Int J Pharm Bio Sci 2015 April; 6(2): (P) 404 - 408

Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICACY OF *ALLIUM SATIVUM LINN.*, *ALLIUM AMPELOPRASUM LINN.* AND *ZINGIBER OFFICINALE ROSCOE.* AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

ANANTHAN ANUSWEDHA[*1] AND USHA ANANDA RAO[2]

*[*1]Post Graduate Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India*
[2] Professor of Research, Department of Microbiology, Ragas Dental College and Hospital, Chennai.

ABSTRACT

Garlic (*Allium sativum* L.) and ginger *(Zingiber officinale R.*) has been used for thousands of years for medicinal purposes. Historically, garlic has been used worldwide to fight bacterial infections.Multi-drug resistant bacteria and their incidence has risen dramatically in recent years. Allium vegetables, particularly garlic (*Allium sativum* L.) exhibit a broad antibiotic spectrum against both gram-positive and gram-negative bacteria.Methicillin Resistant *Staphylococcus aureus* (MRSA) are the major cause of nosocomial infections including pneumonia, post-operative wound infection,bacteremia and other infections worldwide. The *in vitro* antibacterial activity of crude extracts and fresh juice of *Allium sativum L.* (garlic),*Allium ampeloprasum L.*(elephant garlic) and *Zingiber officinale R.*(ginger) was determined and compared against clinical strains of methicillin resistant *Staphylococcus aureus*. *Allium ampeloprasum* fresh juice showed maximum inhibition against all the MRSA strains at a concentration of 100µl/ml followed by the fresh juice of *Allium sativum*.The crude extracts and fresh juice of *Zingiber officinale* showed no activity at the concentration tested. This study confirms the higher therapeutic potential of *Allium ampeloprasum* and *Allium sativum* against MRSA.

KEYWORDS: MRSA, antibacterial, Allium vegetables

*Corresponding author

ANANTHAN ANUSWEDHA
Post Graduate Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India

INTRODUCTION

Antibiotic resistance has rapidly increased during the last decade, creating a serious threat to the treatment of infectious diseases. Treatment of infectious diseases in the current scenario has become difficult due to increase in drug resistance. In addition to resulting in a significant increase in costs and toxicity of newer drugs, antibiotic resistance is eroding our therapeutic armamentarium[1]. One of the most serious contemporary challenges to the treatment of hospital-acquired infections worldwide is the appearance and global spread of MRSA, which carries a uniquely effective drug-resistant mechanism that can protect these pathogens against all the members of the large β-Lactam family of antibiotics. Methicillin resistant *Staphylococcus aureus* is an important hospital pathogen, the incidence of which is increasing every year especially in high risk groups.The organism has a differential ability to spread and cause outbreaks in hospitals which represent substantial burden of morbidity and mortality[2].Plant derived products have been used for medicinal purposes for centuries. Currently, it is estimated at about 80% of the world's population uses herbal preparations to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments[3].Garlic (*Allium sativum*), a member of the liliaceae family, is highly regarded throughout the world both for its medicinal and culinary properties. Garlic exerts broad spectrum antimicrobial activity against many different species of bacteria, virus, protozoans and fungi[4]. When garlic is chopped, crushed or bruised the alliin in the bulb is converted to allicin, which exhibits the antimicrobial activity[5]. Allicin exhibits its antimicrobial activity mainly due to S-S and S-O bond which has the ability to react with thiol containing enzyme to form S-thiolation product, which inhibits thiol dependent enzymatic systems.[6] Ginger has been used as a medicine from the vedic period. *In vitro* studies have shown that the active constituents of ginger inhibits the growth of *E.coli*, *Proteus* species, *Staphyloccocci, Streptococci and Salmonella*[7].

The present study was done to determine the antibacterial efficacy of three medicinal herbs, *Allium sativum L.* (garlic), *Allium ampeloprasum L.* (Elephant garlic) and *Zingiber officinale R.* (ginger) against methicillin-resistant *Staphylococcus aureus* (MRSA). We also compared the antibacterial efficacy of the fresh juice and powdered crude extracts of the medicinal herbs against MRSA.

