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Review Article

Antibacterial, Antioxidant and Cytotoxic Properties of Single Clove Garlic in Kashmir Himalayas

Ab. Rouf Wani[1] and Sugandha Singh[2]

[1]*Department of Botany, Madhyanchal Professional University, Ratibad, Bhopal, M.P.*
[2]*Department of Biotechnology, Madhyanchal Professional University, Ratibad, Bhopal, M.P*

Abstract

Aim: The primary aim of this research investigation was to evaluate the pharmacological potential of aqueous and acetone extracts of single clove garlic of Kashmir valley like antibacterial, antioxidant and cytotoxic. **Introduction:** The garlic or *Allium* sativum (growing wild in Kashmir Valley) is a species is a medicinally and pharmacologically important species. It has been reported of significant antioxidant, antimicrobial, hepatoprotective, anti-cold and cytotoxic activities. **Experimental:** The two different solvent extracts of single clove garlic were prepared using water and acetone. The antioxidant activity of the extracts was evaluated by DPPH scavenging and hydrogen peroxide scavenging assays. Antibacterial activity was tested against gram positive *Styplococcus* aureus and *Bacillus* subtilis and gram negative bacteria *Pseudomonas* aeruginosa by performing well diffusion technique. Finally, the cytotoxic activity of the extracts was investigated against A549 lung cancer cells using WST-1 assay. **Results:** The results indicated that both the extracts showed significant DPPH scavenging and hydrogen peroxide scavenging activities with acetone extract showing close activity to that of reference control. The well diffusion method involved in the antibacterial analysis of the extracts revealed higher inhibition of gram positive bacteria than that of gram negative bacterial strains but highest inhibition was here recorded for *Styplococcus* aureus by acetone extract of the single clove garlic. Moreover, the results from WST-1 assay indicated that acetone extract has a significant potential to inhibit proliferation of A549 cancer cells by inducing cytotoxicity. Acetone extract reduced the viability of A549 cells to 10% at higher concentration. **Conclusion:** We concluded that the single clove wild garlic growing in Kashmir Himalaya has a remarkable pharmacognostic features. The results showed aqueousand acetone extracts exhibit antibacterial, antioxidant and cytotoxic activities.

Keywords: *Allium sativum, extract, antioxidant, cytotoxic, antibacterial.*

Introduction

The term medicinal plants include plants that are being involved in herbalism and possess medicinal properties [Sofowora, A. *et al.*, 2013]. Medicinal plants are of significant importance to human beings and other beings on earth as well. Medicinal plants are the "backbone" of traditional medicine, which suggests quite 3.3 billion people within the less developed countries consume medicinal plants on a daily basis [Davidson-Hunt, I. *et al.*, 2000]. Medicinal plants are diverse in chemical content which aid pharma companies in drug design and discovery [Principe, P. P. 1991]. These plants have significant importance in different human cultures worldwide. India is a huge country area wise and comprises of wide range of ecosystems nurturing thousands of plant species. Above 17,000 plants have been identified and recognized in India out of which over 8,000 species belong to the class of medicinal plants [Poorani, N. *et al.*, 2016]. Kashmir Valley hasa

***Corresponding Author:**
Ab. Rouf Wani;

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very much soothing environmental conditions for growth and development of medicinal plants and high altitude as well [Gupta, S.K. *et al*., 2013]. Kashmir Valley also shows huge variety in flora. *Allium* sativum is one of the medicinal and edible plants growing in Kashmir Valley. Different varieties of this plant are cultivated in the area and grow in wild as well. Single clove garlic grows wild in the mountainous regions of Kashmir Valley and is used as an ingredient in folk medicine. Garlic has been reported of significant pharmacological applications like immune modulatory activities, antioxidant, antidiabetic and anticancer activities [Abd El-Halim, S.S. *et al*., 2012; Taha, N. *et al*., 2013]. It has been revealed to prevent LPO and dose- dependent induction of endogenous antioxidants in rat kidney and liver [Khanum, F. *et al*., 2004]. The higher concentration of organosulfur compounds in garlic, in comparison to other Allium species is responsible for both garlic's pungent odor and flavor as well as many of its medicinal effects [Vazquez-Prieto, M.A. *et al*., 2010]. Moreover, garlic has chemopreventive potential against cyclophosphamide induced chromosomal mutations in Swiss albino mice [Shukla, Y. *et al*., 2002]. Therefore, keeping in view the pharmaceutical activities of garlic current study was designed to evaluate the pharmaceutical abilities of aqueous and acetone extracts of single clove garlic growing in Pir-Panchal range of Kashmir Valley. We investigated antioxidant, antibacterial and cytotoxic potential of the plant extracts.

