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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF AN ISOLATED COMPOUND (AV-2) FROM THE LEAVES OF TITEYPATI (*Artemisia vulgaris Linn.*)

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ABSTRACT

Objective: To know the antibacterial activity of a compound (AV-2) isolated from the leaves of Titeypati (Artemisia vulgaris Linn.), a medicinal plants of North Eastern Himalaya, against four Gram- negative bacteria like Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa and Salmonella typhi. as well as four Gram - positive bacteria viz. Bacillius subtilis, Bacilus megaterium, Staphylococcus aureus and Streptococcus pyogenes. **Method:** Disc diffusion technique was used for in vitro antibacterial screening. **Results:** Result showed that AV-2 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 Bacillus subtilis and lowest was found for Salmonella typhi. The MIC (minimum inhibitory concentration) values of AV-2 against the bacteria ranged from 4 – 32 microgram/mL. **Conclusion:** AV-2, thus, had well anti bacterial activity against the tested bacteria.

Keywords : Antibacterial activity, AV – 2, Artemisia vulgaris Linn, Disc diffusion technique, Zone of inhibition, Minimum inhibitory concentration..

INTRODUCTION

Several plants showed antimicrobial property [1-14]. In our laboratory we screened various medicinal plants for their antimicrobial property. Titeypati (*Artemisia vulgaris* Linn.) is one such plant. It 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, Dhama naga 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. Water extract of the plant is good larvicide like kerosene. It has also feeble insecticidal property. It is antibacterial and antifungal too [15-16].

In screening program we noted anti bacterial activity of the leaves of Titeypati [17]. We intended to isolate the active ingredient(s) from the leaves responsible for anti bacterial activity. Four compounds (AV-1, AV-2, AV-3 and AV-4) were isolated. Anti bacterial activity of AV-1 was reported elsewhere (under communication). Anti bacterial activity of AV - 2 is reported here.

MATERIALS AND METHODS

Artemisia vulgaris Linn.

Leaves of *Artemisia vulgaris* Linn. were collected from the Medicinal Plant Garden of the University of North Bengal and authenticated by the taxonomist 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.

2.2 Isolation of AV-2 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^{0}C 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 shook for 10 minutes on a rotary shaker and re chromatographed using polyamide as adsorbent. Four bands were separated. Bands were eluted by n-butanol, ethyl acetate mixture (20:1, v/v) . Eluents collected separately were reduced to about 10 ml by a rotary evaporator and repeated crystallizations were done by n-butanol, ethyl acetate mixture(10:1, v/v). From each fraction crystals appeared, provisionally given names AV-1, AV-2, AV-3 and AV-4 obtained from the first, second, third and forth fraction respectively. Crystal AV-2 was evaporated to dryness *in vacuo* with rotary evaporatorat 40 – 50 degree centigrade. A brownish mass was obtained. 500 micro gram of the mass is extracted with 1 ml water and the solution obtained there from was used to evaluate the anti bacterial activity against the tested bacteria.

Bacteria

Four Gram - positive bacteria *viz. Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus* and *Streptococcus pyogenes* and four Gram-negative bacteria *viz*. *Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa* and *Salmonella typhi* were employed to determine antibacterial activity and minimum inhibitory concentration. All these bacteria were collected from the department of Microbiology, North Bengal Medical College Hospital.

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 [18]. 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-2 isolated from thee leaves of *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 the solution prepared from AV-2 were compared with that of standard antibiotic kanamycin at a dose of 40 µg per disc; Each sample was used for five times for the 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 and Haque [11]. According to this method, 1.0 mg of AV-2 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^{0}C. Growth of 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 was analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions. Differences between means were tested employing Duncan's multiple comparison tests and significance was set at $p < 0.05$.

RESULTS

In vitro antibacterial activity of AV-2, compound isolated from the leaves of *Artemisia vulgaris* Linn. and kanamycin is given in Table – 1. Results showed that AV-2 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 40 µg 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. Highest activity was noted against *Bacillus subtilis* and lowest activity was found for *Salmonella typhi*. Kanamycin, on the other hand, showed maximum activity against *Staphylococcus aureus* and minimum activity against *Escherichia coli.*

Table – 2 indicates results of minimum inhibitory concentration of AV-2 and kanamycin. The MIC (minimum inhibitory concentration) values of AV-2 against Gram-positive and Gram-negative bacteria ranged from 4 to 16 and 8 to 32 microgram/mL respectively. Kanamycin showed MIC values ranged from 2 to 8 and 8 to 16 microgram/mL for Gram-positive and Gram-negative bacteria respectively.

