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A STUDY OF ANTIMICROBIAL EFFECT OF TUVARAKA

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ABSTRACT

In the present time different antimicrobial agents are found. But tuvarak is a great ayurvedic medicin that show the antimicrobial property. The presence of some compounds or groups in the extract with similar mechanism of action to that of standard drug used. The highest activity was exhibited by crude extract against *Staphylococcus aureus* (13.13±0.41mm) and the lowest exhibited by residual portion against *Pseudomonos aeruginosa* (11.5±0.40 mm). The broad antibacterial activities of this extracts could be as a result of the plant secondary metabolites (triterpenoid , steroid, various amino acid , fatty acids like hydnocarpic and chaulmogric acid) present in the extracts. Methanolic extract was tested against the organisms namely *E.coli ATCC*, (12.96mm) followed by *Pseudomonas aeruginosa* (11.5, 13.03), *Salmonella typhi* (12.96, 11.53mm), *Staphylococcus aureus* (11.73, 13.12mm).

KEYWORDS: Antimicrobial, Tuvarak, *Staphylococcus aureus, Pseudomonos aeruginosa.*

1. INTRODUCTION[1]

When microbes shows resistance against antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more

expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area. The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

2. PRINCIPLE[2]

The principles of determining the affectivity of a noxious agent to a bacterium were well enumerated by Rideal, Walker and others at the turn of the century, the discovery of antibiotics made these tests (or their modification) too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field. The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface. With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when

necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

3. Factor affecting antimicrobial testing

pH

The pH of each batch of Müeller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

- Macerate a sufficient amount of agar to submerge the tip of a pH electrode.
- Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
- Use a properly calibrated surface electrode.

MOISTURE

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

Effects of Thymidine or Thymine

Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Müeller-Hinton agar that is as low in thymidine content as possible should be used. To evaluate a new lot of Müeller-Hinton agar, *Enterococcus faecalis* ATCC 29212, or alternatively, *E. faecalis* ATCC 33186, should be tested with trimethoprim/sulfamethoxazole disks. Satisfactory media will provide essentially clear, distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Müeller-Hinton agar must conform to the control limits.

Testing strains that fail to grow satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented Müeller-Hinton agar should be tested on that medium. Certain fastidious bacteria such as *Haemophilus* spp., *N. gonorrhoeae*, *S. pneumoniae*, and viridans and ß-haemolytic streptococci do not grow sufficiently on unsupplemented Müeller-Hinton agar. These organisms require supplements or different media to grow, and they should be tested on the media described in separate sections.

MATERIALS AND METHODS

Plant materials

The seed of Hydnocarpus *lourifolia* were collected from Goladeenanath ayurvedic market Varanasi (UP) India.

Extraction of plant materials

The shade dried fruit were powdered and then extracted with methanol at room temperature by cold extraction method with occasional shaking and preparation of aqueous extract by decoction method. The methanolic extract was concentrated in a rotary evaporator and the residue was dried in a desiccators over calcium chloride and aq. Extract concentrated at hot plate.

Screening of antibacterial activity

Test microorganism

Total four bacterial strains namely *Escherichia coli* ATCC 25922, *Pseudomona aeruginosa*, *Salmonella Typhi*, and *Staphylococcus aureus* and two fungal strains namely *Candida albicans* and*Candida tropicalis* were used throughout the investigation. All cultures were obtained from American Type Culture Collection (ATCC), MTCC, clinical strain preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of sample extract for microbiological assay

About 1g of each extract was dissolved in 10 ml (100 mg ml-1) of DMSO to obtain a stock solution; the working solution was prepared. The extract was diluted as 1:10 equivalent to 100 mg ml-1 and 1:5 dilution equivalent to 50 mg ml-1, from which 5µl was dispensed into a sterile disc for susceptibility testing.

Antimicrobial Susceptibility Test

The disc diffusion method was used to screen the antibacterial activity.[3] and antifungal activity.[4] Muller Hinton agar (MHA) plates were prepared by pouring 15 - 20ml of molten media into sterile petriplates. The plates were allowed to dry for 5 min. The different concentrations of extract (100, 200, 300, 400mg/ml) were loaded on 6 mm sterile disc of Whatman filter paper No.1. The loaded disc were placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 hr. At the end of incubation, inhibition zones were looked for around the disc if present were measured with transparent ruler in millimeters. This study was performed in triplicate.

Determination of minimum inhibitory concentration (MIC)

Agar dilution tests were performed according to standard methods.[5,6] Media consisted of Mueller-Hinton agar with extract incorporated at concentrations. Inoculate were prepared by suspending growth from overnight cultures in Mueller-Hinton broth (BBL) to a turbidity of a 0.5 McFarland standard. Final inoculate contained 10.[4] organisms/spot. Plates were incubated overnight at 35°C. The lowest concentration of methanolic and aqueous extract showing no growth was read as the MIC.

MEDIA USED

Muller-Hinton agar and broth (Hi-media, Mumbai, India), Sabouraud dextrose agar pH 7.3±0.2 (Hi-media), was used for antibacterial and antifungal activity respectively.

