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Research Article

STUDIES ON ANTIMICROBIAL AND ANTICANCER ACTIVITY OF *SOLANUM TRILOBATIUM*

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ABSTRACT

The present study is aimed to focus on antimicrobial and anticancer activity plant extract *Solanum trilobatum* which was collected from Thirukovilur, Villupuram district. The cancer cell line HT- 29 obtained from King's Institute, Guindy Chennai. The plant extracts were prepared in ethanolic extracts to check the anti- microbial and anti-cancer activity against bacterial pathogens (*E. coli, Staphylococcus aureus, Pseudomonas, Klebsilla, Bacillus*) and fungal pathogens (*A. niger, A. flavus, Fusarium*) by agar well diffusion method. The leaf of *S. trilobatum* were used for antimicrobial analysis, out of the best activity was observed *E. coli* with maximum zone of inhibition. On the other hand maximum antifungal activity was recorded with *Aspergillus niger*. The phytochemical analysis of *S. trilobatum* were performed such as alkaloids, flavonoids, sugars, glycosides, spannins, tannins, proteins, aminoacids and terpenoid were analyzed. Among the phytochemical test alkaloids showed the highest activity compared to other tests. Anti-cancer activity showed the least MIC value at concentration ranging from 2.7µg/ml to 60 µg/ml. A lowest MIC value (2.4 OD at conc. of 60 µg/ml). The results clearly evident that the *S. trilobatum* extract are very useful improving survival and quality of life in patient suffering from advanced cancer.

Keywords: Anticancer; Antifungal; Phytochemical; *S. trilobatum*; Antifungal

INTRODUCTION

The herbal drugs used throughout the world have received greater attention recent times because of their diversity of curing disease, safety and well tolerated remedies when compared to the conventional medicines. Natural products have been extensively used for the last few years ago as a source for the search of antibacterial agents. This was done on the basis of fact that drugs developed from plant products are safe and reliable when compared with synthetic drugs which are toxic and costly for the general masses (Gordon and David, 2001). Nature has a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on the using traditional medicine. Obtaining adequate nutrients from various foods play a vital role in maintaining normal function of the human body. With reason advances in medicinal and nutrition sciences, natural products and health promoting foods have received extensive attention from both health professionals and the common population.

New concepts have appeared with this trend, such as nutraceuticals nutritional therapy, phytonutrients and phytotheraphy. This functional or medicinal foods and phytonutrients or phytomedicines play positive role in maintaining well being, enhancing health and modulating immune function to prevent specific disease (Vinayaka *et al.,* 2009) The history of plants being used for medicinal purpose is probably as old as the history of mankind. According to world health organization, medicinal plants are the best source to obtain a variety of newer herbal drugs about 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties safety and efficacy (Prusti *et al.*, 2008).

Infectious diseases are the leading cause of death worldwide. Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to hold and newly produced drugs in on the rise. The increasing failure of chemotherapeutics and antibiotic résistance exhibited by pathogenic microbial infections agents has led to the screening of several medicinal plants for their potential antimicrobial activity Colombo and (Basisio 1996; Scazzocchio *et al.,* 2001). The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health has been widely observed (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs, chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. The results of investigation performed in the 19 and 20th century and the advent streptomycin and other antibiotics provide the ground for experimentation of a vast of plants for antibiotic or antimicrobial activities that are useful to man (Doss *et al*., 2008).

The genus Solanum was established by Carl Linnaeus in 1753. *Solanum trilobatum* (Solanacea) is a thorny shrub widely distributed in Bengal, Utterpradesh, Southern India and Srilanka in moist place. This plant is well known in Ayurvenda and Siddha system as "Alarka" and Tuduvelai; respectively. The sidda system of medicine uses a ghee prepared from this plant for treatment of tuberculosis (Annamalai *et al.,* 2009). *Solanum trilobatum*, a thorny creeper with bluish violet flower, more commonly available in southern India (Mohan *et al.,* 1998; Swaphalatha, 2006). *Solanum trilobatum* (Solanaceae-herbs) is an amount of calcium, iron, phosphors, carbohydrates, fat, crude fiber and minerals in the leaves (Jawahar *et al.,* 2004; Doss, 2009).

