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TO STUDY THE ANTIMICROBIAL PROPERTIES OF GARLIC AGAINST GRAM POSITIVE & GRAM NEGATIVE BACTERIA

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ABSTRACT

Garlic (Allium sativum) has been known to have inhibitory activity on various pathogenic bacteria. Garlic oil (GO) and extracts were prepared by hydro distillation technique and solvent extraction methods. For this purpose different solvents were used for extraction which includes methanol, ethanol, ethyl acetate and n- butyl alcohol. Disc sensitivity test was used to determine inhibitory action of oil and extracts at different concentrations which were from gram-positive strain (Staphlococcus coli). It was observed that the oil and extracts inhibit the growth of Staphlococcus aureus, Escherichia coli and Enterococcus faecalis. There was however, no effect of garlic oil on Enterococcus faecalis. It was evident that all the extracts exhibited the antimicrobial activity but their activity varied. The most susceptible bacteria against garlic antibacterial activity was Staphylococcus aureus which is gram positive bacteria followed by Escherichia coli which is gram negative bacteria. The antibacterial activity of garlic extracts increases consequently with the increment in garlic concentration from 10% to 100%. The maximum zone of inhibition was observed against Staphylococcus aureus (32mm) and minimum zone of inhibition (23.38mm) was noticed against Escherichia coli at 100% concentration.

KEY WORDS: Garlic, zone of inhibition, gram-positive and gram-negative bacteria.

INTRODUCTION

Garlic (Allium sativum belongs to the Alliaceae family. It is a bulbous perennial herb, closely related to the onion. It has a tall, erect flowering stem that reaches 2-3 feet in height (Block, 2010). It is grown as an annual plant that lasts only for a year. It has adventitious roots, condensed, flattened stem and narrow flat leaves. The part used medicinally is the bulb. The bulb consists of 6 to 35 bulblets called cloves with glistering and transparent covering (Ferland et al., 1950) each colve consists of a protective cylindrical sheath, a single thickened storage leaf sheath and a small control bud. The leaf blade is linear, solid 2-5cm wide and folded lengthwise. Flowers are variable in number or sometimes absent and may wither in the bud (Aiyer et al., 1954). The bulbs are mainly composed of water (approximately 84.09%), organic matter (13.38%0 and inorganic matter (1.53%). The leaves consist of more or less the same components with slightly different ratios (water 87.14%), organic matter 1.27% and inorganic matter 1.59%) (Bilyk and Sapers,) 1985. The organic matter is mostly carbohydrates while the inorganic matter is compounds such as sulphur and iron. The large number of sulfur compounds contributes to the smell and taste of garlic. Garlic is found worldwide as a cultivated crop. Plants are propagated by separating and planting individual bulbs (Kemper et al., 2000). It grows under a wide range of climatic conditions. It prefers moderate temperature in summer as well as in winter (Singh et al., 1984). It is a frost hardy plant requiring a cool and moist period during growth and a relatively dry period during bulb maturity (Yamaguchi, 1983). Galic is grown but its highest producer is China accounting for its 77% of Worlds output followed by India (4%), South Korea (2%), Egypt and Russia (1.6%) and then United States (1.4%) (FAO, 2010). In India, it is cultivated on a large scale in Uttar Pradesh, Gujrat and Madhya Pradesh (FAO, 2010). There are different varieties of Garlic grown in India like Yamuna SAFEED, Bhima Omkar, Agrifond White (G-41), G-282 etc (J. Hort. Sc. 2009). Garlic has a long tradition purposely function as food and as medicinal plant (Weber et al,. 1962). Garlic potentially active chemical constituents at least 33 sulphur compounds (alliin, allicin, ajoene, allypropyldisulfide, diallyltrisulphide, Sallylcysteine, vinyldithiines etc), several enzymes (allinase, peroxidases, myrosinase and others), 17 amino acids and their glycosides (arginine and others) and minerals such as Selenium, Germanium, Tellurium and other trace minerals (Newall et al., 1996). It contains a higher concentration of sulphur compounds than any other Allium species. The sulphur compounds are responsible both for garlic's pungent odour and many of its medicinal effects. Allyl sulfur compounds are the major active constituents founds in crushed garlic. It is hypothesized that much of therapeutic effects of garlic come from sulphur containing

