Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*

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ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 15 January 2011
Accepted 15 March 2012
Available online 20 May 2012

Keywords:
Allium sativum
Insecticidal activity
Antimicrobial activity
Antioxidant property

ABSTRACT

Objective: To evaluate the insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum* (*A. sativum*). **Methods:** Dried bulbs of *A. sativum* were extracted with different solvents and evaluated for insecticidal, antimicrobial and antioxidant activities. **Results:** Aqueous and methanol extracts showed highest insecticidal activity (mortality rate of 81% and 64% respectively) against the larvae of *Spodoptera litura* (*S. litura*) at a concentration of 1 000 ppm. With regard to antimicrobial activity, aqueous extract exhibited antibacterial activity against gram positive (*Bacillus subtilis*, *Staphylococcus aureu*,) and gram negative (*Escherichia coli* and *Klebsiella pneumonia*) strains and antifungal activity against *Candida albicans*. While methanol extract showed antimicrobial activity against all the tested micro organisms except two (*Staphylococcus aureus* and *Candida albicans*), the extracts of hexane, chloroform and ethyl acetate did not show any anti microbial activity. Minimum inhibitory concentration of aqueous and methanol extracts against tested bacterial and fungal strains was 100–150 μg/mL. Antioxidant activity of the bulb extracts was evaluated in terms of inhibition of free radicals by 2, 2′-diphenly-1-picrylhydrazyl. Aqueous and methanol extracts exhibited strong antioxidant activity (80%–90% of the standard). **Conclusions:** Antioxidant and antimicrobial activity of *A. sativum* against the tested organisms therefore, provides scientific basis for its utilization in traditional and folk medicine. Also, our results demonstrated the insecticidal efficacy of *A. sativum* against *S. litura*, a polyphagous insect.

1. Introduction

Allium sativum (A. sativum) , commonly known as garlic, is a species in the onion family Alliaceae. It is a perennial herb with a tall, erect flowering stem that grows up to 3 feet. The garlic plant′s bulb is the most commonly used part of the plant. With the exception of the single clove types, the bulb is divided into numerous fleshy sections called cloves. The cloves are used for consumption (raw or cooked) or for medicinal purposes. Garlic has been used throughout history for both culinary and medicinal purposes[1,2]. The bulbs of the plant have been used in many parts of the world as a stimulant, antiseptic, anthelminthic, antihypertensive, carminative, diaphoretic, expectorant, diuretic, antisorbutic, aphrodisiac and antiasthmatic and for the relief of rheumatic pains[3]. Physicians prescribed the herb during the middle ages to cure deafness and the American Indians used garlic as a remedy for ear aches, flatulence and scurvy. Recent research revealed that garlic is not only beneficial as medicinal plant, but it can be used as repellent to some plant pests and diseases[4]. Transgenic rice cultivars containing ASAL protein (*A. sativum* leaf lectin based) have been found to exhibit increased resistance against sap sucking insects such as brown plant hopper and green leafhopper.

A. sativum is a versatile herb that contains numerous vitamins, minerals and trace elements. The presence of two trace elements, germanium and selenium has been postulated to play a role in the herb′s antitumor effect[5]. The volatile oils present in garlic possess sulfur containing

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compounds such as diallyl disulphide, diallyl trisulphide and methylallyl trisulphate[5]. Allicin, derived from amino acid allin gives the pungent characteristic odour to crushed garlic and is believed to be responsible for some of the pharmacologic activity of the plant[1,2]. Inspite of several traditional uses of garlic there seems to be little literature about the insecticidal and antimicrobial properties of *A. sativum*, which makes it important to investigate in this direction[6, 7].

2. Materials and methods

2.1. Isolation of plant extracts

The cloves of *A. sativum* bulbs were chopped into pieces, shade-dried for about one week at room temperature and powered. 100 g of powder was taken in a separate container, to this 400 mL of hexane was added and kept for 24 h in a shaker. This was filtered through eight layered muslin cloth and the extract was collected. The extraction process was repeated twice and the collected extracts were pooled. Chloroform, ethyl acetate, methanol and aqueous extracts were prepared as that of hexane extract. The solvent extracts were concentrated under reduced pressure using rotavapor and finally freeze dried.

2.2. Phytochemical analysis

Phytochemical screening of bulb extracts was carried out qualitatively for the presence of sterols, triterpenes, tannins, flavonoids, saponins, alkaloids, carbohydrates, glycosides and steroids[8-10].

