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Research Article

EVALUATION OF IN VITRO ANTIMICROBIAL POTENTIAL OF MEDICINAL PLANT: *SOLANUM VIRGINIANUM* L.

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ABSTRACT

Over the past few decades the use of antibiotics is under threat as many commonly used antibiotics have become less effective against certain illnesses due to emergence of drug-resistant bacteria. The continuous evolution of bacterial resistance to available antibiotics has necessitated the search for novel and effective antimicrobial compounds over a wide range of microorganisms. Development of bacterial resistance and increasing popularity of traditional medicine has led researchers to investigate the novel antimicrobial compounds in plants. One way to prevent antibiotic resistance is by exploring new bioactive compounds from traditional medicine which is not based on the existing synthetic antimicrobial agents. Solanum virginianum L. belongs to family Solanaceae is an important plant used in traditional medicine system. In current investigation the antimicrobial potential of Solanum virginianum L. was estimated against different pathogenic microorganisms and preliminary phytochemical study was performed. The results obtained during investigation indicated that the plant possesses considerable antimicrobial activity against a wide range of microorganisms giving a zone of inhibition ranging from 12-21mm in diameter. Ethanolic extract shows minimum Inhibition concentration ranges from 50-350µg/ml. Phytochemical screening revealed the presence of various phytochemicals including Alkaloids, Tannins, and Saponins etc.

Keywords: Traditional medicine, Drug-resistant bacteria, Solanum virginianum L., Phytochemicals.

INTRODUCTION

India has a high range of medicinal plants that are used in ancient as well as in modern pharmaceutical preparations. They have used in a preparation of drugs since centuries ago in ayurvedic, siddha and unani system.[1] Due to the potent therapeutic value, easy avability and mode of action these medicinal plants have attended more pharmacological exploration in modern medicinal practices. The microorganisms have developed multiple drug resistance against many antibiotics due to indiscriminate use of many antimicrobial drugs.[2,3] The continuous evolution of bacterial resistance to available antibiotics has necessitated the search for novel and effective antimicrobial compounds over a wide range of microorganisms.[4,5,6] Development of bacterial resistance and increasing popularity of traditional medicine has led researchers to investigate the novel antimicrobial compounds in plants. [7,8]

Due to increased emergence of number of antibiotic resistant microorganisms, there is necessity of searching for less toxic antimicrobial agents from plant sources. Antimicrobial agents from natural, especially plant sources may be easily accessible and might be cheaper with minimal side effects. [9] The medicinal value of drug plants is due to the presence of some chemical substances in the plant tissues which produce a definite physiological action on the human body. These chemicals include alkaloids, flavanoids, glucosides,

tannins, gums, resins, essential oils etc. [10,11] Solanaceae is a large plant family containing two thousand and three hundred species, nearly half of which belong to a single genus, Solanum. There are herbs, shrubs and small trees under this genus. This family comprises a number of plants widely known for the presence of variety of natural products of medicinal significance. Solanum virginianum L. (Synonyms:- Solanum xanthocarpum Schrad. and H. Wendl, Solanum Surattense)[12] is 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 diameter, yellow or white.[13]

The plant possesses a steroidal alkaloid solasodine as the principal alkaloid along with solasonine, solamargine, beta-solamargine, solanocapine, and solanocarpidine. Dry fruit contains traces of isochlorogenenic, neochronogenic, chronogenic and caffeic acid.[14] Crude plant extract is beneficial in bronchial asthma and non-specific cough, influenza, painful and difficult urination, bladder stones and rheumatism.[15] Plant possesses antiurolithiatic and natriuretic, tumoricidal, anti-allergic and anticancerous activity. [16,17,18]

METHODS AND MATERIALS

Collection and processing of plant material:

The whole plant of Solanum virginianum including leaves, stems, fruits and root were collected from the locality around Pusad city and taken care for its freshness, healthy and free from any deformation. The plant was brought to the laboratory, dried under shade at ambient temperature (32°C) for 7 to 10 days and then pulverized. The plant material were broke into small pieces and then blended into powder by electronic blender which then passed through a No. 20 standard mesh sieve to get the equal size particles. The powder was aseptically kept in air tight container at the moisture free place prior to extraction.

