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Biotherapeutic Antibacterial Potential of *Schleichera oleosa* Against Drug Resistant Isolates

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Aerial parts of the plant *Schleichera oleosa* were studied for antibacterial property. Effects of methanolic extracts of *Schleichera oleosa* were investigated against drug resistant strains of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Salmonella typhii*. The antibiotic sensitivity pattern for all the clinical isolates was studied by Bauer-Kirby method. All the clinical isolates obtained were resistant to one or more than one antibiotic. The plant showed great potential as antibacterial agent.

Key words: *Schleichera oleosa*, antibacterial activity, MIC, drug resistance, phytochemical analysis, agar well diffusion.

In the indigenous health care delivery system, numerous plant species and natural products derived from plants are used to treat diseases of infectious origin. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on ayurvedic medicinal plants. Numerous drugs have entered the international pharmacopoeia through the study of ethnopharmacology and traditional medicine

Emerging antibiotic resistant infections are one of the most serious problems the hospitals face today. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for prevention and cure of different human diseases since they are safe and effective.

Many studies have attempted to shed light on the antibacterial activity of some indigenous medicinal plants. Nonetheless, the investigations have primarily been restricted to screening only. In order to promote herbal drugs there has to be an evaluation of therapeutic potentials of drugs. The medicinal plant *Schleichera oleosa* is widely used by the traditional medicinal practitioners for the treatment of infectious diseases[1]. It belongs to the family Sapindaceae. It is occasionally cultivated throughout India. In traditional medicine, the oil obtained is used for curing itching, acne and other skin afflictions. The bark is stringent and used against skin inflammations and ulcers while an infusion is taken against malaria. Tannin is obtained from the bark.

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The bark is used in leprosy. Hence, it is put to systematic scientific investigation in this study. Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in traditional system of medicine is justified.

MATERIAL AND METHODS

Plant collection

The plant was collected from urban fringe and rural areas of Nagpur region. A voucher specimen (ND 2316) is deposited with the Department of Botany; RTM Nagpur University, Nagpur, (M.S.), India.

Preparation of Plant Extract

Aerial parts of *Schleichera oleosa* were subjected to Petroleum ether, Chloroform, Acetone, and Methanol Soxhlet extraction[2]. The methanolic etracts were found to be more potent than the other solvent counterparts and hence used in this study[3].

Clinical Isolates and Control

Clinical isolates were obtained from Department of Microbiology and Department of Pathology, Jawaharlal Nehru Medical College and Hospital, Sawangi, District-Wardha (M. S), Thirteen strains of *Escherichia coli,* ten strains of *Staphylococcus aureus,* nine strains of *Klebsiella pneumoniae,* and six strains of *Salmonella typhii* were tested against standard antibiotics to get the antibiotic sensitivity pattern. Standard antibiotic discs were purchased from HiMedia, Mumbai. The bacterial cultures were maintained on Nutrient Agar (Himedia, Mumbai) at 4°C and subcultured every two weeks. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 , *K. Pneumoniae* ATCC 10921 and *S. typhii* ATCC 19430 were procured from National Centre for Cell Sciences, Pune were used as controls.

Media

Nutrient Agar (M001), Agar Agar Type I (RM666), Mueller Hinton Agar No. 2 (M1084) and Nutrient broth (M002) were procured from Hi-Media, Mumbai. The preparation of media was done strictly according to the manufacturer's instructions.

Inoculum preparation and antibiotic sensitivity test

Antibacterial activities of 20% to 100% methanolic extracts of *S. oleosa* were studied by agar well diffusion method[4] using the Mc Farland's Standards. The resistance pattern for standard antibiotics was obtained by Bauer-Kirby method[5]. The antibiotic disk susceptibility was performed to get the resistance pattern of the clinical isolates.

