IJMPR 2021, 5(2), 136-140

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

ANTIMICROBIAL ACTIVITYOF METHANOLIC EXTRACT OF SOME MEDICINAL PLANTS AGAINST HUMAN PATHOGENIC BACTERIA

Pal Neha[1], Singh N. P.[3] and Joshi M. D.*[2]

[1,2]Shobhit Institute of Engineering and Technology, Department of Biotechnology, Meerut.
[3]Meerut College, Department of Botany, Meerut.

Received on: 27/02/2020
Revised on: 17/03/2021
Accepted on: 07/04/2021

***Corresponding Author**
Dr. Maya Datt Joshi
Shobhit Institute of Engineering and Technology, Department of Biotechnology, Meerut.

ABSTRACT

Objectives: To calculate the antimicrobial action of medicinal plants against human pathogenic bacteria and observe Minimum Inhibitory Concentration (MIC) of extracts of plant. **Methods**: Rhizome of Curcuma longa, dried buds of Syzygium aromaticum, and seed of *Piper nigrum* and leaves of *Hibiscus-rosa-sinensis*, *Murraya koenigii*were collected from local market of Uttar Pradesh. The plant parts were air-dried at room temperature and crushed to powder form. The ethanolic extracts of medicinal plants were adapted by percolation process using separating funnel and tested against human pathogenic bacteria by disc diffusion method. Then, Minimum Inhibitory Concentration (MIC) of the plant extracts was decisive. **Results**: All plants extracts show antibacterial properties against bacteria under study. However, extract from S. aromaticum (Clove), Hibiscus-rosa-sinensis (Gulhar) and Tinospora cordifolia (Giloy) reveal most promising result against Staphylococcus aureus with zone of inhibition of 14.33*mm*, 12.90*mm* and 16.10*mm* respectively. Likewise, S. aromaticum (Clove), C. longa (Turmeric) and P.nigrum (Black pepper) showed good antibacterial activity against Escherichia coli with inhibition zone of 14.10*mm*, 12.03*mm* and 12.90*mm* respectively. S.aromaticum (Clove) and Tinospora cordifolia (Giloy) showed promising activity against E.coli with inhibition zone of 19.67*mm* and 10.23*mm*. S.aromaticum (Clove) showed good result against P.aeruginosa with inhibition zone 19.53*mm*. MIC values of methanolic extracts of S. aromaticum and T.cordifolia were found to be at the range of 10.80mm. **Conclusion**: This study keep maintained to understand the use of these plants while traditional medicine as an economic and safe alternative to treat infectious diseases.

KEYWORDS: Antibacterial activity, minimum inhibitory concentration, plant extracts, zone of inhibition.

INTRODUCTION

The global spread of drug resistance among common respiratory pathogens, including Streptococcus pneumonia[6,7,3,8,12,16,32] and Mycobacterium tuberculosis[9,17,24,30,31,37,36,4,10,25], and epidemic increases in multidrug-resistant (and, increasingly, truly pan-resistant) gram-negative bacilli[22,5,19,23,35,21,28,34,20,26] Infections created through these and alternative antibiotics-resistant microbes impact clinicians practicing in every field of medicine. Likely their broadness of belongings and important impact on morbidity and mortality, multidrug-resistant microbes are considered a substantial threat to us public health and national security by the National Academy of Science's Institute of Medicine[29], the federal Interagency Task Force on Antimicrobial Resistance (Interagency Task Force).[1]

According to the WHO, over 80% of the world's population calculates on long established fundamental forms of medicine, largely plant based to meet fundamental fitness care needs. In India, collection and prepare of medicinal plants and plant products major part products contributes a major part each year to the national economy as, a source of both full and part time employment.[11] Over ¾ of the world population relies primarily at plants and plant extracts for healthcare. More than 30% of the complete plant species at one time or other was used for medicinal aims. In India drugs of herbal ancestry have been used in traditional system of medicine such as Unani, Ayurveda for as much as ancient times.