The leaves of *Solanum* trilo*batum* revealed the presence of Sugars, Protein, Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycosides, Terpenoids and Lipids (Rangasamy, 2008; Balasubramanian *et al.,* 2010). The root bark is laxative, useful in the treatment of ulcers on the neck, burking of thwart, inflammation of liver and chronic fever. Berries are bitter and pungent useful in the heart disease, piles and dysentery (Kritikar and Basu 1935; Yoganath *et al.,* 2009). Roots, berries and flowers are used for cough (Annamalai, 2008). This herbal plant is used as medicine for asthma, vomiting of blood, reducing blood glucose level and bilious matter phlegmatic rheumatism and several kinds of leprosy it is also antibacterial, antifungal, antimitotic, antioxidant and antitumourous (Shahjahan *et al.,* 2005).

Escherichia coli are a Gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endosperms). Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls (Grant *et al*., 2007).

The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of metagenics, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology and microbiology.

Pseudomonas is a Rod shaped, negative, bacteria, which contains one or more polar flagella providing motility. It is an aerobic bacteria that shows positive towards catalase and oxidase. Although not strictly a plant pathogen, *Pseudomonas tolaasii* can be a major agricultural problem, as it can cause bacterial blotch of cultivated mushrooms. Similarly, *pseudomonas agarici* can cause drippy gill in cultivated mushrooms (Mayer *et al.,* 2007).

Staphylococcus is a genus of Gram-positive bacteria. Under the microscope they appear round (Cocci), and form in grape-like clusters (Harris *et al*., 2005). The *Staphylococcus* genus includes at least forty species. Of these, nine have two subspecies and one has three subspecies. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. The appropriate cell wall structure (including peptidoglycan type and teichoic acid presence) and G + C content of DNA in a range of 30-40 mol%.

Bacillus is a genus of Gram-positive rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species can be obligate aerobes or facultative anaerobes, and test positive for the enzyme catalase. Ubiquitous in nature, *Bacillus* includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera (Madigan *et al.,* 2005).

Klebsiella is a genus of non-motile, Gram-negative, oxidase-negative, rod shaped bacteria with a prominent polysaccharide-based capsule. It is named after the German microbiologist Edwin Klebs). Frequent human pathogens, *Klebsiella* organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, ankylosing spondylitis, and soft tissue infections. *Klebsiella* species are ubiquitous in nature (Ryan *et al.,* 2004).

A fungus is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms (Hibbett *et al.,* 1980). Fungi, which is separate from plants, animals, and bacteria, since the fungi have been used for the production of antibiotics, and, more recently, various enzymes produced by fungi are used industrially and in detergents (Voupoti *et al.,* 2008).

Aspergillus niger grows aerobically on organic matter, therefore it can be found almost everywhere in environments that contain soil. Also, it is found in waste, decaying plant material and compost in outdoor environments. (Deepake *et al.,* 2009)

Aspergillus niger has been exhibited to sustain growth in freezing temperatures, which indicates it as a thermotolerant that can also survive at very high temperatures. It's thermo tolerant abilities that enable growth in a wide temperature range from 6 to 47°C with a preferred optimum temperature at 35-37°C. The fungus is capable of growing over a very wide pH range, from 1.4 to 9.8 pH. The growth ability in various temperature ranges, pH ranges as well as the abundant amount of conidiospores allow the species to be continuously widespread. Conidiospores are distributed by air (Coleman *et al.,* 2002).

