

Full Length Research Paper

Preliminary Photochemical Screening and Antimicrobial activities of Plant extract of *Solanum indicum* Linn.

Tanu Mendiratta, Anshulika Upadhyaya, Mahendra Singh, and Kamal K Pande*

Department of Biotechnology & Allied Science, M.B. Government. Post Graduate College, Haldwani-263139- Uttarakhand), India.

*Department of Chemistry and Department of Biotechnology & Allied Science, M.B. Government, Post Graduate College, Haldwani-263139- Uttarakhand), India.

Article history

Received: 11-12-2015

Revised: 25-12-2015

Accepted: 01-01-2016

Corresponding Author:

Anshulika Upadhyaya,
Department of
Biotechnology &
Allied Science, M.B.
Government. Post
Graduate College,
Haldwani-263139-
Uttarakhand), India.

Abstract

The present study was carried out for phytochemical screening of principle bioactive compounds and antimicrobial activity in *Solanum indicum* linn. Phytochemical analysis revealed the presence of saponin, terpenoid, steroid, saponin, flavonoid, tannin and alkaloid. The petroleum, ether, chloroform, methanol, acetone and aqueous extracts were subjected to antimicrobial activity against bacterial strains *Staphylococcus aureus*, *Pseudomonas*, *E.coli* and *Bacillus subtilis* against anti fungal strains *A.awamori*, *A. fumigatus*, *Rhizopus oryzae*, *Trichoderma viridae* and *C.oryzae*. The antibacterial and antifungal activity was evaluated by disc-diffusion method.

Keywords : *Solanum indicum*, Antifungal activity, Antibacterial activity, Disc-diffusion method.

Introduction

Medicinal plants are the ones whose parts like leaves, seeds, stem, roots, fruits and foliage are used in the preparation of varied extracts, infusions, decoctions and powders in different compositions. The use of different parts of several medicinal parts to cure specific ailments has been in vogue from ancient times (Alavijeh PK, Sharma D, 2012, Zemali D, Ouahrani MR. 2014). These are used in the treatment of different diseases of humans, plants and animals with less side effects. In the last few years plants have been used as antimicrobial agents because of their antimicrobial traits. This property is due to the bioactive compounds synthesized during secondary metabolism in plants (Ahmed A, Tayela E, Wael F et al. 2013). The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic use which is safe and efficient in nature. (Huang W.H. Hsu C.W. & Fang J.T., 2008)

Medicinal plants possess potent medicinal owing to the presence of a variety of phytochemical constituents in the plant tissues, which casts a definite physiological action on the human body. There are reports on plant usage in traditional healing by either tribal people or indigenous community (Sandhy et al., 2006; Ayyanar and Ignacimuthu, 2005; Rajan et al., 2002; Natarajan et al., 1999; Ignacimuthu et al., 1998). The activities of medicinal plants have been selected because of their great medicinal relevance in recent years. Infections have increased to a great extent and resistant against antibiotics makes it an ever increasing therapeutic problem (Austin et al., 1999). Therefore, natural products of higher plants may give a new source of antimicrobial agents. (Samy et al., 1998; Hamil et al., 2003; Motsei et al., 2003).

The plant *Solanum indicum* is a branched perennial shrub gaining upto 1.8m height. It is found mostly throughout warmer parts of India, Africa and Asia, near an altitude of 1500m above sea level. The leaves are 7.5- 15cm long, 2.5-10cm broad, alternate, lobed, entire, spines present on petiole and midrib. *Solanum indicum* Linn. (Solanaceae) is a thorny shrub widely distributed in Assam, Uttarakhand, Karnataka, Himachal Pradesh and Tamilnadu. This plant is commonly known as 'Indian night shade' and 'Brihati', respectively (Chopra et al., 1992).

