Ilkogretim Online - Elementary Education Online, 2021; Vol 20 (Issue 3): pp. 3979-3984
http://ilkogretim-online.org
doi: 10.17051/ilkonline.2021.03.410

Antibacterial Activity Of Allium Sativum On Two Microbial Species

Ramesh. G, Samuel Raja. R and Vignesh. M

PG Department of Microbiology, New Prince Shri Bhavani Arts and Science College, Chennai, Tamil Nadu, India.

E-mail of corresponding author: grameshmku@gmail.com

INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Ayurveda is ancient health care system and is practiced widely in India, Srilanka and other countries (Chopra and Doiphode, 2002). Ayurveda system of medicine use plants to cure the ailments and diseases. Despite the availability of different approaches for the discovery of therapeutically, natural products still remain as one of the best reservoir of new structural types. They are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds (Cowan, 1999). In modern time plants have been sources of analgesics, anti-inflammatory, anti-neoplastic drugs, medicine for asthma, anti arrhythmic agents and anti hypertensive.

Human diseases due directly or indirectly to these infectious agents which infect man have become a primary constraint to productivity and is now severely impacting both economic and socio-economic development in many developing countries of the world (Obasohan et al., 2010). Essential oils are basically extracts which have been concentrated and obtained from different parts of plants. They differ in their active ingredients and are used for various purposes based on the composition of their active ingredients. Some oils are used to promote physical healing, for example to treat swelling or fungal infections (Mercier and Knevitt, 2005).

Allium sativum is a perennial bulbous plant that initially came from middle Asia, and is at present grown globally. Allium sativum can grow up to 2 feet in height or more. The bulb is the main part of the plant which is used for medicine (Steven, 2015). Each Allium sativum bulb is made up of 4 to 20 cloves. Each Allium sativum clove may weigh about 1 gram in weight. Fresh, aged, dried or Allium sativum can be used. Each of the supplements may have

different effects to the body (Sethi et al., 2014). It is commonly used as seasoning. It helps prevent certain heart diseases including atherosclerosis, high cholesterol, high blood pressure and boost the immune system as well as protect against cancer (Steven, 2015). The medicinal potency of Allium sativum is due to glycoside, vitamin B, C, and D allisatin II and I. It also contains volatile sulphur oil, which has a vermifugal action (Arshad et al., 2014).

Antibiotic resistance has become a global concern (Westh et al., 2004). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families. Recent work revealed the potential of several herbs as sources of drugs (Iwu, 2002). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

MATERIALS AND METHOD

Collection of the Plant Material

The plants materials were obtained from the local market, Medavakkam, Chennai. They were identified by an Ethnobotanist in the department of Botany, University of Madras, Chennai.

Preparation of Extracts

The Allium sativum bulbs were separated into cloves. The cloves skins were peeled off and the cloves were sliced and also air dried at ambient temperature for about seven weeks. The dried materials were pounded using a sterile laboratory mortal and grinded using a sterile electric blender to obtain a homogenous sample. 120g of the powdered samples each were then extracted with 750 ml of methanol and n-Hexane by cold maceration method as described by Handa et al. (2008). After extracting the plant materials, it was then concentrated using rotatory evaporator at 40^{o}C. The extract obtained was then freeze dried to remove portion of water present in the extract. It was then stored in sterile sample bottles and preserved in the refrigerator at 4^{o}C until further use.

Antibacterial Assay

The agar well diffusion method was employed to determine the antibacterial activity of the plant extracts according to the Clinical and Laboratory Standards Institute (CLSI) (2006). The standardized suspension was used to inoculate the surfaces of sterile nutrient agar plates using sterile cotton swab. 8 mm diameter wells were bored using sterile core borers in the solidified agar. The bottom of the wells was then closed using 1 ml of sterile nutrient agar. The wells were then filled with desired concentrations of the plant extracts (40 mg/ml and 80 mg/ml). The plates were allowed to stand for about 3 h at room temperature for the

extracts to diffuse and then the agar plates were incubated at 37°C for 24 hours. The antibacterial activities of the plant extracts were evaluated by appearance of zones of inhibition around the wells while lack of activity was observed by absence of zones of inhibition. The antibacterial activity of the plant extracts was compared with amoxicillin (0.25µg/ml) to check its effectiveness. The control plates included extract sterility control (ESC), medium sterility control (MSC) and organism viability control (OVC).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined according to the CLSI (2006). The plant extract was dissolved in 5 ml of solution (0.5 ml DMSO and 4.5 ml of water). 2 ml sterile nutrient broth was transferred into 5 different test tubes and 2 ml of different concentration of extract was added respectively. The test organism was inoculated into the labeled tubes with the exception of the negative control. The tubes were incubated at 37°C for 24h. This procedure was repeated for the remaining extracts and test organisms. The MIC was taken as the lowest concentration that prevented any visible growth.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined according to the CLSI (2006). The test tube that showed no visible growth was subcultured onto sterile nutrient agar and incubated at 37°C for 24 h. The least concentration at which the organism did not grow was taken as the minimum bactericidal concentration.

RESULTS

The Allium sativum extract obtained was a golden-yellow, gummy residue with a pungent offensive smell. Methanol (Met Allium sativum), n-Hexane (Hex Allium sativum) plant extracts was active against the test organisms at 40mg/ml (Table1). The n-Hexane and methanolic Allium sativum extracts showed more activity. Amoxicillin, the standard drug for treating the enteric pathogens inhibited the growth of the test organisms at a standard concentration of 0.25 µg/ml. The methanol extract had MIC values of 5 mg/ml and 10 mg/ml against Escherichia sp., and Klebsiella sp. The n-Hexane extract had MIC values of, 5 mg/ml and 2.5 mg/ml against Escherichia sp., and Klebsiella sp (Table2).