Experimental Plant Material

The single clove garlic *Allium* sativum was collected from the Pir-Panchal mountain ranges of Himalayas in Jammu and Kashmir. The plant was identified by Dr. Kanchan Yadav (Prof. Dept. of Botany, Madhyanchal Professional University, Bhopal, M. P. India).

Extract Preparation

The bulb of *Allium* sativum (single clove garlic) was collected and washed with tap water to remove dirt and soil. Then this material was dried in shade for 30 days at 30°C followed by crushing using a pestle and mortar. The crushed garlic was then placed in water and acetone separately in 20% w/v concentration. Both the mixtures were separately placed on refluxing for 48h followed by evaporation of the solvents used in a rotatory evaporator. The extracts left behind were stored for further use.

Antioxidant Activity

Antioxidant potential of aqueous and acetone extracts of single clove garlic was evaluated by DPPH and H_2O_2 scavenging assays.

DPPH Scavenging

In brief, the various experimental concentrations (50, 100, 200, 300, 400 µg/ml) of aqueous and acetone extracts of single clove garlic were prepared using DMSO. Similarly, the reference control concentrations were also prepared. Separately, we preapered 0.3 mM DPPH solution was prepared with absolute methanol. These two mixtures were mixed and placed under incubation for half an hour at room temperature. Finally, the change in coloration was detected spectroscopically at 518nm of wavelength. DPPH in methanol and methanol were used as positive control and blank, respectively. The free radical scavenging was finally calculated by using following equation:

$$\%\ DPPH\ inhibition = ODcontrol - ODsample/ODcontrol \times 100$$

Where, OD is the optical density.

Hydrogen Peroxide Scavenging

In brief, the various experimental concentrations (50, 100, 200, 300, 400 µg/ml) of extracts and reference control were prepared in DMSO. Separately, a mixture of 40 mM of H_2O_2 was prepared in phosphate buffer of 0.1 M and pH 7.4. The extract and H_2O_2 mixtures were mixed together and incubated for 20 min. the absorbance was recorded at 230 to detect the radical scavenging activity. The percentage inhibition was calculated using following equation:

$$\%\ inhibition = (Absorbance\ of\ reaction\ mixture / Absorbance\ of\ sample) \times 100$$

Antibacterial Activity

The antibacterial activity of the two single clove extracts was detected against two gram positive and one gram negative bacterial strains by using disc-diffusion method. Standardized inoculums of the bacteria (0.1 ml) were plated evenly with the help of a glass spreader onto the Muller-Hinton agar plates. The seeded plates were allowed to dry in the incubator at 37 ^{0}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 ^{0}C for 24 hrs. And zone of inhibition diameter was measured to using nearest millimeter (mm).

Cytotoxic Assay

Cell Culture

The A549 lung cancer cells were procured from American Type Culture Collection (ATCC). Cells were cultured in 10% fetal bovine serum maintains RMPI-1640 medium placed with antibiotics penicillin and streptomycin. The cell culture was placed under humidified environmental conditions within a 5% CO_2 incubator at 37^{o}C.

WST-1 Assay

The proliferation inhibitory effects of aqueous and acetone extracts of single clove garlic of Kashmir against A549 cells were evaluated by WST-1(water soluble tetrazolium) assay. In brief, A549 cells were cultured with a concentration of 1 × 10^5 cells/well of 96- well plates. Each well was then treated with experimental concentrations of the extracts viz 0, 50, 100, 200 and 400 µg/ml for 48 h at 37^{o}C. Afterwards, 10 µL of WST-1 reagent was added to the wells and mixed thoroughly. This mixture was placed on incubation for 2 h at 37^{o}C. Finally, the absorbance of each well was taken with the help of a microplate reader (BioTek Instruments, Inc., Winooski, VT, United States) at a wavelength of 430 nm.