The flavanoid rich extract of *Solanum indicum* leaves possesses antibacterial and antifungal activity. It is effective against Gram positive bacteria and fungi such as *Aspergillus flavus* and some *Candida* species. Leaves are considered to be laxative and diuretic, and are used in treating leprosy, sexually-transmitted diseases and malaria (Adam G, et, al. 1979). Leaves possess expectorant property and are used to treat hemorrhoids, scrofula and leucorrhoea. Heated leaves are applied to the forehead as an

analgesic for headaches while the leaf decoction is used for vertigo. An extract of root is used to treat dysentery, fever, diarrhea, digestive problems and body pains (Huang ST, Su YJ, Chien DK et al. 2009). Its root bark is used as an anti inflammatory and used in the treatment of arthritis. The berries of *S. indicum* (Solanaceae) have been reported in the ancient Indian medicinal literature with beneficial effects in inflammation, tuberculosis, diuretics etc. Fruits are said to be poisonous due to the presence of glycol alkaloids. Steroidal alkaloids from *Solanum indicum* are useful in industries as steroidal precursors. Solasodine present in fruit is useful for the production of medicinal steroids like anti inflammatory corticosteroids, contraceptive steroids and anabolic steroids (Blomqvist MM, and Nguyentien B. 1999., Adam G, et, al. 1979). Literatures reveal the presence of phytochemicals like Tannins, saponins, alkaloids, flavonoids, steroid and terpenoids found in the extracts of *S. xanthocarpum*, *S. surattense*, *S. nigrum* and *S. trilobatum* (Udayakumar R, Velmurugan K, Raghuan K. 2004). The antibacterial property of *S. torvum* against *B. subtilis* and *P. aeruginosa* is well established (Wiar C, Mogana S, Khalifah S et al., 2004). Similar results were also reported in *S. surattense*, *S. xanthocarpum* and *S. trilobatum* on *P. aeruginosa*, *B. subtilis* and *S. typhi* (Sheeba E. 2010., Doss AH, Mohammed M, Dhanabalan R. 2009). The present study was aimed to evaluate antimicrobial activity of plant parts like leaf, fruit (berries) and stem extracts of *Solanum indicum* against some bacterial and fungal strains.

Materials and Methods

Plant Material

The medicinal plants *Solanum indicum* Linn were collected around Haldwani, District Nainital, Uttarakhand, India. The place has an average elevation of 424 meters and is located at 29.22 N and 79.52 E.

Extraction of plant material

Collected fresh field-grown plants were washed with running tap water followed by distilled water and thoroughly dipped in 70% ethanol for disinfecting and removing the adhered dust particles. After blotting, the sample was air dried in shade, ground to fine powder and stored in clean air tight containers. The powdered mixture was then soaked in different solvents ethanol, methanol, acetone, petroleum ether and chloroform for 72 hrs. After filtering the contents using Whatman No 1 filter paper, the filtrate was left at room temperature for 48 hrs to evaporate partially. Greenish brown, dark green, light green, yellowish green and greenish black residues were obtained. All the extracts were dried in vacuum rotary evaporator at 40 °C under reduced pressure, weighed and stored at 4 °C.

Phytochemical studies

The fractions obtained were subjected to various qualitative tests for the identification of constituents like flavonoids, alkaloids, saponins, steroid, terpenes, glycosides coumarin , reducing sugar, phenol and carotenoids.

Test for flavonoids

A few drops of 1 % NH₃ solution was added to 1 ml of the antimicrobial fraction in a test tube. The appearance of yellow coloration shows the presence of flavonoids compound (Andzouana and Mombouli, 2011).

Test for reducing sugars

Take a test tube and add 2 ml of crude plant extract and add 5 ml of distill water and filter. The filtrate was boiled with 3-4 drops of felhings solution A and B for 2 minutes. Observe for orange red precipitate which indicates the presence of reducing sugars.

Test for Steroids

To the plant extract add 2 ml of acetic anhydride and add 0.5 gm of ethanolic extract of each sample with 2 ml of sulphuric acid .Observe for the color change from violet to blue or green in samples indicating the presence of steroids.