MATERIALS AND METHODS

The garlic bulbs were purchased from commercial sources. Freshly peeled and chopped garlic(500 gms) was dried in an oven at 40ºC for two days, after which it was ground to a fine powder[8].Ginger powder was procured from IMPCOPS (the Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd.), Chennai.

i) Preparation of Crude Extracts[9]

Hot method was used for extracting the heat-stable compounds where about 1 gram of the powdered plant material was mixed with 10 ml of the corresponding solvent, placed in a shaker incubator at 250 rpm at 37º C for 3 hours.The contents were transferred to a water bath held at 60ºC for 2 hours after which the contents were filtered and the filtrate was dried in air at room temperature.In the cold method of extraction, employed for extracting the heat-labile compounds, about 1 gram of the powdered plant material was mixed with 10 ml of the corresponding solvent, placed in a shaker incubator at 250 rpm at 37º C for 3 hours. The contents were filtered and the filtrate dried in air at room temperature. The solvents employed were water, methanol, ethanol, ether, hexane and dichloromethane. After drying, the material was resuspended in the corresponding solvent at the required concentration (100mg/ml) and refrigerated for further use.

ii) Preparation of Juice[9]

About 500 grams of garlic, elephant garlic and ginger rhizome were purchased. The individual cloves of garlic and elephant garlic and ginger rhizome was peeled, chopped and crushed in a grinder using aseptic techniques to get the garlic paste. This paste was squeezed out through a double cheese cloth to obtain a pale extract which was filtered

through cellulose nitrate membrane filter (pore size 0.2 µm) to make the extract sterile. The extracted juice was stored in vials at 4ºC.

iii) Microbial cultures used

The test organisms used for the screening were eight laboratory isolates of Methicillin resistant *Staphylococcus aureus* (MRSA).

iv) Screening for Antibacterial Activity[10, 11]

The extracts were screened using agar disc diffusion assay. Discs (6mm) prepared from Whatmann No.1 filter paper was sterilised and impregnated with various crude solvent extracts and fresh juice (100µl/per disc).Concentration of the crude extracts were 100 mg/ml and that of the fresh juice was 100µl/ml. Broth cultures of the isolates were prepared by transferring two or three isolated colonies to nutrient broth and incubating the culture at 37ºC for 4 hours. The culture was checked for turbidity by comparing with 0.5 MacFarland Standard. A lawn culture of the organisms to be tested was made on the corresponding media. The prepared discs were placed on the medium in such a way that each disc was at least 20mm from each other. The plates were incubated at 37ºC for 24 hour after which the plates were observed for the zone of inhibition around the discs and the diameter of the inhibition zones were measured and recorded.

v)Determination of Minimum Inhibitory concentration/Minimum Bactericidal Concentration[12,13]

Broth Dilution assay was performed on a micro titre plate. Doubling dilutions of the fresh juice was prepared in Mueller Hinton Broth. Bacterial cultures of 10^8 organisms/ml were prepared with MacFarland Standard (0.5) and 10 µl were added to each well and shaken well. The plate was incubated at 37ºC overnight and a loopful of the culture was streaked on to Nutrient agar plate. Plates were incubated at 37ºC overnight. The growth/no growth pattern of the organisms corresponded to the MIC/MBC the fresh juice.

RESULTS

TABLE 1

Preliminary Screening of crude extracts with Agar Disc Diffusion Assay

ORGANISMS TESTED	MATERIAL TESTED	ZONE OF INHIBITION IN MM
1. MRSA strain 1-8	Crude solvent extracts of *Allium sativum*, *Allium ampeloprasum* and *Zingiber officinale*	No zone of inhibition

Preliminary screening using the crude solvent extract of Allium sativum, Allium ampeloprasum and Zingiber officinale at a concentration of 100mg/ml showed no antibacterial activity for all the MRSA strains tested.

TABLE 2

Preliminary screening using fresh juice with agar disc diffusion assay

ORGANISMS TESTED	*ALLIUM SATIVUM* FRESH JUICE	*ALLIUM AMPELOPRASUM* FRESH JUICE	*ZINGIBER OFFICINALE* FRESH JUICE
		ZONE OF INHIBITION IN MM	
1.MRSA-1	18	17	-
2.MRSA-2	-	7	-
3.MRSA-3	15	15	-
4.MRSA-4	12	15	-
5.MRSA-5	15	16	-
6.MRSA-6	15	14	-
7.MRSA-7	-	14	-
8.MRSA-8	18	17	-

Preliminary screening using the fresh juice of garlic showed an inhibition zone ranging from 12-18mm,fresh juice of Allium ampeloprasum showed an inhibition zone ranging from 7-17mm whereas Zingiber officinale fresh juice showed no inhibition zone at a concentration of 100µl/ml.

