International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue A16, March 2021
ISSN:2320-7817(p) 2320-964X(0)

Research Article Open Access

Efficacy of extracts of *Ocimum sanctum* (Tulsi) & *Azadirachta indica* (neem) leaves against multidrug resistant pathogens

Nagrale AP [1] and Mendhe SN[2]

[1]Department of Microbiology, Shri. D.M. Burungale Science and Art's College Shegaon Dist: Buldana 444203, MS, India
[2] Department of Microbiology, Shri Shivaji Science and Arts College, Chikhli, Dist. Buldana, MS, India
[1]Email: amol.nagrale8@gmail.com | [2]Email: snmendhe@gmail.com

Manuscript details:

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Cite this article as:
Nagrale AP and Mendhe SN (2021) Efficacy of extracts of *Ocimum sanctum* (Tulsi) & *Azadirachta indica* (neem) leaves against multidrug resistant pathogens, *Int. J. of. Life Sciences*, Special Issue, A16: 135-138.

Article published in Special issue of National Conference on "Recent Trends in Science and Technology-2021 (RTST-2021)" organized by Department of Environmental Science, Shri. Dnyaneshwar Maskuji Burungale Science & Arts College, Shegaon, Bhuldhana**,** and Department of Botany Indraraj Commerce and Science College Shillod, DIst. Aurangabad, Maharashtra, India date, February 22, 2021.

ABSTRACT

Present work mainly focuses on to determine the efficacy of methanol extracts of plants against multidrug resistant Gram-negative bacteria. These bacteria were isolated from clinical samples by standard microbiological procedure. Multi drug resistant bacteria selected on the basis of antibiotic susceptibility test by Kirby-Bauer disc diffusion method. Methanol extracts of *Oscimum sanctum(tulsi)* and *Azadirachta indica* (neem), were tested for antibacterial activity against the selected multidrug resistant bacteria by agar well diffusion method. From different samples multidrug resistant gram-negative bacteria isolated *Escherichia coli, klebsiela pneumonia and pseudomonas spp*. *E. coli* dominant in number. *Ocimum sanctum* were found to be most effective with zone of inhibition rang (11-15nm) than *Azadirachta indica*. these medicinal plants having antibacterial activity can be used as a alternative source of medicine.

Keywords: medicinal plants, pathogen, multidrug resistant, antibacterial activity**.**

INTRODUCTION

Drug discovery has always been a constant process from time to time. Evocative. Antimicrobial products have been used since the advent of antibiotics in the 1950s. With an optimistic view of chemotherapy, chemotherapy has made significant progress. In the near future, conquering infectious diseases. Furthermore, in reality, their challenge of emerging and re-emerging diseases poses a major threat to mankind and survival.

The discovery of antibiotics was a milestone in the medical record. Which could save innumerable lives. Antibiotics have already been deemed to be in the history of medicine, one of the most effective types of chemotherapy.

(Aminov, 2010). The efficacy of existing antibiotics is being threatened by the emergence of Multidrug-resistant (MDR) bacteria. (Dahiya and Purkayastha 2013). Thus, with the decrease in the speed of discovery, the latest approaches to the production of innovative novel antimicrobial drugs to fight the spreading resistance of antibiotic pathogens. As indicated by the World Health Organisation over 80% of the world's population depend on conventional medica-tion for their essential medical care needs (WHO 1993).

Asian peoples continuously use the herbal medicines from decades. This is a long history of human interactions with the environment and is a rich source of antimicrobial agents and potent antibiotics. (Ahmad et al. 2002). Many commercially available drugs used in modern medicine were initially used in crude forms in conventional or folk healing practices, or for other purposes that suggested potential biological activity (Anthony and Livermore 2015).

Plants produce huge diverse types of small molecules classified as Phytochemical. Which is very important for the plant's immunity? (Rathanyaka and Obreshkova, 2013). The conventional system of medicine, i.e. Ayurveda, Homoeopathy, Unani, Sridhar, Armchair et al. in India supported herbal drugs (Mukherjee and Wahile, 2006). Plants are well-thought-out the greatest source to get hold of new antimicrobials. They produce secondary metabo-lites, Phytochemicals, which defend the plant against pathogens. (Thapa, et al., 2018). Plant extracts from medicinal plants have been used in the treatment of infectious disea-ses due to their availability and affordability (Lee et al., 2007).

