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ANTIBACTERIAL ACTIVITY OF LEAVES OF *SOLANUM VERBASCIFOLIUM LINN.*

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ABSTRACT

On the planet there are numerous medicinal plant having antibacterial activity. So, *Solanum verbascifolium* is one of them. It is an important medicinal plant reputed for its dilatory as well as therapeutic uses. The aim of present study is to do, an *in vitro* evaluation of antibacterial activity of extracts of leaves of *Solanum verbascifolium* plant, on various microorganisms having different strain, with the help of agar well diffusion method. The results obtained by using extract of leaves having different concentration against different test bacteria, showed that the leaves of *Solanum verbascifolium* possess an antibacterial activity against above mentioned microorganism.

INTRODUCTION

General introduction

Infectious diseases are the leading cause of death world-wide. Medicinal plant has been used as exemplary source for treating human disease because they contain numerous active constituents of therapeutic value. About three quarter of world population relies on plant and plant extract for their healthcare [1].

The cost of production of synthetic drug is high as well as they produce adverse effects as compared to plant derived drug. Hence much attention has been paid recently to the biologically active compounds derived from plant used in herbal medicine. The antimicrobial drug of plant origin are not associated with any side effect and have an enormous therapeutic potential to heal many infectious diseases e.g. vincristine (antitumor drug), digitalis (a heart regulator) & ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plant[2]. Plant with possible antimicrobial activity should be tested antimicrobial activity should be tested against an appropriate microbial model to confirm the activity & ascertain the parameter associated with it. Not all bacteria are bad. There are many type of beneficial bacterial that live in our bodies & help to protect us against harmful ones. The use of plant extracts and phytochemical both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years a numbers of studies have been conducted in different countries to prove such efficiency many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in secondary metabolism of the plant. These products are known by their active substance, for example the phenolic compounds which are part of essential oils as well as in tannin.

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogen & that increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infection. (e.g. Pencillin resistant and multi resistant pneumococci) has forced scientists to the screening of several medicinal plants for the new antimicrobial compound. There has been an alarming increase in the incidence of new and re-emerging infectious diseases. The plants like Neem and Indrayana etc. are used in India by many of the common people & traditional medicinal practitioners for the treatment of various ailments including bacterial diseases. Extract of different part of these plant (fruits, flowers, leaves, stem and root) in hot water are used in the form of infusion, decoction. Plant phytochemical is non-nutritive plant chemical that have protective or disease preventive

property. There are more than thousand known phytochemical. It is well known that plant produce these chemical to protect itself but research demonstrate that they can protect human against disease. Some of the well-known phytochemicals are lycopene in tomatoes, isoflaones in soya bean and flavonoids in fruits.

So, Aim of this study is to check antibacterial activity of extract of leaves of *Solanum verbascifolium* linn. The Methanolic and Aqueous extracts are tested against clinical isolates by using 'Agar well diffusion method'.

Introduction to plant [3, 4]

Scientific Name: *Solanum verbascifolium* linn.

Synonym: *Solanum verbascum ,Solanum verbascifolium, Solanum erianthum.*

Scientific classification

Kingdom: Plantae

Order: Solanales

Family: Solanaceae

Genus: Solanum

Subgenus: Brevantherum

Section: Brevantherum

Species: S. erianthum

Botanical description

An unarmed shrub or small tree up to 4(-10) m tall with a dense indumentum of soft stellate hairs, stem up to 20 cm in diameter. Leaves simple, ovate-elliptical, (7-)10-20(-29) cm x 3.5-15 cm, margin entire or slightly wavy, base rounded to cuneate, apex acute to acuminate.

Inflorescence appearing terminal, a compound cyme; calyx campanulate, 5 mm long, lobes ovate, corolla stellate, about 1.5 cm in diameter, white, anthers oblong, about 2 mm long, opening with apical pores, ovary densely pubescent, style 4-6 mm long, glabrous. Fruit globose, 8-12 mm in diameter, pubescent, dull yellow when ripe. Seeds many, compressed, 1-2 mm in diameter. Adding to the taxonomic confusion is the fact that S. erianthum has been extensively referred to as S. verbascifolium L., which actually proved to be identical with a South American species.

