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Research Article

STUDIES ON ANTIMICROBIAL ACTIVITY OF GARLIC (*ALLIUM SATIVUM* L.) EXTRACT AGAINST *BACILLUS SUBTILIS* AND *ASPERGILLUS NIGER*

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A B S T R A C T

Garlic (*Allium sativum* L) is a species of monocot, bulb-forming perennial scented herb belonging to family Amaryllidacea. It is has anti-inflammatory properties. It stimulates heart and helps in maintain the proper functioning and also helps in expelling out of the extra amount of mucus that gets accumulated in the respiratory tract. The antibacterial potential of two extracts from leaf and bulb of garlic against pathogenic bacteria such as Bacillus subtilis and *Aspergillus niger* were evaluated by agar well diffusion method. The organic (Ethanol, Methanol, Ethyl acetate, Chloroform, Hexane and Petroleum ether) extracts of the leaves and bulbs were found to possess strong antibacterial activity against a range of pathogenic bacteria. The ethyl acetate leaf extract of garlic showed pronounced inhibition than chloroform, petroleum ether and hexane. The leaf extracts activity being more on Bacillus subtilis than *Aspergillus niger*.

INTRODUCTION

Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases. (Samy and Ignacimuthu 2000). Higher and aromatic plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin *et. al.,* 1998). Garlic (*Allium sativum* L. *var.* aggregatum is a medicinal plant by Indian stages write from Vedic period because of its medicinal properties.

Garlic is not found in the wild "sativum" means "cultivated" but appears to have originated in mountainous regions in Central Asia (Brewster 2008). Garlic has been cultivated for over 5000 years. Diverse representations in Mesopotamian writings from ~3000 B.C. and Egyptian art dating to 2700 B.C suggest that garlic was already widely used (Block 2010). Hippocrates, Galen, Pliny the Elder, and dioscorides recommend garlic for conditions including parasites, respiratory problems, poor digestion, and low energy. It is described in Chinese writings from A.D. 510 (Hahn 1996), and is mentioned in the Bible and the Koran (Block 2010). Garlic produces various sulfur compounds that, together with their breakdown products, yield a characteristic pungent taste and odor, which may persist on the breath and body for up to 30 hours as garlic is metabolized (Block 2010). These compounds have documented antimicrobial and antifungal effects. Allicin, derived from garlic, combats fungal infections and parasites, lowers blood cholesterol, treats artero sclerosis, and promotes circulatory function (Block 2010). Garlic preparations are used to treat insect stings and improve scar healing (Block 2010). Epidemiological studies suggest that dietary garlic consumption lowers the risk of various cancers.

Garlic bulbs contain separate fleshy sections (cloves), each covered with a papery skin (tunic). The plants produce a leafless flower stem (a scape), but the flowers are sterile and produce bulbils (small cloves) rather than seeds; the species is propagated clonally from cloves and bulbils (Block 2010). Hundreds of cultivars are divided into two subspecies: 1) Hardneck garlic (*A sativum* ssp. *ophioscorodon*); and 2) Softneck garlic (*A. sativum* ssp. *sativum*, Block 2010). Presently the extract of the bulb and leaf is consider to be having many alkaloids capable of curing many human diseases including diseases of Lungs, Lever, Intestine and Blood Circulatory.

Antimicrobial Activity

Garlic extracts have long been established to exhibit wide spectrum antimicrobial activity. They are active against several genera of bacteria (*Staphylococcus*, *Streptococcus*, *Brucella*, *Vobrio spp*) ; Viruses (*Herpes simplex* type 1 and type 2,

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Parainfluenza virus type 3, *Vaccinia virus, Vesicular stomatitis virus* and *Human rhinovirus type* 3) Fungi (*Candida albicans*, *Cryptococcus neoformans*) and Worms (*Ascaris lumbricoies*); various hookworms). *Bacillus subtilis* has a rod shaped appearance and belongs to the family of the gram positive bacteria. *Aspergillus niger* is a filamentous fungus that is ubiquitous and commonly found on decaying plant material. *A.niger* has a saprophytic lifestyle an plays an important role in the degradation and recycling of dead plant material In the present study we established antibacterial activity of Garlic (*Allium sativum* L.) *var* aggregatum against pathogenic bacteria. The study confirms that organic solvent leaf and bulb extracts possess strong antibacterial properties against various pathogenic bacteria.