DISCUSSION

The Allium sativum extracts gave the widest zones of inhibition compared to the ginger extracts against all the bacterial isolates. The activity of these plants may be attributed to the presence of secondary metabolites within them (Patra and Saxena, 2009). It was also observed that the solvent of extraction affected the degree of sensitivity of the test organisms as reported by Abdullahi et al. (2014). In this study however, Allium sativum extracts had low MIC values as compared to those of ginger extracts when test against the bacterial isolates. However, n-hexane Allium sativum extract had the lowest MIC value (2.5 mg/ml)

against Klebsiella sp. The Allium sativum extracts were cidal against Klebsiella sp when compared to Escherichia sp. The Allium sativum extract had higher activity in vitro against the test organisms as reported by Aliyu et al. (2017). The n-hexane extracts were found to be more potent than the methanolic extract which is in contrast to findings by Garba et al. (2013). This accounts for the effect of the solvent system, which greatly affects the antibacterial activity of the crude extracts.

CONCLUSION

This study showed that E. coli and K. pneumoniae were susceptible to the Allium sativum extracts which invariably means that the plant have antibacterial activity and could be a source of active antimicrobial agent for the development of drugs for the treatment of these infectious microorganisms.

Table 1: Concentration and zone of inhibition of n-Hexane and methanolic extracts of Allium sativum

Organism	Concentration of extracts (mg/ml)/ Zone of inhibition(mm)			
	40 mg/ml	80 mg/ml	40 mg/ml	80 mg/ml
Escherichia sp	25.0	28.0	26.0	30.0
Klebsiella sp	23.0	25.0	21.0	23.0

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanolic and n-Hexane by the extracts of Allium sativum

Organism	MIC (mg/ml)		MBC (mg/ml)	
Escherichia sp	5.0	5.0	10.0	10.0
Klebsiella sp	10.0	2.5	10.0	2.5

REFERENCES

1. Abdullahi, D.K., O.O. Michael and I.I. Indabawa, 2014. Antibacterial activities and phytochemical screening of aloe vera, garlic and ginger. Journal of Emerging Trends in Engineering and Applied Science, 5(3): 172-178.
2. Aliyu, M.S., M.B. Tijjani, M.H.I. Doko, I. Garba, A.B. Ajimego, U.A. Hanwa and M.M. Ibrahim, 2017. Phytochemical and antimicrobial screening of ethanol extracts of zingiberofficinale, allium sativumand syzygiumaromaticumagainst some food associated bacteria and fungi. UJMR Journal of Microbiology, 2(1): 22-27.
3. Arshad, H.R., M. Fahad, S. Al and M.A. Salah, 2014. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. International Journal of Physiology, Pathophysiology and Pharmacology, 6(2): 125-136.
4. Clarke, P.H. and S.T. Cowan, 1952. Biochemical methods for bacteriology. Journal of General Microbiology, 6(1-2): 187-197.Available at: https://doi.org/10.1099/00221287-6-1-2-187.
5. Clinical and Laboratory Standards Institute (CLSI), 2006. Methods for dilution, antimicrobial susceptibility test for bacteria that grow aerobically. Approved standards. 7th Edn., Italy: Villanova. pp: 112-115.
6. Dixon, D. and G. Jeena, 2017. Comparison of different solvents for phytochemical extraction potential from datura metel plant leaves. International Journal of Biological Chemistry, 11(1): 17-22.
7. Ehigbai, O.I., E.S. Omoregie, F.E. Oviasogie and K. Oriakhi, 2016. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food Science & Nutrition, 4(1): 103-109.Available at: https://doi.org/10.1002/fsn3.268.
8. Garba, I., A. Umar, A. Abdulrahman, M. Tijjani, M. Aliyu, U. Zango and A. Muhammad, 2013. Phytochemical and antibacterial properties of garlic extracts. Bayero Journal of Pure and Applied Sciences, 6(2): 45-48.
9. Handa, S.S., S.P.S. Khanuja, G. Longo and D.D. Rakesh, 2008. Extraction technologies for medicinal and aromatic plants. 1st Edn., Trieste (Italy): Earth, Environmental and Marine Sciences and Technologies. pp: 22.
10. Iram, G., S. Mariam, S. Halima, M.A. Shahbaz, Q.S. Zahoor and M.A. Amin, 2012. Inhibitory effect of allium sativumand pathogenic bacteria. Annals of Clinical Microbiology and Antimicrobials, 11(1): 8-15.
11. James, H.W., L.N. Roberts and E.K. Gary, 1983. Rapid inoculum standardization system: A novel device for standardization of inocula in antimicrobial susceptibility testing. Journal of Clinical Microbiology, 17(6): 1114-1119.
12. Patra, A. and J. Saxena, 2009. The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutrition Research Reviews, 22(2): 204-219.

13. Ponmurugan, K. and S. Rajaram, 2012. Antibacterial effect of allium sativum cloves and zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific Journal of Tropical Biomedicine, 2(8): 597-601.Available at: https://doi.org/10.1016/s2221-1691(12)60104-x.
14. Sethi, N., K. Kaura, N. Dilbaghi, M. Parle and M. Pal, 2014. Garlic: A pungent wonder from nature. International Research Journal of Pharmacy, 5(7): 523-529.Available at: https://doi.org/10.7897/2230-8407.0507106.