Like other *Aspergillus* species, *Aspergillus flavus* has a worldwide distribution. This probably results from the production of numerous airborne conidia, which easily disperse by air movements and possibly by insects. Atmosphere composition has a great impact on mould growth, with humidity being the most important variable (Gibson *et al.,* 1994). *Aspergillus flavus* grows better with water activity between 0.86 and 0.96 (Maraqa *et al*., 2007). The optimum temperature for *Aspergillus Flavus* to grow is 37 ^{0}C, but fungal growth can be observed at temperatures ranging from12 to 48 ^{0}C. Such a high optimum temperature contributes to its pathogenicity in humans.

Fusarium is a filamentous fungus widely distributed on plant and in the soil, filamentous fungi are used in many industrial processes of enzymes and metabolites (Adrio *et al*., 2003). *Fusarium* is a large genus of filamentous fungi widely distributed in soil and in association with plants. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if they enter the food chain. The main toxins produced by these *Fusarium* species are fumonisins and trichothecenes. The genus includes a number of economically important plant pathogenic species (Howard *et al.,* 2003).

Plants have a long history of use in the treatment of cancer hartwell in his review of plants used against cancer, lists more than 3000 plants species that have reportedly been used in the treatment of cancer. In many instances, however , the cancer " is undefined, or reference is made to condition such as "hard swelling" abscesses, callues, corns, warts, polys, or tumors to have a few, these symptoms would generally apply to skin tangible or visible condition, and may indeed sometimes corresponds to a conditions .

The search for anticancer agents from plant source started in earnest in the 1950s with the discovery and development of the vinca, alkaloids, vinblastin and vincristine, and the isolation cytotoxic podophyllotoxin . Although many anticancer compounds such as alkaliating compounds anti metobiltes, redimimetics, hormones and antagonist have been developed (Calabresi and Chapner 1991; Hoppe *et al.,* 1982). None of them give satisfaction and no side effects (Green *et al.,* 1982; Herzieg *et al.,* 1987).

The world health organization estimates that approximately 80% of the world`s inhabitants regular on traditional medicine of their primary health care (Farnsworth *et al.,* 1985). Cancer is a major public health burden in estimated that there were 10.9 million new cases 6.7 million deaths and 24.6 million persons living with cancer around the world in 2002 (Parkin *et al.,* 2005). Cancer is the second leading cause of death in the United States (Hoyerd *et al*., 2005), where one in four deaths is due to cancer. Plants have long been used in the treatment of cancer (Hartwell 1982).

The systematic anticancer remedies are beyond the reach of common man because of cost factor. Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical. Chattisgarh has been declared as the Herbal State of India (Sakarkar *et al*., 2011).

The selection of the plant species for the present study was mainly based on the traditional uses of these species for the treatment of various disease including skin infections, Ulcer, tumor and cancer (Ankur *et al*., 2007).

The *solanum trilobatum* commonly used in traditional medicine because of its derivate having antipyretic effects, and has a long history of use in the treatment of inflammation, edema, martitis, cirrholis, of liver in oriental medicine (Heoks *et al.,* 2004; Jainu *et al.,* 2006). The whole plant of *Solanum trilobatum* contains many steroidal glycosides, steroidal alkaloids, polyphenols, flavanoids, fatty acids and essential oils (Hu *et al*., 2008).

Cancer (Malignant tumour) is an abnormal growth and proliferation of cells. It is a frightful disease because the patient suffers pain, disfigurement and loss of many physiological processes. Cancer may be uncontrollable and incurable and may occur at any time at any age in any part of the body. It is caused by a complex poorly understood interplay of genetic and environmental factors (Somukumar *et al*., 2003; Pandey *et al*., 2006). It continues to represent the largest cause and kills about 1500 per million populations annually around the world. A large number of chemopreventive agents are used to cure various cancers, but they cause side effects that prevent their extensive usage. Although more than 1500 anticancer drugs are in active development with over 500 of the drugs under clinical trials. There is an urgent need to develop much effective and less toxic drugs (Boopathy *et al*., 2006).

