Research Article

Phytochemical, Antibacterial and Cytotoxic Screening of *Salicia fruticosa* Heyne Ex Lawson., Aerial Parts - *in vitro* Model

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ABSTRACT

The present study was carried out to evaluate the phytochemical, antibacterial and cytotoxic properties of medicinal plant frequently used in Indian traditional medicine. *Salicia fruticosa* Heyne ex Lawson belonging to the family Celestraceae is an edible plant found in India. The following materials were employed: The aerial part of plant was washed thoroughly with tape water, shade dried, homogenised to fine powder. The dried powder *Salicia fruticosa* Heyen ex Lawson is subjected to extraction using different solvents of increasing polarity (Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water) adopting cold maceration using conical flask. The extracts were concentrated under few reduced pressure to yield semi solid mass. Preliminary phyto chemical studies was carried out for each of aerial part of *Salicia fruticosa* to determine the presence of various phytochemical constitutents. Preliminary anti-bacterial screening carried by agar well diffusion method. Short term in vitro cytotoxicity studies in EAC and DLA cell line by tryphan blue dye exclusion method. We discussed about phyto chemical screening of various extracts of *Salicia fruticosa* and their constitutents present in extract to identified presence or absence. *In vitro* antibacterial study was carried out by well diffusion method using various concentration and there micro organisms were used are *E.coli*, *S.aureus*. short term I*n vitro* cytotoxic activity of EAC and DLA cell line using different concentration to find the % cell death by various extract. This study has shown that the ethanolic extract of *Salicia fruticosa* contains some active constituents which are responsible for cytotoxic and antibacterial activity. Further characterisation and isolation of the extract has to be carried out to reveal the exact pharmacological activity based upon *In vivo* cytotoxic studies.

Keywords: EAC, DLA, tryphan blue, cytotoxic.

INTRODUCTION

Natural products especially plants have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient times and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Acadians[1]. Cancer is a general term applied of series of malignant diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. The main forms of treatment for advance stage cancer in humans are surgery, radiation and drugs (cancer chemotherapeutic agents). Cancer chemotherapeutic agents can often provide temporary relief of symptoms, prolongation of life, and occasionally cures[2]. In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs. Many hundreds of chemical variants of known class of cancer chemotherapeutic agents have been synthesized but have a more side effects. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side effects when under-going treatment[3].

Types of Cancers[4]

1) Cancers of Blood and Lymphatic Systems

a) Hodgkin's disease, b) Leukemia's, c) Lymphomas, d) Multiple myeloma, e) Walden strom's disease.

2) Skin Cancers

a) Malignant Melanoma

3) Cancers of Digestive Systems

a) Esophageal cancer b) Stomach cancer c) Cancer of pancreas d) Liver cancer e) Colon and Rectal cancer f) Anal cancer

4) Cancers of Urinary system

a) Kidney cancer b) Bladder cancer c) Testis cancer d) Prostate cancer

5) Cancers in women

a) Breast cancer b) Ovarian cancer c) Gynecological cancer d) Choriocarcinoma

Although adjuvant CTX is frequently used for primary tumors, its main use is to control overt disseminated

disease. The excessively active growth-signaling pathways in cancer cells makes them susceptible to a wide range of drugs which target growth-signaling molecules and/or processes involved in cellular replication and expression. However, as these processes also drive normal cells, the effect is preferential and not exclusive, which results in the unwanted side effects seen with these agents. Cells which are normally actively dividing, in particular the bone marrow constituents and those of the intestinal lining, are particularly susceptible. Disregulated cell cycle events, due to mutations in cancer cells, do sometimes offer opportunities to target those cells without affecting normal cells. The relatively wide spectrum of activity of cytotoxic drugs makes them a rather harsh and non-specific form of treatment that can only be tolerated for short periods. Indeed the effects of the treatment may sometimes cause more distress than the disease. Mechanism These side-effects include dry flaky skin, loss of hair, nausea and vomiting, changes in taste and appetite, blood clotting problems, fatigue, depressed immune system and possible sterility.[5] Two sets of genes are controlling cancer development. Oncogenes are the first set of genes and are involved in different cell activities including cell division. However, over expression of these genes transforms a normal cell into a cancer cell. On the other hand, the second set of genes (tumor suppressor genes) inhibits cancer cell formation by different mechanisms. Tumor suppressor genes are under expressed in cancer cells while, oncogenes are over expressed[6]. Oncogenes and their products represent good targets for Cancer therapy. Other targets include enzymes involved in cell division like topoisomerases that unwind the DNA during replication. The diversity of plant derived natural products can provide therapeutic products attacking different targets in cancer cells[7].