Uses

Medicine: Solasodine is found in S. erianthum. The total alkaloid content of air-dried leaves and fruits is respectively 0.37% and 0.39% for S. erianthum. The solasodine content in Solanum fruits from Indian samples is 0.01-0.70% in S. erianthum. Leaf samples of S. erianthum from Vietnam contained 0.26% solasodine, 0.05% tomatidine and 0.01% solaverbascine. S. erianthum also has steroidal saponins and free genins. The aqueous leaf extract of S. erianthum did not produce any significant suppression of Plasmodium berghei infection in mice. The flavonoid-rich extract of S. erianthum possesses antibacterial and antifungal activity. Solasodine is a nitrogen analogue of diosgenin, a compound often used as raw material for the production of medicinal steroids. The synthetic steroids have three main applications in medicine: as anti-inflammatory corticosteroids, as contraceptive sex steroids and as anabolic steroids.

Need and objective of the study

Need of study

Since ancient time, mankind has been facing many problem related to its health. Many disease & ailment are curable by using synthetics & herbal medicines but still there are certain diseases which do not have proper treatment of their cure. Amongst these diseases most of diseases are occurring due to several infection bacteria. The diseases are like typhoid, tuberculosis & cholera etc. occurring due to some harmful bacteria some of them are mentioned as follows:

1. *Salmonella typhi*-typhoid.
2. *Mycobacterium tuberculosis*-Tuberculosis (T. B.).
3. *Escherichia coli*-Urinary tract infection, traveller's diarrhoea.
4. *Candida albicans*-oral infection.
5. *Entamoeba histolytica*-Amoebiasis.

As mentioned previously problem of microbial resistance is growing world-wide therefore action must be taken to reduce this problem either by controlling the use of antibiotics or by developing the research to better understand the genetic mechanism of resistance and by developing new drug either synthetic or natural. The ultimate goal should to offer appropriate & efficient antimicrobial drug to the patient.

Objectives:-

1. To extract the active phyto constituent from leaves of *Solanum verbascifolium* linn. in aqueous methanol solvent by maceration method.
2. To explore the possibility of using traditional medicine with proper chemical, pharmacological profiles.
3. To evaluate sensitivity of vegetative bacterial species by in vitro evaluation method against selected plant part which is considered to passes antibacterial activity.
4. To conduct the systematic phytochemical investigation of leaves of solaneum verbascifolium linn perform detail study of antibacterial activity of leaves of solaneum verbascifolium linn using different parameters.
5. To perform detail study of antibacterial activity of leaves of solaneum verbascifolium linn.

MATERIALS AND METHODS

These sections include two parts.

Collection of plant material

The leaves of *Solanum verbascifolium* linn. were collected from nearby area of Satara, Maharashtra, during the month of September and authenticated at Botany department, Y. C. Institute of Science, Satara.

Extraction of plant material:-

The leaves of *Solanum verbascifolium* linn. was extracted by using distilled water & methanol by maceration method.

A. Preparation of aqueous extracts:

About 500 gm. of leaves of *Solanum verbascifolium* linn. was extracted with 500ml of distilled water by using maceration. Then extract was filtered. Aqueous solvent was allowed to evaporate by heating. The residue was obtained as aqueous crude extract.

B. Preparation of methanol extracts:

About 500gm of leaves of *Solanum verbascifolium* linn was extracted with 500ml of methanol by using maceration. Then extracted was filtered. Methanol solvent was allowed to evaporate by heating. The residue was obtained as methanol crude extract.

Micro-organism used in study:-

Both Gram positive & Gram negative bacterial strains are used.

A. Gram positive strain-

Bacillus subtillis, *Staphylococcus aureus*.

B. Gram negative strain-

Klebsiella pneumoniae, *Salmonella typhi*, *Escherichia coli.*

Determination of antibacterial activity:-

The evaluation of antibacterial activity can be done by following method

1. Cup plate method/ agar well diffusion method
2. Disc plate method
3. Filter paper method

From the above method the cup plate method is most suitable method. The antibacterial activity of all the extract was accurately determined by the cup & plate method.

Preparation of agar medium

The composition of agar medium used to prepare agar plates is as follows

Table no. 1 composition of nutrient agar medium

Sr.no.	Name of Component	Quantity
1	Peptone	2.5 gm
2	Beef extract	0.8 gm
3	Sodium chloride	1.5 gm
4	Agar	7.5 gm
5	Distilled water	250 ml

Procedure

1. All ingredients were weighed as per given proportion in required quantity.
2. All ingredients were then dissolved except agar- agar by warming and constant stirring.
3. After that PH of medium was adjusted to 6.8-7 by adding dilute acid or dilute alkali and then added agar-agar powder and dissolved.
4. The conical flask was plugged and subjected for sterilization process at 15 psi pressure 121˚c temperature for 30 min.

