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Investigation of Chemical Composition, Antimicrobial and Antioxidant Activities of Allium Wallichii Kunth (Garlic) Bulb

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Abstract

Garlic can high fully be called one of nature's wonderful plants with leading power. In this research work, the bulb of Allium wallichii Kunth (garlic) was collected from Panglong area, southern Shan state. It is known that garlic (Allium wallichii Kunth.) possesses antimicrobial, antiprotozoal, antimutagenic, antiplatelet and antihyperlipidermic properties. From the presence research work, the following inference could be deduced. The preliminary phytochemical investigation indicated that alkaloids, □-amino acids, flavonoids, saponins, phenolic compounds, glycosides were present and tannin, starch and reducing sugar were absent. The nutritional value of garlic were determined by AACC method, it was show that (60.22%) of moisture, (1.25%) of ash, (5.15%) of proteins, (0.69%) of fibre, (0.1%) of fat and (32.63%) of carbohydrate. Elemental analysis of garlic sample was determined by EDXRF method. It was found that Ca (56.489%), K (40.207%), S (0.927%), Si (0.926%), Mn (0.656%), Fe (0.434%), Cu (0.131%), Zn (0.130%), and Rb (0.100%) were present in garlic. The vitamin C contents was observed (27.455mg) in the samples by using titration method. Antioxidant activities of two solvent extracts, such as ethanol and water extracts, were determined by DPPH assay. Water extract exhibits higher DPPH radicals scavenging activity (IC50 = 1.21□gmL-1) than that of ethanol extract (IC50 = 2.10 □gmL-1). In vitro, antimicrobial activity of some crude extracts (EtOH, MeOH and H2O) of garlic was screened by agar-well diffusion method. H2O extract of garlic was found to exhibit potent of antimicrobial activity (25mm) against on B.sub, S.sureous, P.seudomonus, B.pumalis, Candida and E.coli.

Keywords: Allium wallichii Kunth; Phytochemical; DPPH and EDXRF elemental Main text.

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1. Introduction

Garlic (Allium wallichii Kunth.) is a plant from the family of arcs (Alliaceae). It is herbaceous plant with height of 20-40 cm, a bulb of strong odour and pungent taste. Sulphur compounds in garlic are responsible both for its strong smell, and for its medicinal properties. . It can also boost the immune system to fight-off potential disease and maintain health. It is also considered an effective antioxidant to protect cells against free radical damage. Garlic contain at least thirty-three sulfur compound, several enzymes and seventeen amino acids. It contains higher concentration of sulfur compounds than any other Allium species. The sulfur compounds are responsible both for garlic's pungent odour and many of its medicinal effects .Garlic has been a favourable additive in food for many years in various cultures. For a long time, plants have been a valuable source of natural products for maintaining human health. Garlic tea has long been recommended for fever, headache, cholera, dysentery, and prolonging longevity and has been used for the treatment of hemorrhoids, rheumatism, dermatitis, abdominal pain, cough and as an antiseptic lotion for washing wounds and ulcers, due to its antibacterial properties Garlic is also highly rich in vitamins (especially, vitamin B complex, and Vitamin C), antioxidant, flavonoids, minerals, (especially P, K and Se) [13], being even considered a rich sources of other non-volatile phytonutrients with important medicinal and therapeutic properties, from which a particular emphasis is given to flavonoids, saponins and sapogenins, phenolic compounds, nitrogen oxides and amides and proteins [7,8]. Garlic produces a variety of volatile sulfur-based compounds which are effective as insect repellents and insecticides. Diallyl disulfide is one of such compounds which has a strong odor and acts as a powerful insecticide [6]. Commercial preparations of garlic are certified as insecticide against mites, nematodes and mosquito larvae-affecting a variety of crops [2, 5]. Intact garlic cloves contain only a few medicinally active compounds. The main chemical constituent of intact garlic is the amino derivative of cysteine alkyl sulfoxide, which may varies from 0.2 to 2.0% fresh weight [10]. The efficacy of chemical constituents of garlic chiefly depends on the mode of preparation of its extract. Crushing, chewing or cutting (or exposing dehydrated, pulverized garlic to water) of garlic cloves release the vascular enzyme allinase that rapidly lyses the cytosolic cysteine sulfoxide to form sulfanic acid (R-SOH) which immediately condenses to form allicin: the compound which produces the odor of fresh-cut garlic? Garlic contains at least 100 sulfur-containing compounds basic to medicinal uses [7]. Allicin represents 70-80 % of the total thiosulfates .Garlic contains 0.1-0.36% of a volatile oil these volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic .Beside sulfur compounds garlic contains 17 amino acids and their glycosides, arginine and others. The odor is formed by the action of the enzyme allinase on the sulfur compound allin. This enzyme is inactivated by heat, which accounts for the fact that cooked garlic produces neither as strong an odor as raw garlic nor nearly as powerful physiological effects. The two major compounds in aged garlic, S-allyl cysteine and S-allylmercapto-L-cysteine, had the highest radical scavenging activity. In addition, some organo sulfur compounds derived from garlic, including S-allylcysteine, have been found to retard the growth of chemically induced and transplantable tumors. Garlic in short consists primarily of alliin which, by means of enzyme allinase, is converted into allicin, a powerful antibiotic and anti-fungal compound, ajoene, enzymes, vitamin B, E, and C, folic acid, panthotenic acid and niacin, minerals (Mn, K, Ca, P, Mg, Se, Na, Fe, Zn, Cu) [15], amino acids (glutamic acid, arginine, aspartic acid, leucine, lysine, valine etc). Garlic is one of the most investigated medicinal plants. During 1960 to 2007, more than three thousand research papers have been published on the