Test for alkaloids

A quantity of 2 ml of Drangendroff's reagent was added to 1 ml of the antimicrobial fraction. The appearance of a turbid orange color shows the presence of alkaloids (Veerachari and Bopaiah, 2011).

Test for carotenoids

A quantity of 1 ml of the antimicrobial fraction was put in a test tube and dried under Fume Hood. A quantity of 10 ml of chloroform was added to the residue obtained and shaken vigorously. The resulting mixture was filtered and 85% sulphuric acid was added. The appearance of a blue color at the interface shows the presence of carotenoids (Ajayi et al., 2011).

Test for saponins

A quantity of 10 ml of the antimicrobial fraction was shaken vigorously, sat aside for 10 min. The appearance of a stable froth shows the presence of saponins (Veerachari and Bopaiah, 2011).

Test for Phenol Compound

Ferric chloride test: The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenol compounds.

Lead acetate test: The extract (50 mg) was dissolved in 5 mL of distil led water. To this, 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compound.

Test for glycosides

To 1 ml of the antimicrobial fraction, 1 ml of FeCl₃ reagent (mixture of 1 volume of 5% FeCl₃ solution + 99 volume of glacial acetic acid) and a few drops of concentrated H₂SO₄ were added. The appearance of a greenish blue color within few minutes shows the presence of glycosides (Trease and Evans, 1989).

Antimicrobial activity

Antibacterial assay was carried out on *Bacillus subtilis* (gram positive), *Staphylococcus aureus* (gram positive), *Pseudomonas* (gram negative), *E.coli* (gram negative) were procured from American Type Culture Collection (ATCC), and Microbial Type Culture Collection (MTCC) Institutes. All the organisms were sub cultured and maintained on nutrient media at 37 °C.

Antifungal assay was conducted on *Aspergillus fumigatus*, *Rhizopus oryzae*, *Culbularia oryzae*, *Trichoderma virid*, *Aspergillus awamori* were procured from American Type culture collection (ATCC), and Microbial Type Culture Collection (MTCC) Institutes. Fungal cultures were maintained on Sabouraud dextrose agar at 30 °C.

Determination of Antibacterial and Antifungal Activity

The antibacterial and antifungal activity of all the solvent extracts of *S.indicum* Linn (stems leaves and fruit) was evaluated by disc-diffusion method. When media is solidified or set, the disc (6 mm) of whatman no 1 filter paper was soaked in crude solvent viz. methanol, ethanol, chloroform, petroleum ether, and acetone and placed carefully in the centre of Petri plates containing the solidified media. To compare the antimicrobial activity same concentration of the solvent using disc is placed in plate which acts as control to our crude solvents. Same procedure is applied for the remaining Petri plates (for different solvents). The plates were incubated at 37 °C for 24 hrs for bacterial culture and at 28 °C for 48 hrs for fungal culture. The plates were observed for inhibition of bacterial growth that was indicated by the clear zone around the well. The size of zone of inhibition (including well) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity. All experiments were carried out in triplicates.

Results and Discussion*Phytochemical analysis*

The preliminary photochemical test revealed that crude, leaf extracts of *S.indicum* contains all the photochemical constituents like reducing sugar, saponins, steroids, coumarin, and phenol. The stem extract contains steroids and coumarin while reducing sugar, saponin and phenol were not found in stem extract. The fruit extract contains reducing sugar, coumarin, phenol and saponins whereas, steroids were not found in fruit extract.