compound. Allicin (C_3H_5SS (O) C_3H_5) is sulphur containing compound and oxygeneated sulphur compound formed when garlic cloves are crushed. Allicin (Diallyl thiosulfinate) play an important role in the antibiotic activity of garlic. The antimicrobial activity of garlic was completely abolished when allicin were removed from the extract (Hughes and Lawson, 1991). Thus, upon reduction of allicin to other form of diallyl disulfide, the antibacterial activity was greatly reduced (Reuter et al., 1996). Allicin has been found to be the compound most responsible for the "hot" sensation of raw garlic (RG))Macpherson et al., 2005). Allicin, along with its decomposition products diallyl disulphide and diallyl trisulphide, are major contributors to the characteristic odour of garlic, while other allicin-derived compounds, such as vinyldithiins and ajoene show beneficial *in vitro* biological activity (Block, 2010). Despite having a minimal amount of ions and other compounds, those that are present play a very important role in the composition and overall beneficial effects that garlic potentially possesses (Prasad, 2010). When crushed, Allium sativum yields allicin, an antibiotic (Focke et al., 1990) and antifungal compound (phytoncide) discovered by Cavallito and colleagues in 1944. Fresh or crushed garlic also has enzymes, B vitamins, proteins, minerals, saponins, flavonoids, and Maillard reaction products. Furthermore, a phytoalexin (allixin) was found, a monsulfur compound with a y-pyrone skeleton structure with antioxidant effects, antimicrobial effect (Ankri and Mirelman, 1999) antitumor promoting effects, inhibition of aflatoxin B2 DNa binding and neurotrophic effects (Aaron, 2001). Garlic contains a chain of complex biochemistry reactions that is not fully understood by scientists. Allicin does not ocour in ordinary garlic but produced when garlic is finely chopped or crushed. It is very sensitive to crushing, slicing or even bruising which all set up a sequence of biochemical events. The finer the chopping and the more intensive the crushing, the more allicin is generated and the stronger the medicinal effects will be. As a result of the mechanical action, the cell integrity is lost, thus the enzyme allinase comes into contact with alliin amino acid. The slightest bruise will release an enzyme called allinase which convert alliin amino acid into allicin (Thompson, 2006). Allicin starts to degrade immediately after it is produced, so its medicinal effectiveness decreases overtime. Allicin can also be broken down when heated. Conversely, its break down can be slowed by lower temperature. When allicin degrades, it produced several diallyl sulphides. This breakdown occurs within hours at room temperature and within minutes during cooking (Blania et al., 1991). Although the diallyl sulphides do not have the strong antibacterial and antifungal properties like allicin, they are still believed to have medical benefits especially to improve blood circulation and lower or reduced cholesterol level in human body (Song and Milner, 2001). So for the most

powerful medicinal effect, cut or crushed a little raw garlic and combined with the cooked food shortly before serving. Pure allicin is said to be highly volatile, poorly miscible with water and has the odours of freshly crushed garlic. Allicin is considered to be the most potent antibacterial agent when garlic cloves are crushed but it can be very unstable within 16 hours at 23^0C (Hughes and Lawson, 1991). The pure allicin can be stored for months without losing its effectiveness. In contrast, allicin, extracted normally loses its beneficial properties within hours because it begins to react with other garlic components as soon as the clove is crushed. For alternative, the use of water based extract of allicin can stabilize the allicin molecules. This may be due to the hydrogen bonding of water to the reactive oxygen atom in allicin which can reduce its instability and there was also a water soluble component in crushed garlic which stabilized the allicin molecules (Hughes and Lawson, 1992). Allicin is relatively unstable molecule that spontaneously decomposes into a group of odoriferous compounds that are rarely found in nature. Garlic is notorious for the lingering odours it produces even days after consumption. The new compounds that are formed when garlic is disrupted are what provide the medicinal nature. There are a myriad of ways to prepare garlic and different compounds are performed depending on the way it was prepared, for examples garlic can be consumed fresh, aed, raw, cooked, natural, processed in pills or even as extracts and capsule (Belman, 1983). Garlic oil, aged garlic and steam-distilled garlic do not contain significant amounts of allin or allicin, but instead contain various products of allicin transformation; none appears to have as much physiological activity as fresh garlic or garlic powder (Lawson et al., 1991). Research has revealed that garlic and its lipid or water soluble components have many pharmacologic properties. However, studies also demonstrate that heating has negative influence on these beneficial effects with the assumption that if you can smell strong garlic odour when cooking, that means you have destroyed a lot of beneficial compounds in garlic (Brodnitz, 1971. Garlic (Allium sativum), even from Aaoristic times, has been used in all parts of the world not only as a spice or a food, but also for treatment of many diseases. The famous herbal doctors Hippocrates, Paracelsus and Lonicerus recorgnized garlic as a diuretic, an emmengogue, and used it for the treatment of stomach chills, flatulence, colic etc (Stoll and Seeback, 1951). Garlic was an important medicine to the ancient Egyptians listed in the medical text Codex Ebers (ca. 1550 BC) especially for the working class involved in heavy lobor (Lawson et al., 1998; Moyers et al., 1996) There is evidence that during the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina (Lawson et al., 1998). The great herbalists and physicians of the ancient world record garlic historical use. “Garlic has powerful properties and is of great benefit against changes of water and of