Phytochemical analysis of *Solanum trilobatum* extract was conducted by (Evans, 1989; Harbone, 1998). By this analysis, the presence of several phytochemical like sugar, protein, alkaloids,

flavonoids, saponins, tanins, terpenoids and lipids were tested. Preliminary phytochemical analysis of ethanolic extract of *Solanum trilobatum* by silica gel thin layer chromatography showed presences of simple phenols, phenolic acids, isoflavones and lignins. The present study reveals the determination of antimicrobial and anticancer activity of *Solanum trilobatum*.

MATERIALS AND METHOD

Collection of sample

The leaves of *Solanum trilobatum* (Solanaceae) were collected during the month of March, 2010 from Thirukovilur, Villupuram district, Tamil Nadu, India.

Ethanolic extract of *Solanum trilobatum*

The leaves were dry shaded for 15 days and it was powdered by using saw mill. Then the powdered sample was wrapped in Whatmann No – 1 filter paper by using thread. Then the powdered sample was placed in Soxhlet apparatus and to that 150ml of ethanol. After three and half hours the ethanolic extract was collected and it was used for further studies (Ramalingam *et al.,* 2009).

Organisms used for Antimicrobial activity

Bacteria: E. *coli, Staphylococcus aureus, Pseudomonas, Klebsiella, Bacillus*

Fungi: *Aspergillus niger, Aspergillus flavus, Fusarium*

Assessment of antibacterial activity of *Solanum trilobatum*

Antibacterial activity of ethanolic extract of *Solanum trilobatum* was tested using an zone of inhibition assay, described (Nair and Chanda, 2005), on Muller – Hinton agar plates seeded with *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebiella* species. The ethanolic extract of *Solanum trilobatum* (50 µl) were added to the wells of Muller – Hinton agar plates inoculated with bacterial cells. The plates were incubated overnight at 37º C. the zone of inhibition were measured and calculated from the diameter of the zones obtained next day.

Assessment of antifungal activity of *Solanum trilobatum*

The Antifungal activity of was tested using an inhibition zone assay on Potato Dextrose agar plates seeded with *Aspergillus niger*, *Fusarium* sp, and *Aspergillus flavus.* The ethanolic extract (50 µl) was added to the wells of Potato Dextrose agar plates seeded with fungi. The plates were incubated at 28ºC for three days. The zone of inhibitions were measured and calculated from the diameter of the zones obtained.

Phytochemical Screening Procedure

These tests were carried out using standard procedures of (Bharathi *et al.,* 2009).

Protein Estimation from the Ethanolic extract of *Solanum trilobatum*:

Lowry's Method

Protein content was determined according to the method of (Lowry *et al.,* 1951). A stock solution of standard protein, bovine serum albumin (BSA), at a concentration of 1000µg/ml was made. From this solution, 0.2 to 1ml of working standard solution at concentration of 100µg/ml was taken in test tubes .The volume was made up to 1ml of Folin ciocalteau reagent was added to each test tube. After 30 mins of Incubation, the absorbance was measured at 660nm using a double beam UV-visible spectrophotometer and the protein content was determined.

DEAE cellulose Anion Exchange Chromatography

A chromatography column (2 x 40cm) was packed with DEAE cellulose and equilibrated with 0.1 M Tris-HCL buffer containing 2mM Magnesium chloride (pH 8.6). The extract was loaded into the top of the column and eluted with linear gradient Tris-HCL buffer composed of sodium chloride (0-1M) at room temperature. The crude extract was and loaded on the column equilibrated with Tris – HCl buffer at a constant flow rate of 1ml/minute and eluted with 20ml of buffer. All the fractions were collected and assessed for antimicrobial actgivity. The 14, 15 and 16th fractions which showed good antimicrobial activity were pooled as single active fraction. The phytochemical analysis revealed that the active fraction contained alkaloids.

