ANTIBACTERIAL ACTIVITY OF *MURRAYA KOENIGII, AMARATHUS SPINOSUS* AND *DAUCUS CAROTA* ON NOSOCOMIAL INFECTIONS

PRAJITHA N. KUTTY, PADMA V. DESHMUKH

Abstract: Nosocomial infection is a major health problem throughout the world and is the most common complication affecting hospitalized patients. It has been associated with an increased morbidity, mortality rates and excess health care cost that has huge economic impact. The side effects and rapidly emerging resistance of the pathogens towards the wide range of antibiotics has resulted in the shift of attention from modern chemotherapy to traditional herbal medicines. In the present study, the antibacterial activity of *Murraya koenigii Linn, Amaranthus spinosus Linn* and *Daucus carota Linn*,.were checked against multi drug resistant strains isolated from the air flora of hospitals, fomites as well as clinical samples from indoor patients.

Keywords: Nosocomial infection, multi drug resistant, antibacterial activity, *Murraya koenigii Linn, Amaranthus spinosus Linn* and *Daucus carota Linn*.

Introduction: Nosocomial infections occur worldwide, both in the developed and developing world. They are a significant burden to patients and public health. The World Health Organization offers several definitions of a nosocomial infection/ hospital acquired infection. An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission is called a Nosocomial infection. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility. As a general timeline, infections occurring more than 48 hours after admission are usually considered nosocomial.

Widespread use of antibiotics has spurred evolutionary changes in bacteria allowing them to flourish in the presence of powerful drugs thus, leading to multi drug resistance. Nosocomial infections due to multidrug resistant strains pose a formidable challenge for healthcare practitioners as patients often need to be treated empirically and a delay in the appropriate initial therapy is known to increase mortality rates significantly[1]. New antimicrobials are coming up for the treatment of these multi-drug resistant bacterial infections all over the world. Unfortunately, many of these are costly and several may have serious side effects[2].

The use of herbal medicines for the treatment of diseases remains the main stay of health care system. It is gaining in popularity especially among the rural population in the developing countries since it is an efficacious and cheap source of medical care[3]. **Parekh et al., 2007** also mentioned that plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur **.**

In the present study, the antibacterial activity of some commonly found plants were checked against multi drug resistant strains isolated from

the air flora of hospitals, fomites as well as clinical samples from indoor patients. The plants selected for the study along with their common names are mentioned in the table no.I below :

Table no. I, scientific and common names of the selected plants.

Sr.No.	SCIENTIFIC NAME OF THE PLANT	COMMON NAME OF THE PLANT
1	*Murraya koenigii Linn*	Curry leaves
2	*Amaranthus spinosus Linn*	Spiny amaranth
3	*Daucus carota Linn*	Carrot

Materials and methods:

Specimen collection: Samples collected for this study included air samples, swabs from fomites and clinical samples from patients admitted in hospital.

- **Air samples** were collected from various units in the Hospital by exposing sterile agar plates for about 10 minutes to study the flora of hospitals[4].
- Using sterile cotton tipped applicators, swabs from different kinds of **fomites** and from various wards in the Hospital Complex were obtained.
- The samples were also collected from **patients admitted to the hospital** for various clinical symptoms using sterile swabs. The swabs with samples were carried to the laboratory dipped in Ringer's solution.

Specimen processing:

The samples collected were then subjected to further processing which included isolation and identification.

- **Isolation of pathogens:**

The collected samples were processed by direct streaking on various selective and differential media like Nutrient agar medium, Mac conkey agar medium, Nutrient agar + 0.3 % casein agar medium, Cysteine lactose electrolyte deficient agar medium, Super imposed blood agar medium, Charcoal yeast extract agar medium, Mannitol salt agar medium and Cetrimide agar medium.

- **Identification:**

The identification of the isolates was done on the basis of Gram nature, cultural characteristics observed on selective media, pigments, hemolysis and biochemical properties as per Bergey's Manual of Determinative Bacteriology, 8th edition.

Antibiotic susceptibility testing:

The disc diffusion susceptibility methodbeing a simple, practical and well-standardized method wasused to check the susceptibility of the isolates against a variety of antibiotics as sensitive, resistant or intermediate[5]. Ten different antibiotics [Tetracyclin (30 mcg), Trimethoprim (05 mcg), Nalidixic acid (30 mcg), Ciprofloxacin (05mcg), Clindamycin (02 mcg), Ceftazidime (30 mcg), Gentamicin (10 mcg), Amikacin (30 mcg), Methicillin (30mcg) and Nitrofurazone (30 mcg)] were used for the study, based on their use in clinical treatment.Those isolates which showed resistance to more than 50% of the antibiotics used (MAR Index - > 0.5) in the antibiotic sensitivity testing were selected

for further screening tests.