The Ayurveda system of medicine uses about 700 species, Unani 700, Siadha 600, Amchi 600 and modern medicine around 30 species.[14] The plant-based traditional medicine system continuously plays an essential role in healthcare. Plants endure one of the most important sources of medicines. The application of plants as medicines was date back to pre-historic period. In

India, the remark to the therapeutic properties of some herbs in the rig-veda seems to be the earliest records of use of plants in medicines. The medicinal plants are largely utilized all over the world in two specific areas of health authority; traditional system of medicine and modern system of medicine. The traditional system of medicine mainly functions through two distinct steams[11] local or folk or tribal steam and,[13] codified and organized Indian system of medicines like Ayurveda, Siddha and Unani etc. Over the periods the use of medicinal herbs has turn into an important part of daily life despite the growth in modern medicinal and pharmaceuticals research. Approximately three thousands plants species are known to have medicinal properties in India.[27] The Rigveda (3700 B.C) acknowledged the use of medicinal plants. In traditional systems of medicines viz., Ayurveda, Siddha, Yunani and Homeopathy etc use 17 herbs for treatments. It is estimated that 40% of the world populations depends directly on plant based medicine for their health care.[33]

In India, medicinal plants attempt low cost and intact well-being care result. The world depends upon the herbal medicine for the largest source of the plant bio-diversity still about 70% to 80% of world population which are being used since the ages as the traditional health care system.[18] The medicinal plants contain bioactive phyto-chemical constituents that produce specific physiological effects on human body.[15] The natural compounds of distinct plants containing phytochemicals protect the human body from various diseases.[2]

In this study, an attempt has been made to calculate the antimicrobial activity of extracts of selected medicinal plants against human pathogenic bacteria and observe minimum inhibitory concentration of plant extracts. This is with a view to contribute knowledge for usage of locally available medicinal herbs in resemblance to the antibiotics which can cause various side effects.

MATERIALS AND METHODS

Collection of plant samples and bacterial strains: The plant samples were collected from the areas of Aligarh district of Uttar Pradesh, India and the study was carried out in the laboratory of Aligarh Muslim University, Aligarh. MDR strains Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae obtained from Ram Manohar lohia Hospital, Delhi were used for antimicrobial activity of plant extracts.

Extraction of antimicrobial compounds: The methanolic extracts of medicinal plants were prepared by dissolving 20*gm* of dried powder into 100*ml* of ethanol separately and then kept in the separating funnel for 7 days with continuous shaking in every 24 hours. After 1 week extract was filtered where the sediment settled down at the bottom and left ethanol was evaporated at evaporator. The left over filtrate called as extract containing the chemical constituents of each medicinal plant were dried in the hot air oven at 40o C and stored under refrigeration for further use. Whattman's No 3 filter paper was punched into 2*mm* disc and was sterilized. Each disc was drowned in the particular concentration of the extracts that was prepared by using DMSO and methanol in the ratio of 10:90 i.e. 200*mg/ ml*, 100*mg/ml*, 50*mg/ml*, 25*mg/ml* and 12.5*mg/ml*.

Disc diffusion method: The 20*ml* of sterilized Muller Hinton Agar was discharge into sterile petri plates, later solidification, 100μl of fresh culture of human pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps and incubated at 37° C for 24 hours. After incubation the diameter of inhibitory zones formed were measured.

Standardization of bacterial suspension: McFarland standard was used as a reference to adjust the turbidity of bacterial suspensions. The bacterial suspensions were standardized following the CLSI guidelines for aerobic bacteria. All of the tested bacteria were grown in Mueller Hinton broth for 24 h, followed by the matching of bacterial suspension to the turbidity equivalent to 0.5 McFarland solutions (1-2×108 *cfu/ ml*).

