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ANTIBACTERIAL ACTIVITY OF AN ISOLATED COMPOUND (AV-1) FROM THE LEAVES OF TITEYPATI (*Artemisia vulgaris* Linn.)

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ABSTRACT

Antibacterial activity of an isolated compound (AV-1) from the leaves of Titeypati (*Artemisia vulgaris* Linn.) was evaluated against four Gram-negative bacteria viz.*Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli and Shigelladysenteria*as well asfour Gram - positive bacteriaviz. *Bacillus megaterium, Staphylococcus aureus, Bacillussubtilis,* and *Streptococcus pyogenes.* Disc diffusion technique was used for *in vitro* antibacterial screening. Result showed that compound AV-1 had large zone of inhibition in disc diffusion against the said bacteria. Antibacterial activity was more in Gram - positive bacteria than Gram - negative bacteria. Highest activity was noted against *Bacillussubtilis* and lowest was found for *Salmonella typhi.*The MIC (minimum inhibitory concentration) values of AV-1 against the bacteria ranged from 4 – 32 microgram/ml. Results, thus, suggest thatthe compound (AV-1) isolated from the leaves of *Artemisia vulgaris* Linn.had good anti bacterial activity against the tested bacteria.

Keywords: Antibacterial activity, *Artemisia vulgaris* Linn., Disc diffusion technique, Zone of inhibition, Minimum inhibitory concentration.

INTRODUCTION

Titeypati (*Artemisia vulgaris*Linn.) is a perennial shrubby aromatic plant throughout the hills of India. The plant is abundant in Sikkim and Darjeeling Himalayas in the middle and upper hill forest up to the height of 2000- 5000 ft. The plant has different names : Titeypati

in Nepali, Tuk – gnyel in Lepcha, Dhamanaga in Tibetan, Dona in Hindi, Nagdamini in Bengali, Barha in Sanskrit and Indian worm wood in English. The whole plant has medicinal values. Medical uses of the plant as recorded in Ayurvedic literature are : used as appetizer, cures "kapha", asthma and itching, prevents convulsion etc. Water extract of the plant is good larvicide like kerosene. It has also feeble insecticidal property. It is antibacterial and antifungal too [1,2].

Recently we have noted anti bacterial activity of leaves of *Artemisia vulgaris* Linn.against different Gram-positive and Gram-negative bacteria [3]. We tried to isolate the active compound present in the leaves of *Artemisia vulgaris* Linn.responsible for anti bacterial activity. By solvent extraction and chromatographic techniques a compound (AV-1) was isolated from the leaves of *Artemisia vulgaris* Linn. Antibacterial activity of AV-1 was studied against few Gram-positive and Gram-negative bacteria. In this communication results of experiments are being reported.

MATERIALS AND METHODS

Plant Material

***Artemisia vulgaris*Linn.**

Leaves of *Artemisia vulgaris*Linn.were collected from the medicinal plants garden of the University of North Bengal, Siliguri, West Bengal, India and authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department for future reference. Leaves were shed dried and powdered. The powder was used as the test drug.

Isolation of AV-1 from the leaves of *Artemisia vulgaris* Linn.

50g of the test drug, as prepared above, were extracted with 500 ml of ethanol : water mixture (10 : 1, v/v) for 30 minutes in a soxlet apparatus at room temperature. The extract

was then filtered. Filtrate was refluxed with 10 ml 1(N) hydrochloric acid at 100^0C for 15 minutes. The content was cooled and neutralized with 1(N) sodium hydroxide. Volume was reduced to 10 ml under reduce pressure by a rotary evaporator. This was then filtered and the filtrate was subjected to column chromatography using silica gel G mesh (200 – 400 size) as adsorbent. Nine bands were separated. Elusion was done by 30% ethanol – chloroform mixture . Eluent of the first band (about 100 ml) was reduced to dryness under controlled temperature. Brown dry mass was obtained. 10 ml n –butanol was added to it. The mixture was shaked for 10 minutes on a rotary shaker and re chromatographed using polyamide as adsorbent. Four bands were separated. First band was eluted by n-butanol, ethyl acetate mixture (20:1, v/v) . Eluent was reduced to about 10 ml by a rotary evaporator and repeated crystallizations were doneby n-butanol, ethyl acetate mixture(10:1, v/v). Crystal appeared, provisionally given the name AV-1.