Soxhlet extraction of plant material:

100gm of powder is accurately weight and is transferred to the cup made up of 'Whatman filter paper' and placed into the extraction thimble. The powder was primarily defatted by using petroleum ether and then successively extracted by using solvents chloroform, methanol and ethanol for 48 hrs. At the end of the extraction process, individual solvent extract was separated and concentrated under reduced pressure at 50° C using a rotary vacuum evaporator. The weight of extract was measured and percentages of yield of the plant material were calculated. Vacuum dried extract was stored at 40C for further work.[19]

Isolation of test organisms:

Pure cultures of the test organisms used for antibacterial activity were isolated from the water and soil sample by using selective media (Himedia). The characterization of the test organisms was done by using IMVIC tests. All the test organisms were cultured on nutrient agar slant. The cultures were maintained by sub-culturing periodically and preserved at 40C prior to use.[20]

The gram negative bacteria includes; Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Shigella flexneri, Klebsiella pneuminiae, Enterobactor aerogenes etc. While the gram positive bacteria includes; Bacillus subtilis, Bacillus megaterium, Staphyloccus aureus, Streptococcus faecalis, Bacillus fusiformis, Streptococcus pneumoniae, Streptococcus pyogenes etc.

Preparation of Inoculums:

The suspensions of all the organisms were prepared using nutrient broth to a density of 9×108 cfu/ml as per Mac-Farland Nephelometer Standard. A 24 hrs old culture was used for the preparation of bacterial suspension. Suspensions of organisms were made in sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted.[21]

Screening for antibacterial activity:

All the test organisms were screened for the antibacterial activity against ethanolic extract of Solanum virginianum by agar well diffusion method. With the introduction of variety of antimicrobials it becomes necessary to perform the antimicrobial susceptibility test. For this the antimicrobial agent was allowed to diffuse out into the medium and interact in a plate freshly speeded with the test organism. Stock solution of ethanolic extract of Solanum virginianum was prepared to carry out the antimicrobial activities against selected cultures for the further process. For the preparation of the stock solution 1 gm of ethanolic extract was accurately weight and dissolved in 10 ml of DMSO; giving concentration

of the stock solution as 100 mg/ml. this solution is then centrifuged and supernatant liquid was collected in a separate test tube, covered with paraffin wax and stored at 40C for further use. [22]

Agar well diffusion method:

The Muller-Hinton agar plates for the bacteria were prepared and 0.1 ml of fresh 18 hours old broth culture was spread on the respective media. After spreading the culture, wells of 6 mm in diameter was made at the centre of the plate by using sterile cork borer. The wells were open with the help of sterile forceps. Then 100 µl of extract from stock solution was added by using micropipette in each well. The final concentration in the well was 10 mg/ml. The extract was allowed to diffuse; hence the prepared plates were kept in deep refrigerator for 25 minutes. After this plates were incubated at 370C for 24-48 hours. The zone of inhibition was measured in mm and recorded. The diameter of the zone of inhibition around each well was taken as measure of antibacterial activity. Each experiments was carried out in triplicates and mean diameter of the inhibition zone was recorded.[23] All results were expressed as means ± S.D. The significance between means was determined using student's t-test and results were regarded as significant when $P < 0.05$.