Preparation of extracts:

The plant materials of *Murraya koenigii Linn, Amaranthus spinosus Linn* and *Daucus carota Linn* were directly obtained from the local market, washed, dried in sunlight and powdered. The powders were extracted using hot water, cold ethanol by plain decoction method and hot ethanol by continuous hot extraction using the Soxhlet apparatus[6] [7].

Screening of extracts:

- **Antibacterial screening of the extracts:**

Preliminary antibacterial screening of the extracts was carried out using Agar ditch method at a concentration of 3 % each[8].The innocula prepared from the test organisms were streaked perpendicular to the ditch containing extract of 3% concentration and parallel to each other.

- **MIC Determination:**

MIC was determined by disc diffusion method [9].The range of extract concentration used for MIC determination was 0.3 % to 3 %.

- **Bactericidal activity:**

Efficacy for certain antibacterials can be optimized by dosing strategies that

maximize the duration of antibacterial exposure (time dependent bactericidal activity). Cold ethanolic extracts of *Daucus carota Linn*, *Murraya koenigii Linn* and *Amaranthus spinosus Linn* were found to be the most active against the MDR's and therefore used for the formulation with 1% extract concentration in Aloe vera gel as the base.The cidal activity time of the above extracts were determined by carrying out the bactericidal activity testing from 0 to 7 hrs and 24hrs [10].

- **Biovailability of gels:**

Further, the antibacterial activity of the prepared herbal gel against the isolated MDR's was checked using 6mm borer and measuring the zone size of inhibition and comparing the zone with standard ointment and the gel base by **Agar well diffusion method** [8,11,12].

In vivo studies:

Staphylococcus aureus and *Pseudomonas aeruginosa* being the most prevalent in most cases were selected for in vivo studies.The efficiency of the extractswere tested in vivo using white Wistar rats.Three sets each containing 6 mice were defined for the in vivo studies. A daily dose of about 100mg was applied on the skin surface and observed for necrosis, edema, erythema or irritation [13].

Results and discussions:

A total of 155 isolates obtained from thirty five clinical samples, fomites and air flora included *Staphylococcus aureus (32%), Pseudomonas aeruginosa (25%), Staphylococcus epidermidis (15%), Micrococcus luteus (10%), Proteus vulgaris (10%), Klebsiella pneumonia (8%), Citrobacter spp. (8%), Escherichia coli (7%), Serratiamarcescens (5%), Xanthomonas spp. (4%), Enterobacter spp. (2%) , Bacillus subtilis* (9%), *Bacillus cereus (5%) and Candida albicans(5%) as in* **fig no. I**.

Isolate obtained from air flora included *staphylococcus aureus, pretus vulgaris,klebsiella pneumonia, Bacillus subtilis*, *Micrococcus luteus*, *Candida albicans*, etc. Isolates obtained from various fomites included *Staphylococcus aureus, Proteus vulgaris , Bacillus subtilis*, *Bacillus cereus, Citrobacter diversus*, *Candida albicans*, etc. Isolates obtained from the clinical samples included *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Escherichia coli, Serratia marcescens, Xanthomonas maltophilia, Enterobacter aerogenes, Bacillus subtilis, Bacillus cereus and Candida albicans*.

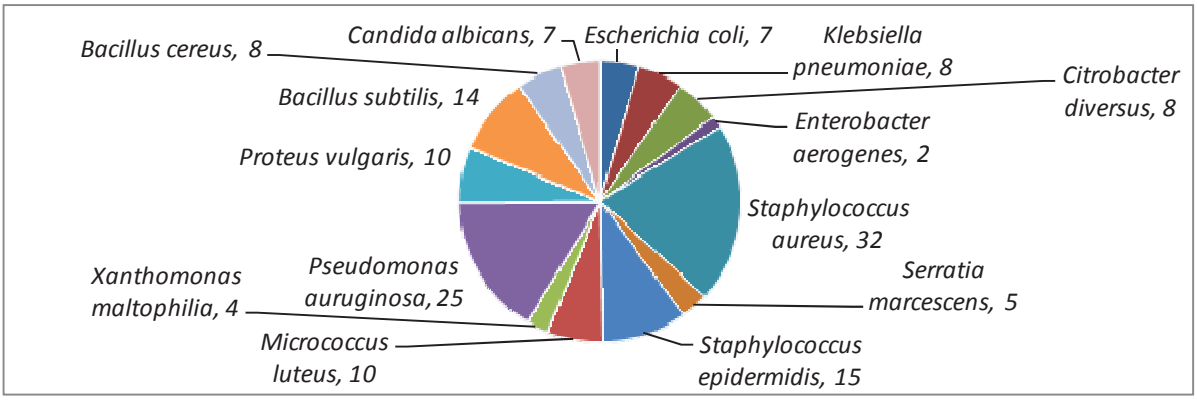

Fig. no.I, Percentage wise distribution of all the identified isolates

The dominance of *staphylococcus aureus* and *Pseudomonas aeruginosa* in this study confirms the finding of **Qadar et.al., (2010) and Azimi et al., (2011).**

The isolates were then screened against ten different antibiotics to study the antibiotic susceptibility pattern. Thus, the 38 multi drug resistant strains were obtained.