Antibiotic discs

Commercially available standard antibiotic discs were obtained from Hi-Media, Mumbai. The abbreviations and strength of the antibiotics are given in brackets. The antibiotic discs used were Amoxycillin SD 076 (Ac-30 mcg), Ampicillin SD 002 (A-10 mcg), Chloramphenicol SD 006 (C-30 mcg), Erythromycin SD 013 (E-15 mcg), Penicillin-G SD 028 (P-10 mcg), Kanamycin SD 017 (K-30 mcg), Tetracyclin SD 037 (T-30 mcg), Cephalexin SD 048 (Cp-30 mcg), Ciprofloxacin SD 060 (Cf-5 mcg), Co-trimoxazole SD 010 (Co-25 mcg), Gatifloxacin SD 737 (Gf-5mcg), Norfloxacin SD 057 (Nx-10 mcg), Ofloxacin SD 087 (Of- 5mcg), Pe-floxacin SD 070 (Pf-5 mcg), Sparfloxacin SD 162 (Sc-5 mcg) and Streptomycin SD 031 (S-10 mcg)

Antibacterial susceptibility test

A suspension (0.1 ml) of the test organisms from the 18 hour cultures was thoroughly mixed with 20 ml of sterile Mueller Hinton Agar maintained at 45-50° C. The seeded M.H. Agar is poured in presterilized petri plates and set aside. After solidification, the seeded agar was punched with a flamed (sterile) 10mm cork borer in order to obtain a well of 10mm diameter in the center of the petri plate. 100 ul of the methanolic plant extract is loaded into the well accurately with a micropipette (with presterilized tips) to obtain concentration of 20, 40, 60, 80 and 100%. The petri plates were delicately handled and kept in refrigerator for 30 minutes and then at room temperature for 30 minutes which facilitated diffusion of the plant extract. The petri-plates were then incubated at 37° C for 24 hours. The zone of inhibition was measured with HiAntibiotic ZoneScale (PW096), HiMedia, Mumbai.

Minimum inhibitory concentration

The determination of MIC was done by agar dilution method (NCCLS 1990). Stock solution of 100mg/ml of methanolic extract of selected plant was prepared in DMSO-Tris buffer (3:7). This was diluted to achieve final concentrations of 0.5mg/ml, 1mg/ml, 2mg/ml,

5mg/ml, 8mg/ml, 10mg/ml and 15mg/ml. These were poured in petri plates and allowed to solidify. The reverse side of the plate was divided into checker board blocks by glass marker to accommodate bacterial culture. A bacterial inoculum of all the test organisms was prepared as discussed in bacterial testing section. All the plates including control plate without plant extract were inoculated with 10 ul of bacterial innoculum using sterile micropipette. The Petri plates were incubated at 37 °C for 24 hours. The result was read as presence or absence of bacterial growth. Complete suppression of growth was required for an extract to be declared active. MIC was determined as the least concentration of extract inhibiting the growth of the test organisms.

RESULTS AND DISCUSSION

The pattern of resistance obtained after performing antibiotic sensitivity tests are shown in respective tables. The clinical isolates were found to be resistant to one or more than one antibiotic. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, *K. Pneumoniae* ATCC 10921 and *S. typhii* ATCC 19430 *K. Pneumoniae* ATCC 10921 and *S. typhii* ATCC 19430 were used as controls. Table 1.

E.coli is a parasite living only in human or animal intestine. Urinary tract infections, diarrhea, pyogenic infections and septicemia are caused by *E.coli*[6-9].

Clinical isolates of *E.col*i which show resistance to commonly used antibiotics like Amoxycillin, Penicillin, Cephalexin, Streptomycin etc., when treated with 100% MeOH extract of S. oleosa shows a zone of inhibition of 16 mm and diameter. Table 2.

S. aureus are ubiquitous and form the most common cause of localized suppurative lesions in human beings. Their ability to develop resistance to penicillin and other antibiotics enhances their importance as human pathogen, especially in the hospital environment[10-11]. In this study, the clinical isolates of S. aureus show resistance against most commonly used antibiotics such as Amoxycillin, Co-trimoxazole, Cephalexin, Ampicillin, Kanamycin, Penicillin etc. Interestingly, the MeOH plant extracts show commendable activity against multi-drug resistant

