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Research Article

Selective In Vitro Antimicrobial Properties of *Solanumvirginianum Linn* Crude Extract

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ABSTRACT:

The antibacterial activities of *Solanumvirginianum*was investigated against *Escherchia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *B.subtilis* using the Streak plate, Broth dilution methods. The solvent type extracts were obtained by three extractions with Petroleum ether, Ethyl acetate, Ethanol, and aqueous extract were obtained by cold maceration, respectively. Solvents were removed in vacuo to and which were made up to a concentration of 5 ppm in distilled water. These were tested in varying volumes of 0.2-0.6 ml/plate. The solvents were used as control whereas ampicillins were used as references for bacteria. The solvents had no effect on the microorganisms whereas ampicillin inhibited microbial growth. *Solanumvirginianum* showed antimicrobial inhibitory activity most prominent with the Ethanoic extracts and negligible with the aqueous extract. This study suggests that the Ethanolic extract of *Solanumvirginianum*, can be used as herbal medicines in the control of all the bacterial species i.e. *Escherchia coli*, *Pseudomonas aeruginosa*, *S.aureus*and *Bacillus subtilis* following clinical trials.

Keywords: *Solanumvirginianum,Escherchia coli, Pseudomonas aeruginosa, Staphylococcus aureus, B.subtilis*.

INTRODUCTION

There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. The number of resistant strains of microbial pathogens is growing since penicillin resistance and multiresistancepneumococcicaused a major problem in South Africa in 1977 [1]. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem. The search for new effective antimicrobial agents is necessary due to the appearance of microbial resistance. The screening of plant extracts and plant products for antimicrobial

activity has shown that higher plants represent a potential source of novel antibiotic protypes[2-5].*Solanum*is a large, cosmopolitan genus, comprises 1,500 species, about 75% of the Solanaceae, but in the absence of a recent critical assessment these figures may well be too high. The genus is mainly tropical but a few are temperate. The greatest diversity of species is to be found in South and Central America; Australia and Africa are less rich but have a significant number of endemics. Eurasia is poor in species numbers. Many of the species in the genus are food plants, ornamentals, weeds or medicinal. It is important that the classification and nomenclature of a genus with such an extensive interaction with man should be understood and stabilized. The genus S*olanum* however, has a certain taxonomic notoriety, derived in part from the large number of species names described, which complicates specimen identification, but also in part from the difficulty of identifying natural species groups. A number of factors are responsible for this and these include the poor definition of the generic limits, the occurrence of suites of attributes in varying combinations throughout the genus, the phenotypic plasticity andgenetic variation in many of the species, and the continual reclassification of parts of the genus which has ended innomenclatural confusion[6,7].*Solanumvirginianum*L. a prickly, diffuse under shrub, somewhat woody at the base; stem somewhat zigzag. Prickles compressed, straight, yellow, often exceeding 1.3 cm long. Leaves 5-10 cm long, ovate or elliptic, sinuate or subpinnatifid, obtuse or subacute, armed on the midrib and nerves with long yellow sharp prickles. Flowers are in extra-axillary few-flowered cymes; corolla white, 2 cm long. Berry 1.3-2 cm diam., yellow or white with green veins, surrounded by the enlarged calyx. Roots are diuretic and expectorant; employed in cough, asthma, chest painand catarrhal fever. Fruit juice is useful in sore throat and rheumatism. Stem, flowers and fruits are carminative. Paste of the leaves is applied on painful joints to relieve pains. Seeds are given as an expectorant in asthma and cough. Decoction of the plant is useful in gonorrhea. The plant also possesses cardioactive and antipyretic activities[8-10]. Crude plant extract caused hypotension which has been attributed to release of histamine by some constituents. In the present study, In *vitro* antimicrobial screening methods could provide theneeded preliminary observations necessary to selectamong crude extracts, those with potentially usefulproperties for further chemical and pharmacologicalinvestigations[11]. This study was aimed at investigating theantimicrobial property of S. *virginianum*by preliminarybioassay screening.

MATERIALS AND METHODS

Plant material:-

Collection, Authentication

Solanumvirginianum drug was collected from Botanical garden, Yash Institute of Pharmacy, Aurangabad and it was identified by Head of Department, Department of Botany, Dr. B.A.M. University, Aurangabad. The specimen was deposited in the Department of Botany and accession number is given 0551.

