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SCREENING FOR IN VITRO ANTIMICROBIAL ACTIVITY OF SOLANUM AMERICANUM MILLER

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ABSTRACT

The plant *Solanum americanum* Miller of family *Solanaceae* is traditionally used as a medicinal plant widely antiseptic cadalgia and gripe. Present study has tried to *In vitro* antimicrobial study (well diffusion method) of petroleum ether, ethyl acetate, methanol, chloroform and aqueous extracts of *Solanum americanum* leaves were investigated individually. Four bacterial species (two Gram positive and two Gram negative bacterial species) and two fungal strains were used for study these are *Bacillus subtilis, Escherichia coli, Pseudomonas aerugenosa, Staphylococcus aureus, Aspergillus niger* and *Candida albicans*. High antibacterial activity was found in methanol plant material extracts, followed by other extracts. Aqueous extract showed no antimicrobial activity. While no extract showed anti bacterial activity against *Candida albicans*. The results of this study indicates that the leaf extract have more potential of antimicrobial activity and is concentration dependent.

KEY WORDS: *Antimicrobial activity, Solanum americanum, plant material extract, bacteria, fungus. Agar well diffusion*

INTRODUCTION

Solanum americanum of family *Solanaceae* plants are known to contain innumerable biological active compounds[1]. Plants may offer a new source of antimicrobial agents for use and they produce great deal of secondary metabolites, many of them with antifungal activities. Angiosperms are reported to have a reservoir of effective therapeutants and constitute inexhaustible sources harmless protect ants[2]. In recent years, a number of studies have been reported; dealing with antimicrobial screening of extracts of medicinal plants [3-6.] One of the plants known for having many medicinal uses in traditional system of medicine is *Solanum americanum* an important medicinal plant widely used as an antiseptic and is given internally for cardalgia and gripe. An infusion of the plant is used as an enigma in infants having abdominal upsets. Freshly prepared extract of the plant is effective in the treatment of cirrhosis of the liver and also serves as an antidote of opium poisoning. In China, the leaves of this plant are applied to wounds and sores. The juice of fresh is reported to produce dilation of the pupil[7]. The plants, especially the leaves and green fruit, are poisonous and contain the glycoalkaloid solanine as well as the tropane alkaloids scopolamine (hyoscine) and hyoscyamine (an isomer of atropine).The present study was done to determine the antimicrobial activity of the *Solanum americanum*.

MATERIALS AND METHODS

Plant material

The plant material *(Solanum americanum)* powder was used for the study, were collected from University campus, Kakatiya University, Warangal, Andhra Pradesh. The plant material was air dried at room temperature for 10 to 15 days and ground into uniform powder using a milling machine and stored in airtight container for further use.

Preparation of extraction

Dried plant materials (100gr) were extracted with 500 ml of petroleum ether, ethyl acetate, methanol and chloroform in a soxhlet apparatus. Aqueous extract was prepared by hot maceration. The extraction process was completed 72 cycles of 8 hours per day from 9 days. When the solvent was driend colourless, the extract was stopped. The solvent was completely removed by using rotary flash evaporator or water bath to obtain semi solid mass except water extract which was obtained as dried powder. These extracts were resuspended in petroleum ether, ethyl acetate methanol and chloroform to yield 100 mg residue /100 ml solvent

Table 1: Data showing antimicrobial activity of different extracts and standard drug by Agar well-diffusion method. (*Solanum americanum*)

	Zone of inhibition (in mm) SD					Zone of inhibition (in mm) Standard drugs/control	
Test Microorganism	Petroleum ether	Ethyl acetate	Methanol	Chloroform	Aqueous	Gentamycin	Amphotericin B
Bacillus subtilis **ATCC-6633**	Nil	Nil	Nil	Nil	Nil	15.33	Nil
Escherichia coli **ATCC-2343**	56 ± 0.6	6.0 ± 0.8	7.5 ± 0.5	6.5 ± 0.4	Nil	17.33	Nil
Pseudomonas aerugenosa **MTCC-1034**	6.7 ± 0.3	5.6 ± 0.9	7.2 ± 0..3	4.8 ± 0.4	Nil	14.67	Nil
Staphylococcus aureus **ATCC-29737**	5.9 ± 0.4	6.5 ± 0.8	8.2 ± 0.2	6.8 ± 0.3	Nil	16.33	Nil
Aspergillus niger **KUCCC-A-11**	6.0 ± 0.2	5.7 ± 0.8	7.8 ± 0.4	6.2 ± 0.3	Nil	Nil	16.33
Candida albicans **KUCCC-C-8**	7.3 ± 0.5	5.3 ± 0.6	8.7 ± 0.6	5.8 ± 0.7	Nil	Nil	16.00

