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STUDIES ON ANTIBACTERIAL ACTIVITY OF *NELUMBIUM SPECIOSUM*-WILD SEEDS EXTRACTS

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Seeds of *Nelumbium speciosum*-Wild were studied for their antibacterial activity against Gram + ve and Grame -ve bacteria *in vitro*. Ethanolic extract "C" was more active against Gram -ve bacteria than "A" and "B" (aqueous extracts), while extract "A" was more active than "B". Their actions were comparable to the action of Erythrocin, Ampiclox and Penbritin.

Key words: *Nelumbium speciosum*- seeds, Antibacterial activity.

Introduction

Nelumbium speciosum-Wild, commonly known as Kanwal ghatta belongs to the family Nymphaeceae. It is large aquatic herb widespread in India [1-4] and Pakistan [5]. Its seeds are hard, dark grey, round, oval or oblong, with a white albuminous and slightly sweetish kernel [6]. The chemical constituents of the seeds included oil [7], resins, metarbin and fat [1], starch [8], glucose and alkaloids [1,9,10]. Seeds are demulcent nutrient [1,9], prevent vomiting, diuretic, refrigerant and nervine tonic [4,8], as antidote for poisons [4]. It is beneficial for leprosy and skin diseases [1,4].

It was, therefore, considered worthwhile to look for some cheap herbal medicine, capable of antibacterial activity and which may be easily procureable. *Nelumbium speciosum* Wild was selected to evaluate its antibacterial activity and also compared with Erythrocin, Ampiclox and Penbritin.

Materials and Methods

AQUEOUS EXTRACT OF *NELUMBIUM SPECIOSUM* (SEEDS).

Extract "A". *Nelumbium speciosum* (Kanwal ghatta) seed (without cover) indentified by the Pharmacognosy Section of these Laboratories, were washed with distilled water and dried at room temperature. The material (1.5 kg) was ground to fine powder and soaked in 95% ethyl alcohol (2.5 lit.) for one week, and filtered. The solvent was removed under reduced pressure and the brownish viscous residue was partitioned between petroleum ether and water (1:3 v/v). The aqueous phase was withdrawn and water was removed under reduced pressure below 70° to furnish a dark brownish syrup "A" (11 g).

Extract "B". The powdered material (500 g) was soaked in distilled water (1 lit.) for two weeks. After filtration the solvent was removed under reduced pressure to furnish a brownish viscous residue "B" (22 g).

ETHANOLIC EXTRACT OF *NELUMBIUM SPECIOSUM* (SEEDS).

Extract "C". The powdered material (2.5 kg) was soaked in 95% ethyl alcohol (2.5 lit) for 2 weeks and filtered. From the filtrate, solvent was removed under reduced pressure to afford a brownish viscous material "C" (165 g).

Antibacterial activity. Cultures of pathogenic and non-pathogenic bacteria employed during this study were: five Gram +ve i.e., *Bacillus subtilis, Streptococcus feacalis, Streptococcus lactis, Streptococcus pyogenes* and *Staphylococcus aureus;* and nine Gram -ve bacteria, i.e. *Escherichia coli, Pseudomonas* sp., *Salmonella para* B, *Salmonella typhi, Salmonella typhimurium, Enterobacter* sp., *Klebsiella pneumoniae, Vibrio cholerae* and *Aeromonas* sp.

All the bacteria used for these tests were inoculated on nutrient agar slants incubated at 37° for 24 hrs. The 24 hrs old cultures were grown on nutrient broth tubes (1 ml in each tube) and incubated at 37° for 24 hrs.

The well-method (11) was employed for testing antibacterial activity of the Kanwal ghatta extracts (ethanolic extract "C" and aqueous extract "A" & "B") against fourteen bacterial (five Gram +ve and nine Gram-ve). The zones of inhibition were measured from the centre of the boarer to four different points on the margin of the zone. Three antibiotics, namely Erythrocin, Ampiclox and Penbritin were used for comparasion.

Results and Discussion

Antibacterial activity against Gram +ve bacteria in vitro. Aqueous extract "A" and "B" inhibited the growth of *B. subtilis, S. lactis, S. faecalis, S. pyogenes* and *S. aureus.* (Table 1). Ethanolic extract "C" was found active against *B. subtilis, S. faecalis* and *S. pyogenes* in 2.5 mg/ml concentration. In 5 mg/ml concentration it showed activity against all the five Gram +ve bacteria used. Aqueous extract "B"

showed activity in 0.5 mg/ml concentration against *S. pyogenes, S. lactis, S. faecalis* and *S. aureus* while in case of *B. subtilis,* it was found active only in 10 mg/ml concentration. The ethanolic extract was found active against *B. subtilis, S. faecalis* and *S. pyogenes* in 2.5 mg/ml concentration.

