

Antibacterial and anatomical study of two *Allium sativum* L. Cultivars

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Abstract

Garlic (*Allium sativum* L.) plant is medicinally very important and is being used since time immemorial. In this research antibacterial activity and anatomy of two cultivars were tested by using dedicated methods. Diffusion method was used to test the antibacterial activity of the bulb of two different garlic species i.e. Desi garlic (DG) and white garlic (WG) against different bacteria such as (*S. aureus*, *B. sibirica*, *E. coli*, *P. aeruginosa*). The ethanol, methanol, chloroform, and distilled water extracts of bulb of both species has tested which showed significant antibacterial activities on both gram positive and gram negative in contrast to white garlic. DG showed maximum antibacterial activity in chloroform and methanol extracts against *E. coli* and *S. aureus*. The chloroform bulb extract of DG showed maximum activity against *E. coli* that was 36.33 ± 0.28 . The methanolic bulb extract of DG showed maximum activity against *S. aureus* that was 32.33 ± 0.571 . The chloroform bulb extract of DG showed less inhibitory effect against *P. aeruginosa* that was 11.58 ± 0.02 . The chloroform bulb extract of WG showed maximum activity against *E. coli* that was 27.67 ± 0.04 . The methanolic bulb extract of WG showed less activity against *P. aeruginosa* that was 9.33 ± 0.57 . For anatomical study two samples of *Allium sativum* L. were collected from two different sites of District Bhimber. the DG (*Allium sativum* L.) stomatal size of Bandala showed maximum size as compared to DG (*Allium sativum* L.) stomatal size of Bhimber. The maximum stomatal size of DG of Bandala was $9.41 \pm 0.74 \mu\text{m}$ and the minimum size of DG of Bhimber was $5.71 \pm 0.74 \mu\text{m}$. As it is WG (*Allium sativum* L.) stomatal size of Bandala showed maximum size as compared to WG (*Allium sativum* L.) stomatal size of Bhimber. The maximum stomatal size of WG of Bandala was $6.67 \pm 0.72 \mu\text{m}$ and the minimum stomatal size of WG (*Allium sativum* L.) was $2.55 \pm 0.85 \mu\text{m}$. The DG (*Allium sativum* L.) stomata index of Bandala showed maximum stomata index as compared to DG (*Allium sativum* L.) stomata index of Bhimber. The maximum stomata index of DG of Bandala was 97.74 and the minimum stomata index of DG of Bhimber was 92.89. As it is WG (*Allium sativum* L.) stomata index of Bandala showed maximum stomata index as compared to WG (*Allium sativum* L.) stomata index of Bhimber. The maximum stomata index of WG of Bandala was 85.63 and the minimum stomata index of WG (*Allium sativum* L.) was 47.33. Anatomical studies showed changes in stomatal size and index in collected species. It was concluded that DG extract was better than WG because former had stomatal index was higher than later and same depicted higher antibacterial activity than others, that is due to presence of more quantity of bio-constituents in taxa with numerous stomata.

Keywords: garlic, antibacterial activity, anatomy; bhimber; azad Kashmir; Pakistan

1. Introduction

Garlic (*Allium sativum* L.) is the oldest of all cultivated plants. That played a role in folk medicine from ancient period. Garlic has been used as culinary and medical purpose for thousands of years. It is a remarkable plant, which has multiple beneficial effects. Garlic is cultivated and used worldwide in all cultures. Its common name is associated to the Anglo Saxon 'garleac.' China is by far the largest producer of garlic, with almost 10.5 million tones (23 billion pounds) annually, accounting for over 77% of world output (Bentley and Tremen). Garlic comprises at least 33 sulfur compounds, several enzymes and the minerals (Josling, 2005) [4]. It has a higher concentration of sulfur compounds than any other *Allium* species which are responsible both for garlic's pungent odor and many of its medicinal effects. One of the most biologically active compounds in garlic is allicin (diallyl thiosulfinate or diallyldisulfide at 10 and 30 mg/g in fresh and dry garlic, respectively (Lawson, 1998) [5].