Preparation of agar plate

After the preparation of medium agar plates were prepared. About 20 ml of medium was poured in each petri plate for one bacterial suspension two plates were prepared About five bacterial suspension used and ten plates were prepared. After the pouring of medium in

plates, the plates were kept at room temperature for cooling and solidification.

Preparation of dilutions

Dilutions were prepared in sterilized distilled water the concentrations of dilution were 200mg/ml, 400mg/ml, 600mg/ml, 800mg/ml and 1gm/ml the dilution for both aqueous and methanol extract was prepared under aseptic condition.

Preparation of saline

100ml of saline was prepared by dissolving 0.85gm sodium chloride in 100ml of distilled water & then sterilized by autoclaving. Then different strains of micro-organism were inoculated into saline for the preparation of bacterial suspensions, so as to prepare standard suspensions.

Spreading of bacterial suspension

For determination of antibacterial activity gram positive and gram negative organism were used. With the help of glass spreader the suspension was spread on media plate uniformly under aseptic condition.

Preparation of cups

With the help of sterile cork borer of 8mm diameter three cups were prepared in each petri plate. For one bacterial species two plates were prepared and the cups are labelled accordingly as,

1. 200 mg/ml
2. 400 mg/ml
3. 600 mg/ml
4. 800mg/ml
5. 1000 mg/ml
6. Std. Antibiotic(ciprofloxacin 600mg/ml)

Addition of solution in cups:-

0.1ml of different dilutions of extract was added in the cups and plates were kept for diffusion in refrigerator for 1-2 hrs.& finally plates were kept for incubation in incubator at 37 ^{0}C for 24 hrs. After 24 hrs, the diameter of zone of inhibition was measured in mm.

RESULT AND DISCUSSION

When the respective extract of leaves of *Solanum verbascifolium* linn. were obtained & subjected for antibacterial activity by using nutrient agar at optimum time & temperatures, then following observation were obtained. Different concentration 200mg/ml, 400mg/ml, 600mg/ml, 800mg/ml, 1000mg/ml has shown zone of inhibition in following manner.

Table no .2 Antibacterial activity of leaves of *Solanum verbascifolium* linn. Aqueous Extract

Sr. No.	Bacterial species	Concentration(mg/ml)	Zone of inhibition (mm)
1.	*Bacillus subtillis*	200	13
		400	-
		600	-
		800	14
		1000	16
		600 Std (ciprofloxacin)	38
2.	*Salmonella typhi*	200	-
		400	-
		600	-
		8000	-
		1000	-
		600 Std (ciprofloxacin)	44
3.	*Escherichia coli*	200	-
		400	-
		600	-
		800	-
		1000	-
		600 Std (ciprofloxacin)	39
4.	*Klebsiella pneumonae*	200	-
		400	-
		600	-
		800	-
		1000	-
		600 Std (ciprofloxacin)	34
5.	*Candida albicans*	200	-
		400	-
		600	-
		800	-
		1000	-
		600 Std (ciprofloxacin)	-

Methanol Extract

Sr.No.	Bacterial species	Concentration(mg/ml)	Zone of inhibition (mm)
1.	*Bacillus subtillis*	200	11
		400	13
		600	15
		800	-
		1000	-
		600 Std (ciprofloxacin)	16
2.	*Salmonella typhi*	200	-
		400	-
		600	-
		800	-
		1000	-
		600 Std (ciprofloxacin)	46
3.	*Escherichia coli*	200	-
		400	-
		600	-
		800	16
		1000	22
		600 Std (ciprofloxacin)	42
4.	*Klebsiella pneumonae*	200	16
		400	18
		600	17
		8000	-
		1000	-
		600 Std (ciprofloxacin)	31
5.	*Candida albicans*	200	-
		400	-
		600	-
		8000	-
		1000	-
		600 Std (ciprofloxacin)	-

From above obtained result it was found that aqueous extracts of leaves of *Solanum verbascifolium* linn. exhibited antibacterial activity against gram positive bacteria i.e. *Bacillus subtillis*. It was found that methanol extract of leaves of *Solanum verbascifolium* linn. exhibited antibacterial activity against both gram positive bacteria *Bacillus subtillis* & gram negative bacteria i.e. *Eschrchia coli* & *Klebsiella pneumoniae.*

The ciprofloxacin was used as standard drug which exhibited wider zone of inhibition than that of both aqueous & methanol extract.