chemistry and biological effects of garlic and garlic preparations. These studies mainly focus on the cardiovascular, anti-microbial and anti-cancer effects of garlic and, to a lesser extent, on the therapeutic indications for the treatment of hypoglycemia, heavy metal poisoning and liver dysfunction and hyperthyroidism.

2. Materials and method

2.1 Collection of bulbs

The bulb of medicinal plant Allium wallichii Kunth. (Garlic) was chosen for the present research. The sample was collected from Panglong Township, Loilem District, Southern Shan State, Myanmar. The collected sample was identified in Department of Botany, University of Pang Long.

2.2 Chemicals

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed [16]. DPPH (2,2-diphenyl,1-picrythydrazyl) radical, gallic acid ,ascorbic acid and Folin – Ciocalteau reagent were obtained from Sigma-Aid-rich, USA. All other chemicals and reagents used were of analytical grate.

2.3 Preparation of bulb extracts

Allium wallichii Kunth.(Garlic) was collected sample and it cloves were separated and peeled. The sample was cut into small pieces and ground into purely fine powder by using an electric grinder. The powder sample was labeled and stored separately in air tight plastic bottle to prevent moisture and other contaminations. The powder bulb material (15)g was extracted with 100mL of water, ethanol, and methanol separately. The contents were kept as such in room temperature for 48h with constant stirring at regular intervals. Then, the contents were filtered through Whatman No.1 filter paper. Then the filtrate was vacuum dried using rotary evaporator and was stored at 4°C. The residues were redissolved with the appropriate solvents from which they were prepared and used for further studies.

2.4 Preliminary Phytochemical analysis

Qualitative phytochemical analyses were performed the filtrates of Allium wallichii Kunth. The preliminary phytochemical tests were carried out according to determine the prescence of phytochemicals the alkaloid, □-amino acids, flavonoids, phenolic compounds, glycosides, and saponins as described by standard procedure.

2.5 Test organism

Screening of antimicrobial activity was investigated by Agar Well Diffusion Methods for various crude

extracted samples such as 95% EtOH, MeOH and watery extract of Allium wallichii Kunth. In the present work, the test microorganisms were Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli.