Results and Discussion

Antioxidant Activity

Antioxidants or oxidation inhibitors are those agents which inhibit or hurdle the process of oxidation and enhance the life of oxidizable matter [Neha, K. *et al*., 2019]. Free radicals and oxidantsare those agents which to oxidize everything coming in their way and have short life span with higher damaging potential and reactivity towards macromolecules including lipids, nucleic acids and proteins [Hemnani, T.A. *et al*., 1998]. Reactive oxygen species and reactive nitrogen species are both oxidants in nature. In living cells free radicals and oxidant species are constantly formed but are quenched by the naturally occurring antioxidants in living cells. The overproduction of these species contributes in cellular and tissue damages and even carcinogenesis [Ziech, D. *et al*., 2011]. The naturally occurring antioxidants in medicinal plants play a significant role in quenching these oxidants and are considered as an alternative source of medicine to target these oxidative species [Atta, E.M. *et al*., 2017]. Herein, we investigated single clove garlic for its antioxidant potential via DPPH and H_2O_2 scavenging assays.

DPPH Scavenging Activity

Results indicated that both the aqueous and acetone extracts of single clove garlic showed significant antioxidant activity against DPPH radicals (Figure 1). The antioxidant potential of these extracts showed concentration dependence and high percentage of inhibition was noted down at higher extract concentrations. The acetone extract showed higher antioxidant activity than that of aqueous extract. In case of the aqueous extract the DPPH inhibition increased from 26% to 63% (50-400 µg/ml) while for acetone extract it reached to over 79% from 30%. In comparison to that of reference control (Ascorbic acid) comparable and significant antioxidant activity was noted for acetone extract.

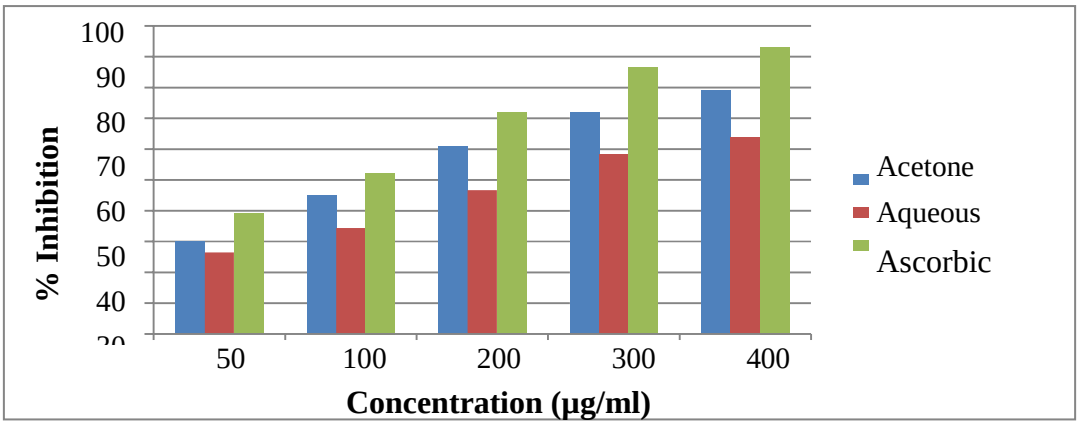

Figure 1: The DPPH scavenging activity of aqueous and acetone extracts of single clove garlic growing in Kashmir

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide radical scavenging assay results also predicted remarkable capability of aqueous and acetone extracts of single clove garlic to quench hydrogen peroxide radicals (Figure 2). The H_2O_2 scavenging capabilities of the extracts were found to be concentration dependent. Higher scavenging activity was reported foracetone extract to that aqueous extract of the garlic bulbs. The H_2O_2 scavenging of acetone extract reached to just above 90% at 400 µg/ml while aqueous extract reached to 69%. Comparing both the extract with reference control ascorbic acid significant activity was shown by acetone extract.