Table 1. Antibiotic sensitivity vs control

S. No	Control Micro-organisms	A	Am	C	E	P	K	T	Cp	Cf	Co	Gf	Nx	Of	Pf	Sc	S
1.	*E. coli* ATCC 25922	18	22	24	19	17	19	22	15	33	24	29	32	32	30	32	12
2.	*S.aureus* ATCC 25923	32	33	26	27	22	26	27	29	28	29	19	19	26	26	32	15
3.	*K. peumoniae* ATCC10921	20	22	25	22	25	22	22	16	32	20	26	28	26	22	30	12
4.	*S.typhii* ATCC 19430	18	20	21	20	18	17	20	14	22	18	20	20	30	30	30	16

Table 2. Eeffect of methanolic plant extracts on multidrug resistant strains of *E.coli*

S. No.	Clinical isolates	Resistance pattern	100ul of MeOH S.oleosa leaf extract				
			20%	40%	60%	80%	100%
1	*E. coli* 1 (EC1)	E,P,K,Co,S,Cp,Gf	11	13	13	15	16
2	*E. coli* 2 (EC2)	Am,E,P,Co,S	11	12	12	15	16
3	*E. coli* 3 (EC3)	Am,A,E,P,T,Gf,Nx,S	12	12	13	14	15
4	*E. coli* 4 (EC4)	E,P,Cp,Nx,S	-	-	13	14	17
5	*E. coli* 5 (EC5)	Am,C,P,K,S,G	11	12	12	14	16
6	*E. coli* 6 (EC6)	Am,E,P,K,T,S	12	13	13	14	17
7	*E. coli* 7 (EC7)	Am,E,P,K,Co	12	13	12	15	16
8	*E. coli* 8 (EC8)	Am,E,P,K,Cp	13	13	13	14	15
9	*E. coli* 9 (EC9)	Am,A,P,Co,Nx	-	11	13	15	15
10	*E. coli* 10 (EC10)	E,P,K,Nx,S,Gf	-	12	13	15	16
11	*E. coli* 11 (EC11)	Am,A,E,P,Of	11	12	12	15	16
12	*E. coli* 12 (EC12)	E,P,Cp,Co,Of	-	-	12	15	16
13	*E. coli* 13 (EC13)	Am,A,E,P,K,K,Cp,Of	-	-	13	14	16
	E. coli ATCC 25922	-	15	18	21	24	26

Table 3. Effect of methanolic extracts of plants on multidrug resistant strains of *S.aureus*

S. No.	Clinical isolates	Resistance pattern	100ul of MeOH S.oleosa leaf extract				
			20%	40%	60%	80%	100%
1	*S. aureus* 1(SA1)	Am,A,P,K,T,Cp,Co	18	19	21	23	25
2	*S. aureus* 2(SA2)	Am,A,C,P,K,Cp,Co,Nx,Sc	18	18	20	22	25
3	*S. aureus* 3(SA3)	Am,A,P,K,Cp,Co	16	19	20	23	24
4	*S. aureus* 4(SA4)	Am,C,P,K,Cp,Cf,Co,Nx	17	19	21	22	24
5	*S. aureus* 5(SA5)	Am,A,E,P,K,Cp,Co	16	18	20	23	25
6	*S. aureus* 6(SA6)	Am,A,P,K,Cp,Co	18	19	21	22	25
7	*S. aureus* 7(SA7)	Am,A,P,K,Cp,Co,Sc	17	18	21	23	24
8	*S. aureus* 8(SA8)	Am,A,P,K,T,Cp,Co	18	19	20	23	25
9	*S. aureus* 9(SA9)	Am,A,P,K,Cp,Cf,Co,Sc	17	18	20	22	25
10	*S. aureus* 10(SA10)	A,C,P,K,T,Cp,Co	18	19	20	23	24
	S. aureus ATCC 25923	-	24	30	36	38	40

Table 4. Effect of methanolic plant extracts on multidrug resistant strains of *K.pneumonia*