2.6 Preparation of inoculum

The microorganisms were inoculated into nutrient broth and rose Bengal broth for bioassay and incubated for 24 and 48 h at 37°C. The turbidity of the medium indicates the growth of organisms.

2.7 Antimicrobial studies

The agar well diffusion method was employed for the determination of antimicrobial activity of extracts. Lawn culture of E.coli, Candida albican, Bacillus puimilus, psrudomonus aeruginosa, Staphylococcus aureus and Bacillus subtils were spread on nutrient agar and A.niger &A flavus spread on rose bengal agar using sterile cotton swabs. The wells (6mm in diameter) were cut from the agar plates using a cork horer.30µlof the extracts(7mg/mL)were poured into the well using a sterile micro pipette .The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity .The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

2.8 DPPH (2,2-Diphenyl-1-picryl-hydrazyl) radical scavenging activity

The ability of the extract to scavenge DPPH radical was determined according to the method described by [3]. One mL of a 0.3 mM DPPH methanol solution was added to a solution of the extract or standard (250 µg/mL, 2.5 mL) and allowed to read at room temperature for 30 min. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity (AA%).Methanol (1.0 mL) plus extract solution (2.5 mL) was used as a blank 1 mL of 0.3 mM DPPH plus methanol (2.5mL) was used as a negative control.

Solution of ascorbic acid served as positive control

2.9 Proximate analysis

Garlic samples were evaluated for moisture, crude protein crude fat, crude fiber, ash and nitrogen free extract (NFE) according to their respective methods as mentioned in AACC [1]. All the tests were carried out in triplicates. Principle of each method is briefly described as follow:

2.10 Moisture Content

The moisture content of garlic was determined by [1]AACC Method No. 934-01 accordingly .10 g sample was dried in hot air oven (Model: DO-1-30/02, PCSIR, Pakistan) at a temperature of 105± 5 °C for the duration until weight was constant.

2.11 Crude Protein

The Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) was used for the determination of nitrogen percent in garlic using [1]AACC Method No. 984-13. Accordingly, garlic was digested with concentrated H_2SO_4 by using digestion mixture (K_2SO_4:$FeSO_4$:$CuSO_4$ i.e. 100:5:10) until the color was light greenish. The digested material was diluted up to 250 mL in volumetric flask. 10 mL of 40% NaOH as well as 10 mL of digested sample was taken in distillation apparatus where liberated ammonia was collected in beaker containing 4% boric acid solution using methyl red as an indicator. This resulted in formation of ammonium borate that was used for nitrogen determination in sample. Thus percentage of nitrogen in sample is assessed by titrating distillate against 0.1N H_2SO_4 solution till color is light golden. Crude protein content was estimated by multiplying nitrogen percent (N %) with factor (6.25).

2.12 Crude Fat

The crude fat content in garlic sample was estimated following guidelines of Method No 920-39 in [1]. Dried sample (3 g) was refluxed in soxhlet apparatus (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) using n-hexane as a solvent.

2.13 Crude fiber

The garlic sample was subjected to crude fiber content by elaborating Method No. 978-10 outlined in [1] AACC . Fat free sample was digested with 1.25% H_2SO_4 followed by 1.25% NaOH solution in Labconco Fibertech apparatus (Labconco Corporation Kansas, USA). After filtration and washing with distilled water reaming residues was weighed and ignited in muffle furnace at temperature of 550-650 °C till grey or white ash was obtained. The crude fiber percentage was estimated according to the expression given below.

Crude fiber = weight loss on ignition (g) X 100 /

Weight of sample (g)

Total ash

The ash content of peel was estimated according to the procedure mentioned in [1] AACC Method No. 942-05. For which, 5 g sample was directly charred on flame in crucible until there was no fumes coming out. Afterwards sample was ignited in muffle furnace (MF-1/02, PCSIR, Pakistan) at 550-600ºC for 5-6 hours or until grayish white residues were obtained.