Bacteria

Four Gram - positive bacteria *viz.Staphylococcus Aureus, Bacillus subtilis, Bacillus megateriumand Streptococcus pyogenes*as well as four Gram-negative bacteria *viz. Shigelladysenteriae, Pseudomonas aeruginosa, Escherichia coli*and *Salmonella typhi*were used to determine antibacterial activity and minimum inhibitory concentration. Bacteria were collected from the department of Microbiology, North Bengal Medical College Hospital,Siliguri, West Bengal, India.

Media

Nutrient agar media (Difco laboratories) pH 7.2 and nutrient broth media (Difco laboratories) pH 6.8 were used for antibacterial screening and minimum inhibitory concentration determination.

Antibacterial screening

In vitro antibacterial screening was carried out by disc diffusion method [4]. According to this method , 20 ml quantities of nutrient agar were placed in a petri dish with 0.1 ml of 10^{-2} dilution of bacterial culture of 20 hours old. Filter paper discs (6 mm diameter) impregnated with 60 µg per disc and 120 µg per disc concentration of the solution prepared from AV-1, isolated compound from *Artemisia vulgaris* Linn., were placed on test bacteria-seeded plates. Blank disc impregnated with water was used as negative control. Zone of inhibition was recorded after 18 hours of incubation. Diameters of zone of inhibition produced by AV-1 were compared with that of standard antibiotic kanamycin 40 µg per disc.Each sample was

used for five times for determination of anti bacterial activity.

Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration is defined as the lowest concentration of antibiotic completely inhibiting visible growth of bacteria after 18 – 24 hours of incubation at 37 ^{0}C. This was done by the method of Mosaddik*et al.* [5]. According to this method, 1.0 mg of AV-1, the compound isolated from *Artemisia vulgaris* Linn.leaves,was dissolved in 2 ml nutrient broth media to obtain a stock solution of concentration 500 µg/ml. 3 drops of Tween 80 was added in nutrient broth to facilitate dissolution. Serial dilution technique was followed to obtain 250 µg/ml concentration of the compound. One drop (0.02ml) of prepared suspensions of organism

(10^6organism/ml) was added to each broth dilution. These dilutions were then incubated for 20 hours at 37^0C. Growthof bacteria was examined by noting turbidity of the solution. The nutrient broth media with 3 drops of Tween 80 was used as negative control while kanamycin was used as positive control.

Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

RESULTS

In vitro antibacterial activityof AV-1, the compound isolated from the leaves of *Artemisia vulgaris* Linn., and kanamycin is given in Table – 1. Results showed that AV-1 exerted anti bacterial activity both at 60 µg per disc and at 120 µg per disc concentrations for all the tested bacteria which were comparable to that of reference drug kanamycin at 40µg per disc concentration. Large zone of inhibition in disc diffusion was found out. Antibacterial activity was more in Gram - positive bacteria than Gram - negative bacteria. Zone of inhibition with AV – 1,in the dose of 60 µg per disc, came 25 ± 0.7, 23 ± 0.6, 24 ± 1.1, 22 ± 0.7 for *Bacillus subtilis,Bacillus megaterium, Staphylococcus aureus, Streptococcus pyogenes* respectively

and 20 ± 0.7, 19 ± 1.0, 19 ± 1.1, 17 ± 0.6 for *Escherichia coli, Shigelladysenteriae, Pseudomonas aeruginosa, Salmonella typhi* respectively. In the dose of 120 µg per disc, AV – 1 gave Zone of inhibition 36 ± 0.8, 30 ± 1.0, 35 ± 1.1, 29 ± 0.6 for *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Streptococcus pyogenes* respectively and 22 ± 1.1, 28 ± 1.3, 28 ± 1.0, 25 ± 0.7for *Escherichia coli, Shigelladysenteriae, Pseudomonas aeruginosa, Salmonella typhi* respectively. Highest activity was thus noted against *Bacillus subtilis* and lowest activity was found for *Salmonella typhi.* Kanamycin (40 µg per disc), however, produced maximum zone of inhibition (39 ± 1.2) for *Staphylococcus aureus*and minimum zone of inhibition (28 ± 1.0) for *Escherichia coli.*

Table – 2 indicates results of minimum inhibitory concentration of AV-1, the compound isolated from the leaves of *Artemisia vulgaris* Linn., and kanamycin. The MIC (minimum inhibitory concentration) values ofAV-1 against Gram-positive and Gram-negative bacteria ranged from 4 to16 and 16 to 32 microgram/ml respectively.MIC withkanamycin, however, came 2 to 8 for Gram-positive bacteria and 8 to 32 for Gram –negative bacteria.

Table -1 : *In vitro* antibacterial activity of AV-1, the compound isolated from the leaves of *Artemisia vulgaris* Linn.and kanamycin [Zone of inhibition (diameter in mm)].