S. No.	Clinical isolates	Resistance pattern	100ul of MeOH S.oleosa leaf extract				
			20%	40%	60%	80%	100%
1	*Klebsiella pneumoniae*1 (KA1)	Am,C,Cp,S	-	-	15	16	19
2	*Klebsiella pneumoniae*2(KA2)	E,T,Co,S	11	12	14	17	18
3	*Klebsiella pneumoniae*3 (KA3)	Am,E,T,Cp,S	13	14	15	15	16
4	*Klebsiella pneumoniae*4 (KA4)	A,T,Cp,Co,S	-	13	15	17	185
5.	*Klebsiella pneumoniae*5 (KA5)	Am,Cp,Co,S	12	13	16	16	19
6	*Klebsiella pneumoniae*6 (KA6)	A,E,T,Cp,S	-	-	16	17	19
7	*Klebsiella pneumoniae*7 (KA7)	Am,T,Cp,Co,S	14	15	14	16	17
8	*Klebsiella pneumoniae*8 (KA8)	A,T,Cp,Co,S	11	13	15	16	18
9	*Klebsiella pneumoniae*9 (KA9)	A,E,Cp,Co,S	13	14	15	16	18
	K. pneumoniae ATCC 10921	-	16	20	26	28	30

Table 5. Effect of methanolic extracts of plants on multidrug resistant strains of *S.typhii*

S. No.	Clinical isolates	Resistance pattern	100ul of MeOH S.oleosa leaf extract				
			20%	40%	60%	80%	100%
1	*S. typhii* 1 (ST1)	Am,A,C,E,P,Cp,S,Of	-	-	18	20	23
2	*S. typhii* 2 (ST2)	Am,A,P,S,T,Cp,T,Of	11	13	17	21	24
3	*S. typhii* 3 (ST3)	Am,A,P,S,T,Cp,Of	14	15	18	22	24
4	*S. typhii* 4 (ST4)	Am,A,P,S,Cp,Of	13	16	19	20	23
5	*S. typhii* 5 (ST5)	Am,A,P,S,E,Cp,Of	12	14	15	18	21
6	*S. typhii* 6 (ST6)	Am,A,P,S,K,Cp,T	11	13	14	16	19
	*S. typhii*ATCC 19430	-	14	16	20	24	28

Table 6. Minimum inhibitory concentration (mic) of *S.oleosa* meoh extracts on test microorganisms

S.No	Test microorganisms	MIC in mg/ml
1	E. coli 13 (EC13)	1
2	S.aureus 1(SA1)	10
3	Klebsiella pneumoniae8 (KP8)	5
4	S.typhii 4 (ST4)	8

A=Ampicillin T=Tetracycline
Nx=Norfloxacin Am=Amoxicillin
Cp=Cephalexin Of=Oflaxacin
C=Chloramphenicol Cf=Ciprofloxacin
Pf=Pefloxacin E=Erythromycin
Co= Co-trimoxazole Sc=Sparfloxacin
P=Penicillin-G Gf=Gatifloxacin
S=Streptomycin K=Kanamycin

S. oleosa= Schleichera oleosa
* 100 ul of aerial parts of *Schleichera oleosa* methanolic extracts
* Observations are mean of triplicates studied.

S. *aureus* strains. Worth mentioning is the extraordinary activity shown by the plant extracts at all the concentrations Table 3.

K. pneumoniae is the second most populous member of human intestine. It causes pneumonia, urinary infection, other pyogenic infections such as abscesses, meningitis, septicemia and rarely diarrhea. [12] The clinical isolates of *K. pneumoniae* show resistance to more than one antibiotic as clearly seen from the resistance pattern in Table IV.

S. oloesa MeOH extracts are effective at >80% for most of the clinical isolates tested.

The genus Salmonella consists of bacilli that infect human beings, leading to enteric fever, gastroenteritis, septicemia. Enteric fever is endemic in all parts of India. resistance. hospital outbreaks of neonatal septicemia caused by multi drug resistant Salmonellae have occurred. Mortality in neonates is very high unless early treatment is started with antibiotics to which the infecting strain is sensitive[13-14].

The clinical isolates of S. typhi show a common resistance pattern for Am, A, P, and Cp. The *S.oleosa* MeOH extract showed great potential as an antibacterial agent. Table 5.

CONCLUSION

This study validates *Schleichera oleosa* methanolic extract as g a potential biotherapeutic antibacterial agent.

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