Determination of MIC: The minimum inhibitory concentration (MIC) of the aqueous extract was determined by serial dilution method. For MIC, two-fold serial dilutions of the extracts were prepared (200, 100, 50, 25 and 12.5$\mu g/ ml$). Incubation of the tubes was carried out at 37o C for 24 hours for bacteria and was observed for any visible growth. The bacterial interruptions were used as decisive control and extracts in broth were used as adverse control. The MIC was explained as the lowest concentration of the extract that conclude no more display any visible growth when compared to control tubes that contained only the extracts.

RESULTS

One highest effective antibiotic which showed highest zone of inhibition against MDRs was selected from each isolated MDR bacteria in every clinical sample category studied viz., blood and throat swabs and further compare to the tested plant extracts. Total two E.coli MDRs were isolated from blood sample, three were isolated from throat swab, onepseudomonas aeruginosa and three S.aureus which were isolated from clinical blood samplescompared to all tested plant extracts.

Extract of S.aromaticum showed highest antibacterial activity against E.coli with zone of inhibition 19.67*mm*. Extracts of T.cordifolia and C.longa showed most promising result against E.coli and S.pneumoniae with the zone of inhibition of 14.90 and 12.90 *mm*. K.pneumoniae was found highly sensitive to the action S.aromaticum (14.10 mm) followed by C.longa and P. nigrum with zone of inhibition of 12.03 and 12.90 *mm* respectively.

At the clinical MDRs sample of blood and throat swabs, extracts of S.aromaticum, M.koenigii and P.nigrum were effective against P.aeruginosa with significant zone of inhibition 19.53, 3.77 and 5.13 *mm* respectively. T.cordifolia was found most sensitive against S.pneumoniae with zone of inhibition 16.10mm. On the contrary T.cordifolia was resistant against E.coli and K.pneumoniae. S.pneumoniae and K.pneumoniae were resistant against P.nigrum.

Among the six medicinal plants, the crude extracts of S.aromaticum, T.cordifolia, M.koenigii, C.longa, Hibiscus-rosa-sinensis and P.nigrum showed good antimicrobial activity against all tested microorganisms. S.aromaticum showed goodresult against E.coli with zone of inhibition 19.67 *mm*. M.koenigii was effective against S.pneumoniae (5.03 *mm*). MIC values of methanolic extracts of S.aromaticum and T.cordifolia were found to different clinical blood and throat swabs MDR samples.

Table 1: Activity against of plant extracts against human pathogenic bacteria by disc diffusion method.

Extracts	MDR	T.cordifolia	S.aromaticum	M.koenigii	C.longa	H.rosa-sinensis	P.nigrum
E.coli	APRCBM3	9.70	19.67	0.00	5.07	0.00	4.60
	RMLBM3	14.90	19.23	0.00	2.33	0.00	3.07
K.pneumoniae	APRCTSF1	3.73	14.10	1.60	12.03	1.77	9.77
	RMLTSF2	1.70	13.70	1.23	11.73	2.67	11.90
	JNMCBM1	4.83	10.80	4.03	10.80	3.23	12.90
P.aeruginosa	RMLBF1	0.00	19.53	3.77	0.00	0.00	5.13
S.pneumoniae	APRCBM2	14.03	12.40	2.73	4.80	12.90	6.10
	APRCBF2	16.10	14.33	1.73	4.13	11.00	3.97
	RMLBM1	12.97	10.93	5.03	8.40	12.90	7.33