Preparation of plant extracts

Aqueous extracts of *Solanumvirginianum*were prepared by cold maceration. For preparation of aqueous extracts of*Solanumvirginianum*, 400 g of

plant powder was soaked in 1000 ml of distilled water. Homogenate was kept for one weeks at room temperature (25 ± 2ºC) in extraction bottle. After 1 weeks, for aqueous extract the mixture was filtered twice; the filtrate was concentrated in rotary evaporator then was air dried and the percentage yield was 9.3 %.Petroleum ether (500 ml), Ethyl acetate (500 ml) and Ethanolic (500 ml) extract was prepared by Soxhlet extraction process. The temperature was maintained between 60^0C and 70 ºC. The extraction was carried out for one week for each extract and the extract was filtered, concentrated in rotary evaporator and then was air-dried. The percentage yield was calculated as 7.6 %, 2.9 %, 6.4 % for Petroleum ether, Ethyl acetate, Ethanolic extract respectively[12].

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Microorganisms used

The test organisms *Staphylococcus aureus*(ATCC 25923),*Escherchia coli* (ATCC 25922)*Pseudomonas aeruginosa*ATCC (27853) and *Bacillus subtilis*(ATCC 6633) were obtained from Department of Botany, Institute of Science, Dr. BabasahebAmbedkarMarathwada University, Aurangabad and was stored in a refrigerator at the Microbiology Laboratory, Department of Pharmaceutics , Yash Institute of Pharmacy, Aurangabad.

Streak Plate Method:

Nutrient agar was prepared as described above and 10 ml was poured into plates. Plant extracts dissolved in solvent at a final concentration of 0.035g/0.01L were pipette out into three sterilized plates under aseptic conditions at different volumes (0.2-0.4-0.6 ml), using a micropipette. The plates were allowed to cool and then the bacteria were streaked onto the surface of the solidified agar/plant extract medium. A flame loop was used to inoculate the bacteria from their cultures These plates were left for 24 hours in a dessicator.The plates with inhibition were used in further experiments. A reference experiment was setup using an antibiotic (ampicillincapsule) at the same concentration as plant extracts (0.035g/0.01L) at different volumes (0.2-0.4-0.6ml). Controls were also setup using solvents: Petroleum ether, Ethyl acetate, Ethanol, Methanol, Distilled water at the different volumes[13].

Broth Dilution Method:

This method was used to test the plant extracts for antimicrobial activities against bacteria by investigating whether there was turbidity or not. Turbidity represents microbial growth, while no turbidity represents inhibitionof microbes. One set of tubes containing Nutrient broth was inoculated with *Staphylococcus aureus*and the second set was inoculated with *Escherichia coli* using a loop, flame and alcohol. Under aseptic conditions, the plant extracts (dissolved in solvent at concentration 0.035g/0.01L) and which showed inhibition in the streak plate were added to the one set of test tubes containing *E. Coli* and the other set, *S.aureus*with Nutrient broth(medium) in differing volumes (0.2-0.4-0.6ml). Two sets of four tubes each were treated with the four solvents (Petroleum ether, Ethyl acetate, Ethanol, Methanol, Distilled water). One set was inoculated with *S.aureus*and the other with *E.coli*, Fig 3.0. Cotton wool was used to plug test tubes. The tubes were observed after 24 hrs[13].

RESULT

Streak Plate Method:

Table-1: Results obtained from Streak plate method for the bacteria's *Escherchia coli, Pseudomonas aeruginosa, Staphylococcus aureus, B.subtilis against* different volumes of dissolved plant extracts at a final concentration of 5 ppm and controls.

Plant extract In solvent	Volume of Dissolved plant Extract used in (ml) at concen Tration 5 ppm	Inhibition or no growth of microbe, *Escherichia coli*	Inhibition or no growth of microbe, *Pseudomonas aeruginosa*	Inhibition or no growth of microbe, *Staphylococcus aureus*	Inhibition or no growth of microbe, *Bacillus subtilis*
Solanumvirginianum with Petroleum ether	0.2	+++	++	+++	+++
	0.4	+++	+++	+++	+++
	0.6	+++	+++	+++	+++
Solanumvirginianum with Ethyl acetate	0.2	++	++	++	+++
	0.4	++	++	+++	+++
	0.6	+++	+++	+++	+++
Solanumvirginianum With ethanol	0.2	+++	+++	+++	+++
	0.4	+++	+++	+++	+++
	0.6	+++	+++	+++	+++
Solanumvirginianum with Distilled water	0.2	_	_	+++	
	0.4	+ ++++	++		
	0.6	++	+	+++	+++
Reference	0.2	++	++	++	++
	0.4	+++	+++	+++	+++
	0.6	+++	+++	+++	+++
Petroleum ether/ Ethyl acetate/ Ethanol/Methanol/ Water	0.2	_	_	_	_
	0.4	_	_	_	_
	0.6	_	_	_	_

Inhibition or no growth of microbes were represented by a positive sign (+), while the negative sign (-) represents no inhibition or growth of microbes.