Therefore, these result indicate remarkable antioxidant potential and health benefits of the wild single clove garlic growing in Kashmir.

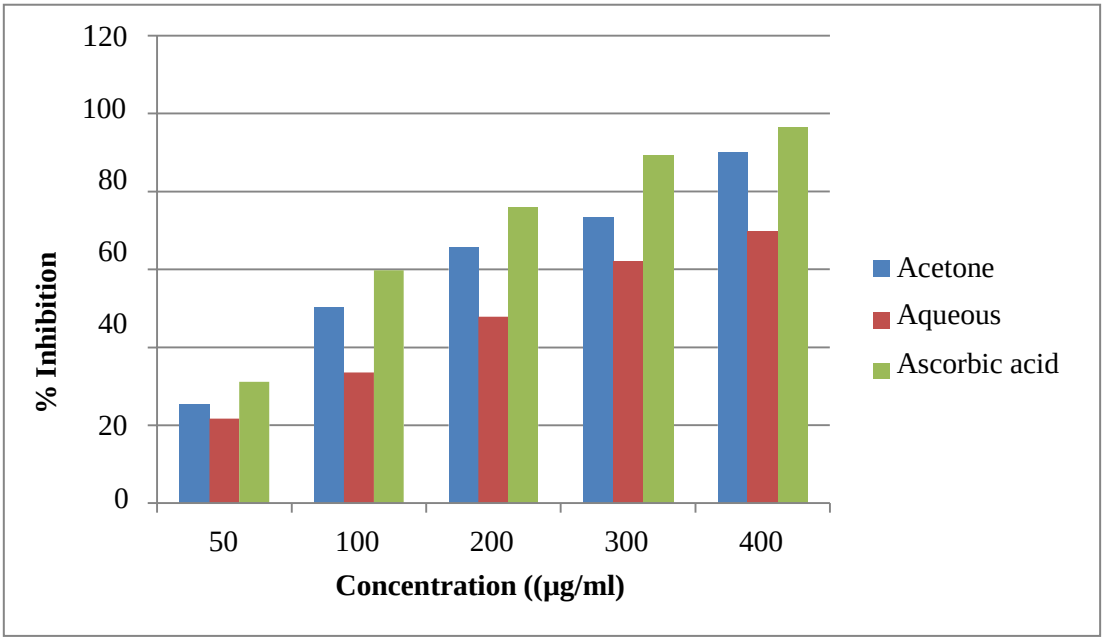

Figure 2: The H2O2 scavenging activity of aqueous and acetone extracts of single clove garlic growing in Kashmir

Antibacterial Activity Bacteria are the pathogens that both act as beneficial and hazardous to human and other forms of life on earth [Chaudhary, P.P. *et al.*, 2018]. Several medicinal plants have been identified with protective effects against harmful bacteria in humans and food as well [Hossan, M.S. *et al.*, 2018]. Herein, we investigated the efficacy of aqueous and acetone extracts of single clove garlic growing in Kashmir against two gram

positive bacterial strains and one gram negative strain.

Both the extracts exhibit protective effects against bacteria in a concentration- dependent manner. Results shown in table 1 indicate that acetone extract of the single clove garlic showed highest inhibitive effects against both gram positive and gram negative bacterial strains but against *A. aureus*, it reported maximum inhibition.

Table 1: The antibacterial activity of aqueous and acetone extracts of single clove garlicgrowing Wild in Kashmir

S. No	Name of microorganism	Zone of Inhibition (mm)	
		Aqueous	Acetone
1.	*S.aureus (gram +ve)*	32.51±0.35	35.63±0.45
2.	*B.stubtils (gram +ve)*	21.33±0.37	29.17±0.43
3.	*P.aeruginosa (gram –ve)*	17.37±23	26.22±0.45

Cytotoxic Activity

Unfortunately, the cancer cases are rising globally and each year thousands of people suffer death from this malignancy [Islami, F. *et al*., 2018]. Due to high structural diversities and medicinal potential of plants, scientists and researchers have turned to plants in search of potent cytotoxic and anticancer agents [Khursheed, A. *et al*., 2020]. Numerous phytochemicals isolated from plants have been identified of significant anticancer and cytotoxic activities. Currently, the potent chemotherapeutic agents like taxol, vinblastine etc. have been either derivedfrom medicinal plants or based on chemicals derived from them [Gupta, A.K. *et al*., 2019].