Preparation of Mac Farland Standard

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standards may be prepared as follows:

Table no.1

MF STD No	0.5	1	2	3	4
1.0% barium chloride(ml)	0.5	0.1	0.2	0.3	0.4
1.0% sulphuric acid(ml)	9.95	9.9	9.8	9.7	9.6
Approx cell density 1×10^8 FU/ml	1.5	3	6	9	12
Transmitance*	74.3	55.6	35.6	26.4	21.5
Absorbance*	0.132	0.257	0.451	0.582	0.669

Antibacterial activity measured by zone of inhibition (in mm) of methanolic and aqueous extract of *Hydnocarpus lourifolia* seed.

Table 2: Antimicrobial activity of plant extracts.		
Microorganism	**Zone of inhibition (in mm)**	
	Extract concentration (100 mg/ml)	
	W1	**M1**
E. coli ATCC 25922	12.96 ± 0.73	12.40 ± 0.30
P. aeruginosa	11.5 ± 0.40	13.03 ± 0.66
S. aureus	11.73 ± 0.56	13.12 ± 0.41
S.typhi	12.56 ± 1.30	11.53 ± 0.40
C.tropicalis	10.12 ± 0.94	11.27 ± 0.75
C. albicans	-	12.65 ± 0.84

W_1- Aqueous extract, M_1 - Methonalic extract.

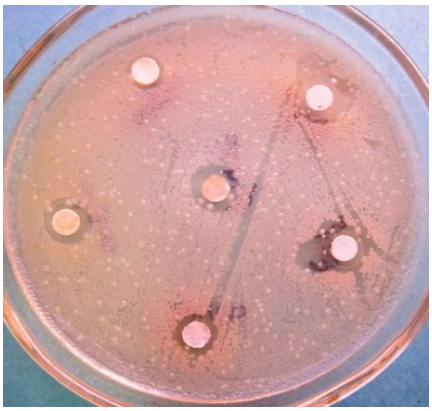
Determination of Minimum inhibitory concentration

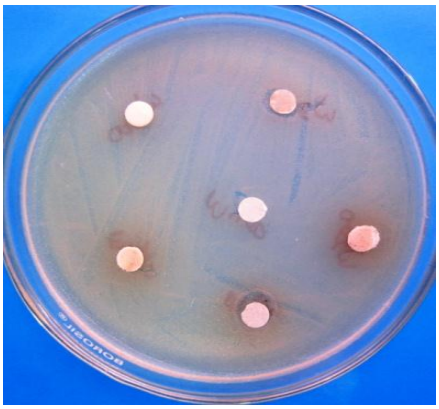
Table no.3: Determination of MIC (mg/ml) of seed extract *H. lourifolia.*			
S.No	**Bacterial Strains**	**W1**	**M1**
1	*E. coli ATCC 25922*	6.25	12.5
2	*P. aeruginosa*	12.5	12.5
3	*S. aureus*	12.5	6.25
4	*S.typhi*	6.25	12.5
5	*C.tropicalis*	12.5	12.5
6	*C. albicans*	6.25	12.5

RESULTS AND DISCUSSIONS

In vitro antimicrobial test results presented in Table 1.shows the susceptibility test against various bacteria as well as antifungal activity. The methanol extract exhibited considerable level of inhibition against all the test organism compared to standard drug. This is suggestive of the presence of some compounds or groups in the extract with similar mechanism of action to that of standard drug used. The highest activity was exhibited by crude extract against *Staphylococcus aureus* (13.13±0.41mm) and the lowest exhibited by residual portion against *Pseudomonos aeruginosa* (11.5±0.40 mm) listed in Table 2. However, it may be suggested that plant extracts exhibiting diameters of zones of inhibition > 10mm considered active. In

line with this, it is believed that the extract was better antibacterial agents for various pathogenic bacteria. *S. aureus* has been known to play a vital role in invasive skin diseases including superficial and deep follicular lesion. The broad antibacterial activities of this extracts could be as a result of the plant secondary metabolites (triterpenoid, steroid, various amino acid, fatty acids like hydnocarpic and chaulmogric acid) present in the extracts. Methanolic extract was tested against the organisms namely *E.coli ATCC*, (12.96mm) followed by *Pseudomonas aeruginosa* (11.5, 13.03), *Salmonella typhi* (12.96, 11.53mm), *Staphylococcus aureus* (11.73, 13.12mm) and fungal strain such as Candida tropicalis (10.12, 11.27mm), Candida albicans (12.65mm) at concentrations 100 mg/ml. The MIC value of 12.5, 6.25 mg/ml against *Staphylococcus aureus* and 6.25, 12.5 mg/ml for *Escherichia coli* and 6.25 – 12.5 mg/ml against remaining organism tested listed in Table 3.





CONCLUSION

On the basis of the result the tuvaraka crude methanolic and aqeuous extracts of *H.lourifolia* found to have significant antibacterial activity and may be used for treatment of several infectious diseases. This study support why crude extracts of *H.lourifolia* may be used for treatment of several infectious diseases.

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