reducing Power (µg/ml)	Total Flavonoids (µgRutin/g)	Total phenolics (mg GAE/g)
Hot water	66.3	61.6	185.7	508	40.65
Cold water	59.1	57.3	130.3	463	32.30
Ethanol	60	56.6	188.5	544	44.13
L.S.D 5%	N.S	N.S	2.1	20.1	1.8

Reducing Power

The reducing power of garlic extracts was evaluated and expressed as gallic acid equivalent, which was used for standard curve preparation. The highest amount of reducing power 188.5 µg/ml was significantly found in ethanol extract followed by hot water extract (185.7 µg/ml.), while the lowest amount of reducing power was observed in cold water extract 130.3 µg/ml (Table3). The reducing power is mostly used as an indicator of electron donor activity which is a

remarkable technique for testing the antioxidant action of total phenolics. These results agreed with those of Sultana *et al*.(2009) who found that extracts of Aloe Vera which containing a high level of total phenolics, also showed a great reducing power.

Total flavonoids

Flavonoids are widely known antioxidant of plants, it has a large spectrum of biological and chemical activities including scavenging of free radical (Miliauskas *et al*., 2004). Total flavonoids of garlic extracts were determined by relation as rutin equivalent (µg). The highest amount of total flavonoids (544 µg rutin/g D.W) was significantly observed for ethanol extract followed by hot water extract (508 µg/g). The lowest amount was found for cold water extract (463 µg/g) (Table 3). The results suggested that garlic extracts can be a favorable source of powerful antioxidants. These results are in accordance with those Bhandari *et al*. (2014).

Total phenolic

Total phenolic of garlic extracts is shown in (Table 3). Ethanol extract of garlic showed a significantly higher total phenolic content (44.13 mg GAE/g) than hot water (40.65mg/g) and cold water (32.30 mg/g) extracts of garlic. It is found that the antioxidant activity of plants is attributed to the existence of total phenolic (Hernandez and Beltran, 2014). The phenolic is very strong in scavenging free-radicals due to their rapid electron transfer process while hydrogen atom removal becomes a secondary reaction path (Foti *et al*., 2004). Several previous studies showing a strong correlation between antioxidant activity and total phenolic (Bertoncelj *et al*., 2007). These results agreed with those of Abdul Qadir *et al*., (2017), high antioxidant activity of garlic extracts is closely related to the high level of total phenolic and flavonoids in garlic extracts.

Table 4. Anticancer activity of garlic extracts against (MCF-7) cell line

Garlic extracts	Extracts Concentration (µg/ml)					
	0.1 µg	1 µg	10 µg	30 µg	60 µg	IC_{50} µg/ml
	Dead %					
Hot water	0	0	63.1	61.7	62.2	33.4
Cold water	0	19%	61.3	60.4	59.5	33.2
Ethanol	8.1	64	64	62.2	61.3	15.3

Anticancer activity against (MCF-7) cell line

After overnight incubation of the MCf-7 cells (Breast cancer) with garlic extracts, the cytotoxicity on the tumor cell line was evaluated by the Neutral red uptake assay. The highest percentage inhibition of cell growth was (8.1, 64, 64, 62.2 and 61.3% dead cells) with IC50 15.3 µg/ml using concentrations of ethanol extract (0.1, 1, 10, 30 and 60 µg/ml) respectively, Followed by cold water extract which gave (0, 19, 61.3, 60.4 and 59.5% dead cells) with IC50 33.2 µg/ml using same of concentrations mentioned above from cold water extract, respectively. While hot water extract of garlic possesses moderate anticancer activity with IC50 33.4 µg/ml. The results in vitro cytotoxicity of ethanol, cold and hot water extracts of garlic demonstrated the strong dose-dependent inhibition of cancer cell. The extracts have very high cytotoxic activity on the MCF-7 (Breast cancer) cell lines (Table 4).Anticancer activities of garlic extracts on cancer cell lines in vitro might differ from the effects attainable in vivo. Nevertheless, different garlic extracts are shown to have favorable anticancer activities in various cancer models (Yagdi *et al*., 2016).These results are consistent with those of Ghazanfari *et al*. (2011); Modem *et al*. (2012) and Petrovic *et al*. (2018) who found that garlic extract prevents the growth of many various cancer cells in vitro as well as cancer growth in vivo in breast cancer model.

CONCLUSION

In this study, garlic contains more than 80% total carbohydrates and proteins. Also, garlic extracts by ethanol, hot water and cold water contain many phytochemicals. Moreover, antioxidant activity of garlic extracts showed a strong effect on the activity of scavenging of free radicals. In addition, garlic extracts also showed very high cytotoxic activity against human breast cancer cell line type (MCF-7) especially ethanol extract, which makes us recommend that garlic is useful as a rich antioxidant and has great strength against cancer cells.

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فحص المواد الكيميائية النباتية ونشاط مضادات الأكسدة والسرطان لمستخلصات الثوم(*Allium Sativum*)

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الهدف من هذه الدراسة هو تحديد المواد الكيميائية النباتية للثوم ومضادات الأكسدة ونشاط مضادات السرطان ضد بعض أنواع الخلايا السرطانية (MCF-7) لمستخلصات الماء الساخن والماء البارد والإيثانول لنبات الثوم (*Allium Sativum*). أظهرت النتائج أن الثوم يحتوي على أكثرمن 80٪ كربوهيدرات كلية وبروتينات. كما أشارالتحليل الكيميائي النباتي لمستخلصات الثوم إلى وجود مركبات الفلافونيدات والقلويدات والتانينات والفينولات والصابونينات والتربينويدات والستيرويدات والفيتوستيرولات في مستخلصات الإيثانول والماء الساخن ، في حين أن القلويدات والصابونينات كانت غائبة في مستخلصات الماء البارد. علاوة على ذلك ، أظهرالنشاط المضاد للأكسدة لمستخلصات الثوم بطريقة DPPH تأثيرًا قويًا وفعالاً لمستخلصات الثوم على نشاط كسح الشقوق الحرة. بالإضافة إلى ذلك، كان لمستخلص الإيثانول القيم الأعلى في القوة الاختزالية والفلافونيدات الكلية والفينولات الكلية مقارنة بمستخلصات الماء الساخن والماء البارد. أخيرًا ، أظهرت مستخلصات الثوم قدرتها على تثبيط نمو خلايا سرطان الثدي من النوع (MCF-7) وخاصة مستخلص الإيثانول ، مما يجعلنا نوصي بأن الثوم مفيد حيث انه غنى بمضادات الأكسدة وله فعالية كبيرة ضد خلايا سرطان الثدى.