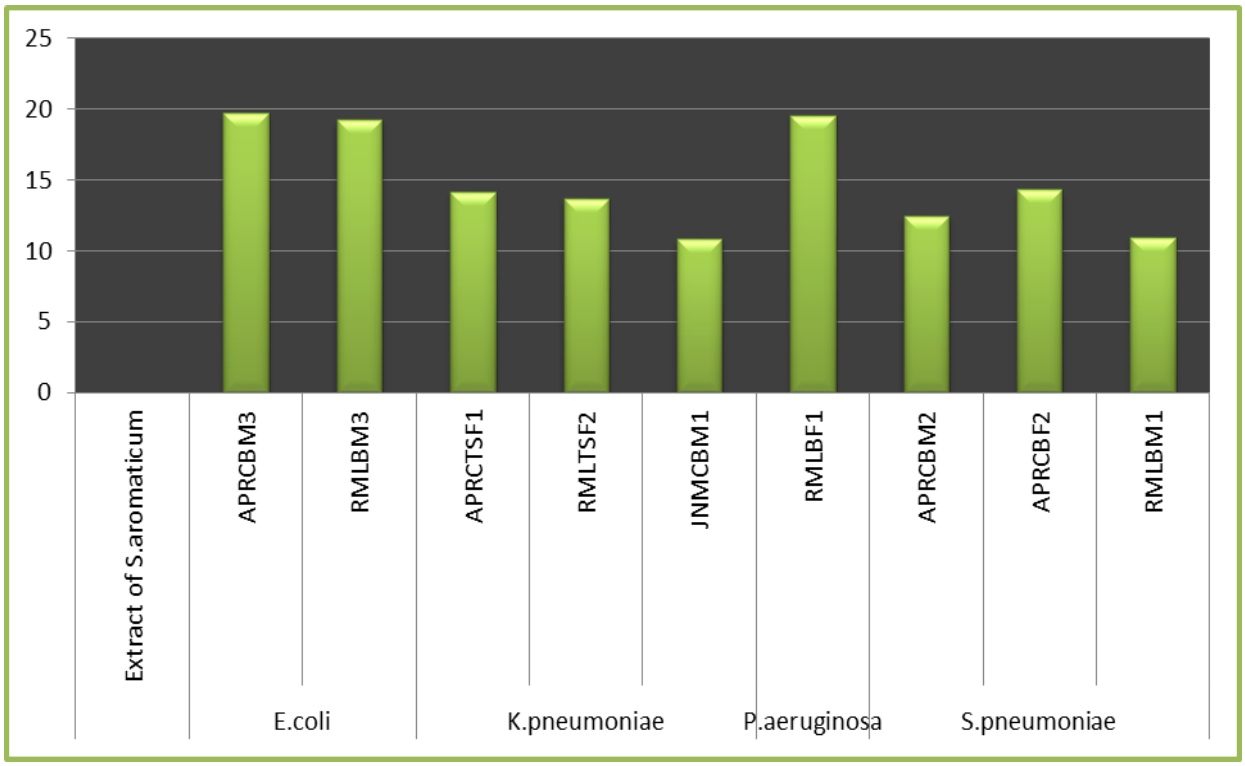

Figure 1. Minimum inhibitory concentration (MIC) of S.aromaticum.

