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Effect of *Allium sativum* paste against Antimicrobial, Antioxidant and Cytotoxicity activity

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Abstract: In the present work, *Allium sativum* paste has been prepared and analyzed to determine their antioxidant, *in vitro* antibacterial, *in vitro* cytotoxicity and apoptosis activities. Butylated hydroxy toluene has been used as standard. Results show the highest % of inhibition in DPPH assay 97.06±0.001 and metal chelating assay 82.35±0.005 at 100µg concentration. Extracts exhibited strong antimicrobial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* than against *Salmonella typhi*. 3- (4-5-dimethyl thiazol-2-yl) -2,5-diphenyl tetrazolium Bromine (MTT) assay demonstrated the inhibition of 43.18% the proliferation of the Siha cervical cancer cell in time and dose dependent manners.
Key words: *Allium sativum*, Antioxidant, Antimicrobial, cytotoxicity.

Introduction

Now a day's ready to use products are attracted by consumers just because of their availability and to save time. *Allium sativum* is a bulbous plant belonging to the family Liliaceae and Subfamily Allioideae[1]. It is a fundamental component in many dishes of Southeastern Asia, the Middle East, Northern Africa and Southern Europe. The bulb has a characteristic spicy and pungent flavors, noticeably sweeter with cooking[2]. Garlic has the ability to prevent the viral, fungal and helminth infection. Garlic treated with meat leads to diminished the bacterial counts[3],[4]. The present study characterizes the phenolic compound of the *Allium sativum* paste and evaluates their antioxidant activities, *In vitro* cytotoxicity activities, and assess their antimicrobial activities against eight pathogenic strains using the well diffusion method.

Materials and Methods

Plant materials

Allium sativum samples were collected in Coimbatore and authenticated by the Botanical Survey of India, TamilNadu.

Preparation of sample

Allium sativum paste was prepared using the standard procedure[5]. In a typical procedure, the outer peel of the garlic was removed then it's cut into small pieces. Fresh garlic paste is prepared by grinding smaller pieces using a blender mixer. The pH of fresh paste was found to be 5.3. Then, 100g of the fresh paste is mixed with 10g sodium chloride and 0.5g of citric acid. Then the paste was extracted with ethanol using soxhlet apparatus and the solvent was separated by rotatory evaporator. The yield of the extract was found to be 10.8%.

Collection of bacterial strains

The bacterial strain *E. coli* (MTCC-1303), *K. pneumonia* (MTCC-109), *P. aeruginosa* (MTCC-429), *S. typhi* (MTCC-733), *B. subtilis* (MTCC-121), *S. aureus* (MTCC-737), *A.niger* (ATCC -9029), *C.albicans* (MTCC-227) were procured from MTCC, Chandigarh, India. Above mentioned strains was maintained in nutrient agar slants at 37°C. Bacteria and fungus were reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth, potato dextrose agar broth and incubated at 24h at 37°C also 48h at 37°C for fungus. *In vitro* antibacterial activity was done by standard method[6]. **Antioxidant activity**

Antioxidant activity was determined by DPPH assay[7], Phosphomolybdneum assay[8], Metal Chelating assay[9].

Cytotoxicity analysis

The *in vitro* cytotoxicity analysis was carried out using MTT assay[10]. To each well of the 96 well plates, 10000 cells were added and incubated in a CO_2 incubator for 24 hours at 37º C. The medium was flicked off, and 95 µl of fresh medium, 5 µl of the drug were added. The plate was incubated in the CO_2 incubator for 48 hours at 37º C. The 20 µl mediums were removed and 20 µl of MTT was added. The plate was incubated in the CO_2 incubator for 4 hours at 37º C. The entire medium was carefully removed and the formazan crystals were dissolved in DMSO. The plate was read at 570 nm. The percentage growth inhibition was calculated by using the formula, % Cell inhibition = 100- Abs (sample) / Abs (control) × 100).

Apoptosis

800µl of RPMI media is mixed with 100 µl of DLA cells; 15µg/ml drug was added and kept for 24hrs in CO_2 incubator. The sample was allowed to centrifuge at 13000rpm for 15 minutes. The pellet has smear with glass slides. Haematoxylin and Eosin were sprayed over the slides for staining.