3. Results and Discussion

Phytochemicals detected in Garlic (Allium wallichii Kunth) extract According to the experiments alkaloids, □-amino acids, flavonoids, phenolic, glycosides, and saponins are present and reducing sugars, starch and tannins are absent. Alkaloids, phenolics, terpenoids and cardiac glycosides detected in the extracts are compounds that

have been documented to possess medicinal properties and health–promoting effects [12,4,11,9]. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extracts [14]. The determination of some nutritional values such as moisture, ash, fat, proteins, fiber, carbohydrates content and energy values were carried out in section (2.5). In this sample, the carbohydrate content (32.63%) was observed to contain the highest amount. In addition, protein (5.15%) and fiber (0.69%) were also found to be higher than the other nutrients, moisture (60.22%) and ash (1.25%). The fat content (0.1%) was found to be the lowest amount in Allium wallichii Kunth. The energy value was observed to be 153 kcal/100g. The result of nutritional values from Allium wallichii Kunth are mentioned in Table (1) and Figure 2. Elemental analysis of dried powder was carried out by EDXRF technique. Cu and K were found to as major constituents and Pb as trace element. The results of qualitative elemental analysis are shown in Table (2) and Figure (3). The vitamin C content was observed 27.455 mg in the sample by using titration method. From Screening of antimicrobial activity, it was found that the antimicrobial activity against six microorganisms with the inhibition zone diameter ranged between 15 mm -20mm. In addition, watery extracts was more potent antimicrobial activity against six microorganism MeOH and EtOH extracts are found to exhibit antimicrobial activity 15mm-18mm against antimicrobial activity against six microorganisms. The result of inhibition zone diameters are described in Table (3) and Figure (4).The antioxidant activities of 95% ethanol extract and watery extract were studied by DPPH method. This method is based on the reduction of colored of free radical DPPH in ethanolic solution by different concentration of sample. The antioxidant activity was expressed as 50% oxidative inhibitory concentration (IC50). The lower the IC50 values, the higher the antioxidant activity of the sample. In this experiment, each sample was dissolved in ethanol to get 0.2mg/mL concentration and then it was diluted with ethanol to obtain 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 □g/mL concentration. After mixing with DPPH solution, the absorbance of each solution was measured at 517nm.The antioxidant potential of sample can be determined by IC50 (50%inhibition concentration). The IC50 value for each sample were determined by linear regressive excel program. By using DPPH free radical scavenging assay, watery extract was found to the most potent antioxidant activity than 95% ethanol extract. The results of antioxidant activity are shown in Table (4) and Figure (5, 6).

4. Conclusion

In conclusion the results of phytochemical parameters in the present study would be the evidence for the identification and purity of the garlic (Allium wallichii Kunth). The presence of various secondary metabolites such as alkaloids, □-amino acids , flavonoids , saponins , phenolic compounds, glycosides were providing the promising effect on the antimicrobial activity against tested pathogenic organisms. The allicin inside garlic having anti-oxidant feature is useful for human diseases and has antibacterial properties that is used for repelling insects and/or removing insects and is used as one plant agrochemicals as pesticides that is safe for human and environment. Emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low values of antioxidant indicates in plants do not translate to poor medicinal properties. In the present review, antioxidant, antimicrobial, immune function, cardiovascular activity, antineoplastic actions of garlic has been shown.

Table 1: Some Nutritional Value of Allium wallichii Kunth. (Garlic)

	Nutrient Parameter	Percentage of Content (%)
1.	Moisture	60.22
2.	Ash	1.25
3.	Fat	0.1
4.	Protein	5.15
5.	Fiber	0.69
6.	Carbohydrate	32.63

Table 2: Quantitative Result of Some Element in Garlic

No.	Analyte	Result (%)
1.	Cu	56.489
2.	K	40.207
3.	S	0.927
4.	Si	0.926
5.	Mn	0.656
6.	Fe	0.434
7.	Cu	0.131
8.	Zn	0.130
9.	Pb	0.100

Figure 1: Bulb of Allium wallichii Kunth.