MATERIALS AND METHODS

Preparation of Leaf and bulb Solvent Extracts

Healthy plants of garlic were collected and washed thoroughly in tap water and they are surface sterilized by keeping them in 1% Mercuric Chloride for 5 minutes. They are then ground into a paste in a grinder. A crude extract is prepared from this paste with distilled water and sterilized water in equal proportion (1.0g/1ml) this extract is used as a *Aspergillus niger* (fungi) and *Bacillus subtilis* (Bacterium). Pure cultures of these two micro organisms are prepared by technique of repeated Sub-culturing. These pure cultures are employed in this study

Inoculums

The test microorganisms *Bacillus subtilis* (Bacteria) and *Aspergillus niger* (Fungi) were obtained from culture repository of Best Biotech culture collection, Department of Microbiology Kakatiya University Warangal. The Fungal organisms were inoculated onto NB (Nutrient Broth), ($NaNO_3$ 2.0 g, KH_2PO_4 1.0 g, KCl 0.5 g, $MgSO_4.7H_2O$ 0.5 g, $FeSO_4$ 0.01g Sucrose 30g Agar Agar 20g Distilled Water 1000 ml 0.15% Yeast extract; pH 7.4). Nutrient Agar medium with the following composition is used as a bacterial medium. (Beef extract 0.3g Peptones 0.5g Bacto Agar Agar 1.5 g Distilled water 0.1 liter). Approximately 0.3 mg. Streptomycin Penicillin is added to the medium to inhibit the growth of bacterium and incubated at 37°C for overnight for the growth of the fungal organism and bacteria both the Medias are sterilized in an autoclave at 1 5 lbs pressure at 121^{o}C temperatures. The pH of the medium is maintained 7.0 by the addition of Bromo ethymolblue. Sterilized petriplates are used for each medium and micro organism. Pour Plate Method is employed for culturing the micro organisms in the petriplates. The antibacterial activity of the leaf and bulb extracts was determined using agar well diffusion method with slight modification of (Perez *et.al.,* 1998). Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (5 mm diameter) were punched in the agar with a cork borer and filled with plant extracts. Control wells containing neat solvents (negative control) and standard antibiotic solution (positive control) viz., Chloromphenicol (100 µg/ml) were also run parallel in the same plate. The plates were incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The antibacterial activity of different extracts of leaf and bulb of Garlic was made in triplicate. The diameter of the zones of inhibition in the triplicate was measured by calculating the difference between cork borer (5 mm) and the diameters of inhibition.

RESULTS AND DISCUSSION

The various crude extracts of Garlic showed significant activity against two bacteria tested (*Bacillus subtilis* and *Aspergillus niger)*. The antibacterial activity of the Garlic was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated and represented in (Table-1 and Figure 1).

Table 1 Susceptibility of test bacterial strain and fungal strain to leaf and bulb extracts of Garlic (*Alium sativum* L.) and standard antibiotics

Sl.No	Type of extracts/antibiotics used	Bacillus subtilis		Aspergillus niger	
	Leaf	50µl	100µl	50µl	100µl
1	Ethanol	2.0	6.5	1.8	4.8
2	Methanol	3.6	5.0	3.0	4.2
3	Ethyl acetate	6.0	8.0	4.8	8.0
4	Chloroform	4.0	7.0	4.2	6.0
5	Hexane	2.3	4.0	2.0	3.8
6	Petroleum	1.8	3.2	1.0	3.0
7	Ether	1.0	3.0	1.2	2.6
	Bulb				
1	Ethanol	2.3	2.0	2.0	6.0
2	Methanol	3.4	9.0	3.2	8.5
3	Ethyl acetate	5.8	12.0	5.0	12.0
4	Chloroform	5.4	10.0	4.2	10.2
5	Hexane	2.5	4.8	2.0	4.2
6	Petroleum	2.0	3.5	1.8	3.0
7	Ether	1.3	3.2	1.0	3.0
	Standard antibiotics				
1	Penicillin	12.0	20.2	11.0	17.0
2	Kanamycin	10.7	18.5	8.5	6.8
3	Tetracycline	6.8	12.6	6.2	11.5
4	Cefotaxime	8.5	14.0	7.5	13.5

Among all the extracts, methanol, ethyl acetate and chloroform bulb extracts showed high activity (3.4 - 12.0 mm of zone of inhibition) on bacteria and fungi. The entire leaf extracts (ethanol, methanol, ethyl acetate and chloroform) except hexane petroleum and ether showed high activity (3.0 mm) when compared to the bulb extracts (ethanol, methanol, ethyl acetate, chloroform and hexane) which showed a moderate activity (2.0 – 12.0 mm).