Garlic consists of several individual bulblets, known as "cloves." These individual bulblets are enclosed in a membranous bag that is whitish or purplish in color. In folk

medicine, garlic has been used to treat bronchitis and respiratory problems, gastrointestinal problems, flatulence, leprosy, high blood pressure, diabetes and has been used externally for warts, corn, arthritis, muscle pain, neuralgia, and sciatica. Recently, science has started to approve some of garlic long-standing medicinal uses. Garlic has been shown to lower blood cholesterol, blood pressure and blood sugar in studies and clinical trials and has also demonstrated anticancer, antibacterial, anti-fungal and antioxidant effects (Nadkarni, 2001) [6]. Due to high potential of medicinal use, an experiment was designed to explore scientific basis of its medicinal value. Aim of the research was based on (i) to make comparative study in order to investigate efficacy of local (desi) and introduced (white) Kashmiri cultivars and (ii) to see effect of presence of stomata numbers in two cultivars and then its correlation with antibacterial potential.

2. Materials and Methods

2.1 Antibacterial Activity

Plant Sample Selection

Desi garlic (*Allium sativum* L.) and white garlic (*Allium*

sativum L.) were selected to observe antibacterial activity. Deshi garlic was collected from Tehsil Samahni (Bandala) and white garlic was purchased from the local vegetable market of Bimber Azad Kashmir.

Preparation of Plant Extracts

Plant material was collected and then washed under tap water for the removal of extra mud. It was then cut into small pieces and dried carefully under shade and then homogenized to fine powder and stored in airtight bottles separately. Ten gram portion of each dried powdered plant part material was soaked separately in 100 ml Chloroform, Methanol, Distilled water, Ethanol. The extraction was carried out by maceration for 7 days in each solvent at room temperature. The solvent extracted material was filtered in separate beakers (Rawlins and Tindall, 1977) [8]. All extracts were then dried at room temperature, weight and stored until further analysis.

Test Organisms

In the present study gram positive bacteria i-e *Stylococcus aureus*, *Bacillus subtilis*, gram negative bacteria i-e *Escherichia coli*, *Pseudomonas aeruginosa*, were used to evaluate the antibacterial potential of different extracts of the selected plant material.

Protocol Optimization

Screening of the anti-bacterial activity was performed by well diffusion technique (Saeed *et al.*, 2005). The Mueller-Hinton agar plates were seeded with 0.1 ml of the standardized inoculums of bacteria. The inoculums were spread evenly over plate with sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37 °C for 20 minutes. A standard crack border of 5 mm diameter was used to cut uniform wells on the surface of the plates, and 0.1 ml of each concentration was introduced in the well with ethylene glycol as a control. The inoculated plates were incubated at 37 °C for 24 hrs. And zone of inhibition diameter was measured to using nearest millimeter (mm).

2.2 Anatomical Analysis

Collection of Experimental Material

Two samples of desi variety of garlic (*Allium sativum* L.) and the two samples of white variety of garlic (*Allium sativum* L.) were collected from two different areas of District Bimber

Azad Kashmir (Bandala, Bimber).

Procedure Adopted

Fresh leaves were used directly for anatomical studies. Fresh leaves were placed in a test tube filled with 88% lactic acid kept hot in boiling water bath for about 5 to 10 minutes. Lactic acid softens the tissue of the leaf to make peeling off possible, Anatomical studies were conducted on leaves taken from middle portion of plant. The epidermal peelings of leaves were done in six accession of each plant. When leaves become soften they were poured in Petri dish and washed with water for 2 or 3 times. The leaf was then placed on glass slide. The epidermis was peeled with sharp razor and the residues left were then removed. Then 2 or 3 drops of bleach was put on the peeled epidermis and then it was left for 30 to 40 seconds remove spare traces of chloroplast and again washed. At one end one or two drops of lactic acid were poured over it and then covered with cover slip. Anatomy of leaf epidermis was studied using microscope. Microphotograph was done by using CCD digital camera (Model: DK 5000). No of stomata were count 3-4m times in order to ensure accuracy. Different magnifications were used to obtain finer details of epidermis.