CONCLUSION

This study has helped in demonstrating the potential bioactive compound of natural plant extracts that are economical. Among six plant extracts examines Syzgium aromaticum, Tinospora cordifolia and Curcuma longa showed the best antibacterial activity against E.coli, S.pneumoniae and K.pneumoniae respectively. Based on the consequences we may conclude that secondary components of these plants reveal antibacterial activity that can be used for the treatment of diseases caused by the organism. This study has helped us to understand the importance of traditional medicine in the treatment of different bacterial disease than antibiotics as it shortens the length of treatment, increase patient compliance as well as reduce overdose which may lead to toxicity or other side effects.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to Jawaharlal Nehru Medical College for providing us with laboratory facilities that are required for smooth and efficient passage of work required for the completion of the Research work. We are also thankful to the supervisors for the proper guidance, support, and encouragement throughout the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Antibiotic/antimicrobial resistance: action plan. Atlanta: US Department of Health and Human Services Centers for Disease Control and Prevention. 1999. Available at: http://www.cdc.gov/drugresistance/actionplan
2. Apparao, R.K., Kaladhar, D.S.V.G.K. and Santosh, K.S. 2011. Evaluation of antimicrobial activity of *Lawsoniainermis*(Henna) on aquapathogens. *JPBMS.*, 7(02): 1–3.
3. Centers for Disease Control and Prevention. 2001. Resistance of *Streptococcus pneumoniae* to fluoroquinolones—United States, 1995–1999. MMWR *Morb. Mortal. Wkly. Rep*., 50: 800–4.
4. Centers for Disease Control and Prevention. 2005. Multidrug-resistant tuberculosis in Hmong refugees resettling from Thailand into the United States, 2004–2005. *MMWR Morb. Mortal. Wkly. Rep*., 54: 741–744.
5. Douglas, M.W., Mulholland, K., Denyer, V. and Gottlieb, T. 2001. Multi-drug resistant *Pseudomonas aeruginosa* outbreak in a burns unit-an infection control study. *Burns.*, 27: 131–135.
6. File, T.M. Jr. 2006. Clinical implications and treatment of multiresistant *Streptococcus pneumoniae* pneumonia. *Clin. Microbiol. Infect*, 12(Suppl 3): 31–41.
7. File, T.M. Jr. 2004. *Streptococcus pneumoniae* and community-acquired pneumonia: a cause for concern. *Am. J. Med*., 117(Suppl 3A): 39S-50S.
8. Gordon, K.A., Biedenbach, D.J. and Jones, R.N. 2003. Comparison of *Streptococcus pneumoniae* and *Haemophilusinfluenzae*susceptibilities from community- acquired respiratory tract infections and hospitalized patients with pneumonia: five-year results for the SENTRY antimicrobial surveillance program. *Diagn. Microbiol. Infect. Dis.*, 46: 285–9.
9. Grant, G.R., Lederman, J.A, Brandstetter, R.D. and Heaton, T.G. 1997. Tuberculosis and artificial pneumothorax: once again, back to the future? *Chest*., 112: 7–8.
10. Granich, R.M., Oh, P., Lewis, B., Porco, T.C. and Flood, J. 2005. Multidrug resistance among persons with tuberculosis in California, 1994–2003. *JAMA.*, 293: 2732–2739.
11. Holley, J. and Cherla, K. 1998. The Medicinal Plants Sector in India. International Development Research Centre. South Asia Regional Office, IDRC, Canada. Medicinal and Aromatic Program in Asia (MAPPA), New Delhi, India.
12. Hyde, T.B., Gay, K. and Stephens, D.S. 2001. Macrolide resistance among invasive *Streptococcus pneumoniae* isolates. *JAMA.*, 286: 1857–62.
13. International Union for Conservation of Nature, IUCN, Red List of Flora, cited 1998, updated August, 2010.
14. Joy, P.P., Thomas, J., Mathew, S. and Skaria, P. 1998. Medicinal plants, Kerala Agricultural University Publications; Kerala, India, 3.
15. Kaladhar, D.S.V.G.K., Narsinga, R.V. and Sreenu, B. 2010. Comparative Antimicrobial Studies of *Dioscorea Hamiltonii* hook.f. tubers with *Azadirachtaindica* Stem. *Journal of Pharmaceutical Science and Technology*, 2(8): 284–287.
16. Kays, M.B., Smith, D.W., Wack, M.E. and Denys, G.A. 2002. Levofloxacin treatment failure in a patient with fluoroquinolone-resistant *Streptococcus pneumonia* pneumonia. *Pharmacotherapy*, 22: 395–9.
17. Kir, A., Tahaoglu, K., Okur, E. and Hatipoglu, T. 1997. Role of surgery in multidrug- resistant tuberculosis: results of 27 cases. *Eur. J. Cardiothorac. Surg*, 12: 531–4.
18. Lale, N.E.S. 2003. Bio-activity and Limitation against wide spread use of neem products for the management of insect pests. *Nigerian J. Applied Biol.*, 3: 115–125.
19. Levin, A.S., Barone, A.A. and Penco, J. 1999. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and Acinetobacter baumannii. *Clin. Infect. Dis.*, 28: 1008–1011.
20. McDonald, L.C. 2006. Trends in antimicrobial resistance in health care–associated pathogens and effect on treatment. *Clin. Infect. Dis.*, 42(Suppl 2): S65–71.
21. McGowan, J.E. Jr. 2006. Resistance in nonfermenting gram-negative bacteria: multidrug resistance to the maximum. *Am. J. Med*., 119: S29–36; discussion S62–70.
22. Mirza, S.H., Beeching, N.J. and Hart, C.A. 1996. Multi-drug resistant typhoid: a global problem. *J. Med. Microbiol*, 44: 317–319.
23. Muller, M., McGeer, A. and Willey, B.M. 2002. Outbreaks of multi-drug resistant *Escherichia coli* in long-term care facilities in the Durham, York and Toronto regions of Ontario, 2000–2002. *Can. Commun. Dis. Rep*., 28: 113–118.
24. Nachega, J.B. and Chaisson, R.E. 2003. Tuberculosis drug resistance: a global threat. *Clin. Infect. Dis*., 36: S24–30.
25. Nettleman, M.D. 2005. Multidrug-resistant tuberculosis: news from the front. *JAMA*, 293: 2788–2790.
26. Paterson, D.L. 2006. The epidemiological profile of infections with multidrug resistant *Pseudomonas*