Antibacterial screening of the extracts at 3% concentration against all the MDR's was carried out. Further, the Minimum Inhibitory Concentration (MIC) of the extracts was determined to range between 0.3 % to 3%and the results obtained are mentioned in the **table no.II**.

Table no. II, MIC values of the extracts against the isolated MDR strains

Organism	MIC of the extracts in percentage (%)								
	Murrayakoenigi Linn			***Amaranthusspinosus Linn***			***Daucuscarota Linn***		
	HAE	**HEE**	**CEE**	**HAE**	**HEE**	**CEE**	**HAE**	**HEE**	**CEE**
Staphylococcus aureus	**0.3**	**0.3**	**0.3**	**0.9**	**0.3**	**0.3**	**0.9**	**0.3**	**0.3**
Proteus vulgaris	**0.3**	**3**	**0.3**	**0.9**	**3**	**3**	**0.9**	**3**	**3**
Pseudomonas aeruginosa	**0.3**	**0.3**	**0.3**	**1.2**	**0.3**	**0.3**	**1.2**	**0.3**	**0.3**
Escherichia coli	**0.3**	**3**	**0.3**	**3**	**3**	**0.3**	**3**	**3**	**0.3**
Klebsiella pneumoniae	**0.3**	**3**	**0.3**	**3**	**3**	**0.3**	**3**	**3**	**0.3**

Key: HAE – Hot Aqueous Extract, HEE – Hot Ethanolic Extract, CEE – Cold Ethanolic Extract

Findings of **Thalwal et al., 2013** had proved that methanolic extract of *Amaranthus spinosus Linn* was the most effective against all the bacteria. **Khan et al., (2008)** reported that 3,5-diacetyltambulin derived from *Amorphophallus campanulatus Linn* showed significant antimicrobial activity against both Gram positive and Gram negative organisms.

Bactericidal efficacy of cold ethanolic extracts of *Daucus carota Linn*, *Murraya koenigii Linn*and *Amaranthus spinosus Linn* with respect to time was carried out against the MDR strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Amaranthus spinosus Linn*, *Murraya*

koenigii Linn and *Daucus carota Linn* were found to show similar results against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as shown in **table.no.III**, that is cidal activity after 3 hrs.

Table no.III, Bactericidal efficacy of the extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa in hours.*

TEST COMPOUNDS	*Staphylococcus aureus*				
	GEL BASE	**STANDARD OINTMENT**	**FORMULATION**		
			Amaranthusspinosus Linn	***Murrayakoenigi Linn***	***Daucuscorota Linn***
HOURS	**24hrs**	**5 hrs**	**4 hrs**	**4 hrs**	**4 hrs**
Pseudomonas aeruginosa					
HOURS	**24hrs**	**5 hrs**	**4 hrs**	**4 hrs**	**4 hrs**

Amaranthus spinosus Linn and *Murraya koenigii Linn* were further selected for in vivo studies as they showed best and similar results as compared to *Daucus carota Linn.* Hence, they were further subjected to bioavailability studies. A formulation with unprocessed aloe vera gel and 1 % of each extract was prepared for proper topical application purpose. *Amaranthus spinosus Linn* showed a zone of about 20 mm against *Staphylococcus aureus* and a zone of 18 mm against *Pseudomonas aeruginosa.* Similiarly, *Murraya koenigii Linn* showed a zone of 18 mm against *Staphylococcus aureus* and a zone of 19 mm against *Pseudomonas aeruginosa* as shown in **table no.IV.**

Table no.IV, Results of bioavailability testing.

Sr.No.	ORGANISM	GEL BASE	STANDARD OINTMENT	FORMULATION	
				Amaranthusspinosus Linn	***Murrayakoenigi Linn***
1	***Staphylococcus aureus***	No inhibition	12 mm	20 mm	18 mm
2	***Pseudomonas aeruginosa***	No inhibition	14mm	18mm	19 mm

Further,in vivo testing of the herbal extracts was done to check for the skin toxicity and healing

power on white Wistar rats. The formulations did not show any toxicity or cumulative irritation even after 21 days and both the formulations showed healing on 5th day.The results of the same are shown in **table V .**