Antimicrobial resistance is a one of the major problems face worldwide public health. The effectiveness of anti-microbial drugs has been lost due to the development of resistance in the pathogen. Continuous exposure of bacteria antibiotics resulted in the development of resistant microbial strains. This had created a curiosity in finding alternatives for antibiotics. In a quest to find substitutes for antibiotics, the medicinal plants which are widely used by medical practitioners for the treatment of infectious disease is put to systematic scientific investiga-tions. The appearance and increased number of antibiotic-resistant microorganisms also triggered this type of plant investigation. Thus, it was deemed important to consider for investigation of antimicrobial activity of herbal plants against MDR Gram-negative isolates from the clinical samples In this study commonly available medicinal plants *Ocimum sanctum* (tulsi), *Azadirachta indica* (neem) were selected based on their common uses in Traditional Indian systems.

MATERIALS AND METHODS

The different clinical sample like urine, pus, sputum, etc. will be collected from hospitalized and outdoor patients. Isolates were identified by Standard microbiological procedure (Gram staining, cultural characteristics, catalase test, oxidase test, motility, biochemical test). Using Bergey's Manual of Systematic Bacteriology.

Gram negative bacteria separated and selected for antibiotic susceptibility pattern. Antibiotic susceptibility tests of the identified bacteria were performed by modified Kirby-Bauer disk diffusion method in Mueller Hinton agar (MHA) according to CLSI guideline (2014). MDR bacteria were categorized as per the defination given by Magiorakos et al. (2012).

Leaves of *O. sanctum* (tulsi) and *A. indica* (neem) collected, thoroughly washed with tap water and left to air dry for 13 to 15 days. Fine powders of dried samples were obtained by grinding in an electrical grinder. Extraction from approximately 60-80 gm of powdered form was carried out by Soxhlet extraction method using methanol as solvent. Crude extracts was collected, dried and further assayed for antimicrobial activity.

Antimicrobial activity of extracts was performed by using agar well diffusion method. Fresh culture of Gram - negative MDR bacterial inoculums was prepared and 100µl of test culture were swabbed uniformly on the surface of nutrient agar plates. 6mm diameters well were cut in each agar plates with the help of cork borer. These well were filled with 50µl of plant extracts of 100 mg/ml concentration and solvent blank (DMSO) separately with the help of micropipette. (Redfern *et al* 2014). After that these plates were incubated in the incubator at 37ºC for 24h. After overnight incubation zone of inhibition were observed.

RESULTS AND DISCUSSION

Table 1: Number of antimicrobial effects of methanol (ME) extracts against multidrug resistant Strain of *Escherichia coli, klebsiela pneumonia and pseudomonas spp*.

Plants	extracts	*Escherichia coli (n=34)*		*Klebsiela pneumonia(n=24)*		*Pseudomonas spp. (n=22)*	
A. indiaca	ME	Active	Inactive	Active	Inactive	Active	Inactive
		3	31	2	22	1	21
O. sanctum	ME	6	28	4	20	2	20

Out of different clinical samples processed and three different MDR Gram negative bacteria isolated were *Escherichia coli, klebsiela pneumonia and pseudomonas spp*. With highest percentage 27% of *E. coli*. Similarly, 19.2% of *K. pneumonia* and 17.6% of Pseudomonas spp. As shows in table.

Extracts were prepared and their antimicrobial activity medicianal plant *A. Indica and O. Sanctum* when tested against Gram negative MDR bacteria, very only some *E. coli* isolates inhibited by the plants extracts. Out of 34 isolates only 3 were inhibited by *A. Indica* and 6 were inhibited by *O. Sanctum* but others didn't show any activity.

Along with 24 *Klebsiela pneumonia tested*, only 2 and 4 were shows the inhibition zone against extracts of *A. Indica* and *O. Sanctum* respectively. Whereas remaining isolates didn't shows any activity. Similarly, Among the extracts, *O. Sanctum* was found be effective against 2 and *A. Indica* extracts only against single isolates of *Pseudomonas spp*. While other unaffected from the extracts. From this observation isolates shows strong resistant power against antimicrobial compound.

Different plants produced different types of secondary metabolites which are having beneficial effects on the human health. when these plant material extract prepared were found rich in phenols, saponins, flavanoids , alkaloids, steroids, terpenoids resins and tannins. Because of these compound extracts shows antimicrobial activity. (Cowan 1999; Padayana et al., 2011).