Aqueous extract "A" was found active in 2.5 mg/ml concentration against *B. subtilis, S. faecalis, S. pyogenes* and *S. aureus*. The growth of *S. lactis* was inhibited in 5 mg/ml concentration.

Aquoeus extract "B" inhibits the growth of all the four Gram +ve bacteria tested except *B. subtilis* in 5 mg/ml concentration (minimum inhibitory concentration, MIC). Its MIC against *B. subtilis* was 1 mg/ml.

According to the above observations, MIC of ethanolic extract "C" was found 2.5 mg/ml. The MIC of aqeous extract "A" was also 2.5 mg/ml against *B. subtilis, S. pyogenes, S. faecalis*. All the fractions (A, B and C) were less active than the antibiotics, tested.

Antibacterial activity of aqueous and ethanolic extracts against Gram -ve bacteria. All the extracts were tested for their antibacterial activity against nine Gram-ve bacteria; *E. coli, Pseudomonas* sp., *S. para* B, *S. typhi, S. typhimurium, Enterobacter* sp, *K. pneumoniae, V. cholerae* and *Aeromonas* sp.

Aqueous extract "A" was found to inhibit growth of all the nine Gram -ve bacteria, tested. The MIC for *E. coli, Pseudomonas* sp., *S. para* B, *S. typhimurium, Enterobacter* sp. and *V. cholerae*, was 2.5 mg/ml while in case of *S. typhi* and *K. pneumoniae* the MIC was 5 mg/ml. Against *Aeromonas* sp. MIC was 1.25 mg/ml.

Aqueous extract "B" produced zones of inhibition around *E. coli, Pseudomonas* sp., *Aeromonas* sp., *E. Coli, Enterobacter* sp. and *V. cholerae* in 2.5 mg/ml concentration (MIC). The MIC for *S. typhi, S. typhimurium* and *K. pneumoniae* was 5 mg/ml but in case of *S. styphi* the MIC was 10 mg/ml.

Ethanolic extract "C" was active against all the Gram-ve bacteria tested except *S. typhi*. The MIC was found 1.25 mg/ml against *E. coli, Aeromonas* sp. *Enterobacter* sp. and *V. cholerae*. The MIC for *Pseudomonas* sp., *S. para* B and S. *typhimurium* was 2.5 mg/ml. The MIC for *K. pneumoniae* was 5 mg/ml. All the extracts (A, B &C) showed more activity than Penbritin and Ampiclox against *V. cholerae*, while these extracts were found active against *Pseudomonas* sp. as compared to Penbritin, Ampiclox and Erythrocin. Extract "C" was more active than Penbritin against *E.coli* and Erythrocin proved to be less active than extract "A" when tested against *S. para* B in 10 mg/ml concentration. These seeds were already in use against some

TABLE 1. ANTIBACTERIAL ACTIVITY AGAINST GRAM +VE BACTERIA ZONES OF INHIBITION IN mm.

Culture used	Concentration mg/ml	Kanwalghatta aqueous extract 'A'	Kanwalghatta aqueous extract 'B'	Kanwalghatta ethanol extract 'C'	Penbritin	Eryth-rocin	Ampiclox
Bacillus subtilis	10	16	11	15	14	24	22
	05	14	10	13	13	23	16
	0.5	11	10	12	12	22	14
	Control	10	10	10	10	10	10
Streptococcus lactis	10	12	13	-ve	16	29	22
	05	11	12	-ve	14	25	20
	2.5	10	10	-ve	13	23	18
	Control	10	10	-ve	10	10	10
Streptococcus faecalis	10	14	12	15	13	24	21
	05	12	11	12	11	21	18
	2.5	11	10	11	10	19	16
	Control	10	10	10	10	10	10
Streptococcus pyogenes	10	14	13	15	15	23	22
	05	12	11	13	14	20	20
	2.5	11	10	11	12	18	18
	Control	10	10	10	10	10	10
Starephylococcus aureus	10	14	14	-ve	20	29	40
	05	13	13	-ve	18	25	36
	2.5	12	10	-ve	16	21	32
	Control	10	10	-ve	10	10	10

diseases and it can easily be purchased from local market in rural and urban areas.