Fig. No.2- Antibacterial activity shown by aqueous extract of linn.

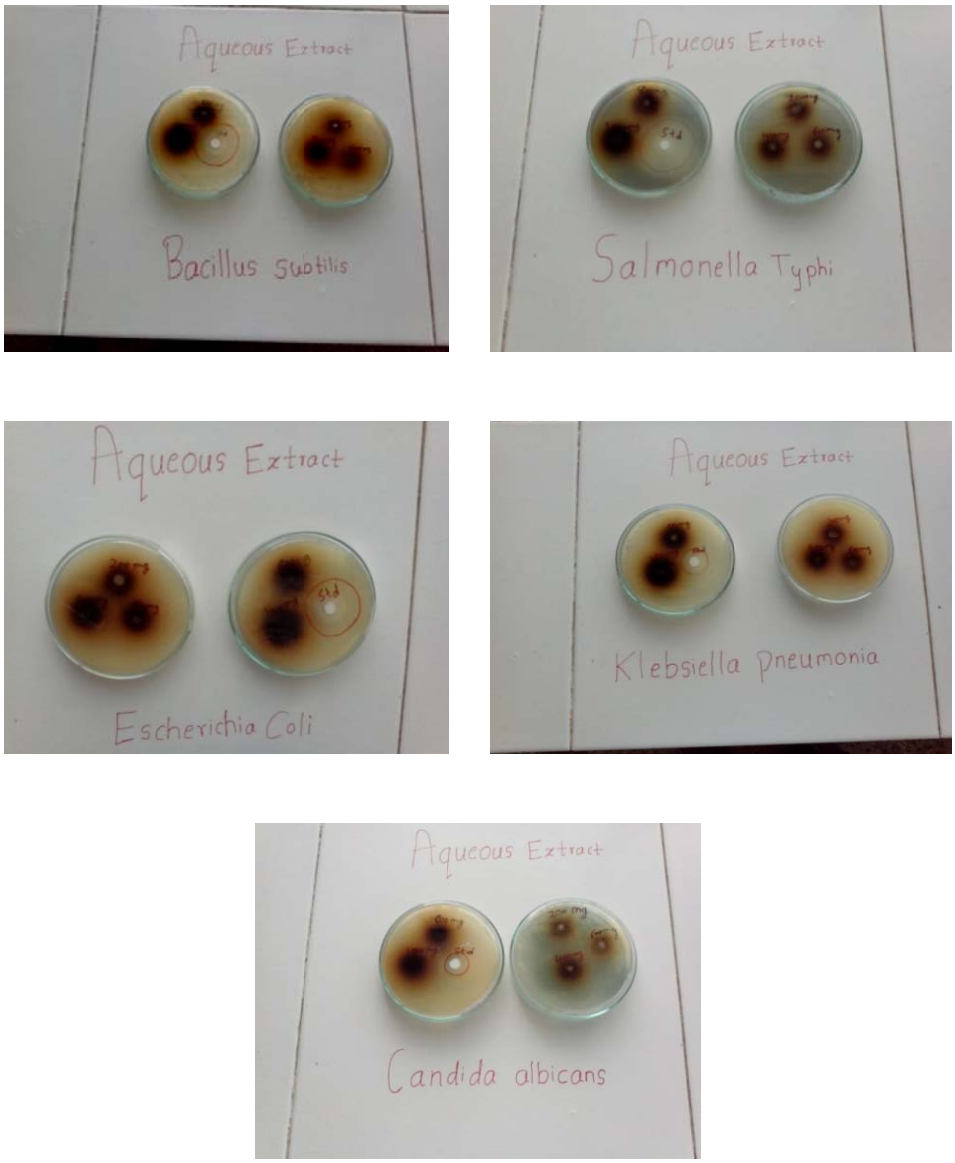
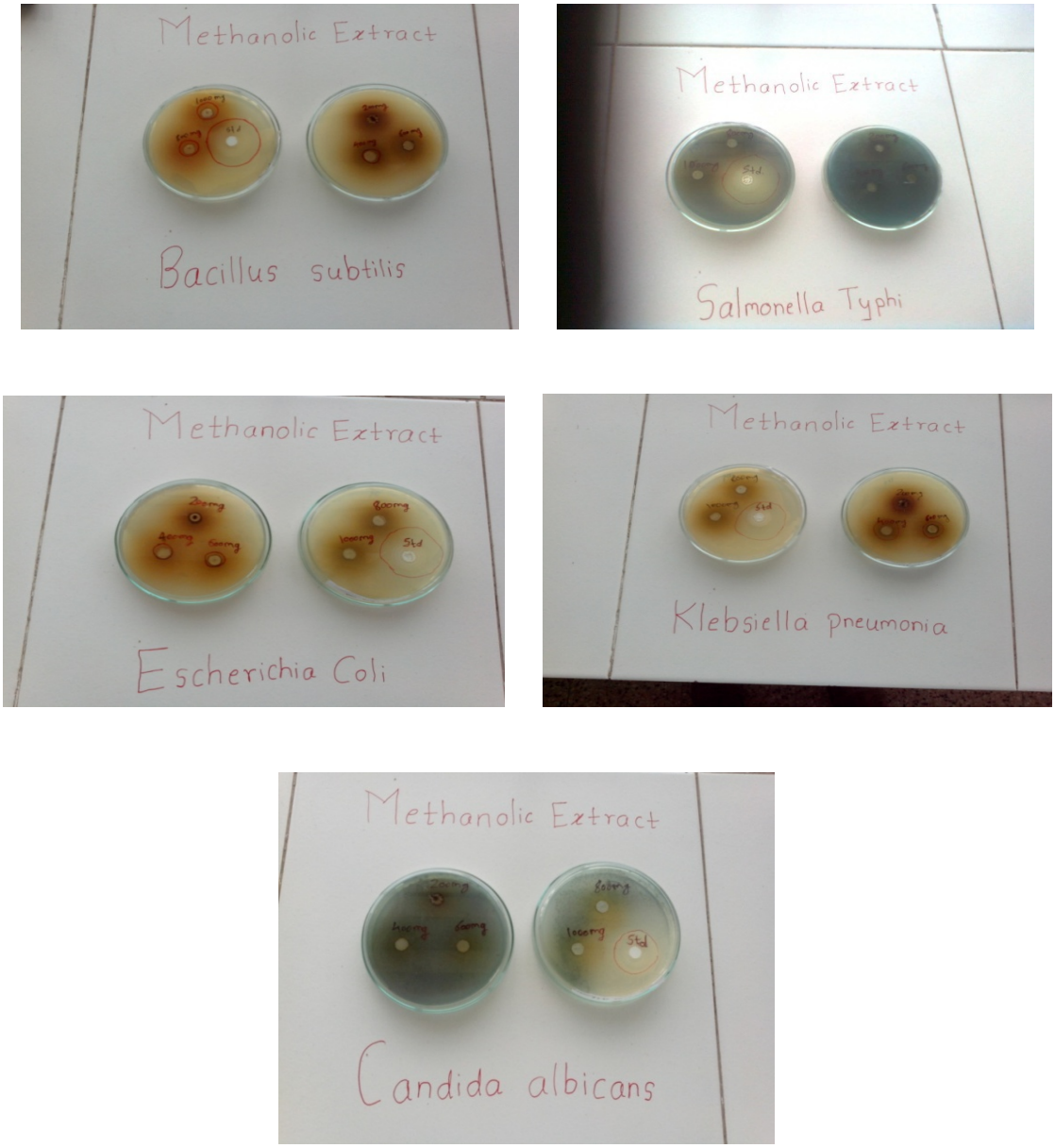


Fig. No.3 Antibacterial activity shown by methanol extract of *Solanum verbascifolium* linn .



CONCLUSION

The result of this study strongly suggests that extract of leaves of *Solanum verbascifolium* linn. possess antibacterial activity. It also can be confirmed that leaves of *Solanum verbascifolium* linn. can be used in case of infection of test bacterial strain. As we know thin layer chromatography provide the good evidence for the presence of different phytochemical in exatract. So, it further needs the investigation to determine phytochemically active compound form the leaves of *Solanum verbascifolium* linn.

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