TABLE 3
Determination of MIC/MBC for Allium sativum fresh juice

ORGANISM TESTED	VOLUME OF *ALLIUM SATIVUM* FRESH JUICE (IN µL) USED							
	375	187.5	93.75	46.88	23.43	11.71	5.85	2.92
MRSA -1	-	-	+	+	+	+	+	+
MRSA-2	-	-	+	+	+	+	+	+
MRSA-3	-	-	+	+	+	+	+	+
MRSA-4	-	-	+	+	+	+	+	+
MRSA-5	-	-	+	+	+	+	+	+
MRSA-6	-	-	+	+	+	+	+	+
MRSA-7	-	-	+	+	+	+	+	+
MRSA-8	-	-	-	+	+	+	+	+

+: denotes presence of bacterial growth,-: denotes absence of bacterial growth
In the Microbroth dilution assay for determining MIC, Allium sativum fresh juice showed an MIC value of 93.75 µl/ml for a single strain (MRSA-8) and other strains showed MIC value of 187.5µl/ml.

TABLE 4
Determination of MIC/MBC for Allium ampeloprasum fresh juice

ORGANISM TESTED	VOLUME OF *ALLIUM AMPELOPRASUM* FRESH JUICE (IN µL) USED							
	375	187.5	93.75	46.88	23.43	11.71	5.85	2.92
MRSA -1	-	-	-	+	+	+	+	+
MRSA-2	-	-	+	+	+	+	+	+
MRSA-3	-	-	-	+	+	+	+	+
MRSA-4	-	-	-	+	+	+	+	+
MRSA-5	-	-	+	+	+	+	+	+
MRSA-6	-	-	+	+	+	+	+	+
MRSA-7	-	-	-	+	+	+	+	+
MRSA-8	-	-	-	-	+	+	+	+

In the Microbroth dilution assay for determining MIC, Allium ampeloprasum fresh juice showed an MIC value of 46.88µl/ml for a single strain(MRSA-8),four strains showed MIC value of 93.75 µl/ml and three strains showed MIC value of 187.5µl/ml.

DISCUSSION

Antimicrobial drug resistance is an important global public health concern. Recent epidemiological reports from India states that there is an alarming increase in antimicrobial resistance. *Staphylococcus aureus* continues to be a persistent and dangerous pathogen for both community- acquired and hospital-associated infections. The control of MRSA is important to curtail the introduction and spread of infection[14]. In order to control these infections, there is a need for the development of alternative therapeutic compounds from cheaper natural resources with marked antibacterial activity and less toxicity. The antimicrobial and other medical benefits of garlic have been widely recognised. In the present study, the crude solvent extracts have shown no antibacterial activity whereas the fresh garlic juice had shown maximum antibacterial activity. Allicin is considered to be the most potent antibacterial agent in crushed garlic extracts, but it is unstable, breaking down with 16 hours at 23ºC[15].Exposing garlic extracts to temperatures of 80ºC for 5 minutes completely destroys the antibacterial activity. The loss is due to volatilisation and due to the physical and chemical changes that take place during heating. This may explain the reason for the marked antibacterial activity exhibited by fresh garlic juice but not by the crude extracts. According to studies done by Vimalin Hena, allicin is bactericidal against MRSA at concentrations of 130 to 138µg/ml[16] whereas in our studies the antibacterial activity of the crude garlic juice was analysed at a concentration of 100µl/ml. The antibacterial efficacy of *Allium ampeloprasum* was comparatively higher than *Allium sativum* which is confirmed by previous studies[17].Though ginger extracts have been reported to show antibacterial efficacy against many bacteria at a concentration of 2000mg/ml[18], in the present study neither the ginger crude extracts nor the fresh juice showed any antibacterial activity against the organisms tested at a concentration of 100mg/ml and 100µl/ml respectively. Further studies involving higher concentrations of the ginger extracts need to be explored for its antibacterial efficacies.