aeruginosa and *Acinetobacter species. Clin. Infect. Dis*., 43(Suppl 2): S43–48.
27. Prakasha, H.M., Krishnappa, M., Krishnamurthy, Y.L. and Poornima, S.V. 2010. Folk medicine of NR PuraTaluk in Chikamaglur district of Karnatka. *Indian Journal of Traditional Knowledge*, 9(1): 55-60.
28. Rhomberg, P.R., Fritsche, T.R., Sader, H.S. and Jones, R.N. 2006. Clonal occurrences of multidrug-resistant gram-negative bacilli: report from the Meropenem Yearly Susceptibility Test Information Collection Surveillance Program in the United States (2004). *Diagn. Microbiol. Infect. Dis*., 54: 249–257.
29. Smolinski, M.S., Hamburg, M.A and Lederberg, J. 2003. Microbial threats to health: emergence, detection, and response. Washington, DC: *Institute of Medicine*.
30. Strambu, I. 2000. Therapeutic pneumothorax—an effective adjuvant method in treating multidrug-resistant tuberculosis. *Pneumologia*, 49: 129–36.
31. Sung, S.W., Kang, C.H., Kim, Y.T., Han, S.K., Shim, Y.S. and Kim, J.H. 1999. Surgery increased the chance of cure in multi-drug resistant pulmonary tuberculosis. *Eur. J. Cardiothorac. Surg*, 16: 187–93.
32. Whitney, C.G., Farley, M.M. and Hadler, J. 2000. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N. Engl. J. Med*., 343: 1917–24.
33. World Health Organization. Guidelines for the Assessment of Herbal Medicine Programme on Traditional Medicine. 2003. Doc. WHO/TRM/91.4. WHO, Geneva.
34. Wright, M.O. 2005. Multi-resistant gram-negative organisms in Maryland: a statewide survey of resistant *Acinetobacter baumannii*. *Am. J. Infect. Control*, 33: 419–421.
35. Zansky, S., Wallace, B. and Schoonmaker-Bopp, D. 2002. From the Centers for Disease Control and Prevention: outbreak of multi-drug resistant Salmonella Newport—United States, January–April 2002; *JAMA*. 288: 951–953.
36. Zazueta-Beltran, J., Muro-Amador, S., Flores-Gaxiola, A., Llausas-Magana, E., Leon-Sicairos, N. and Canizalez-Roman, A. 2006. High rates of multidrug- resistant *Mycobacterium tuberculosis* in Sinaloa State, Mexico. *J. Infect*, 54: 411–412.
37. Zignol, M., Hosseini, M.S. and Wright, A. 2006. Global incidence of multidrug resistant tuberculosis. *J. Infect. Dis*., 194: 479–85.