Thin Layer Chromatography

TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted developing chamber so that only the very bottom of near the bottom of this plate. (Bharathi *et al.,* 2010).The TLC plate is then placed in a shallow pool of a solvent in the plate. The eluent mobile phase slowly rises up the TLC plate by capillary action. As the solvent (Choloroform10 : Ethanol 1) moves past the spot that was applied, an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. . The solvent was allowed to run till it reaches about half a centimeter below the top of the plate. After running, the plate was kept in room temperature for the complete drying of the plate. The plates were kept in a beaker containing iodine pellets. After running the spots were appeared Rf value of the spot on the TLC plate was determined.

Rf value= Movement of solute / Movement of solvent

Assessment of antibacterial activity of alkaloids:

Antibacterial activity of alkaloids scraped from TLC was tested using zone inhibition assay (Barbosa *et al.,* 1991) described on Muller – Hinton agar plates seeded with *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebiella* species. The Ethanolic extract of *Solanum trilobatum* (50 µl) were added to the wells of Muller – Hinton agar plates inoculated with bacterial cells. The plates were incubated overnight at 37º C. the zone of inhibition were measured and calculated from the diameter of the zones obtained next day.

Assessment of antifungal activity of alkaloids:

The antifungal activity of was tested using an inhibition zone assay on Potato Dextrose agar plates seeded with *Aspergillus niger*, *Fusarium* sp and *Aspergillus flavus.* The pure alkoids (50 µl) was added to the wells of Potato Dextrose agar plates seeded with fungi. The plates were incubated at 28ºC for three days. The zone of inhibitions were measured and calculated from the diameter of the zones obtained

Characterization of alkaloid present in the active fraction using High Performance Liquid Chromatography:

The purity of the alkaloid in active fractions of column chromatography and their chemical nature were analyzed using High Performance Liquid Chromatography. The active fraction from column chromatography of alkaloid collected using the solvent of acetonitrile/tetrafluroaceticacid/water (0.05%:70:30) by Waters Breeze HPLC equipped with C18 reverse phase Column and UV wavelength detector set at channel where 210nm absorbance (Jiang *et al.,* 2006).

Maintenance of Cancer cells (HT – 29 cells)

Cancer Cell lines and Chemicals

Cancer cell lines HT – 29 were obtained from King's Institute of Preventive Medicine, Guindy, Chennai. Dulbecco's Modified Eagle Medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum (FBS), 3(-4,5-dimethyl thiazol-2yl) 2,5-diphenyltetrazolium bromide (MTT), Sodium bicarbonate, Dimethyl sulphoxide (DMSO), and antibiotic solution. 96 well plates, 6 well plates, Tissue culture flasks (25 and 75 mm), centrifuge tubes (15 and 50ml).

Cell culture reagents

DMEM

10gms of DMEM was dissolved in 990ml of sterilized double distilled water. To this solution, 1.5g of sodium bicarbonate and 10ml of penicillin-streptomycin cocktail (100-units/ml penicillin and 10-units/ml streptomycin) were added and mixed thoroughly. The pH was adjusted to 7.4 using 1N HCl or 1N NaOH. Then the medium was filter sterilized using (0.22µm) membrane filter and dispensed into sterilized container and stored at 4ºC. 10% FBS was added to this medium and used for culturing the cells.

Phosphate Buffered Saline (PBS) (pH 7.4)

Sodium dihydrogen phosphate (0.63 g), 170mg of disodium hydrogen phosphate and 4.5g of sodium chloride were dissolved and made up to 500ml of sterile distilled water. The pH was adjusted to 7.4 using 1N HCl or 1N NaOH, filtered and then stored in a sterile container.

Trypsin-EDTA solution:

Trypsin-EDTA solution (0.25% per 0.02%) in PBS was used. This was aliquoted and stored frozen until use. (Note: Freeze/thaw process does not affect the enzyme activity. Thawing was done at room temperature).

Maintenance of cell lines:

Changing Medium:

The condition of the cells was observed under the inverted microscope. If the color of the medium became yellow indicates the medium is acidic, the media was aspirated and then fresh media was added into the flask.

Checking Contamination and Cell health:

The flask was removed from the CO_2 incubator and examined under the microscope for contamination (bacterial, fungal) and cross contamination. The general cell health was checked under the microscope (normal condition of cells and floating on media).