Table 1. Preliminary photochemical test

Phytochemical constituent	Fruit	Leaf	Stem
Steroids	–	+	+
Reducing sugar	+	+	–
Phenol	+	+	–
Coumarin	+	+	+
Saponins	+	+	–

Antibacterial and Antifungal Activity:

The results of the antimicrobial activity tests of crude extracts are shown in table 2 and fig. 1. It was found that ten crude extracts of *Solanum indicum* Linn at 100mg/mL concentration exhibited various antibacterial and antifungal activity. The result revealed that crude extracts of stem and fruit of *S.indicum* Linn were less potent than crude extracts of its leaves as they inhibited the growth of only two bacterial strains viz. stem extract inhibited the growth of *Bacillus* and *S.aureus*. However, fruit extract inhibited the growth of *E.coli* and *S.aureus*. In case of fungal strains it is concluded that fruit, stem and leaf crude extract showed activity against only two fungal strains viz. *A.fumigatus* and *R.oryzae* while none of the extract showed activity against three fungal strains i.e. *A.awamori*, *Trichoderma viridae* and *C.oryzae*. Plants belonging to the *Solanum* genus have been reported to have remarkable pharmacological activity (Thambiraj J, Paulsamy S. 2011). Wiart *et al* reported the antimicrobial activity of *S.torvum* against *B. subtilis* and *P. aeruginosa* (Wiart C, Mogana S, Khalifah S et al., 2004). Similar results were also reported in *S. surattense*, *S. xanthocarpum* and *S. trilobatum* on *P. aeruginosa*, *B.subtilis* and *S. typhi* (Doss AH, Mohammed M, Dhanabalan R. 2009).

Screening of antibacterial activity of leaf showed that it is potent antibacterial agent against *S.aureus*, *B.subtilis*, *Pseudomonas* and *E.coli*. Chloroform extract showed maximum activity while crude extract in Acetone showed no activity against all the bacterial strains. Crude extracts of stem showed that it is potent antibacterial agent against *S.aureus* and *B.subtilis*. Maximum activity was shown by ethanol crude extract against *S.aureus* and *B.subtilis* respectively. However, none of the crude extract of stem was able to inhibit the growth of *E.coli* and *Pseudomonas*. Fruit (berry) crude extracts showed that it is potent antibacterial agent against *S.aureus* and *E.coli*. Maximum activity was shown by petroleum ether against both the bacterial strains. Ethanolic and chloroform extract of fruit was not able to inhibit the growth of any of the bacterial strain.

Study of antifungal activity of leaf showed that maximum activity was found against *A.fumigatus* and *Rhizopus.oryzae*. Further investigation revealed that ethanolic crude extract was unable to inhibit the fungal growth. Maximum activity was shown by chloroform crude extract followed by petroleum ether extract. Screening of antifungal activity of stem showed that crude extracts of stem were able to inhibit growth of two fungal strains viz. *A.fumigatus* and *Rhizopus.oryzae*. Ethanolic crude extract showed significant zone of inhibition against *A.fumigatus*. Chloroform crude extract showed maximum activity against *R.oryzae*. The crude extracts of fruit were found effective against only two fungal strains i.e *A.fumigatus* and *R.oryzae*. The leaf, stem and fruit revealed that none of the crude extracts showed activity against the three fungal strains viz. *A.awamori*, *Trichoderma.viridae* and *C.oryzae*.

Table 2. Antimicrobial activity tests of crude extracts against Pathogenic Microorganisms) CH- Chloroform ,MT- Methanol, ET- Ethanol, PE- Petroleum ether, AC-Acetone

Pathogenic Microorganisms	Zone of inhibition in mm									
	Leaf Extracts					Fruit Extracts			Stem Extracts	
	CH	MT	ET	PE	AC	CH	MT	PE	CH	ET
Gram positive Bacteria										
<i>B.subtilis</i>	0	0	0	0	0	0	0	0	0	0
<i>S.aureus</i>	9	8	9	12	0	0	0	8	0	9
Gram negative Bacteria										
<i>Pseudomonas</i>	14	0	13	0	0	0	0	0	0	0
<i>E.coli</i>	13	9	0	13	0	0	0	19	0	0
Fungi										
<i>A.fumigatus</i>	9	0	0	8	0	0	0	10	0	10
<i>R.oryzae</i>	10	10	0	0	10	0	12	0	12	0
<i>A.awamori</i>	0	0	0	0	0	0	0	0	0	0
<i>Trichoderma viride</i>	0	0	0	0	0	0	0	0	0	0
<i>C.oryzae</i>	0	0	0	0	0	0	0	0	0	0