residence," wrote Pliny the elder, the first century Roman naturalist (23-79 AD) (Foster, 1996; Koch and Lawson, 1995). Garlic has been used from the ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease (Kris-Etherton et al., 2002: Koscielny et al., 1999; Yu-Yan and Liu, 2001; Gardner et al., 2003), and regular use of garlic may help to prevent cancer, to treat malaria, and to raise immunity. Garlic has also proposed to treat asthma, candidiasis, colds, diabetes, nd antibacterial effects against food borne pathogens like Salmonella, Shigella and S. aureus (Teferi and Hahn, 2002). Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different micro organisms. For example; the antibacterial (Sato et al. 1990; Waqar et al., 1994), antifungal (Barone and Tansey, 1977; Moore and Atkins 1977; Sato et al. 1993), antiviral (Rees et al. 1993), larvicidal (Amonkar and Banerji, 1971) and enzyme inhibitory (Wills 1956) activities of garlic have been widely studied. The active inhibitory principle of garlic is allicin or diallyl thiosulphinic acid (Saleem and Al-Delaimy, 1982). Moreover, garlic extracts exhibited activity against both gram negative (E. coli, Salmonella sp. And Citrobacter enterobacter, Pseudomona kilabsella) and gram positive (S. aureus, S. pneumonia Group A streptococcus and Bacillus anthrax) all of which are cause of morbidity worldwide Gram-positive bacteria:Gram positive bacteria are bacteria that give a positive result in the Gram stain test. They take up the crystal violet stain used in the test, and then appeared to be purple-colored when seen through a microscope the reason behind this is that the thick peptidoglycan layer in the bacterial cell wall retains the stain after it is washed, in the decolorization stage of test. (a) Gram negative bacteria: Gram negative bacteria that cannot retain the violet stain after the decolorization step this is due to the degration of outer membrane of gram-negative cells making it more porous and incapable of retaining the crystal violet stain. Their thinner peptidoglycan layer causes them to take up the counterstain (safranin or fuchsine) and appear red or pink. There is extensive literature on the antibacterial effects of fresh garlic juice, aqueous and alcoholic extracts, steam distilled oil and other commercial preparations of garlic. Fenwick and Hanely, (1958) understood a thorough review of the antibacterial effects of garlic and other allium vegetables up to mid-1984; more recently, the antibacterial effects of garlic have been studied by Reuter et al., (1996). The aim of this proposed study is summarized as below:- (i) To extract the oil from garlic using different methods. (ii) Hydro distillation technique (iii) Filtration technique (iv) To analyze the antimicrobial activity of the garlic against the potent pathogenic bacteria *Staphylococcus aureus, Escherichia coli and Enterococcus faecalis.*

MATERIALS AND METHODS

Procurement of raw material:-Fresh garlic bulbs were procured from local market of Kashmir.

Sample Preparation:-The sample (garlic extract and garlic oil) was prepared by means of Solvent extraction technique and Hydro distillation technique.

Solvent Extraction Technique:- Fresh garlic cloves were washed, peeled, sliced having wet weight (600gm) and then dried in hot air oven (Model NSW-101 Narang Scientific Works Pvt. Ltd. New Delhi) at 45^0C for 2 days to remove the moisture and the samples were then grounded to fine powder using electric blender (Model HL 1632). Fine garlic powder was divided into four equal portions (70gm) each. Weighed garlic powder was put into flasks having tight lid. Organic solvents (methanol, ethanol, ethyl acetate and n-butyl alcohol) about (200ml) were added to these flasks. The flasks were placed in shaking incubator (Model CIS 24 BL) at 25^0C for 72 hours. The solutions were then filtered by using Whatman filter paper no. 1 to obtain pure garlic extract. The ethanol, methanol and ethyl acetate extracts were evaporated at 50^0 C while as n-butyl extract was evaporated at 123^0C to remove all the solvent from the extracts. The extract samples were put in separate vials and stored at 4^0C. The extracts in this experiment were labeled as GaM, GaE, GaEA and GaBA for extracts of methanol, ethanol, ethyl alcohol and butyl alcohol respectively.