Methonolic extracts of *Azadirachta indica* leaf against *E. coli* reported a inhibition activity having inhibition zone 18mm in diameter Sethi *et. al* (2012). Nandedkar et al., (2013) evaluated the antimicrobial actibity methanol extract of *Azadiracta indica* and *O. sanctum* were they found that 10.2 and 12.5 zone of inhibition respectively. Methanol has polarity index of 5.1 which responsible for making it an efficient solvent. Therefore, it is easily available commonly used in extraction of plant material. Besides it is cheap compare to other solvents (Crag et. al. 2013).

In present study of antibacterial assay, selected plants exhibited different clear or inhibition zone ranges form 11-15mm. in the same way 11-14 zone of inhibition obtained in the study of Ahmad et al. (2002).

Jain et al., (2012) conducted study on leaves extracts *of O. sanctum* they were found to be antibacterial effect with 10% activity in which the leaves extract inhibit the growth of *E. coli and S. aureus*. Extracts were found to be inactive against MDR gram negative *E.coli, Psudomonas spp. And P. vullgaris*, different result obtained in the study of Jocoby et al. (2012).

Antimicrobials based on plants have tremendous therapeutic potential as they can serve the purpose with lower side effects frequently associated with synthetic antimicrobials (Vieira and Simon 1999).

CONCLUSION

Selected medicinal plants (*O. sanctum and A. indica*) were successful in exhibiting inhibitory effect on MDR gram negative isolates. Newly emerging bacterial resistant to many antibiotics also called MDR , discovery of novel efficient new with lesser side effect antibacterial agent is essential.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Ahmad IH, Mehmood Z & Mohammad F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of ethno pharmacology*, 62(2):183-193.

Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. *Frontiers in microbiology*, 1:134.

Anthony CM and Livermore DM (2015). Resistance to antimicrobial drugs: a worldwide calamity. *Ann Intern Med* 18: 557-561.

Cowan MM. (1999) Plant products as antimicrobial agents. *Clinical microbiology reviews*, 12(4): 564-582.

Crag GM, Newman DJ and Sander KM (2013) Natural products in drug discovery and development. *J Nat Prod* 60:52-60.

Dahiya P and Purkayastha S. (2013). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian J Pharm Sci* 74:443-450.

Jacoby B, Aghdam BM and Hassanzade Z **(**2012) Antibacterial activities of lemon grass methanol extract and essence in pathogenic bacteria. *American-Eurasian Journal of Agricultural and Environmental Science,*12**:** 1042-1046.

Jain R, Katare N and Kumar V (2012) In vitroantibacterial potential of different extracts of *Ocimum* spp. *Journal of Natural Science Res* 2:84-90.

Li, HB, Wong CC, Cheng, KW, & Chen F. (2007) Evaluation of two methods for the extraction of antioxidants from medicinal plants. *Analytical and Bioanalytical Chemistry*, 388(2):483-488.

Magiorakos MF, Norzials MH and Karim AA **(**2012) Antimicrobial activity and mechanical properties of partially hydrolyzed sago starchalginate edible film containing lemon grass oil. Journal of *Food Science,* 72:324-330.

Mukherjee PK, Wahile A, Kumar VR., Mukherjee K, & Saha, BP. (2006) Marker profiling of botanicals used for hepatoprotection in Indian system of medicine. *Drug information journal: DIJ/Drug Information Association*, 40(2):131-139.

Nandedkar PH & Mulani RM. (2016) Phytochemicals and HPTLC studies of methanolic extract of different germplasms of Cordia dichotoma Frost f. *Journal of Pharmacognosy and Phytochemistry*, *5*(4): 6.

Padayan SA, Ashalatha M, Pranjna PS & Bhatt RM. (2011) Evaluation of antibacterial and antioxidant properties of *Uvaria narum* Wall. International Research journal of Pharmacy, (5):142-144.

Rathnayaka G and Obreshkova D. (2013) Comparitive studies on the activity of basil-an essential oil from *Ocimum basilicum* L.-against multidrug resistant clinical isolates of the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* by using different test methods. *Journal of Microbial Methods,* 54:105-110.

Redfern J, Kinninmonth M D and Verranm J (2014) Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *J App Microbiol* 1: 45-46.

Sethi SS & Gupta BL (2012) Antimicrobial activity of medicinal plants on urinary tract pathogens, 4(2):626-628.

Thapa B, Singh A, & Tuladhar R. (2018) In vitro Antibacterial effect of medicinal plants against multidrug resistant Gram negative bacteria. *Tribhuvan University Journal of Microbiology*, 5: 25-31.

Vieira RF and Simon JE (1999). Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. *Journal of Sociedade Botanical Sao Paulo,* 54: 207-216.

WHO (1993). Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram* 28:13-14.