Determination of Minimum Inhibitory Concentration (MIC):

The extract which showed antibacterial activity in agar assay was subjected to MIC assay. For this purpose cup-plate method was followed. Five clean test tubes were taken and 20 ml of nutrient agar was added each tube aseptically. To these tube a concentration of 20µl-60µl was added from the standard stock of ethanolic extract to give the concentration of extract as 100µg/ml -350µg/ml of media, while the control tube contain only nutrient agar. All the tubes were autoclaved and poured into the sterile petriplates in aseptic condition. After solidifying, the plates were spread with 0.1 ml of 18 hours old respective bacterial culture. All the plates were incubated at 370C for 24-48 hours. After the incubation growth was observed in each plate. The MIC values were interpreted as the lowest concentration of the sample (extract), at which no considerable growth was observed after inhibition. All the test tubes were performed in triplicate and the values of MIC were recorded.[24]

Phytochemical Screening: The ethanolic extract of Solanum virginianum was screened for the phytochemical content by using different chemical test for each component. The ethanolic extract of the plant was used for the phytochemical test to detect the presence of alkaloids, tannins, saponins, flavonoids, cardiac, glycoside, anthraquinones and steroids according to standard method as follows. [25]

a) Alkaloids: A 5ml quantity of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for Hager's test.
b) Flavonoids: Alkaline reagent test: Extract was treated with 10 % NaOH solution; formation of intense yellow color indicates presence of Flavonoids.
c) Steroids: 1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H2SO4 acid was added from the side of test tube. The upper layer turns red and H2SO4 layer showed yellow with green fluorescence .This indicates the presence of steroid.
d) Tannin: 4ml extract was treated with 4 ml FeCl3 formation of green color indicates that presence of condensed tannin.
e) Saponins: 5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponins.
f) Cardial Glycosides: Plant extract treated with 2 ml glacial acetic acid containing a drop ofFeCl3 .A brown color ring indicates the presence of positive test.
g) Anthraquinones: About 0.5gram of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 min. the extract was filtered and the filtrate shaken with equal volume of 100% ammonia solution. A pink violet or red color in the ammonical layer indicates the presence of free anthraquinones.

RESULTS

Table no.1 shows phytochemical screening of ethanolic extract of Solanum virginianum Linn. The presence of different phytoconstituents was determined by using various tests like Mayer's test, Killer Killani test, Salkowski test, Bortrager's test, Alkaline test and Potassium hydroxide test for Alkaloid, Glycosides, Steroids, Saponins, Anthraquinones, Flavanoids and Tannins respectively. The ethanolic extract

Table no.1: Phytochemical screening of ethanolic extract *Solanum virginianum* plant

Sr. No.	Phytochemical constituents	Ethanolic extract
1	Alkaloids	+
2	Saponins	+
3	Tannins	+
4	Steroids	+
5	Flavonoids	-
6	Anthraquinones	-
7	Glycosides	+

(+) = present, (-) = absent

Table no.2: Antimicrobial activity of ethanolic extract of *Solanum virginianum* against gram negative bacteria

Sr. No.	Test Organism	Zone of Inhibition (mm in diameter)
1	*Salmonella typhi*	15.4 ± 0.21 mm
2	*Pseudomonas aerogenosa*	14.8 ± 0.11 mm
3	*Escherichia coli*	19.6 ± 0.09 mm
4	*Shigella flexneri*	21.7 ± 0.19 mm
5	*Vibrio cholerae*	17.8 ± 0.23 mm
6	*Enterobacter aerogenes*	19.3 ± 0.36 mm
7	*Klebsiella pneumoniae*	18.4 ± 0.27 mm

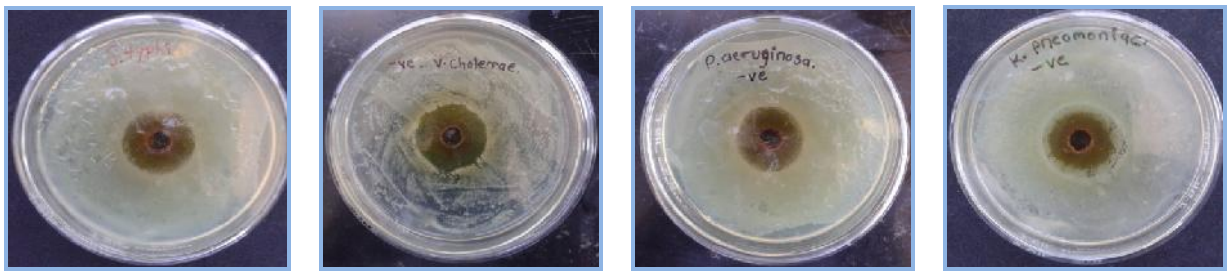

Figure no.1: Zone of inhibition (in mm) of *Solanum virginianum* against gram negative bacteria