Passage of Cells

The flask with 70-80% confluence can be passage. The medium was removed and washed the cells twice with PBS. Five hundred micro liter of Trypsin (0.1% (w/v)) was added to the flask. After 2 to 3 minutes trypsin was discarded. Then the cells were incubated at 37ºC for 30 seconds to 5 minutes. Gently the flask was tapped to loosen all the attached cells. Then 5ml of fresh medium was added into the flask and transferred into 15ml centrifuge tube and centrifuged at 1000rpm for 5 minutes. The pellet was resuspended in 5ml of medium and distributed the cells according to the split ratio of the cell line. The passage number was maintained. The flask was kept in CO_2 incubator.

Experimental procedure

Morphological studies

Cancer cells HT-29 were trypsinized. Then 5ml of growth medium was added to trypsinized cells and cell suspension was mixed well. Then 2ml of cell suspension was added to the sterilized test tubes containing cover slip. The test tubes were placed in a slanting position and kept in CO_2 incubator for two days. The monolayer of cells formed in the cover slip was observed under light microscope and photographed.

Cell viability assay by Dye exclusion method

The cell viability was assessed by the method described by (Rosenberg *et al.,* 1978). The adherent cells were washed with PBS, trypsinized and resuspended the cells in fresh medium in after centrifugation. The cell suspension was mixed by pipeting several times to get uniform single cell suspension. Then 100µl of the cell suspension was added with 100µl of 0.4% trypan blue was added. The content was mixed well and incubated for 5 minutes at room temperature to allow dead cells to take up the dye and the viable cells to pump out the stain by efflux mechanism. The cover slip was placed in the haemocytometer and 10 µl of trypan blue- cell suspension was transferred to haemocytometer by carefully. The viable and non viable cells were counted separately in all four squares

Cells / ml = Average cell count /square x Dilution factor x 10^4

Total viable cells = cells /ml x original volume of cell suspension from which sample is drawn.

$$\text{Percentage of Cell viability} = \frac{\text{Total viable cells (unstained)}}{\text{Total cells (stained and unstained)}} \times 100$$

MTT ASSAY FOR DETERMINATION OF CELL VIABILITY

The cell viability of HT-29 determined by 3(-4, 5- dimethyl thiazol 2-yl) 2,5- diphenyl tetrazolium bromide (MTT) reduction assay. Tetrazolium salts are metabolized by various mitochondrial dehydrogenase mainly by succinate dehydrogenase and reduced to a blue formazon by living cell only.

The HT-29 were pre-incubated overnight in a 24 well plates at a density of 1 X 10^5 Cells per well and were then washed with PBS (pH 7.5) to remove fetal bovine serum. Cells were treated with various concentrations of pure alkaloids appropriate vehicles (DMSO<PBS) for 24 hours. For pure alkaloids for 2 hours after treat cells were washed with PBS (pH 7.5) to remove pure alkaloids followed by washing with PBS. Add 1 ml of MTT (0.5mg/ml) in DMEM medium and incubated for 4 hours, then the medium was removed by inverting the plates and gently tapped on tissue paper. The formazan crystals were dissolved with 1 ml of DMSO. The optical density of each sample was measure in a spectrophotometre at 540 nm against a suitable blank. All determinations were performed duplicate and confirmed at least three independent experiments.

RESULTS AND DISCUSSION

The sample was collected from Thirukovilur (Villupuram district) and it was used for further studies like antimicrobial and anticancer activity.

Ethanolic extract of *Solanum trilobatum*

The ethanolic extract of *Solanum trilobatum* (Leaf) were obtained for analyzing the various activities of the plants by using Soxhlet apparatus and it was used for the further studies and it was shown in the Fig. 1.