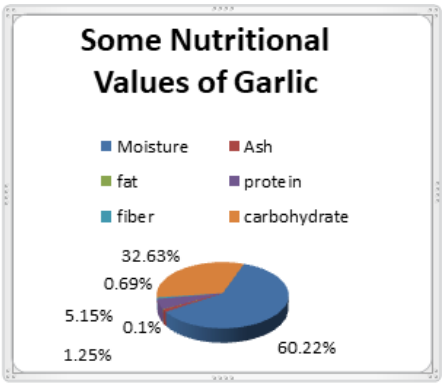


Figure 2: Pie chart Graph of Some Nutritional Values of Allium wallichii Kunth (Garlic)

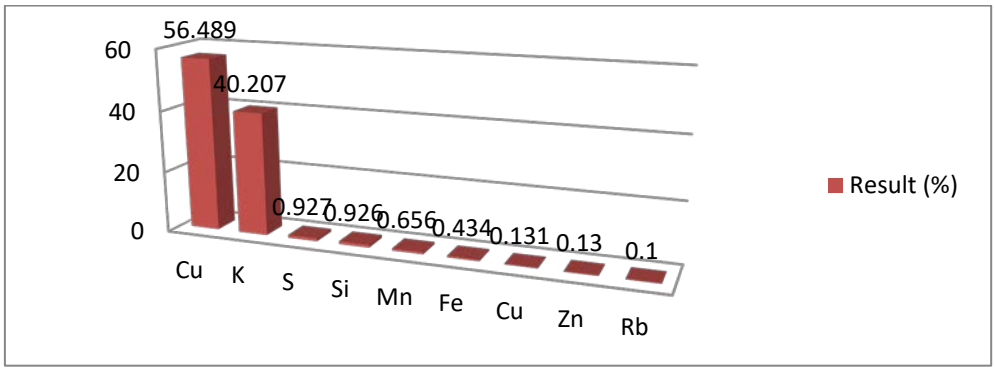
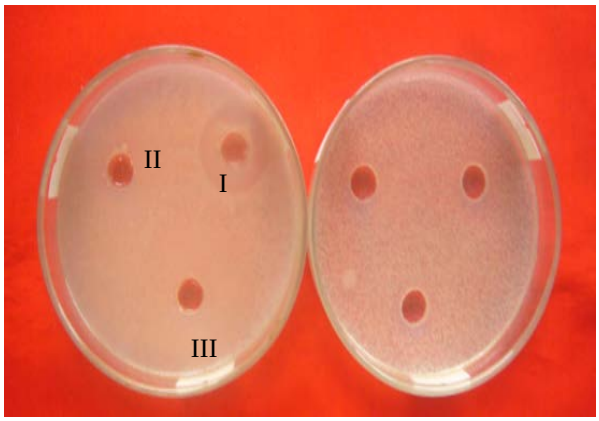


Figure 3: Bar graph of Some Elements in Allium wallichii Kunth by EDXRF

Table 3: Diameter of Inhibition Zone of Crude Extracts on Different Bacterial Strains

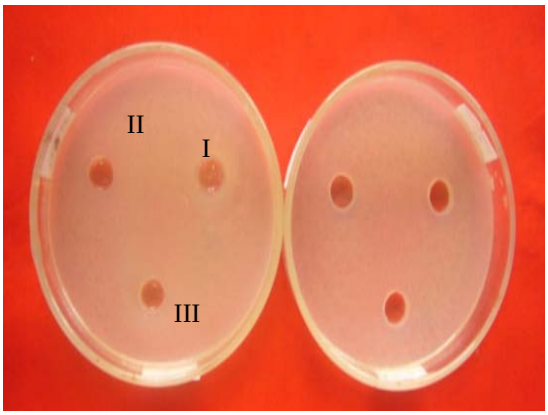
No.	Type of bacteria	Diameter of inhibition zone (mm)		
		H_2O	MeOH	EtOH
1.	*Bacillus subtilis* (N.C.T.C.8236)	25 (+++)	12 (++)	12 (++)
2.	*Staphylococus aureus* (N.C.P.C.6371)	24 (+++)	13 (++)	13 (++)
3.	*Pseudomonas aeruginosa* (6749)	20 (+++)	13 (++)	15 (++)
4.	*Bacillus pumilus* (N.C.I.B.8982)	26 (+++)	12 (++)	15 (++)
5.	*Candida albicans*	27 (+++)	12 (++)	13 (++)
6.	*Escherichia coli* (N.C.I.B.8134)	25 (+++)	13 (++)	14 (++)