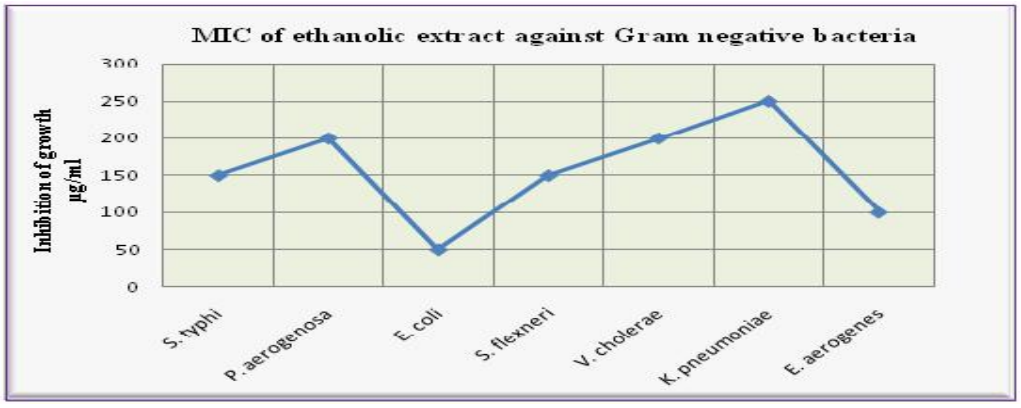

Figure no.2: MIC of ethanolic extract of Solanum virginianum against gram negative bacteria

Table no.3: Antimicrobial activity of ethanolic extract of Solanum virginianum against gram positive bacteria

Sr. No.	Test Organism	Zone of Inhibition (mm in diameter)
1	*Bacillus subtilis*	16.2 ± 0.22 mm
2	*Bacillus megaterium*	15.6 ± 0.08 mm
3	*Bacillus fusiformis*	13.8 ± 0.16 mm
4	*Streptococcus faecalis*	12.3 ± 0.41 mm
5	*Streptococcus pyogenes*	14.7 ± 0.26 mm
6	*Streptococcus pneumoniae*	13.4 ± 0.13 mm
7	*Staphyloccus aureus*	14.4 ± 0.42 mm

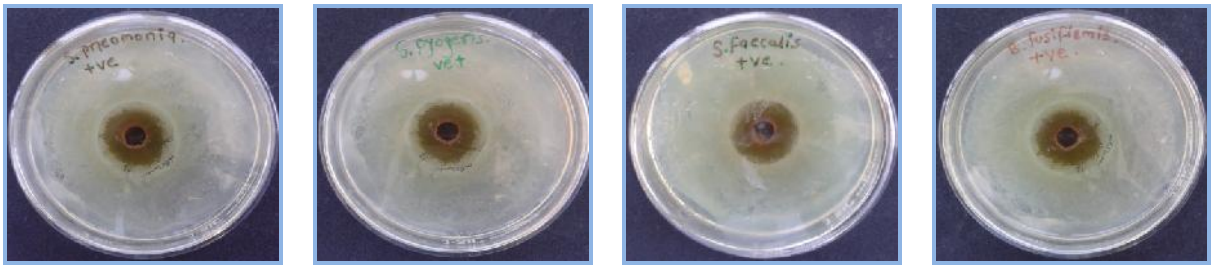

Figure no.3: Zone of inhibition (in mm) of *Solanum virginianum* against gram positive bacteria