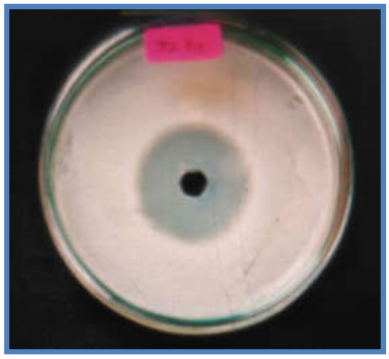
The obtained results of the crude extracts are comparable with the standard antibiotics such as Penicillin, kanamycin, tetra-cycline and cefotaxime. All the tested organisms are highly sensitive to the ethyl acetate leaf and extract of *A.cepa* (6.0 - 12.0 mm) than the standard penicillin, cefotaxime and tetracycline antibiotics. Kanamycin showed more or less similar activity (9.0 - 21.2 mm) on the tested organisms when compared with the ethyl acetate leaf extract (7.0 - 21.2 mm).
Approximately 1 ml of the culture of the each micro organism is poured into the petriplates containing the relevant medium. 10 ml of moltan medium is poured into the petriplates and allowed to solidify. After the medium as solidified at a rate of 4 wells for each plate. Wells are made with the help of a sterilized swab.

Each well is filled with 1 ml of garlic crude extract. Thus prepared petriplates are incubated at 37 ^{o}C temperature for 48 hours in case of bacteria 7 days in case of fungi. Growth pattern of bacterium and fungus is periodically observed during the growth.

The extract is also containing an enzyme allinase which is released when the fresh bulb is damaged and catalyzes the formation of allicin from alliin. Allicin is responsible for the odour of Garlic and together with its numerous degradation products is considered to be the primary active principle.

Numerous pharmacological studies have confirmed the principal activities of Garlic as follows

Anti-inflamatory and immunomodulating activity: Garlic has established anti-inflammatory action in experimental models and anti-oxidant activities particularly related to reducted glutathione (GSH) enhancement. Garlic extracts have been shown to be capable of immune modulation, increasing macrophage oxidative burst and stimulating T-lympheyte blastogenesis *in vitro* the active compound was a protein fraction.



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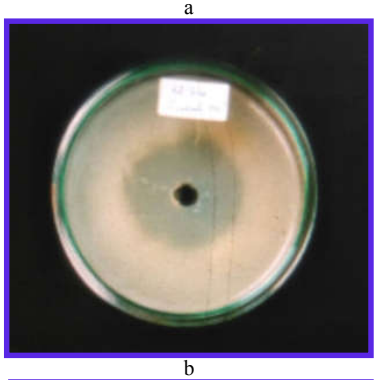

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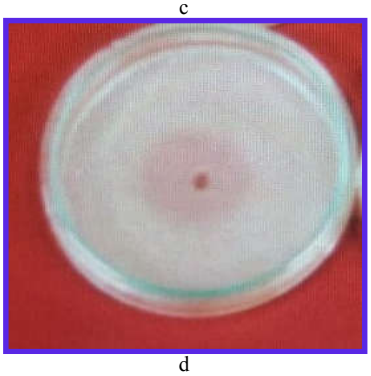

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Miscellaneuous activities: Garlic possesses diuretic, diaphoretic, emmenagogue and expectorant actions on the digestive system. Garlic is also moderately hypoglycemic: the mechanism suggested is competitive binding of OSC's to hepatocyte insulin receptors resulting in a sparing of insulin and potentiation of its peripheral actions.

Above discussion clearly shows that the extract of Garlic has marked antimicrobial property with regard to disease causing bacteria in particular and other micro organisms in general. However beggar information is available as per as its antimicrobial properties against saprophytic bacteria and fungi. On overall, bulb extracts are very active against all the tested bacterium and fungal strains followed by leaf extracts. An antibacterial property of leaf and bulb extracts against these bacterium and fungal suggests that the leaf and bulb extracts can be used for wound healing and septicemia. The preset study clearly shows that Garlic extract is also marked negative effect on the growth of saprophytic bacteria and fungi. The present study also indicates that it has more antimicrobial properties against fungi, than bacteria as revealed by (Table- I)

Figure 1 (A) Highest antibacterial activity exhibited by ethyl acetate leaf extract against *Bacillus subtilis*; (B) Highest antibacterial activity exhibited by ethanol and chloroform leaf extracts against *Aspergillus niger* (C) Highest antibacterial activity exhibited by ethanol and ethyl acetate bulb extracts against *Bacillus subtilis* (D) Least antibacterial activity shown by hexane leaf extract against *Aspergillus niger*.

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