+ = Lightly Inhibited , ++ = Moderately Inhibited , +++ = Strong Inhibition

Dilution Method: Table- 2 : Degree of turbidity of dissolved *Solanumvirginianum*extracts at a concentration of 5 ppm at different volumes against *Escherchia coli*.

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract** 0.2 ml	**Volume ofof dissolved plant extract** 0.4 ml	**Volume ofSolvent dissolved plant extract** 0.6 ml	**Control Solvent(+)**	**Control Ampicillin) (-)**	**Reference**
Solanum virginianum with Pet. Ether	T0	T0T0	T1	T0	T0	
Solanum virginianum with Ethyl acetate	T1	T1T0	T2	T0	T0	
Solanum virginianum with Ethanol	T0	T0T0T2		T0	T0	
Solanum virginianum with water	T3	T2	T1	T1T0	T0	

Table -3: Degree of turbidity of dissolved *Solanumvirginianum*extracts at a concentration of 5 ppm at different volumes against *Pseudomonas aeruginosa*.

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract (ml)** 0.2 ml	**Volume ofof dissolved plant extract (ml)** 0.4 ml	**Volume ofof dissolved plant extract** 0.6 ml	**Control Solvent (+)**	**Control Solvent (-)**	**Reference (Ampicillin)**
Solanum virginianum with Pet. Ether	T1	T0	T0	T2	T0	T0

Solanum virginianum with Ethyl acetate	T1	T0	T2	T0	T0	
Solanum virginianum with Ethanol	T0	T0	T1	T0	T0	
Solanum virginianum with water	T2	T2	T2	T0	T0	

Table -4: Degree of turbidity of dissolved *Solanum virginianum* extracts at a concentration of 5 ppm at different volumes against *Bacillus subtilis.*

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract (ml)**	**Volume of dissolved plant extract (ml)**	**Volume of dissolved plant extract**	**Control Solvent (+)**	**Control Solvent (-)**	**Reference (Ampicillin)**
0.2 ml	0.4 ml	0.6 ml				
Solanum virginianum with Pet. Ether	T0	T0	T0	T1	T0	T0
Solanum virginianum with Ethyl acetate	T0	T0	T0	T2	T0	T0
Solanum virginianum with Ethanol	T0	T0	T0	T2	T0	T0
Solanum virginianum with water	T2	T2	T1	T2	T0	T0

Table – 5 : Degree of turbidity of dissolved *Solanumvirginianum*extracts at a concentration of 5 ppm at different volumes against *Staphylococcus aureus*.

Plant extract dissolved in solvents at concentration of 5 ppm	**Volume of dissolved plant extract (ml)**	**Volume ofof dissolved plant extract(ml)**	**Volume ofof dissolved plant extract**	**Control Solvent (+)**	**Control Solvent (-)**	**Reference (Ampicillin)**
	0.2 ml	0.4 ml	0.6 ml			
Solanum virginianum with Pet. Ether	T0	T0T0T1	T0	T0		
Solanum virginianum with Ethyl acetate	T1	T0	T0T2	T0	T0	
Solanum virginianum with Ethanol	T0	T0T0	T2	T0	T0	
Solanum virginianum with water	T1	T0	T0	T2	T0	T0

T0 = No Turbidity = Strong Inhibition, T1 = Lightly Turbid = Moderately Inhibited T2 = Moderately Turbid = Lightly Inhibited , T3 = Very Turbid = No Inhibition