Antibacterial activity of *Solanum trilobatum*

The Ethanolic extract of *solanum trilobatum* (50 µl) was added to the wells of Muller – Hinton agar plates inoculated with bacterial cells (Nair and Chanda, 2005).The plates were incubated overnight at 37º C. The zone of inhibition were measured and calculated from the diameter of the zones obtained the *E. coli* was shown the highest inhibitory activity when compared to the other species as shown in Table 1 and Fig -2.

Antifungal activity activity of *Solanum trilobatum*

The Ethanolic extracts (50 µl) were added to the wells of Potato Dextrose agar plates seeded with fungi. The plates were incubated at 28ºC for three days. The zone of inhibitions were measured and calculated from the diameter of the zones obtained and the *Aspergillus niger* shows the highest inhibitory activity when compared to the other species as shown in Table 2 and Fig. 3.

Phytochemical tests

The various photochemical tests were performed to know the active components present in the *Solanum trilobatum* such as alkaloids, flavonoids, sugars, glycosides, spannins, tannins, proteins, aminoacids, terpenoids as shown in the Table 3.

Protein Estimation

Protein content was determined according to the method of (Lowry *et al.,* 1951). The absorbance was measured at 660nm using a double beam UV-visible spectrophotometer.

DEAE cellulose Anion Exchange Chromatography

A chromatography column (2 x 40cm) was packed with DEAE cellulose and equilibrated with 0.1 M Tris-HCL buffer containing 2mM magnesium chloride (pH 8.6). The extract was loaded into the top of the column and eluted with linear gradient Tris-HCL buffer composed of sodium chloride (0-1M) at room temperature. The crude extract was loaded on the column equilibrated with Tris-HCl buffer at a constant flow rate of 1ml/minute and eluted with 20ml of buffer. All the fractions were collected and checked for antimicrobial activity.

Phytochemical Screening for Alkaloids

All the phytochemical tests were performed on active fraction. The tests for alkaloids showed positive result. Mayers test, Wagners test, and Dragendorff tests were carried out using standard procedures. The results were shown in Table 4.

Thin Layer Chromatography

The Alkaloid component was separated by using Thin Layer Chromatography by placing the TLC plate in the solvent of Chloroform:Ethanol whose ratio is 10:1 ratio (Bharathi *et al*., 2009). After running, the plate was kept in room temperature for the complete drying of the plate. The plates were kept in a beaker containing iodine pellets as shown in the Fig. 3.

Antibacterial activity of alkaloids

The alkaloids extract of *Solanum trilobatum* (50 µl) was added to the wells of Muller – Hinton agar plates inoculated with bacterial cells. The plates were incubated overnight at 37º C. The zone of inhibition were measured and calculated from the diameter of the zones obtained the Pseudomonas species was shown the highest inhibitory activity when compared to the other species as shown in Table 5and Fig.4.

Antifungal activity of Alkaloids

The pure alkaloids extracts (50 µl) were added to the wells of Potato Dextrose agar plates seeded with fungi. The plates were incubated at 28ºC for three days. The zone of inhibitions were measured and calculated from the diameter of the zones obtained and the *Aspergillus niger* shows the highest inhibitory activity when compared to the other species as shown in Table 6 and Fig. 5.

Characterization of alkaloid present in the active fraction using High Performance Liquid Chromatography

The purity of the alkaloid in active fractions of column chromatography and their chemical nature were analyzed using High Performance Liquid Chromatography. The active fraction from column chromatography of alkaloid collected using the solvent of acetonitrile/tetrafluroaceticacid/water (0.05%:70:30) by Waters Breeze HPLC equipped with C18 reverse phase Column and UV wavelength detector set at channel where 210nm absorbance (Jiang *et al.,* 2006). Fig. 6

Maintenance of Cancer cells (HT – 29 cells)

Cancer cell lines HT – 29 was collected from King's Institute of Preventive Medicine, Guindy, Chennai.

Cell viability assay by dye exclusion method

To the DMEM HT-29 cells were grown and it was mixed with 10% of tryphan blue (0.8gm/10ml). From this 10µl of tryphan blue and 0.5 µl of HT-29 cells were mixed and place a haemocytometer and it was checked for the cell viability

MTT Assay

The Antiproliferative effect was assessed by MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide) method described by Mosmann *et al.* (1983).