2.3. Larvicidal bioassay

The larvae of *Spodoptera litura (S. litura)* were obtained from Regional Agricultural Research Station, Tirupati. From the stock solution of plant extracts, test solutions were prepared in the concentrations of 250, 500, 750 and 1 000 ppm. Polysorbate 80 was used as an emulsifier at the concentration of 0.05% in the final test solution and Lufenuron was used as standard drug. Leaf-dipping method was used to evaluate the larvicidal activity of the test solutions on castor leaf disks[11]. For each dose, five leaf disks, each with a diameter of 6.5 cm were separately dipped in each test solution for 30 s. Solvents were evaporated under a fume hood for 2 h. Fourth-instar larvae were transferred individually on control and treated leaf disks placed in Petri plates. To calculate the larvae feeding activity, the percentage of leaf damage was gravimetrically estimated every 12 h with an additional initial check after 6 h. A total of 50 larvae were exposed in five replicates of ten larvae each. Experiments were maintained at (26±1) ℃, (65±2)% relative humidity and a photoperiod of 16:8 (L/D). Mortality rate was determined 24 h after larvae were placed on disks. All moribund pest larvae were considered as dead.

2.4. Anti microbial assay and minimum inhibitory concentration (MIC)

The bacterial strains used in this study included *Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Klebsiella pneumonia(K.pneumonia)* and fungal species, *Candida albicans (C. albicans)* were obtained from Department of Microbiology, Sri Venkateswara University, Tirupati and from IMTECH, Chandigarh, India. Antimicrobial activity was determined by agar disc diffusion method[12]. The discs (6 mm diameter) impregnated with bulb extracts were placed on the surface of the petri plates containing 20 mL of nutrient agar media for bacterial strains and potato dextrose agar media for fungal strains respectively, seeded with 100 μL of microbial cultures (5×10^5 CFU/mL). Discs of ampicillin, tetracycline and ketconazole (20 μg each, Himedia) were used as standard antibiotics against gram positive, gram negative bacterial strains and fungal strain respectively. The plates were incubated for 24 h at (35±2) ℃ for bacteria and for 48 h at 30℃ for fungi. At the end of incubation, inhibition zones formed around the discs were measured with Himedia zone scale. The study was performed in triplicate and the mean values were presented.

To measure the MIC values, various concentrations of the stock, 0.05-2 mg/mL were assayed against the test microbes. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth [13].

2.5. In vitro antioxidant activity

2.5.1. 2, 2′-diphenly-1-picrylhydrazyl (DPPH) radical scavenging activity

Free radical scavenging activity was determined by using DPPH method described by Burits and Bucar[14]. 1 mL of plant extract was added to 4 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated by using the following equation.

$$I\ \% = [(A\ control - A\ sample) / A\ Control] \times 100.$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

2.5.2.Reducing power assay

The reducing power was determined in accordance with the procedure of Oyaizu[15]. Methanol and aqueous extract of *A. sativum* were prepared and mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated

at 50 ℃ for 20 min and 2.5 mL of trichloroaceticacid (10%) was added to the mixture, which was then centrifuged at 3 000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a standard.

2.6. Stastical analysis

Statistical analysis was carried out using SPSS software.

3. Results

3.1. Phytochemical analysis

In the present study extracts of *A. sativum* bulbs were prepared with hexane, chloroform, ethylacetate, methanol and water and checked for the presence of phytochemicals. As shown in Table 1, phytochemicals were differentially distributed among the solvents extracts. Steroids were present in all the solvent extracts of garlic except hexane and ethyl acetate extracts. While triterpenes were present in chloroform and methanol extracts they were absent in other extracts. Flavonoids, alkaloids and carbohydrates were found to be present in methanol and aqueous extracts but absent in other extracts. Saponins were present only in hexane, ethyl acetate and methanol extracts. Tannins and glycosides were weakly present in methanol and aqueous extracts but absent in other extracts. The presence or absence of particular component(s) plays a major role in deciding the medicinal property of the plant extract.

3.2. Larvicidal activity

With increase in concentration of the bulb extracts from 250 to 1 000 ppm, the mortality rate of the larvae increased in dose dependent manner. The highest mortality rate observed with methanol and aqueous extracts was 81% and 64% respectively at 1 000 ppm as noted. At 250 and 500 ppm, the mortality was (66.0±2.6) and (72.0±6.3) for methanol extracts, and (42.0±8.6) and (55.0±4.6) for aqueous extracts. 10% lufenuron showed (94.0±5.6). The highest insecticidal activity exhibited by methanol extract can be attributed to the presence of a good number of phytochemicals in it.