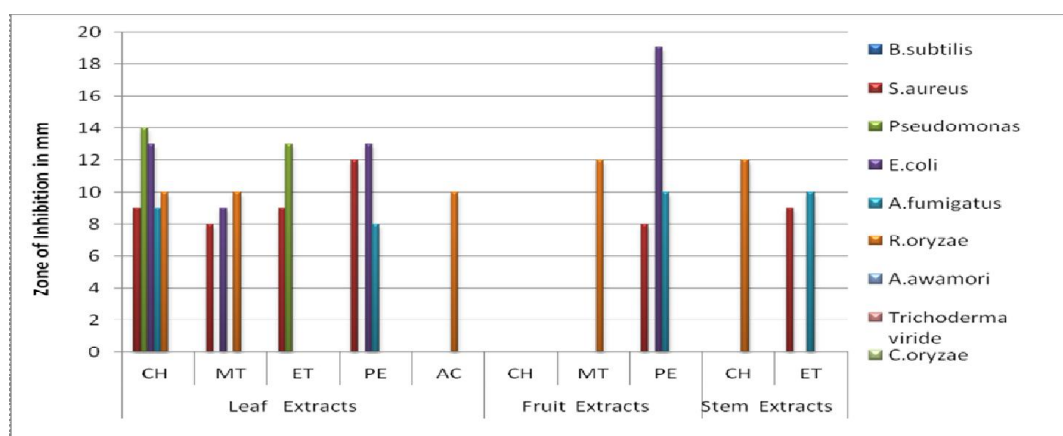


Fig. 1. Antimicrobial activity tests of crude extracts against Pathogenic Microorganisms.

Conclusion

The plant *S.indicum* Linn exhibited the presence of many secondary metabolites and revealed broad antimicrobial activity on the tested microorganisms. This investigation strongly suggests the possibility of this plant as an important source of antimicrobial drug development.

References

- Adam G, Huong HT, Khoi NH. (1979).The constituents of the Vietnamese drug plant *Solanum verbascifolium* L. *Planta Med* 36: 238–239.
- Alavijeh, P. K., Sharma D. A study of antimicrobial activity of few medicinal herbs, *Asian Journal of Plant Science and Research*, 2012; (4):496-502
- Andzouana M, Mombouli, J. B. Chemical composition and photochemical screening of the leaves of *Hymenocardiaulmoides* and *Vitexferruginea*. *Pak. J. Nutr.*, 2011; 10(12):1183-1189.
- Ajayi, I. A, Ajibade O, Oderinde R. A., Preliminary Photochemical Analysis of some Plant Seeds.*Res. J. Chem. Science*, 2011: 1(3).
- Ahmed A, Tayela E., Wael, F et al. Production of anticandidal cotton textiles treated with oak gall extract. *RevArg Mic.*, 2013; 45: 271-276