Statistical analysis

The statistical analysis was performed using one way analysis of variance (ANOVA) followed by DMRT using a software Graph pad, Instat version 3.0, (*$P<0.05$* are considered statistically significant). Values were expressed as mean ± SD (n=3).

Results and Discussion

The inhibition concentration less are considered as good antioxidant due to radical scavenging activity. IC_{50} values of DPPH assay are 38.26±0.001 and BHT 53.87±0.005 at 100µg concentration due to flavonoids and phenolic compounds which in turn increases the radical activity[11]. [12] observed that vitamin and thiol compound would be helpful to maintain the phenolic concentration. The metal chelating assay is given in Figure 1. The percentage of inhibition is 82.35±0.005 compared with standard (BHT) 45.09±0.002. The above results proved that the antioxidant activity is more in sample treated one.

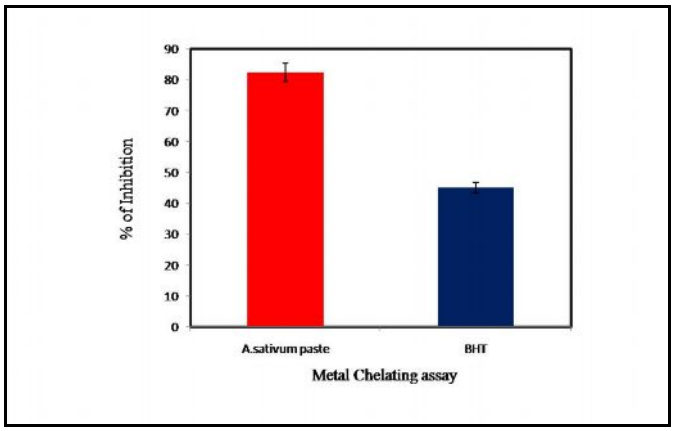

Figure 1. Metal chelating assay

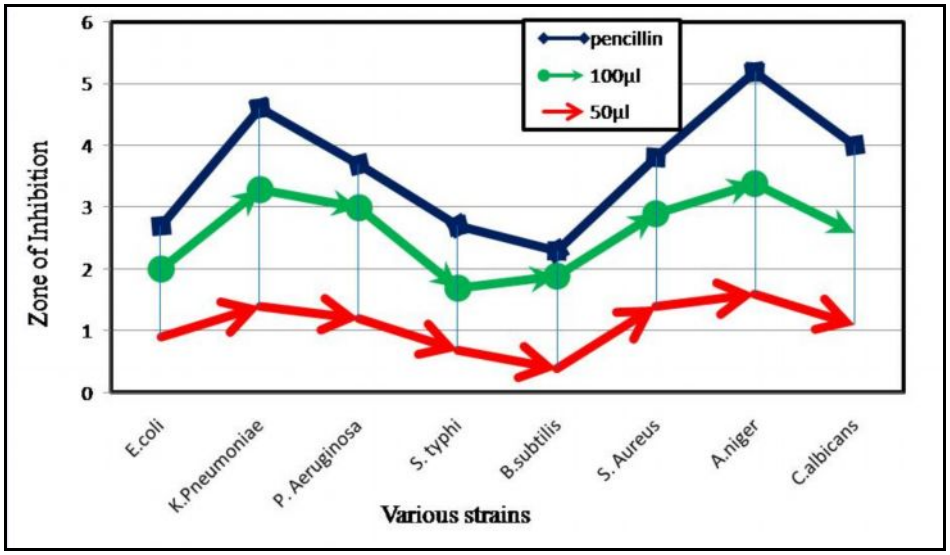

Figure 2. Antibacterial activity of *Allium sativum* paste

The Zone of inhibition is shown in Figure 2. When data are assessed, it can be seen that high zone of inhibition was observed in *K. pneumonia, B. subtilis, A. niger.* But weakest activity found in *Salmonella typhi*. The sulfur containing compound diallyl mono sulfide and diallyl di sulfide is responsible for antibacterial activity[13]. [14] found that oil portion of garlic had the more antibacterial activity. [15]proved that again, a garlic derived sulfur containing compounds, have antimicrobial activity against gram positive bacteria like *Bacillus subtilis* and gram negative bacteria like *E. coli*, *Klebsiella pneumoniae.* [16]revealed that allicin had fungicidal activity against *candida albicans,* ajone inhibit the *A.niger* and *Candida* at less than 20 microorganism/ml.