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as Staphylococcus aureus is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning[8]. Gram negative bacterium such as Escherichia coli is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia[9,10]. Different antibiotics exercise their inhibitory activity on different pathogenic organisms[11].

MATERIALS AND METHODS

In accordance with investigated the aerial part of *Salicia fruticosa* heyne ex Lawson was collected from Tirunelveli, Tamilnadu. The sequential extraction was done in dried powdered *Salicia fruticosa.* Heyne ex Lawson is subjected to extraction using different solvents of increasing polarity (petroleum ether, chloroform, ethyl acetate, ethanol, water) adopting cold maceration using conical flasks. The extract were concentrated under reduced pressure to yield semisolid mass. The preliminary phytochemical studies were carried out for each extract of *Salicia fruticosa* to determine the presence of various phytochemical constituents showed the presence of alkaloids, flavonoids, phenolic compound, steroids, saponins but glycosides and tannins were totally absent[12]. Preliminary anti-bacterial screening was done by agar well diffusion method is widely used to evaluate the antibacterial activity of plant or microbial extracts. the agar plate surface is inoculated by spreading a volume of the microbial innoculum over the entire agar surface. a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or tip a volume of the antimicrobial agent or extract solution is introduced into the well. Then agar plates are incubated at 37^0C for 24 hrs depending upon the test microorganism the anti-microbial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The Staphylococcus aureus and Escherichia coli were obtained from MTCC, Chandigarh were maintained on nutrient agar medium at 4^0C for further experiment.[13] The short *in vitro* cytotoxicity studies in EAC and DLA cell line by tryphan blue dye exclusion method,[14] tryphan blue, fetal bovine serum, phosphated buffered saline ,Dulbecco's modified eagle's medium were obtained from sigma Aldrich co,st Louis USA. Antibiotics from Hi media laboratories Ltd., Mumbai.

For cytotoxicity studies each weighed test drugs were separately dissolved in distilled DMSO and volume made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by filtration serial twofold dilution were prepared from this for carrying out cytotoxicity studies.

The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by Tryphan blue exclusion method. Viable cell suspension (1×10^5 cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 hours at 37 °C. Further cell suspension was mixed with 0.1 ml of 1% tryphan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of tryphan blue while live cells do not take up the dye. The number of stained and unstained cells was counted separately.

$$\% \text{ cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cell + No.of dead cell}} \times 100$$

RESULTS AND DISCUSSION

Today, there is much interest in studying natural products and their derivatives in search of options for disease treatment and other medical applications. Natural products are especially notorious as anticancer and anti-infective agents[15]. With the current decline in the number of new molecular entities from the Pharmaceutical industry, novel anticancer agents are being sought from traditional medicines[16]. Investigations concerning the choice of appropriate constituents, including the extracts

are concentrated under reduced pressure to yield semi-solid mass which is dried in a desiccator and then subjected to preliminary phytochemical analysis. The percentage yield of Pet.ether, Chloroform, Ethyl acetate, ethanol and water were found to be 2.75 % w/w, 10.12 % w/w, 13.68 % w/w, 25.40 % w/w, 17.58 % w/w respectively. Phytochemical analysis shows the presence of much medicinally important the secondary metabolite type of phytoconstituents like Alkaloids, Glycosides, Saponins, Triterpenes which indicates that the plant possesses high profile value and thus can be used to treat various kinds of diseases. Qualitative phytochemical analysis gave valuable information about the different phyto constituents present in the extracts, which helps the investigator in the selection of the particular extract for further investigation. This study revealed that Petroleum ether extract showed presence of steroids and tri terpenoids only, Chloroform extract showed presence of alkaloids, carbohydrate, phenolic compounds and steroids. Ethyl acetate extract showed presence of alkaloids, carbohydrates, phenolic compounds, tannins and proteins. Ethanolic extract showed presence of alkaloids, carbohydrates, phenolic compounds, saponins, steroids, triterpenoids and flavonoids. Aqueous extract showed presence of alkaloids, carbohydrates, phenolic compounds, saponins, steroids and flavonoids. Presence of phenolic compounds, alkaloids and flavonoids in chloroform, ethyl acetate, ethanol, water extracts may influence anticancer activity.