CONCLUSION

In conclusion, the study confirms that fresh garlic juice with effective antibacterial activity and lower MICs may be considered as a functional food in human nutrition for the prevention or can be explored as an alternative/combination treatment strategy for nosocomial infections caused by methicillin resistant *Staphylococcus aureus.*

REFERENCES

1. Swartz M.N.Use of antimicrobial agents and drug resistance. N. Engl. J. Med, 337:491-492, (1997).
2. Kluytmans J, Belkum A.V, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms and associated risk. Clin.Microbiol.Rev, 10 (3): 505-520, (1997).
3. Olukoya D.K, Idika N and Odugbemi.T. Antibacterial activity of some medicinal plants from Nigeria. J. Ethanopharmacol., 39:69-72, (1993).
4. Adetumbi M.A and Lau B.H. Allium sativum – a natural antibiotic.Med Hypotheses, 12:227-237, (1983).
5. Perez-Giraldo .C,Cruz-Villalon G, Sanchez-Silos R, Martinez-Rubio R, Blanco M.T and Gomez-Garcia A.C.*In vitro* activity of allicin against *Staphylococcus epidermidis* and influence of subinhibitory concentrations on biofilm formation. Journal of Applied Microbiology.95; 709-711, (2003).
6. F.Rehman And Samya Mairaj. Antimicrobial studies of Allicin with Paeonol and its oxime. Int. J. Pharm. Bio. Sci. Jan; 4(1): (B) 240 – 249,(2013)
7. Gugnani H.C and Ezenwanze E.C.Antibacterial activity of extracts of ginger(Zingiberofficinale) and African oil bean seed (Pentaclethoramacrophylla). J. Commun. Dis.17; 233,(1985).
8. Pantoja CV, Chiang LC, Norris BC, Concha JB. Diuretic, natriuretic and hypotensive effects produced by*Allium sativum* (garlic) in anaesthetized dogs. J. Ethnopharmacol., 31:325-31, (1991)
9. J.N.Eloff. Which extractant should be used for the screening and isolation of antimicrobial components f
10. rom plants?. J. Ethanopharmacol., 60; 1-8, (1998).
11. Navarro V, Villarreal M.L, Rojas G and Lozoya X. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. Journal of Ethanopharmacology, 53:143-147, (1996).
12. Cutler R.R and Wislon.P. Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin resistant Staphylococcus aureus. British Journal of Biomedical Science 61(2): 71-74, (2004)
13. AyaforJ.F,Tchuendem M.H.K and Nyasse B. Novel bioactive diterpenoids of Aframomumaulacocarpos. Journal of Natural Products, 57:917-923, (1994).
14. Elnima E.I, Ahmed S.A, Mekkawi AG and Mossa J.S.The antimicrobial activity of garlic and onion extracts. Pharmazie 38:747-748, (1983).
15. Pereira V,Riboli D, da Cunha M. Characterisation of the clonal profile of MRSA isolated in neonatal and pediatric intensive care units of a University Hospital.Ann. Clin. Microbiol.Antimicrob. 13(1):50, (2014)
16. Ross S. Feldberg, Stephen C. Chang, Alan N Kotik, Marcus Nadler,Zeev Neuwirth, David C Sundstrom and Nathan H.Thompson. *In vitro* mechanism of inhibition of bacterial cell growth by allicin. Antimicrobial agents and chemotherapy. 32(12):1763-1768, (1988).
17. J.Vimalin Hena. Antioxidant nature of Allicin to cure Methicillin Resistant Staphylococcus aureus. Int. J. Pharm. Bio. Sci.2(1):529-532,(2011)
18. Hughes B.G and Lawson L.D.Antimicrobial effects of *Allium sativum L.*(garlic),*Allium ampeloprasum L.*(Elephant garlic) and *Allium cepa L*(onion) ,garlic compounds and commercial garlic supplement products. Phytotherapy Research, 5:154-158, (1991).
19. James M.E, Nannapaneni R and Johnson M.G.Identification and characterisation of two bacteriocin producing bacteria isolated from garlic and ginger root. J.Food.Prot., 62:899, (1999).