Agar well- 10 mm , 10mm - 14mm (+) , 15 mm -19 mm (++) , 20mm above (+++)

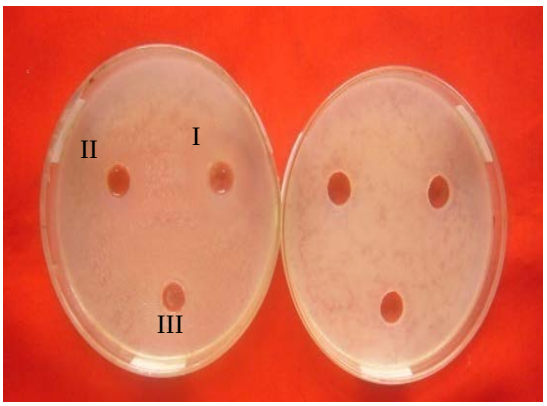


On Bacillus subtilis

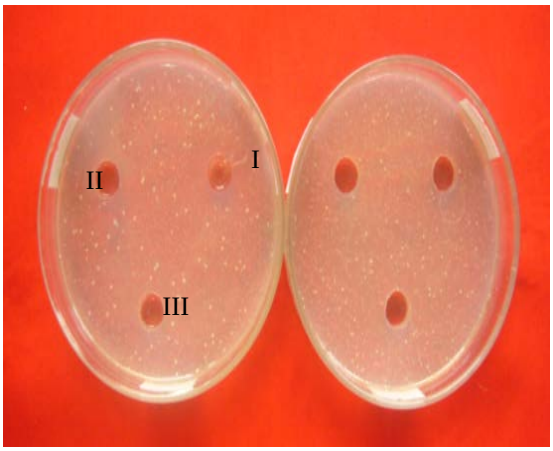
On ***Staphylococus aureus***



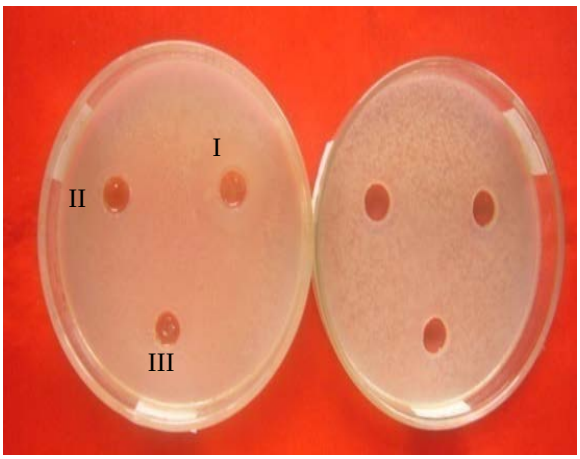
On *Pseudomonas aeruginosa*



On *Bacillus pumilus*



On *Candida albicans*



On *E-coli*

Figure 4: Antimicrobial Activities of Crude Extracts of Garlic by Agar well Diffusion method

I = watery extract , II = Ethanol extract , III = Methanol extract

Table 4: Radical Scavenging Activity (IC50) of Water and EtOH Crude Extracts of Garlic and Ascorbic Acid