DISCUSSION

Bacterial resistance is now a worldwide problem. A large number of antibacterial agents have been discovered but pathogenic bacteria are constantly developing resistance to these agents. This is due to massive and uncontrolled use of the anti bacterial drugs in society. But for this life threatening bacterial infection has been increased worldwide and is becoming an important cause of morbidity and mortality [19] . Under the circumstances, search for antibacterial agent is going on and extended even in the field of medicinal plants [18, 20].

Table 1: *In vitro* antibacterial activity of AV-2, compound isolated from the leaves of *Artemisia vulgaris* Linn. [Zone of inhibition (diameter in mm)]

Bacteria	Strain	AV-2 (60 µg per disc)	AV-2 (120 µg per disc)	Kanamycin (40 µg per disc)
Gram – positive				
Bacillus subtilis	ATCC 19659	22 ± 0.9	34 ± 1.4	36 ± 1.3
Bacillus megaterium	NBMC 1122	20 ± 0.7	32 ± 1.3	34 ± 1.2
Staphylococcus aureus	ATCC 25923	21 ± 1.0	33 ± 1.1	37 ± 1.6
Streptococcus pyogenes	NBMC 1321	19 ± 0.8	30 ± 0.7	32 ± 0.9
Gram – negative				
Escherichia coli	ATCC 25922	20 ± 0.8	27 ± 1.3	28 ± 1.2
Shigella dysenteriae	NBMC 1127	21± 1.0	28 ± 1.2	31 ± 1.1
Pseudomonas aeruginosa	NBMC 1243	21 ± 1.2	28 ± 1.0	32 ± 1.2
Salmonella typhi	MTCC 733	17 ± 0.8	26 ± 0.8	29 ± 1.4

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-2, the compound isolated from the leaves of *Artemisia vulgaris* Linn.

Bacteria	MIC values of AV - 2 (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		
Shigella dysenteriae		
Pseudomonas aeruginosa	16	8
Salmonella typhi	8	8
	16	8
	32	16

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

Hiremath et al. [21]demonstrated antimicrobial activity of the whole plant of Titeypati . We have noticed anti bacterial activity of leaves of Artemisia vulgaris Linn. [17]. 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. Four compounds (AV-1, AV-2, AV-3 and AV-4) were isolated.

Antibacterial property of AV-2 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 determined. Standard antibiotic kanamycin was kept as control drug. It was found out that AV-2 exerted antibacterial activity against the tested bacteria. Maximum activity was found in case of *Bacillus subtilis* while minimum activity was seen for *Salmonella typhi.*The results were comparable to that of standard antibiotic kanamycin.

REFERENCES

1. Venugopal PV and Venugopal TV. Anti dermatophytic activity of neen (Azadirachta indica) leaves in vitro. Indian J Phrmacol. 1994; 26: 141 – 43.
2. Chakraborty A, Chakraborty BK and Bhattacharya P. Clausenol and clausenine -two carbazole alkaloid from Clausena anisata. Phytochemistry. 1995; 40: 295 - 299.
3. Padmaja V, Thankarmany V and Hara N. Biological activities of Amona glabra. J.Ethnopharmacol. 1995; 48:21 – 24.
4. Desmarchelier C, Mongelli E, Coussio J and Ciccia G. Studies on cytotoxicity, antimicrobasis and DNA – binding activities of plants used by Eseejas. Ethnopharmaco. 1996;, 50: 91 - 6.
5. 5. Nair A and Bhide SV. Antimicrobial properties of different parts of Semecarpus anacardium. Indian Drugs. 1996; 33: 323 - 8.
6. Gopalakrishnan G, Banumathi B and Suresh G. Evaluation of the antifungal activity of natural xanthones from Garcinia mangostana and their synthetic derivatives. J Nat Prod. 1997; 60: 519 – 524.
7. Rana B K, Singh U.P and Taneja V. Antifungal activity of kinetics of inhibition byessential oil solated rom leaves of Aegle marmelos. J. Ethnopharmacol. 1997; 57: 29 – 34.
8. Suresh B, Sriram S, Dhanaraj S, Elango K and Chinnaswamy K. Anticandidal activityof Santolina chamaecparissus volatile oil, J Ethnopharmacol. 1997; 55: 151 – 59.
9. Valsaraj R, Pushpangadan P and Smitt UW. Antimicrobial screening of selected medicinal plants from India. J. Ethnopharmacol. 1997; 58: 132-40.
10. Sakharkar PR and Patil AT. Antimicrobial activity of Cassia alata. Indian Journal of Pharmaceutical Sciences. 1998; 60: 311 – 312.
11. Mosaddik MA and Haque ME. Cytotoxicity and antimicrobial activity of goniothalamin isolated from Bryonopsis laciniosa. Phytother Res. 2003; 17: 1155 - 7.