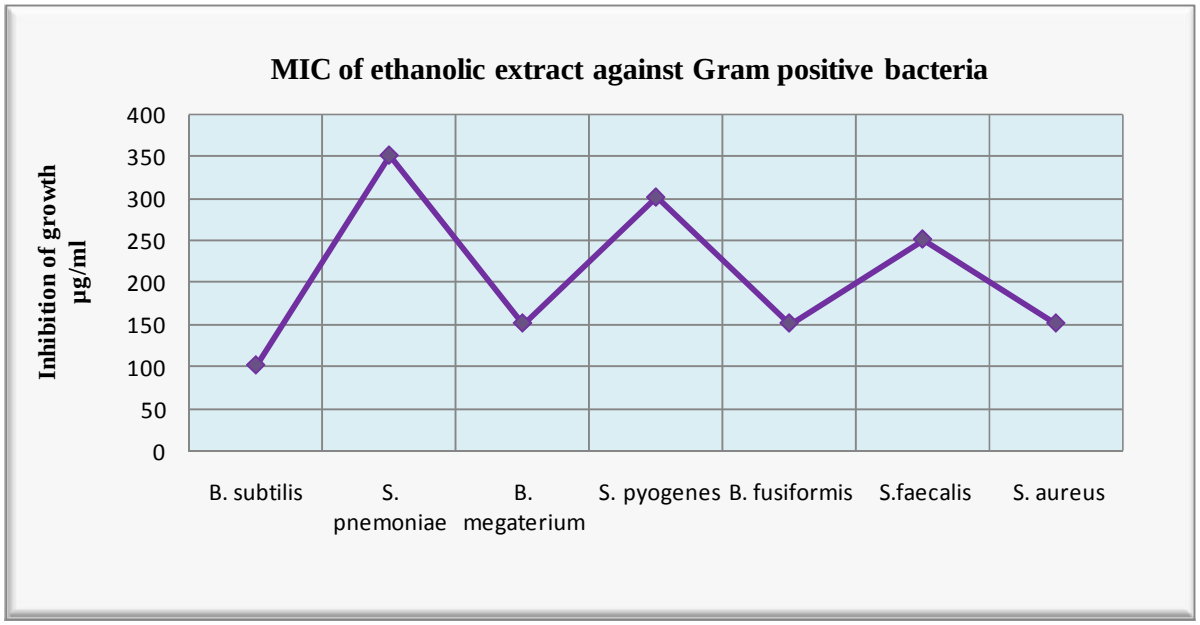

Figure no.4: MIC of ethanolic extract of Solanum virginianum against gram negative bacteria

was found to contain Saponins, Tannins, Alkaloids, Steroids and Glycosides.

Table no.2 shows agar well diffusion method for demonstration of antimicrobial activity of ethanolic extract of Solanum virginianum against gram negative bacteria. The zone inhibition around the well observed for gram negative bacteria varies from 12mm-16mm in diameter with highest for Shigella flexneri at 21.7±0.19 mm and lowest for Pseudomonas aerogenosa at 14.8 ± 0.11 mm. Results show that bacteria are sensitive to ethanolic extract of leaves. (Figure no.1)

In case of gram negative bacteria, the minimum inhibitory concentration for ethanolic extract shows inhibition of growth for Escherichia coli (50 µg/ml), Pseudomonas aeruginosa (200 µg/ml), Salmonella typhi (150 µg/ml), Vibrio cholera (200 µg/ml), Shigella flexneri (150 µg/ml), Klebsiella pneuminiae (250 µg/ml), Enterobactor aerogenes (100 µg/ml) respectively. (Figure no.2)

Table no.3 shows agar well diffusion method for demonstration of antimicrobial activity of ethanolic of from Solanum virginianum against gram positive bacteria. The zone of inhibition around the well observed for gram positive bacteria varies from 10mm-16mm in diameter with highest for Bacillus subtilis at 16.2 ± 0.08 mm and lowest for Streptococcus pneumoniae at 13.4 ± 0.26 mm. Result shows that bacteria are sensitive to ethanolic extract of leaves. (Figure no.3)