In this research, we investigated cytotoxic effects of aqueous and acetone extracts of single clove garlic against human lung cancer cell line A549 using WST-1 assay. The A549 cell line was exposed to altering concentrations of both the extracts ranging from 0-400 µg/ml for 48 h. Results indicated that extracts showed promising ant proliferative effects against the target cell line in a concentration-reliant manner (Figure 3). The proliferation dropped from almost 98% to 10% in case of acetone extract while from 99% to 30% in aqueous extract. Therefore, significant cytotoxicity was induced by both the extracts except acetone extract was extraordinary.

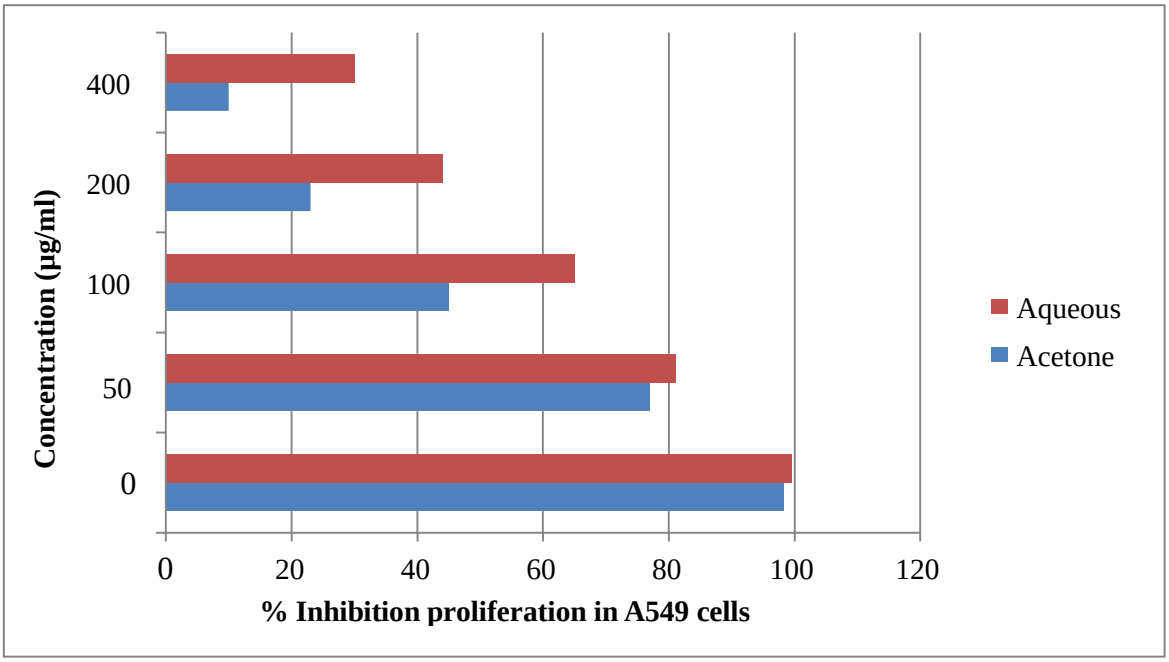

Figure 3: Ant proliferative activity of aqueous and acetone extracts of single clove garlicgrowing in Kashmir against A549 cells

Conclusion

The outcomes of this study indicated that the aqueous and acetone extracts of single clove garlic growing in Kashmir Valley possess significant antioxidant, antibacterial andcytotoxic effects. The acetone extract showed extraordinary pharmacological potential by inducing comparable antioxidant effects to that of reference control and cytotoxic effects.

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