- Awoyemi, O. K, Ewa, E.E., Abdulkarim, I. A., Aduloju, A.R., et al. Ethnobotanical assessment of herbal plants in southwestern Nigeria. *Academic research international*, 2012; 2:50-57
- Ayyanar, M and Iganacimuthu, S.. Traditional knowledge of kani tribals in Kouthalai of Tirunelveli hills. Tamil Nadu, India. *Journal of Enthopharmacology*, 2005; 102: 246-255.
- Austin, D.J., Kristinsson, K.G., Anderson, R.M.. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proc Natl Acad Sci USA*, 1996; 96:1152-6.
- Blomqvist MM, Nguyen B. (1999). *Plant Resources of South-East Asia*, Backhuys Publishers. Leiden, Netherlands; pp 453-460.
- Chopra, R.N., Nayer SL, Chopra I.C., *Glossary of Indian Medicinal Plants*, New Delhi, 1992, PID, CSIR.
- Doss, A. H., Mohammed M., Dhanabalan R. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. *Ind J of Sci and Tec.*, 2009 2: 41-43.
- Huang, W.H., Hsu, C.W., and Fang, J. T., (2008) Central diabetes insipidus following digestion *Solanum indicum* L. concentrated solution. *Clinical Toxicology*, 2008 46(4), 293 –96
- Hamil, F.A., Apio, S., Mubiru, N. K., Bukenya Ziruba, R., Mosanyo, M., Magangi., Ow, et al., Traditional herbal drugs of southern Uganda, II. Literature analysis and antimicrobial assays. *J. Ethnopharmacol*, 2003; 84:57-78.
- Ignacimuthu, S., Sankarasivaraman, K., Kesavan. *Medico-ethno botanical survey among Kanikar tribals of mundentherai sanitary fitoterapia*. 1998; 69: 409-414.
- Lee, S.J., Lim, K.T., Glycoprotein isolated from *Solanum nigrum* Linne stimulates caspase-3 activation and reduces inducible nitric oxide production in HCT-116 cells. *Toxicol. in Vitro*, 2006; 20(7): 1088-1097.
- Motsei, M.L., Lindsey, K.L., Vanstaden, J., Jaeger, A.K.. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *J Ethnopharmacol*, 2003; 86: 235-41.
- Natarajan, B., Paulsen, B.S., Pushpangadan, P. An Enthopharmacological study from the Coimbatore district, Tamil nadu, India. *Traditional knowledge compared with modern biological science, pharmaceutical biology*, 1999; 37: 378-390.
- Rajan, S., M. Sethuraman & Mukherjee. P.K. (2002). Ethnobiology of the Nilgiri Hills, India. *Phytotherapy Research* 16:98–116.
- Samy, R.P., Ignacimuthu, S., Sen, A. Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol*. 1998; 62: 173-81.
- Sandhy, B., Thomas, S., Isabel, W., Shenbagavathai, R. Ethnomedicinal plants used by the valaiyan community of Piranmalai hills (Reserved forest), Tamil Nadu, India. A pilot study, *African journal of traditional complements and Alternative medicines*, 2006; 3:101-114.
- Sheeba E. 2010. Antibacterial Activity of *Solanum Surattense* Burm. F. *Journal of Science, Engineering and Technology*. 2010; 6(1):1-4.
- Subashini R, Mahesh V, Kavitha A et al. Comparative evaluation of antimicrobial activity of selected three herbal plants extract with digital image processing technique. *Eur J of Bio*, 2013 14-26.
- Thambiraj J, Paulsamy S. Evaluation of antimicrobial efficacy of the folklore medicinal plant, *Acacia caesia*. *Asi J Pharm Clin Res* 2011; 4: 103-105.
- Trease GE, Evans, W. C. *A textbook of pharmacognosy*. 12th Edn., Bailliere, Tindall. London, 1989 pp: 388, 480, 502, 535, 546
- Udayakumar R, Velmurugan K, Raghuan K. (2004). Phytochemical and antimicrobial studies of extracts of *Solanum xanthocarpum*. *Anc Sci of Life* 24: 83-87.
- Veerachari U, Bopaiah AK (2011). Preliminary Phytochemical Analysis of some Plant Seeds. *J. Chem. Pharm. Res.*, 2011; 3(5):574-583.
- Wart C, Mogana S, Khalifah S et al. (2004). Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia*, 2004 75: 68-73.
- Zemali D, Ouahrani M R. (2014). Phytochemical study of selected medicinal plant, *Solanum nigrum*, the Algerian desert. *Int Let of Chem, Phy and Ast.*, 2014; 1: 25-30.