In case of gram positive bacteria, the minimum inhibitory concentration for ethanolic extract shows inhibition of growth for Bacillus subtilis (100µg/ml), Bacillus megaterium (150 µg/ml), Staphyloccus aureus (150 µg/ml), Streptococcus faecalis (250 µg/ml), Bacillus fusiformis (150 µg/ml), Streptococcus pneumonia (350 µg/ml), Streptococcus pyogenes (300 µg/ml) respectively. (Figure no.4)

DISCUSSION

In present investigation, all the gram positive and gram negative microorganisms shows considerable inhibition of growth against the ethanolic extract of Solanum virginianum ranging from 12-21mm. The plant is valued for its Solasodine flavonoid which attributes for its many medicinal properties which induce moderate to strong inhibition against all the bacterial species.

In similar studies, E. Sheeba (2010) reported the zone of inhibition for B. subtilis (19mm), Streptococcus sp. (13mm), S. aureus (16mm), S. typhi (16mm), E. coli (14mm) and V. cholerae (14mm) at concentration of 100µg/ml. R.Raja Sidambaram. et al. (2011) reveled that methanolic extract of leaves shows antibacterial activity against E. coli (15mm), S. aureus (15mm), S. typhi (18mm) and V. cholerae (10mm). Almazini et al. (2009) demonstrated that the mechanism of action of this constituent may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis and observed the zone of inhibition for S. aureus (13.20±2.30mm), E. coli (13.20±0.22mm) and P. aeruginosa (12.91±0.22mm) ethanolic extract of solanum nigrum. Chaturvedi and Saxena (2014) shows that ethanolic extract of Solanum surratense Burm F. exhibit inhibition of growth against E. coli (26.6±0.064mm), B. subtilis (22.7±0.116mm), P. aeruginosa (21.4±0.124mm), S. pneumonia (28.4±0.034mm) and S. typhi (18.6±0.113mm) respectively. M. Syed Ali et al (2014) revealed that ethanolic extract of stem shows antimicrobial activity against Bacillus sp. (14.2±0.5), E. coli (24.8±0.36mm), P. aeruginosa (32±0.72mm), K. pneuminiae (13±0.4mm), S. aureus (13.5±0.82mm) and Streptococcus sp. (10±0.32mm).

The minimal inhibitory concentration of ethanolic extract of Solanum virginianum against bacterial strain exhibited varying results. The MIC values for ethanolic extract ranges from 50-250µg/ml for gram positive bacteria and 100-350µg/ml for gram negative bacteria. This variation in the effectiveness of the different extracts against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. It has been suggested that the antimicrobial activity is mainly due to the presence of essential oils, flavanoids and terpenoids and other natural polyphenoilc compounds or free hydroxyl groups.[8]

The results obtained on investigation of phytochemical constituents is similar to that of Sharma et al. (2013) who revealed the presence of Alkaloids, Glycosides, Phenol, Lignin, Saponins, Sterols and Tannins in ethanolic extract of fruit pericarp. Phytochemical analysis of S.xanthocarpum revealed the presence of solasonine, solasomargine, sapogenin and solasodine that are responsible for the medicinal effect of this plant.[29] Lipophilic flavonoids

disrupt microbial cell membranes. Some phytochemicals form a complex with extracellular, soluble microbial proteins which bind to the microbial cell wall resulting in the dissolution of the cell wall. [30]

CONCLUSION

Today, most of the allopathic drugs have side effects but ayurvedic drugs have not side effect. Therefore, most of the medicinal plants raw materials are used for the development of new drugs.[31] A detailed study on biological usefulness of Solanum virginianum was carried out. Crude ethanolic extract was found to contain a variety of secondary metabolites and possess a considerable growth inhibiting effect against a wide range of microorganisms. In addition, the great importance of Solasodine in the pharmaceutical industry, there are many publications concerned with the search for Solasodine glycosides in various plants.[32] Therefore, these compounds may be responsible for the antibacterial activity. The mechanism of action of this constituent may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis. In conclusion, further investigation and phytochemical analysis is needed to isolate secondary metabolites and bioactive compounds responsible for antibacterial activity.

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