

ANTIMICROBIAL ACTIVITY OF GARLIC EXTRACT STUDIED ON *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Candida albicans*

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Abstract: Garlic (*Allium sativum*) is a species in the onion genus *allium*. Garlic is a herb that is grown around the world it is related to onion leekes and chives. It is thought that garlic is native to Siberia but spread to other parts of world over 5000 years ago. Garliic is used for many conditions related to heart ad circulatory system. Conditions includes high BP, low BP, high cholesterol, coronary heart diseases. Garlic is said to be having antineoplastic activity used to treat various cancer conditions it is also having anti inflammatory activity, antimicrobial activity etc. But the studies available on antimicrobial activity are limited as of now so the present investigation is to determine antibacterial and antifungl activity of allicin(sulphur containing organic compound present in garlic and important chemical constituent having antimicrobial properties) against *staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Candida albicans* by using agar well diffusion method and allicin shows clearly antimicrobial activity aganist *staphylococcus aureus*, *kebsiella pneumonia*, *Escherichia coli*, *candida albicans*.

Keywords: Garlic, Allicin, Antimicrobial activity, Extraction, *Staphylococcus aureus*, *E.coli*.

1. REVIEW OF LITERATURE

Seema yadav, Niyati, A., Antimicrobial resistance has been a global concern. Currently, interest has been focused on exploring antimicrobial properties of plants and herbs. One such botanical is *Allium sativum* (garlic).

Amir sasan mozafarri nejad, Shahrokh shabani, Using garlic is widespread in Iran and other countries as a medicine and a natural spice. Garlic is a potential inhibitor for food pathogens. Foods contaminated with pathogens pose a potential danger to the consumer's health. The use of garlic can increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods.

M. Shokrzadeh and A.G. Ebadi Garlic (*Allium sativum* L.) has an important dietary and medicinal role for centuries. It is a large annual plant of the Liliaceae family, which grows in most of Europe and in northern Iran. Iranian garlic is used in traditional medicine for infectious diseases, flu and as an anti-febrile. The present study tested the aqueous extract of garlic in vitro for its antibacterial activity. The extract showed concentration-dependent antibacterial activity against *Staphylococcus aureus* 8327. This activity was heat resistant, but the activity of freeze-dried extract gradually diminished during a 90 days period. The traditional use of Iranian garlic for infectious diseases and for controlling fever appears to be justified.

M.N.Palaksha, Masoor Ahmed, This study focuses the significant antibacterial activity of Garlic (*Allium sativum* Linn.) extract on streptomycin-resistant strains solely and in synergism with streptomycin. Gram-positive *Staphylococcus aureus* ATCC BAA 1026 and gram-negative *Escherichia coli* ATCC 10536 were made resistant to standard antibiotic streptomycin used as a control in the experiment. Zones of inhibition of different treatment groups were measured by agar-well-diffusion assay and compared with control. Statistical comparison of sole extract and streptomycin synergism with streptomycin control had proved it significant.

2. MATERIALS

Table 2.1: Equipments used:

Name of the Equipment	Name of the Manufacturer
Autoclave	REMI Instruments Ltd., Mumbai
Hot air oven	BIOMED INC.
Incubator maintained at 37 ⁰ c	REMI Instruments Ltd.,Mumbai
Orbital Rotary Shaker	REMI Instruments Ltd.,Mumbai
Centrifuge	REMI Instruments Ltd.,Mumbai
Centrifuge tubes	Tarsons products Pvt.Ltd.,Kolkata
Rotary Evaporator	Heidolph (Labrota 4000)
Laminar Airflow Unit	KLENZAIDS
Analytical weighing Balance	Shimadzu (aux 220),Japan
Millipore	Quantum – IX
Cooling Centrifuge	REMI Instruments Ltd.,Mumbai
Cooling Incubator	REMI Instruments Ltd.,Mumbai
Microtips (200 – 1000 _μ L)	Tarsons products Pvt.Ltd.,Kolkata
Micropipette (10 – 1000 _μ L)	Tarsons products Pvt.Ltd.,Kolkata
Deep freezer	Cell Frost(CF – 200)
UV- Spectrophotometer	Elico
Trinocular microscope	Labomed – CX _{R3}

Table 2.2: Glassware used

Borosil	Test tubes,boiling tubes,conical flasks,beakers,petriplates,measuring cylinders,funnels,separating funnels,loops,Borers,pH papers
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Table 2.3: Organic solvents used:

Name of the organic solvent	Name of the Manufacturer
Ethyl acetate	Merck Limited,Mumbai
Ethanol	Merck Limited,Mumbai
Methanol	Merck Limited,Mumbai

Table 2.4: Chemicals used:

Name of the chemical	Name of the Manufacturer
Peptone	Qualigens fine chem.Mumbai
Sodium chloride	Himedia lab.,Mumbai
Meat extract	Himedia lab.,Mumbai
Nutrient Agar	Himedia lab.,Mumbai
Soluble starch	Himedia lab.,Mumbai
Hydrochloric acid solution 0.1M	Qualigens fine chem.Mumbai
Potassium nitrate	Qualigens fine chem.Mumbai
Sodium hydroxide solution 0.1M	Qualigens fine chem.Mumbai
Hydrated Magnesium sulphate	Himedia lab.,Mumbai
Calcium carbonate	Qualigens fine chem.Mumbai
Ferrous sulphate	Himedia lab.,Mumbai
Soya bean meal	Himedia lab.,Mumbai
Dextrose	Qualigens fine chem.Mumbai

Glycerol	Qualigens fine chem.Mumbai
Sodium nitrate	Qualigens fine chem.Mumbai
Zinc sulphate	Himedia lab.,Mumbai
Di potassium hydrogen phosphate	Himedia lab.,Mumbai
Yeast extract powder	Qualigens fine chem.Mumbai

Table 2.5: List of test organisms used:

Test Organism	Short forms	Strain No.	Source
Staphylococcus aureus	S.aureus	NCIM 2079	NCIM,Pune
Escherichia coli	E.coli	NCIM 2065	NCIM,Pune
Candida albicans	C.albicans	MTCC 227	MTCC, Chandigarh
Klebsiella pneumonia	k.p	NCIM	NCIM pune

NCIM: National Collection of Industrial Microorganisms

MTCC: Microbial Type Culture Collection

3. EXPERIMENTAL METHODS

Extraction of allicin from garlic:

- ❖ Fresh Garlic (*Allium sativum*) bulbs were purchased from local market, narsaraopeta.
- ❖ The bulbs were peeled, weighed (200g) and cleaned.
- ❖ Cleaned cloves were surface-sterilised by immersing them into 70% (v/v) of ethanol for 1 min.
- ❖ Residual ethanol on surface was evaporated in sterile laminar air flow chamber followed by crushing of garlic with the use of distilled water aseptically in sterile mortar and pestle.
- ❖ Homogenization of crushed garlic had done and this mixture filtered through sterile muslin cloth.
- ❖ The extract was considered as the 100% concentration of the extract.
- ❖ The concentrated mother extract was further diluted to 75% and 50% by mixing with appropriate sterile distilled water.

Inoculum preparation:

Bacterial inoculums preparation: A pure colony of the test organism was taken using a sterile loop and transferred into tubes having a sterile nutrient broth and incubated with shaking at 35⁰c-37⁰c until the visible turbidity was equal to that of the 0.5 McFarland standard.

Fungal inoculums preparation: Three to seven days slant of fungal culture on potato dextrose agar was taken and scrapped to form a suspension in sterile water. The mixture was vortexed and heavy particles were allowed to settle. The suspension was adjusted to 0.5 McFarland standards.

these inoculums are used to inoculate the petri plates containing the Nutrient agar medium(In case of Bacteria) and Sabaroud agar medium (in case of fungi)

DETERMINATION OF ANTIMICROBIAL ACTIVITY:

Agar well diffusion method/cup plate method:

The active compound obtained after extraction is tested for its antimicrobial activity. Nutrient agar medium was prepared and sterilised and inoculated with the test organisms. This is poured into the petri plates under aseptic conditions allowed to solidify then cups were made, into the cups active compound is added using a micropipette and the petri plates kept into refrigerator for 30 min to improve the diffusion, then the plates were kept for incubation at 37⁰C, 24 hrs in case of bacteria and 28⁰C for 48 hrs in case of fungi by this agar well diffusion method we can assure that the aqueous extract of garlic is having antibacterial and antifungal activity by measuring zone of inhibition.

4. RESULTS

Zone of inhibition were measured in mm and recorded separately for bacteria and fungi in comparison with sterile water as control.

S.NO	EXTRACTS	DOSE	ZONE OF INHIBITION(mm)			
			BACTERIA			FUNGI
			(STAPHYLOCOCCOS AUREUS)	(E.COLI)	(KLEBSIELLA)	(CANDIDA ALBICANS)
1	Distilled water	100µl	–	–	–	–
2	25% garlic extract	100µl	20	22	21	24
3	50% garlic extract	100µl	24	27	23	26
4	75% garlic extract	100µl	28	26	25	28
5	100% garlic extract	100µl	40	45	42	43

5. SUMMARY

The summary of work, initially extraction of garlic had done. Then the fresh cultures of bacteria and fungi were prepared and antimicrobial activity of aqueous extract of garlic was determined by using agar well diffusion method, and it is clearly showing antimicrobial activity.

6. CONCLUSION

Results from antimicrobial activity revealed that our aqueous extract of garlic was found to have broad spectrum of antimicrobial activity on both bacteria (g+ve, g-ve) and fungi. Further studies are required to get complete information and regarding active constituents present in garlic extract and analysis of the extract of garlic for more activities like anti-inflammatory (wound healing), hepatoprotective, anti-ulcer and galactagogue properties.

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