DISCUSSION

Two methods: Streak plate and Broth dilution method were successful in determining *Solanumvirginianum antimicrobial* activities. Several trends are noted. The streak plate method indicated that the Petroleum ether, Ethyl acetate and Ethanol extract induce moderate to strong inhibition against all the bacterial species i.e. *Escherchia coli*, *Pseudomonas aeruginosa*, *S.aureus*and *Bacillus subtilis* at volume of 0.2 to 0.6 ml. However, Aqueous extract of*Solanumvirginianum*induce no inhibition against gram negative bacterial species i.e.*Escherchia coli*, *Pseudomonas aeruginosa*at volume of 0.2 ml-0.6 ml. On other hand, Aqueous extract of*Solanumvirginianum*induce moderate to strong inhibition against gram positive bacterial species i.e.*S.aureusand Bacillus subtilis* at volume of 0.2 to 0.6 ml.The Dilution method was used to test the dissolved plant extracts for antimicrobial activity against bacteria: *Escherchia coli*, *Pseudomonas*

*aeruginosa, Staphylococcus aureus, B.subtilis.*Plant extract that showed positive results for the streak plate was used. Results were recorded in terms of turbidity. In general, no turbidity indicates inhibition. The use of LB or Nutrient broth as a rich medium to foster or stimulate the growth of the bacteria is noted. Yeast extract and tryptone provide vitamins and amino acids respectively for the bacteria to grow. The result indicates that for the Petroleum ether, Ethyl acetate and Ethanol extract induce moderate to strong inhibitionagainst all the bacterial species i.e. *Escherchia coli, Pseudomonas aeruginosa, S.aureus*and*Bacillus subtilis* at volume of 0.2 to 0.6 ml. (No turbidity, T0). However, Aqueous extract of*Solanumvirginianum*induce noinhibition against gram negative bacterial speciesi.e.*Escherchia coli, Pseudomonas aeruginosa*at volume of 0.2 ml-0.6 ml (Turbidity, T3). On other hand, Aqueous extract of*Solanumvirginianum*induce moderate to strong inhibition againstgram positive bacterial species i.e.*S.aureus and Bacillus subtilis* at volume of 0.2 to 0.6 ml (Light to no turbidity, T1-T0). The reference compound ampicillin and the controls i.e. Petroleum ether, Ethylacetate,Ethanol, Water showed inhibition and non inhibition respectively as anticipated.

CONCLUSION

It is clearly seen that *Solanumvirginianum*has antimicrobial properties. However, antimicrobial activity is selective and solvent dependent with the Ethanolic extract, the most potent and aqueous extract the least. In general, the order of antimicrobial activity follows the sequence: Ethanolic extract > Petroleum ether extract > Ethyl acetate extract > Aqueous extract. Thus, the Ethanolic extract and Petroleum ether extract *Solanumvirginianum* of *can* be used as the active constituent of an antimicrobial cream or following clinical trial as herbal medicines. Future work such as isolation and purification of bioactive constituents should target the Ethanolic extract and Petroleum ether extract of*Solanumvirginianum.*

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REFERENCES

1. Ambikakarpathy V, Mahalingam R, et al A.J. Of Plant Sci. and Rese,2011, 1, 56-60,
2. Clinical and Laboratory Standards Institute (CLSI), National Committee for Clinical Laboratory Standards, P29.2009, VIII edition, M07-A8.
3. Cao R, Xue C, I.J. Of. Food. Microbio,2009, 131, 272-276.
4. Kumar VS, Meenukrishnan VG, et al A.J. Of Plant Sci.and Rese,2013, 3, 31-37.
5. Lamyaa FI, Salwa AK, et al Carbohydr. Res, 343, 155-158.,
6. Lu Y, Luo J, et al J.Of. Phytochem,2008, 72, 668-673.

7. Sheeba E, J. of. Sci.Engi. and techno,2010, 6, 1-4.
8. Maurya A, Manika N, Verma KR, , John Wiley & Sons Ltd, 24 (1), 87-92.2013
9. Maurya A, Srivastava SK, J. Brazil.Chem.Soc,2012, 23, 468-472.
10. Talib H, Gupta RK, et al A.paci. J. of Tropi.Medicine, 2012, 5, 686-691.
11. Maurer-Grimes B, Mcbeth DL, et al"Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae" Int. J. Pharmacog, 1996, 34, 243-248.
12. Elloff JN"Which extract should be used for the screening and isolation of antimicrobial components from plants"Ethnopharmacology, 1998, 60, 1-8.
13. Afolayan AJ, Grierson DS, et al"In vitroantifungal activity of some South African medicinal plants" S. Afr. J.Bot, 2002, 68, 72-76.