All the values of inhibitory activity for the agents and extracts tested are significant at 0.05 levels except for the aqueous extract on one hand for *Bacillus subtilis*

Microorganisms, media and standard drugs

Four bacterial (two Gram positive and two Gram negative) strains and two fungal species were obtained from Microbiology Department, Kakatiya University, Warangal. *Bacillus subtilis* (ATCC 11774), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538), *Aspergillus niger* (KUCC-A-11), and *Candida albicans* (KUCC-C-8). Gentamycin was used as standard drug for antibacterial while Amphotericin B for antifungal activity

Experimental Procedure

Well diffusion method or pore method (zone of inhibition)

The plant materials extracts were tested for antimicrobial activity by the well diffusion method[8]. This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of Petri dish to an extract such that growth of the added microorganism is prevented entirely in circular area or zone around the cavity containing the extracts[9]. Using micropipette 0.5 ml of each of the seeded broth containing 10-5-10-6 Efu/ml test organisms were incubated on the four plates of solidified agar and spreaded uniformly with a glass spreader. Then four well were cut out in the agar layer of each plate with an aluminum bore of 5 mm diameter to contain 0.5 ml extract, standard drug and DMSO and Methanol. All the work was carried out in freeze for one day. After addition

to allow diffusion of the solution in to the medium and then incubated for 37^{o}C for 24 hours for antibacterial and 48 hours for antifungal activity. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured which has been shown in Table-1. Antifungal study was carried out through same procedure as used in antibacterial study only different was media used for antifungal study was Sabouraud-Dextrose Agar media (SDA media) instead of Nutrient agar medium which was used for antibacterial study. Gentamycin was used as standard drug for antibacterial while Amphotericin B for antifungal activity.

Statistical Analysis

All data were expressed as the mean ± SE and where applicable, the data were analyzed statically by student's t-test using and the level of significance was from P<0.05

RESULTS AND DISCUSSION

The antimicrobial activity of plant extract was observed using the well diffusion method by measuring the diameter of the growth inhibition zone. The results were shown in the Table.1. Four bacterial species and two fungal strains were used for study there are *Bacillus subtilis Escherichia coli, Pseudomonas aerugenosa*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albican*. *In vitro* antimicrobial study indicated that petroleum ether, ethyl acetate methanol and chloroform extracts showed moderate activity while aqueous extract showed no antibacterial activity against *Bacillus subtilis*.

Similar finding and conclusion were drowned by[10], [11] in their experiment which represent a very good mechanism of biological control of microorganisms. In addition the effectiveness of plant was not the due to one main active constituent, but to the combined action of other chemical compounds involved in it[12]. Some examples include alkaloids, flavonoids triterpenoids, saponins thymol, and other compounds of phenolic nature which are classifieds antimicrobial compounds[13]. The present study showed the efficacy of antimicrobial activity exclusively for bacterial pathogens which really shows the presence of biological principle of the plant extract responsible for antimicrobial activity. The bacterial cultures (two positive and two negative) used in the present study were obtained from ATCC, Chandigarh, India where as the two fungal strains tested were procured from culture collection of Department of Microbiology, Kakatiya University Warangal. The culture accession numbers of bacteria and fungi tested are provided in Table1.

CONCLUSIONS

The methanolic plant material extract of *Solanum americanum* has shown the maximum anti bacterial activity regardless of the solvent system. It also showed maximum inhibitory activity against the fungal strains except *Aspergillus niger* against which the ethyl acetate extracts of had shown highest activity. The antimicrobial activity exhibited by various extracts of *Solanum americanum* plant material was however less then the standard drugs used.

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