3. Results and Discussion

In this research, two cultivars of garlic (*Allium sativum* L.) viz Desi (pink) and Kashmiri (white) were studied for their anatomical variations and their subsequent impacts as antibacterial agent against human pathogens (bacteria) Fig 1 and Fig 2. The research depicted that morphologically and anatomically two cultivars were different. The local community prefers pink (local) garlic for culinary purpose and it has good taste and smell. It is has more antibacterial activity then white garlic population/ accession.

First four strains of bacteria were used as test organism and extracts of four different solvents were used for efficacy verification. The results showed that all solvents' extract do possess less or more activity against bacteria (Table 1). The highest values of zone of inhibition were shown by chloroformic extract against *E. coli* with 32.33±0.57 mm, followed by chloroform extract against *B. subtilis* with 32.43±0.24 mm. While lowest antibacterial potential was found for ethanolic extract as 12.45±0.03 mm against *B. subtilis* (Table 1).

Table 1: Antibacterial Activity Shown as Zone of inhibition (mm) of Bulbs of *Allium sativum* L. (Desi garlic cultivar- Pink One)

Sr.No	Strains	Ethanol	Methanol	Chloroform	Distill Water
1.	<i>E.coli</i>	14.43±0.26	16.00±1.00	36.33±0.28	27.66±0.57
2.	<i>B.subtilis</i>	12.45±0.03	20.25±0.21	32.43±0.24	23.3±0.29
3.	<i>S.aureus</i>	20.24±0.02	32.33±0.57	18.00±0.57	31.2±0.16
4.	<i>P.aeruginosa</i>	30.23±0.20	15.43±0.32	11.58±0.02	28.46±0.06

Table 1 it is evident from the results that all desi garlic (*Allium sativum* L.) bulb extracts showed activity against selected bacteria. The chloroform bulb extract of DG (*Allium sativum* L.) showed maximum activity against *E. coli* that is 36.33±0.28. The chloroform bulb extract of DG (*Allium*

sativum L.) showed maximum activity as compared to other extracts. The chloroform bulb extract of DG showed minimum activity against *P. aeruginosa* 11.58±0.02 as shown in Fig 3. These results were compare able with previous study Gaherwal *et al.*, (2014) [3].

Table 2: Antibacterial Activity Shown as Zone of inhibition (mm) of Bulbs of *Allium sativum* L. (White garlic cultivars)

Sr.No	Strains	Ethanol	Methanol	Chloroform	Distill Water
1.	<i>E. coli</i>	15.66±0.57	12.06±0.04	27.67±0.04	12.6±0.16
2.	<i>B.subtilis</i>	9.56±0.32	11.80±0.04	10.33±0.57	11.33±0.57
3.	<i>S.aureus</i>	26.00±0.00	12.31±0.03	10.02±0.24	21.66±0.57
4.	<i>P.aeruginosa</i>	15.43±0.32	9.33±0.57	11.80±0.04	10.66±0.57

In Table 2 The chloroform bulb extract of White garlic (*Allium sativum* L.) showed maximum activity against *E. coli* that is 27.67±0.04. The chloroform bulb extract of WG (*Allium sativum* L.) showed maximum activity as compared to other extract. The Ethanolic bulb extract of WG (*Allium sativum* L.) showed less activity against *B. subtilis* that is 9.56±0.32. These results are more or less similar to results of Rathee *et al.*, (2010) [7].

In second parameter of research, anatomical analysis, leaf of two cultivars were tested for their epidermal features. It was found that epidermal anatomy of two populations were different with reference of stomata number, types and frequency depending on type of soil and area of experiment. Two zones of climatic were used for comparison analysis for foliar dermal study.