Table no. V, continued……shows the skintoxicity testing

TEST COMPOUND	ERYTHEMA			EDEMA			NECROSIS		
	24 HRS	48HRS	72 HRS	24 HRS	48HRS	72 HRS	24 HRS	48HRS	72 HRS
GEL BASE	-	-	-	-	-	-	-	-	-
STANDARD OINTMENT	-	-	-	-	-	-	-	-	-
HERBAL GEL (*Murrayakoenigi*)	-	-	-	-	-	-	-	-	-
HERBAL GEL (*Amarathusspinosus*L)	-	-	-	-	-	-	-	-	-

Key: (-) No reaction / irritation

Table no. V, continued……shows the skintoxicity testing

TEST COMPOUND	ERYTHEMA			EDEMA			NECROSIS		
	24 HRS	48HRS	72 HRS	24 HRS	48HRS	72 HRS	24 HRS	48HRS	72 HRS
GEL BASE	-	-	-	-	-	-	-	-	-
STANDARD OINTMENT	-	-	-	-	-	-	-	-	-
HERBAL GEL (*Murrayakoenigi*)	-	-	-	-	-	-	-	-	-
HERBAL GEL (*Amarathusspinosus*L)	-	-	-	-	-	-	-	-	-

Key: (-) No reaction / irritation

Conclusion: The present work revealed that cold ethanolic extracts of *Amaranthus spinosus Linn* and *Murraya koenigii Linn* followed by *Daucus carota Linn* showed antibacterial activity against the MDR's (*Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia). Amaranthus spinosus Linn, Murraya koenigii Linn* and *Daucus carota Linn* were found to show similar bactericidal activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, that is growth upto 3 hrs. Thus,the

kill time being more than 3hrs for *Staphylococcus aureus* and *Pseudomonas aeruginosa.*In vivo test results showed complete healing

in 11 days and no erythema, edema or necrosis with both the herbal formulations containing *Amaranthus spinosus Linn* and *Murraya koenigii Linn* each.

Thus the conclusions drawn from this study indicate the *in vitro* and *in vivo* efficacy of the herbal formulations against the bacteria causing nosocomial infections. It is also to be noted that the plants used in the study are regularly used vegetable plants that could possibly be used for medicinal purposes with great potential.

References:

1. V. Chauhan,, A Dissertation submitted in the partial fulfillment for the requirements of the award of degree of masters of science in Biotechnology, 2006.
2. L. Longworth, Microbial drug resistance and the roles of new antibiotics, Cleveland clinic journal of medicine, 68 (6):, 217-225, 2001
3. S. Chusri , N. Chaicoch, W. Thongza-ard , S. Limsuwan, and Voravuthikunchai P., In vitro antibacterial activity of ethanol extracts of nine herbal formulas and its plant components used for skin infections in Southern Thailand, Journal of Medicinal Plants Research,6 (44) :, 5616-5623, 2011
4. F. Ekhaise, E. Isitar, O. Idehen and O. Emoghen, Airborne Microflora in the Atmosphere of an Hospital Environment of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. World Journal of Agricultural Sciences, 6 (2): 166-170, 2010
5. A. Bauer, W., W. Kirby, M. M., Sherris J. C. and M. Truck, Antibiotic susceptibility testing by standardized single disc method,American Journal of Clinical Pathology, 45: 493–496, 1966
6. C.O. Akueshi, C.O. Kadiri, E.U. Akueshi, S.E Agina and B. Ngurukwem, Antimicrobial potentials of *HyptisSauvedens*poit (Lamiaccae), J. Bot.,15:37-41, 2002
7. H. Davis, M.W. Partridge and A.I. Robinson, Bentley's Textbook of Pharmaceuticals, Pub by Balliere, Tindall and Co., London , 5: 209, 1950.
8. D.F. Spooner and G. Sykes, Laboratory assessment of antibacterial activity in "Methods in Microbiology " by Norris, J.R. and Ribbons, D.W. Academic Press,78: 211-272, 1972
9. M. T. Alam, M. M. Karim, and Khan, S.N., Antibacterial Activity of Different Organic Extracts of *Achyranthes Aspera* and *Cassia Alata*, J. Sci. Res.,1 (2), 393-398, 2009.
10. P.M. Dwet, Burns. 16(4): 1990, 302 – 306.
11. G.R.M. Perez, B.R. Vargas, G.S. Perez, S.M.A. Zavala, and G.C. Perez, Antiurolithiatic activity of *Raphanus sativus* aqueous extract on rats. J. Ethnopharmacol. 68:335-338, 1999.
12. C.K. Kokate, Practical Pharmacognosy, New Delhi:Vallabhprakashan 107–111, 1994
13. P. Gupta, P Deshmukh,. and Ravishanker, Antimicrobial and Phytochemical Screening of *Mangifera Indica* against Skin Ailments, Journal of Pure and Applied Microbiology, 4(1): 387-392. 2010.

***

Prajitha N. Kutty/Padma V. Deshmukh

Dept. of Microbiology/ Smt. C.H.M. College/Ulhasnagar-421003

Thane/Maharashtra