Bacteria	Strain	AV – 1 (60 µg per disc)	AV – 1 (120 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive				
Bacillus subtilis	ATCC 19659	25± 0.7	36 ± 0.8	37 ± 1.1
Bacillus megaterium	NBMC 1122	23± 0.6	30 ± 1.0	35 ± 1.1
Staphylococcus aureus	ATCC 25923	24± 1.1	35 ± 1.1	38 ± 1.2
Streptococcus pyogenes	NBMC 1321	22± 0.7	29 ± 0.6	33 ± 0.7
Gram – negative				
Escherichia coli	ATCC 25922	20± 0.7	22 ± 1.1	25 ± 1.0
Shigelladysenteriae	NBMC 1127	19± 1.0	28 ± 1.3	32 ± 1.2
Pseudomonas aeruginosa	NBMC 1243	19± 1.1	28 ± 1.0	33 ± 1.1
Salmonella typhi	MTCC 733	17± 0.6	25 ± 0.7	26 ± 1.2

Data was in mean SEM (n = 5). Control was made with water. It had no zone of inhibition. So data has not been shown.

Table – 2 : Minimum inhibitory concentration of AV-1, the compound isolated from the leaves of *Artemisia vulgaris* Linn. and kanamycin.

Bacteria	MIC values of AV - 1 (microgram/mL)	MIC values of kanamycin (microgram/mL)
Gram – positive		
Bacillus subtilis	4	4
Bacillus megaterium	16	8
Staphylococcus aureus	8	2
Streptococcus pyogenes	16	8
Gram – negative		
Escherichia coli	16	32
Shigelladysenteriae	16	8
Pseudomonas aeruginosa	16	8
Salmonella typhi	32	16

Negative control containing water had no MIC value. Thus, it has not been shown

DISCUSSION

Antibiotic resistance is now a topic of research as several bacteria have developed resistance towards many antibiotics. This is specially applicable for the bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa* etc. This has created immense problem in treatment of infectious diseases [6-10] . To combat the situation continuous effort is going on for synthesis of new chemicals having anti microbial activity [11-13]. Chemists are synthesizing chemical compounds in laboratory and established their anti bacterial activity. Unfortunately, most of these compounds are potentially toxic and are not free from side effects on the host[14]. This has extended the research even in the field of medicinal plants for isolation of natural compounds which will be less toxic and a proper substitute of chemical antimicrobial agents [15].

Several plants were screened to know their antimicrobial property [16 – 23].Hiremath*et al.*(2011) demonstrated antimicrobial activity of the whole plant of Titeypati[24].
Recently we have noticed anti bacterial activity of leaves of *Artemisia vulgaris* Linn [3]. As leaves of *Artemisia vulgaris* Linn. are widely used in folk medicine in West Bengal, Sikkim and adjoining area we tried to isolate the active compound(s) from the leaves of *Artemisia vulgaris.*Linn. responsible for anti bacterial activity. A compound (AV-1) was isolated from the leaves of *Artemisia vulgaris* Linn.

Antibacterial property of AV-1 was evaluated against four Gram – positive and four Gram – negative bacteria. Anti bacterial activity was measured by noting zone of inhibition in disc diffusion. Minimum inhibitory concentration was also noted. Standard antibiotic kanamycin was kept as control drug. It was found out that AV-1 isolated from the leaves of *Artemisia vulgaris* Linn. exerted antibacterial activity against the tested bacteria. Maximum activity was found in case of *Bacillussubtilis*while minimum activity was seen for *Salmonella typhi*.The results were comparable to that of standard antibiotic kanamycin.For *Bacillus subtilis*zone of inhibition produced by AV -1 was 36 ± 0.8 and by kanamycin it came 37 ± 1.1.For *Salmonella typhi*zone of inhibition produced by AV -1 was 25 ± 0.7 and by kanamycin it came 26 ± 1.2. Same trends were also found in case of other bacteria. We are now interested to note the mechanism of anti bacterial property of AV-1. Work is progressing in this direction.

CONCLUSION

Anti bacterial activity of AV-1, a compound isolated from the leaves of *Artemisia vulgaris* Linn., was examined against four Gram-positive and four Gram-negative bacteria. Kanamycin was employed as control drug. AV-1 showed anti bacterial activity against all the tested bacteria. Maximum activity was found against *Bacillus subtilis*and minimum activity was noted for *Salmonella typhi*. Results were comparable to that of kanamycin.Compound AV-1.thus provides a scientific rationale for use as anti bacterial drug.

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