3.3. In vitro antimicrobial activity and MIC

The bulb extracts of *A. sativum* obtained with different solvents were checked for both antibacterial and antifungal activity. However, the results of only methanol and aqueous extracts are mentioned here because other extracts did not show considerable antimicrobial activity. While the aqueous extract was active against all the tested microorganisms, methanol extract was active against all except *S. aureus* and *C. albicans*. The maximum zone of inhibition observed with aqueous extract (100 μg/mL) was 20 mm and methanol extract (100 μg/mL) was 16 mm against *B. subtilis*. The minimum inhibitory concentrations and the diameter of the zones of inhibition of the tested microbes were shown in Table 2.

3.4. In vitro antioxidant activity

Antioxidant efficacy of methanol and aqueous extracts increased with increase in concentration of the bulb extracts. Both the extracts showed 80%-90% antioxidant activity in

Table 1

Secondary metabolites present in bulb of *A. sativum*.

Secondary metabolites	Tests	HE	CE	EA	ME	AE
Steroids	Salkowski test	+	+	+	+	-
	Liebermann test	+	+	+	+	-
Triterpenes	Salkowski test	-	-	+	+	-
	Liebermann test	-	-	+	+	-
Saponins	Foams test	-	-	-	+	+
Alkaloids	Mayer's test	+	+	-	+	-
	Wagner's test	+	+	-	+	-
Carbohydrates	Fehling's test	-	-	+	+	+
	Molisch test	-	-	+	+	+
	Benedicts test	-	-	+	+	+
Flavonoids	Shinoda test	-	-	-	-	-
	Fecl3 test	-	-	-	-	-
	Lead acetate test.	-	-	-	-	-
Tannins	Fecl3 test	-	-	-	-	+
	Gelatin test	-	-	-	-	+
Glycosides	Bal jest test	-	-	-	+	+
	Legal's test	-	-	-	+	+

HE = Hexane extract, CE = Chloroform extract, EA = Ethyl acetate extract, ME= Methanol extract, AE = Aqueous extract, WP = weakly positive, (+) = indicates presence of secondary metabolites, (-) = indicates absence of secondary metabolites.

Table 2

In vitro antimicrobial activity of bulb extracts of *A. sativum*.

Name of the test organism	Methanol extract		Aqueous extract		Standard antibiotics
	ZI (mm)	MIC (μ g/mL)	ZI (mm)	MIC (μ g/mL)	
B. subtilis	16	100	20	100	22[A]
S. aureus	–	–	14	100	21[A]
E. coli	14	150	16	100	22[B]
K. pneumonia	12	150	17	100	22[B]
C. albicans	–	–	12	150	24[C]

ZI: Zone of inhibition in mm, MIC: Minimum inhibitory concentration (μ g/mL), A: Ampiclilin 20 μ g/mL, B: Tetracycline 20 μ g/mL,C: Ketconazole: 20 μ g/mL.

terms of DPPH radical scavenging activity when compared with ascorbic acid, the standard (Figure 1). In reducing power assay, methanol and aqueous extracts showed 40%–50% and 55%–65% antioxidant activity respectively in comparison to ascorbic acid (Figure 2).

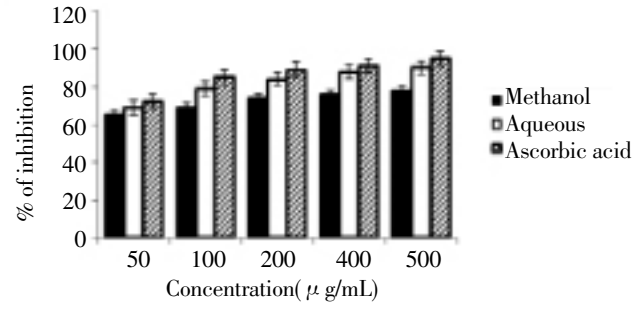

Figure 1. Scavenging activity of methanolic and aqueous extracts of *A. sativum* bulbs on DPPH radical.