Table 3: Stomatal Size of Two Cultivars of *Allium Sativum* L. Leaf from Two Sampling Area of District Bhimber Azad Kashmir

Sr. No	<i>Allium sativum</i> L.	Areas	Stomatal Size (µm)
1.	Desi garlic	Bandala	9.41±0.74
2.	Desi garlic	Bhimber	5.71±0.74
3.	White garlic	Bandala	6.67±0.72
4.	White garlic	Bhimber	2.55±0.85



Fig 1: Two Cultivars of *Allium sativum* L (Left: Desi Cultivar and Right White Cultivar)

It is revealed that the desi garlic’s (*Allium sativum* L.) Stomatal size of Bandala showed maximum size as compared to desi garlic’s (*Allium sativum* L.) Stomatal size of Bhimber. The maximum Stomatal size of desi garlic’s of Bandala was 9.41±0.74µm and the minimum size of desi garlic of Bhimber was 5.71±0.74µm (Table 3). As it is white garlic’s (*Allium sativum* L.) stomatal size of Bandala showed maximum size as compared to white garlic’s (*Allium sativum* L.) stomatal size of Bhimber. The maximum stomatal size of white garlic’s of Bandala was 6.67±0.72 µm and the minimum Stomatal length of white garlic’s (*Allium sativum* L.) was 2.55±0.85 µm. These results more or less similar to results of Anjala and Wang (2014).

Table 4: Stomatal index of Two Cultivars of *Allium sativum* L. Leaf from Two Sampling Area of District Bhimber Azad Kashmir

Sr. No	<i>Allium sativum</i> L.	Areas	Stomata index
1	Desi garlic	Bandala	97.74
2	Desi garlic	Bhimber	92.89
3	White garlic	Bandala	85.63
4	White garlic	Bhimber	47.33



Fig 2: Two Cultivars of *Allium sativum* L (Left: Desi Cultivar and Right White Cultivar)

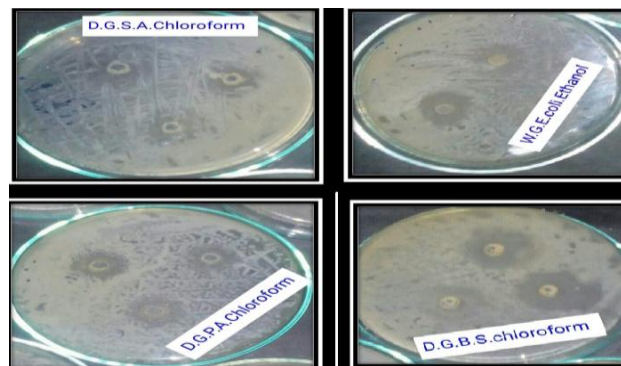


Fig 3: Antibacterial Activity of Two Cultivars of *Allium sativum* L (Left: Desi Cultivar and Right White Cultivar) shown as Zone of Inhibition (mm)

Table 4 revealed that the desi garlic’s (*Allium sativum* L.) stomata Index of Bandala showed maximum stomata index as compared to desi garlic’s (*Allium sativum* L.) stomata index of Bhimber. The maximum stomata index of desi garlic’s of Bandala was 97.74 and the minimum stomata index of desi garlic of Bhimber was 92.89. As it is white garlic’s (*Allium sativum* L.) stomata index of Bandala showed maximum stomata index as compared to white garlic’s (*Allium sativum* L.) stomata index of Bhimber. The maximum stomata index of white garlic’s of Bandala was 85.63 and the minimum stomata index of white garlic’s (*Allium sativum* L.) was 47.33. Desi garlic (*Allium sativum* L. showed more stomatal size due to environmental factors and index as compared to White garlic (*Allium sativum* L.) Due to these DG (*Allium sativum* L.) more antibacterial activity on these basis DG is much better than White garlic (*Allium sativum* L.) These

results more or less similar to results of Saini *et al.*, (2013). It has been proved that DG cultivars has comparatively more antibacterial activity than WG cultivar that might be due to variation in number of stomata and stomatal index. As it has been seen that former has more number of stomata than later one and that is correlated with more quantity of allicin and other phytochemical production due to more and frequent potential of photosynthetic activity depending on stomata size and stomatal index of leaf, which is better in DG population than WG accession.

4. Conclusion

Allium sativum L. (Desi garlic) have more antibacterial activity as compared to *Allium sativum* L. (White garlic) due to large stomata size and stomata index. The antibacterial activity of this species is due to presence of organosulphur compounds. Major constituent of this species is allicin compound. The present study showed that the well diffusion method is better than cup plate method because antibacterial activity was checked by well diffusion method

5. References

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