Aqueous extracts "A" and "B" and ethanolic extract "C" were tested for their antibacterial activity, against nine Grame-ve bacteria Table 2.

Aqueous extract "A" showed activity against *E. coli*, *Pseudomonas* sp., *S. para* B., *S. typhimurium*, *Enterobacter* sp. and *V. cholerae* in 2.5 mg/ml concentration. While against *S. typhi* and *K. pneumoniae*, it was active in 5 mg/ml concentration. In 1.25 mg/ml concentration, it showed activity against *Aeromonas* sp.

Aquous extract "B" was found to inhibit the growth of *E. coli*, *Pseudomonas* sp., *Aeromonas* sp., *V. cholerae* and *Enterobacter* sp. in 2.5 mg/ml concentration. The concentration which inhibited the growth of *S. typhi*, *S. typhimurium* and *K. pneumoniae*, was 5 mg/ml but the growth of *S. para* B was inhibited with 10 mg/ml concentration.

TABLE 2. ANTIBACTERIAL ACTIVITY AGAINST GRAM -VE BACTERIA ZONES OF INHIBITION IN mm.

Culture used	Concentration (mg/ml)	Kanwalghatta aqueous extract 'A'	Kanwalghatta aqueous extract 'B'	Kanwalghatta ethanol extract 'C'	Penbritin	Eryth-rocin	Ampiclox
Escherichia coli	10	14	15	16	15	24	21
	05	13	14	15	14	23	19
	2.5	12	13	14	13	22	17
	Control	10	10	10	10	10	10
Salmonella typhi	10	12	13	-ve	34	18	42
	05	11	12	-ve	30	14	38
	2.5	10	10	-ve	26	12	34
	Control	10	10	-ve	10	10	10
Salmonella para-B	10	16	11	14	38	15	31
	05	14	10	12	31	14	29
	2.5	12	10	11	27	12	27
	Control	10	10	10	10	10	10
Salmonella typhimurium	10	14	13	15	23	25	22
	05	13	12	13	20	22	19
	2.5	12	10	12	18	20	17
	Control	10	10	10	10	10	10
Vibrio cholerea	10	14	14	15	-ve	26	-ve
	05	13	12	14	-ve	21	-ve
	2.5	12	11	13	-ve	18	-ve
	Control	10	10	10	-ve	10	-ve
Enterobacter sp.	10	14	13	14	21	15	23
	05	13	12	13	16	14	20
	2.5	11	11	12	13	13	18
	Control	10	10	10	10	10	10
Pseudomonas sp.	10	13	15	16	-ve	-ve	-ve
	05	12	13	14	-ve	-ve	-ve
	2.5	11	11	12	-ve	-ve	-ve
	Control	10	10	10	-ve	-ve	-ve
Klebsiella pneumoniae	10	13	12	12	15	18	17
	05	11	11	11	13	15	15
	2.5	10	10	10	12	13	13
	Control	10	10	10	10	10	10
Aeromonas sp.	10	17	13	14	22	20	19
	05	15	12	13	20	18	15
	2.5	13	11	12	18	16	13
	Control	10	10	10	10	10	10

Ethanolic extract "C" was found more active against Gram-ve bacteria than "A" and "B" while extract " A" was more active than "B".

Aqueous extracts "A" and "B" and ethanolic extract "C" were also tested for their antibacterial activity against five Gram +ve bacteria. Aqueous extract "A" inhibited the growth of *B. subtilis, S. faecalis, S. pyogenes* and *S. aureus* in 2.5 mg/ml concentration.

Aqueous extract "B" was found active in 0.5 mg/ml concentration against *S. pyogenes, S. lactis, S. faecalis* and *S. aureus* while against *B. subtilis* , its activity was seen in 10 mg/ml concentration.

Ethanolic extract "C" produced zones of inhibitions around *B. subtilis, S. faecalis* and *S. pyogenes* , while it was found in-active against *S. lactis* and *S. aureus.*

In comparison, with antibiotics Penbritin, Erythrocin and Ampiclox, all the extracts were found less active against all the Gram +ve bacteria, used. All the three extracts showed greater activity as compared to Penbritin, Ampiclox and Erythrocin against *Pseudomonas* sp. (Gram -ve). Erythrocin found less active than extract "A" against *S. para* B (Gram -ve). The extract "C" showed greater activity against *E. coli* (Gram -ve) as compared to Penbritin.

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