Hydro distillation Technique:- Hydro distillation apparatus as shown in plate 1, is also known as water distillation. It is a process in which water and plant materials were boiled together in a common round bottom flask. Fresh garlic, weighed about (60gm) was chopped into cubes and put into a 1L flask and water was added approximately to half of the flask. All the apparatus was set up properly and the temperature was set at 55^0C. The vapours thus produced were condensed by means of condenser. The boiling process was continued for 8 hours and constantly monitored for the formation of concentrated garlic extract in oil form in a receiver. The condensed oil essence was carefully collected in a covered receiver flask to prevent the oil from being evaporated into the air because the garlic essential oils are highly volatile. The pure garlic oil was then transferred into vial and was labeled as GO.

Preparation of Growth Media:- The culture media used in this study was nutrient agar, Nutrient agar typically contains peptic digest of animal tissues (5%), sodium chloride (5%), beef extract (1.5%), yeast extract (1.5%) and agar (15%). Distilled water was added to the

nutrient agar powder in 1 litre flask and was autoclaved at pressure of 15 psi at 121^0C for 20 minutes and was then poured into Petri plates under aseptic conditions to prevent air borne contamination of the medium. The agar was then allowed to stand until it solidifies.

Preparation of Bacterial Culture:-This experiment involved three different types of micro organisms which are from gram negative (Escherichia coli), gram positive bacteria (Staphylococcus aureus) and (Enterococcus faecalis). The starter culture was obtained from Microbiology Laboratory of Government Medical College Srinagar. The streaking process was done in a laminar flow cabinet under sterile conditions to prevent contamination and each Petri plate was sealed with Para film. Then the plates were incubated in BOD incubator (Model No. NSW-152) at 37^0C for 24 hours. The single colony as shown in figure. 3.4 were obtained by using streak plating method. One colony of bacteria was isolated and was suspended in 10 ml of saline water and shaken well on vortex (Model SPINIK-1719). It was then allowed to stand at room temperature for ten minutes.

Testing the Garlic Extracts on bacterial Culture using Disc Diffusion method:-The antibacterial assay of garlic was performed by disc diffusion method as described by Kirby-Bauer (1996). All the experiments were performed under aseptic conditions. The nutrient agar plates were inoculated separately with 100 micro litre of tested bacterial culture and evenly spread on entire surface of each plate. Spreading was done carefully and gently to avoid the agar from cracking up. The sterile discs (0.5cm diameter) were placed aseptically over nutrient agar plates seeded with bacterial culture. About 15 micro litres from each extracts were taken by means of Micropipette (Model H6Y008131) and poured over disc. The plates were sealed, incubated at 37^0C to 24 to 48 hours and were observed for zone of inhibition. The zone of inhibition was measured in millimeters using Vernier Caliper (Mode CD-6 CSX) incubated at 37^0C for 24 to 48 hours and were observed for zone of inhibition. The zone of inhibition was measured in millimeters using digital Vernier Calliper (Model CD-6 –CSX). Antibacterial sensitivity tests were performed in triblicates with each bacterial strain.

Control for the Experiment:- One control plate as shown in plate 3 was prepared in each experiment in which the disc paper was soaked into sterilized distilled water instead of garlic extract. The plate was incubated at 37^0C for 24 hrs. Control plate is essential to determine whether garlic extract has antimicrobial properties.

Clear Zone Measurement on Bacteria Culture:- After leaving the plates overnight, the diameters of cleared zone of inhibitions were measured by using a digital Vernier Calliper (Model CD -6CSX). To achieve the objectives so envisaged in the course of present study, the following treatments were undertaken:- (i) T1=10% (10 parts of extract in 90 parts of DMSO) (ii) T2=25% (25 parts of extract in 75 parts of DMSO) (iii) T3=50% (50 parts of extract in 50 parts of DMSO) (iv) T4=100% (100 parts of extract in 100 parts of DMSO) (v) Control=Disk soaked into distilled water.