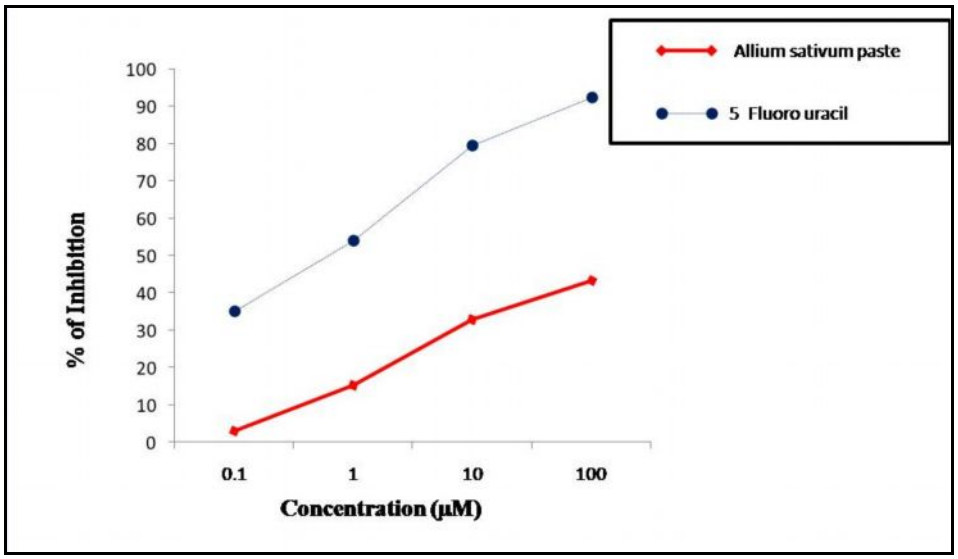

Figure 3. *In vitro* cytotoxicity activity of *Allium sativum* paste

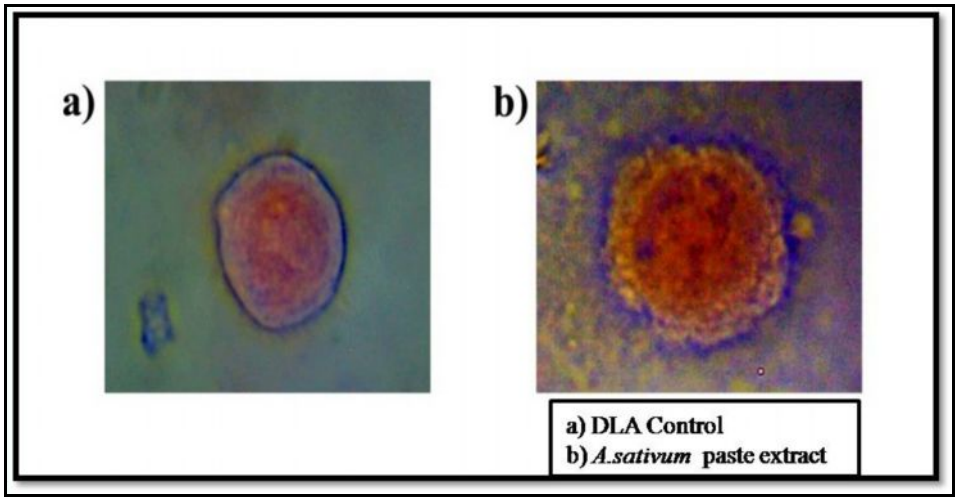

Figure 4. Apoptosis study

In vitro cytotoxicity is given in Figure 3. The Sample inhibits the Siha cancer cell in 43.18% compared with 5-Fluoro uracil. The cytotoxic effect of various concentrations 0.1, 1, 10 and 100µM of extract had 3.06, 15.39, 32.84 and 43.18 % inhibition against the SiHa cervical cancer cell. Total percentage of inhibition was more highly concentrated (100µM) sample. Apoptosis study is shown in Figure 4. It reports that membrane blebbing, nuclear fragmentation and macrophages were observed in samples treated DLA cells.

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