For *Bacillus subtilis zone* of inhibition produced by AV -2 was 34 ± 1.4 and by kanamycin it came 36 ± 1.3. For *Salmonella typhi* zone of inhibition produced by AV -2 was 26 ± 0.8 and by kanamycin it came 29 ± 1.4. Same trends were also found in case of other bacteria.

We are now interested to note the mechanism of anti bacterial property of AV-2. Work is progressing in this direction.

CONCLUSION

A compound AV-2 has been isolated from the leaves of Titeypati (*Artemisia vulgaris* Linn.), Anti bacterial activity of AV-2 was studied against four Gram –positive and four Gram-negative bacteria. AV-2 showed anti bacterial effect for all tested bacteria. Highest activity was noted against *Bacillus subtilis* and lowest was found for *Salmonella typhi*. Results were comparable to that of standard antibiotic kanamycin.

12. Dash S, Nath LK, Bhise S and Bhuyan N. Antioxidant and antimicrobials activity of Heracleum nepalense D. Don root. Trop. J. Pharm. Res. 2005; 4: 341 – 7.
13. Khan A, Rahman M, Islam S. Antibaterial, antifungal and cytotoxic activities of tuberous roots of Amorphophallus campanulatus. Turk. J. Biol. 2007; 31: 167 - 72.
14. Khan Alam, Rahman Moizur and Islam MS. Antibacterial, antifungal and cytotoxic activities of amblyone isolated from Amorphophallus campanulatus. Indian J Pharmaco. 2008;, 40: 41 – 44.
15. Chopra Col Sir RN and Chopra IC. Indigenous drugs of India, U. N. Dhar and Sons Private Limted, Kolkata, 1958, P. 668.
16. Gurung Bejoy. In 'The medicinal plants of the Sikkim Himalaya', Pub. Gurung Jasmin Bejoy, Maples, chakung, West Sikkim, 2002, P. 51.
17. Ghosh Tanaya, Mitra Prasenjit, Mitra Prasanta Kumar, Antibacterial activity of leaves of Titeypati (Artemisia vulgaris Linn). Bioscience Guardian. 2013; 3(1): 107-112.
18. Rahman MM, Mosaddik MA, Wahed MI and Haque ME. Antimicrobial activity and cytotoxicity of Trapa bispinosa. Fitoterapia. 2000; 71: 704 - 6.
19. Al–Bari M, Sayeed MA, Rahman MS and Mossadik MA. Characterization and antimicrobial activities of a phenolic acid derivative produced by Streptomyces bangladeshiensis, a novel species collected in Bangladesh. Respir J. Med. Sci, 2006; 1: 77 - 81.
20. Rahman MM, Wahed MI, Biswas MH, Sadik GM and Haque ME. In vitro antibacterial activity of the compounds of Trapa bispinosa Roxb. Science. 2001; 1: 214 - 6.
21. Hiremath SK, Kolume DG and Muddapur UM. Antimicrobial activity of Artemisia vulgaris Linn. Int. J. Res. Ayur. Phar. 2011; 2(6): 1674 – 1675.