Colored formazan dye by the cleavage of tetrazolium salts corresponding to mitochondrial dehydrogenase activity of cells and hence, the conversion occurs only in viable cells. The formazan crystals are non water soluble in nature when addition of solubilising solution they form a colored complex which can be read at 630nm in an ELISA reader. The values were shown in the graph Fig. 7

SUMMARY AND CONCLUSION

Antimicrobial activity of arial parts of *Solanum trilobatum* was assayed under *in vitro* conditions by agar well diffusion and well diffusion method against five bacterial species, all the solvents ethanol, acetone and ethtylacetate used for extraction were shown significant antibacterial activity expect acetone and ethylacetate extract of leaf against *Klebsiella* and *Pheumoniae* gram positive bacteria was more susceptible to inhibition when compared to gram negative bacterial species. The plant extract are more active against gram positive bacteria then gram negative the inhibition of bacterial growth by *Solanum trilobatum* extract was found to be significantly higher than that of standard antibiotic tested.

Alkaloid isolated from the leaves of *Solanum trilobatum* has shown significant antibacterial activity against tested organism, the inhibition of bacterial growth exbited by the alkaloid fraction was significantly higher shown by the extract. It has been reported that the total alkaloid content present in the plant extract. Several reports are available in support of antimicrobial activity of alkaloid against bacterial and fungal pathogen. In clinical studies the oral administration of *Solanum trilobatum* for three days at a dose of 300mg dry powder, thrice a day was found to be very effective in controlling mild and moderated bronchial asthma.

The MIC (Minimum Inhibition Concentration) of tannins against test organism ranged between1and 4mg/ml while the MBC (Minimum Bacterial Concentration) ranged between 1.5and 4.5 mg/ml. Antimicrobial agents with low activity against an organism add a high MIC while a highly active antimicrobial agents have a low MIC the study support the traditional use of *Solanum trilobatum* as green medicine it also such as that the alkalolids isolate from the plant posses remarkable toxic activity against bacteria, Fungal and Cancer and may assume pharmacological importance.

The genus *Solanum* (Solanaceae) is a rich source of steroidal glycoalkaloids. The plant of this genus mainly contain sterol alkaloids like solamargine, solasonine and solanine and steroidal sapogenins, diosgenin and diosgenone, solanine, isolated from *Solanum* species, has been reported to induce apoptosis in cell, by inhibiting the expression of protein. Another steroidal alkaloid, solamargine has also been report to exhibit the anti-tumor effects. It was previously reported that solamargine and solasodine are cytotoxic to cell.since both Solamargine and solanine reduced the viability of cells, where as p2-solamargine was inactive.

Saponins are an important class of secondary metabolites which have been long recognized to show cytotoxic activity in various cancers cell lines. Apoptosis in an attractive endpoint of cancer therapy.

The anticarcinogenic activity and tumor growth inhibitory effect of Sobatum from *Solanum trilobatum* formed the basis for the assessment on the role of *Solanum trilobatum* on DEN induced hepatocarcinogenesis. The decrease in TSP observed in ccl_4 treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into the decrease hepatic capacity to synthesise protein, but the restoration of the level of TSP after the administration of STE conformed the hepatoprotective

nature of *Solanum trilobatum*. The ethyl acetate extract only contains flavonoids and tannin. It was estimate that the presence of quinine compound in n-Butanol extract in inhibiting cancer cell growth compared by ethyl acetate extract. The anticancer activity of this plants is due to the presence of antioxidants *i.e.,* polysachharides, polyphenols, flavonoids, lignins, xanthones. There is a broad scope to derive the anticancer agent from medical plants, which needs thorough research.

The herbs were helpful against cancer, those outcome require for the conformation with rigorously control the trial particularly herbs are very much useful improving survival and quality of life in patient suffering from advanced cancer.

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