Statistical Analysis:-The whole data was taken from the lab work related to antimicrobial properties of garlic in the Department of Life Science Bhagwant University, Rajasthan, Ajmer. All the readings were taken in triplicates to minimize the error. The data was analyzed by applying one-way ANOVA using MINITAB-11 Software at 5% level of significance.

RESULTS AND DISCUSSION

Table 1 illustrated that the zone of inhibition of garlic oil and methanolic, ethanolic, ethyl acetate, butyl alcohol extracts and lies in the range between (9.44-32mm. It was shown that all the treatments varied significantly at ($p<0.05$). However, the treatments T_2 and T_3 were at par with each other. Also, it was predicted from table 2 that the mean valves of zone of inhibition follows an increasing trend at different concentration of garlic extracts with zone of inhibition ranging from (9.03-26.93mm). However the data of different garlic extracts and garlic oil varied significantly ($p<0.05$) against E. coli) with treatments T_1 and T_2 were statistically at par. From table 3, it was shown that the treatment of garlic extracts against gram-positive bacteria (Enterococcus faecalis) varied non-significantly at ($P>0.05$). However, the mean valves for different extracts ranges from (22.98 -26.81mm). The zone of inhibition follows an increasing trend (fig.4). The susceptibility of garlic extracts and garlic oil against Staphylococcus aureus and E. coli follows the order as GaM>GaEA>GaE>GaBA>GaO, while as the susceptibility order of GaEA>GaBA>GaM>GaE was depicted for Enterococcus faecalis. As was evident from table 4, that the susceptibility effect of all the garlic extracts on all test organisms was inhibited by garlic extracts above 10% concentration and the activity was a linear function of concentration. Figure 5 depicted that at 100%, the maximum zone of inhibition was observed against Staphylococcus aureus, a Gram-positive organism and the minimum was against Escherichia coli, a Gram-negative organism. An experiment had been carried out to test the sensitivity of three bacteria which were from gram positive strain (Staphylococcus aureus and Enterococcus) and gram negative strain (Escherichia coli)

against five different concentration of garlic extract 10%, 25%, 50%, and 100%. It was concluded that all test organisms were inhibited by garlic extracts up to 10% concentration and the activity was a linear function of concentration. Also it was depicted that at 100%, the maximum zone of inhibition was observed against Staphylococcus aureus, a Gram-positive organism and the minimum was against Escherichia coli, a Gram-negative organism. This indicates that garlic extracts had the potential of a broad spectrum activity against both Gram-positive and Gram-negative bacteria. But we can see the variation in the size of the inhibition zone among the different group of bacteria. This may be due to the lipid content of the membranes of the different groups of the micro organisms and the permeability of allicin and other garlic constituent's interface with RNA production and lipid synthesis. If RNA cannot be produced, or produced in less amount then protein synthesis will be severely affected. It would be stopped at every stage due to the absence of messenger RNA, ribosomal RNA and transfer RNA. If amino acids and proteins cannot be produced then growth and development of the organism will not occur as they are essential for all parts of cell structure. The findings were in accordance with Durairaj et al., 2009. The study focused on the garlic antibacterial activity on S. aureus has shown that dilute solutions of garlic can completely inhibit the growth of S. aureus at the concentration of more than 10% However, the zone of inhibitions follows the increasing trend with highest values of zone of inhibition at higher concentrations of 100 % and lower values at lower concentration 10% of garlic extract against Staphylococcus aureus. Basically, the data obtain from the experiment on garlic extract concentration showed the increasing pattern of inhibition zone that merely told us that the optimum concentration of garlic extract which could result in the maximum inhibition zone (diameter) for each bacteria was at the concentration of 100% (). The antibacterial effect of garlic extract against Staphylococcus aureus was proven that it increases with the increase in concentration with maximum zone of inhibition of 12mm at a concentration of 100%. This could be due to the action of biological active ingredient of allicin which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis. Similar results were observed by Cutler and Wilson, (2004) while studying the antibacterial activity of aqueous extract of allicin against methicillin-resistant Staphylococcus aureus and by Deresse Daka, 2011 while studying the effects of garlic extract on Staphylococcus aureus in an in-vitro study. In case of E coli it was found that the zone of inhibition follows an increasing trend. The maximum zone of inhibition was found at a concentration of 100% which could be due to phytoalexin (allixin) a non-sulphur compound with a y-pyroneskeleton structure that has antioxidant effects, antimicrobial effects, inhibits aflation B2 DNA binding,