Tested Sample	% RSA (mean ± SD) In different concentration ((µg/mL)					IC_{50} (µg/mL)
	1.25	2.5	5	10	20	
Garlic (water)	20.37 ±1.46	37.21 ±1.46	57.48 ±1.46	69.86 ±1.46	80.16 ±1.46	1.21
garlic (EtOH)	20.87 ±1.40	37.21 ±2.10	51.56 ±2.80	66.91 ±2.10	82.26 ±0.00	2.10
Ascorbic acid	53.58 ±0.88	65.53 ±1.13	74.82 ±0.59	83.32 ±0.78	91.21 ±0.48	1.17

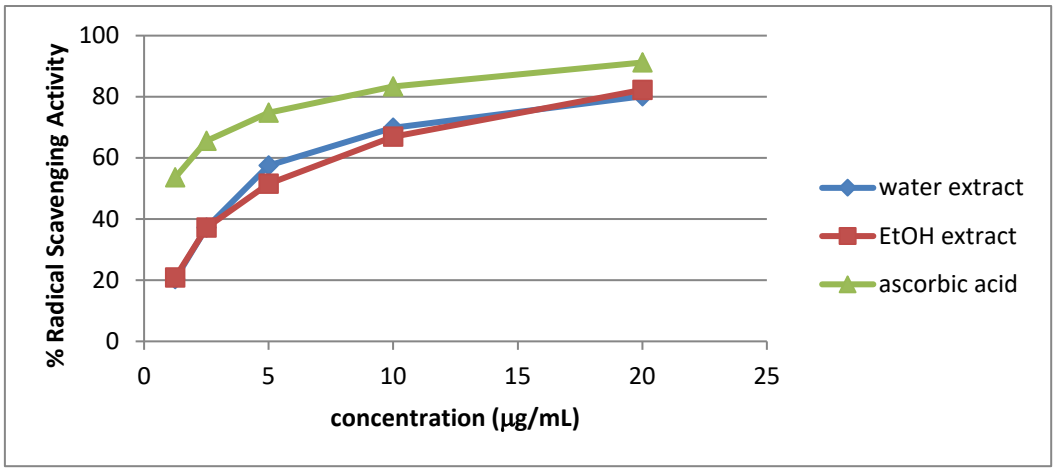


Figure 5: Radical Scavenging Activities of Different Concentrations of Crude Extract of Garlic

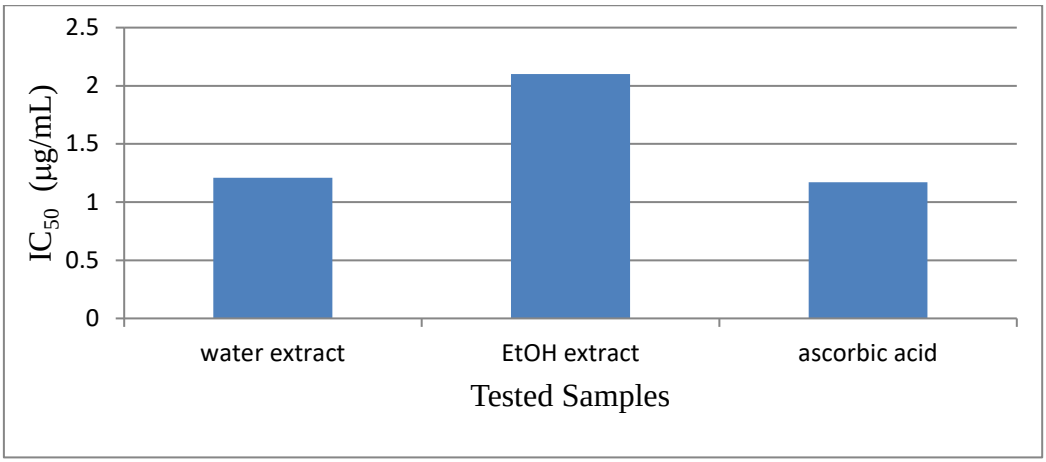


Figure 6: A Bar Graph of IC50 (□g/mL) of Water and EtOH Extracts of Garlic and Ascorbic Acid

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