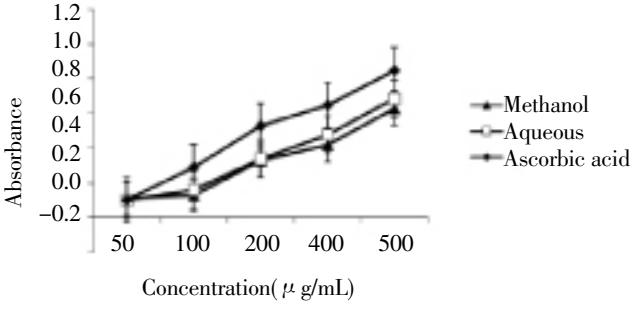

Figure 2. Reducing power assay of *A. sativum* bulb extracts.

4. Discussion

Spodoptera species are polyphagous pests causing economic damage in several agricultural crops throughout the world. Broad spectrum of insecticides have been used for control of *S. litura* which resulted in development of resistance in them[3]. In this scenario newer types of insecticides originating from natural products, targeting *S. litura* could be useful alternative for integrated pest management. Insecticidal activity may be due to interference of garlic compounds that are sprayed over castor leaf. Insecticidal activity against *S. litura* was earlier reported with extracts of *Cassia fistula* (Linn) and *Clerodendron inerme*[16]. In the present study, among different solvent extracts of garlic, methanol extract was highly insecticidal with a mortality rate of (81.0±4.8) followed by aqueous extract showing mortality rate of (64.0±4.3) at 1 000 ppm. Similar reports of insecticidal activity was observed with methanolic extracts of *Ocimum sanctum* and *Rhinacanthus nasutus* on fourth instar larvae of *S. litura* (LC_{50} = 36.46 and 68.84 ppm, respectively)[22]. Ethanolic extracts of *Azadirachta indica* and *Melia azedarach* produced 22.2% and 25% larvicidal activity, respectively against *S. litura* and *Pieris brassicae*[23]. Studies on ethyl acetate and hexane extracts of fruit pulp of *Momordica dioica*, indicated that, both the extracts showed a concentration dependent antifeedant effect on *S. litura* larvae. In another study, aerial parts extracts of *Synedrella nodiflora* Gaertn (Asteraceae) exhibited profound growth reduction and toxic effects on *S. litura*[25]. Hexane extracts of *Porteresia coarctata* produced more than 50% mortality after 24 h of treatment on *S. litura* larvae[26].

Our study indicates that the methanol and aqueous extracts of *A. sativum* are endowed with higher larvicidal efficacy as compared to several other plants. Further characterization of bioactive molecules of *A. sativum* extracts will provide greater clarity about insecticidal nature of these bioactive compounds. This could become alternative to the conventional insecticides used for the regulation of *S. litura*.

Methanol and aqueous extracts of garlic showed a good range of antimicrobial activity against the tested fungal as well as gram positive and gram negative bacterial strains. Similar reports of antimicrobial activity were observed with plant extracts of *Terminalia* sps, *Withania somnifera*, *Cassia auriculata* and *Morinda citrifolia*[17-19]. The antimicrobial activity of *A. sativum* bulbs suggest that, these extracts contain effective phytochemicals responsible for the inhibition of microorganisms.

The antibacterial activity of garlic is widely attributed to its phytochemicals. Allicin, an important constituent of garlic interferes with RNA production and lipid synthesis. If RNA cannot be produced, or produced in less amount then protein synthesis will be severely affected. It would be stopped at every stage due to the absence of messenger RNA, ribosomal RNA and transfer RNA. If amino acids and proteins cannot be produced then growth and development of the organism will not occur as they are essential for all parts of cell structure. Also, as lipid synthesis is affected, other parts of the cell are interfered with. The main effect being that the phospholipid biolayer of the cell wall cannot form correctly,

the synthesis of cell wall components of microbial strains is severely affected.

Our results based on DPPH radical scavenging activity and reducing power assay authenticate the presence of higher levels of antioxidants in garlic extracts which are largely responsible for many of the health benefits attributed to *A. sativum*. A good number of plant species such as *Allium cepa*, *Coriandrum sativum*,*Cuminum cyminum*, *Zingiber officinale*, *Cinnamomum verum*, *Elettria cardamomum* and *Cinnamomum verum* were earlier reported for their high antioxidant activity[20].

From our studies we conclude that, the bulb extracts of *A. sativum* contains an array of phytochemicals in it. Aquous and methanol extracts of garlic bulbs showed insecticidal, antibacterial, antifungal and antioxidant activities providing evidence for its usage in traditional and folk medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors profusely thank DST-FIST, New Delhi, for making their facilities available to us in the department of Biochemistry, S.V.University, Tirupati.

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