and neurotrophic effects (Yamasaki et al., 1991). Similar results were observed by Lawson, (1996) against Escherichia coli; and Safithri et al., 2011 against pathogenic animal bacteria. Also, it was observed that the garlic extract obtained by using ethyl acetate shows maximum inhibitory action on Enterococcus faecalis which could be attributed to non-volatile sulphur containing compounds such as g-glutamyl-S-allyl-L-cysteines and S-allyl-L cysteine sulfoxides (alliin). These sulfoxides are then converted into thiosulphinate (such as allicin) through enzymatic reactions (Amagase, 2006). However, garlic oil was not effective against Enterococcus bacteria which may be due to loss of volatile bioactive compounds. The reason for the increasing zone of inhibition of garlic extract against Enterococcus could be attributed to the bioactive compounds especially the diallyl sulphides which get released after crushing the fresh garlic. The results were in close agreement with Deresse Daka, 2011.

Table-1. Effect of garlic extract and oil on Staphylococcus aureus

Treatment	Methanol	Ethanol	E. acetate	B.alcohol	Garlic oil
T1	18.40^{a}	20.98^{a}	18.60^{a}	13.92^{a}	9.44^{a}
T2	22.40^{b}	22.25^{ab}	24.18^{b}	18.22^{b}	16.60^{b}
T3	29.50^{cd}	23.48^{bc}	29.36^{c}	20.25^{c}	18.42^{c}
T4	32.00^{d}	25.71^{d}	31.90^{d}	23.26^{d}	21.13^{d}

Table-2. Effect of garlic extract and oil on E. coli

Treatment	Methanol	Ethanol	E. acetate	B. alcohol	Garlic oil
T1	13.26^{a}	18.30^{a}	17.93^{a}	15.70^{a}	9.03^{a}
T2	$15.46a^{b}$	20.80^{ab}	21.86^{b}	17.82^{ab}	17.47^{bc}
T3	20.87^{c}	21.82^{c}	24.36^{c}	21.18^{c}	18.11^{c}
T4	32.32^{d}	25.07^{d}	26.93^{d}	24.50^{d}	23.38^{d}

Table-3. Effect of garlic extract and oil on *Enterococcus faecalis*

Treatment	Methanol	Ethanol	E. acetate	B. alcohol	Garlic oil
T1	22.98^{a}	22.60^{a}	23.28^{a}	21.93^{a}	0
T2	23.66^{b}	23.20^{b}	24.12^{b}	23.16^{ab}	0
T3	25.41^{c}	24.13^{c}	25.07^{c}	23.96^{c}	0
T4	25.54^{d}	24.74^{d}	26.81^{d}	26.65^{d}	0

Table -4. Combined effect of garlic extracts and garlic oil on three different bacteria.

Treatments	S. aureus	Enterococucus faecalis	E.coli
T1	20.98^{a}	28.98^{a}	18.30^{a}
T2	24.18^{b}	23.66^{b}	21.80^{b}
T3	29.50^{cd}	25.41^{c}	24.36^{c}
T4	32.00^{d}	26.81^{d}	30.32^{d}

CONCLUSION

Garlic has been used by a wide range of populations for centuries to combat infectious diseases. It can be provided in the form of capsules and powders, as dietary supplement. This study demonstrated many facts and proved that garlic, which was readily available and widely used contained effective components like allicin, thiosulfinates, allyldisulfidesetc, which could inhibit the growth of different micro organisms like Staphylococcus, E.coli, and Enterococcus faecalis. The antibacterial activity of garlic is mainly due to allicin. Among the three Staphylococcus aureus was most susceptible followed by E.coli and Enterococcus faecalis respectively. Aslo, it was concluded that the zone of inhibition increases with the increase in concentration of garlic extract. The garlic oil was totally inactive against Enterococcus. Among the extracts used, methanolic extract was proven very effective against potent pathogenic bacteria especially Staphylococcus aureus and E. coli. The whole work was carried out in micro-biology laboratory in department of Life Science at Bhagwant University, Ajmer. Garlic bulbs were procured from local market and then peeled and finely sliced. Garlic extract was obtained through solvent extraction technique while Garlic oil was obtained through hydro distillation technique. Garlic extracts were obtained by using different organic solvents like methanol, ethanol, ethyl acetate and butyl alcohol respectively. The anti-bacterial activity of garlic extracts and oil were tested by disc diffusion method against both gram-positive and gram-negative bacteria like Staphylococcus, Enterococcus faecalis and E.coli respectively.

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