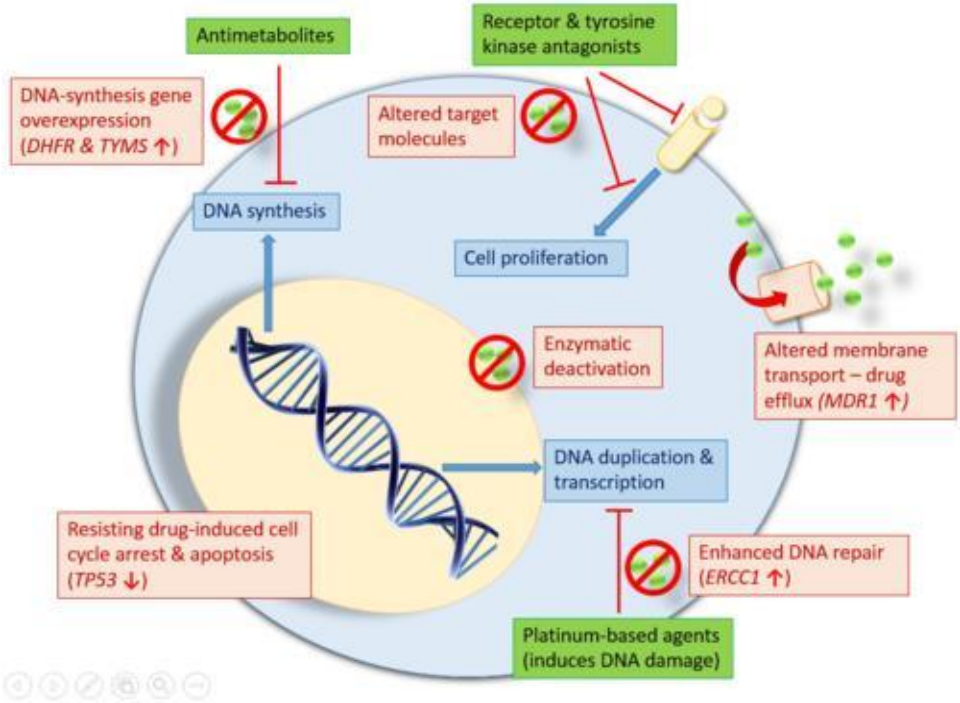

Figure 1: Anti neoplastic resistance Mechanism

***IN VITRO* CYTOTOXICITY STUDY**

EAC CELL LINE: Short term *in vitro* cytotoxic activity of *Salicia fruticosa* Heyne ex Lawson on EAC cell line

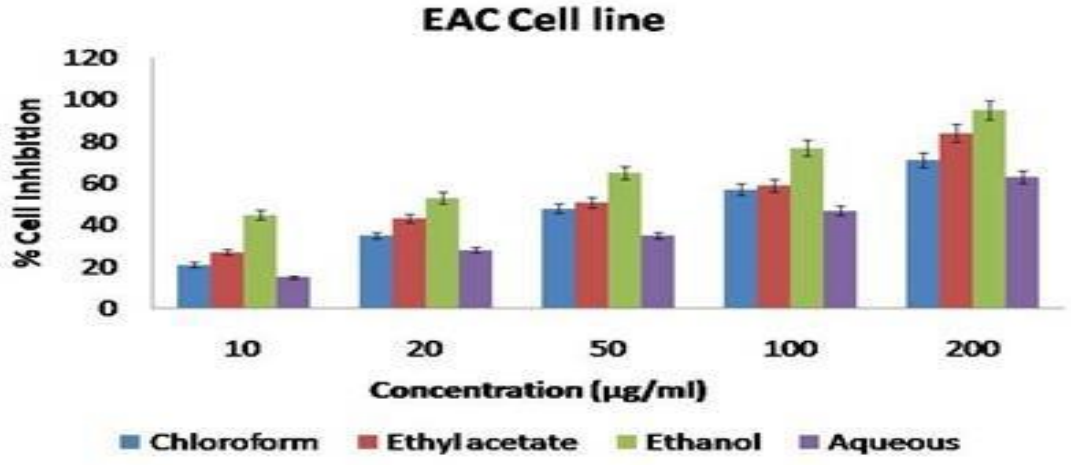

Figure 2: Cytotoxic activity by EAC cell line

DLA CELL LINE: Short term *in vitro* cytotoxic activity of *Salicia fruticosa* Heyne ex Lawson on DLA cell line

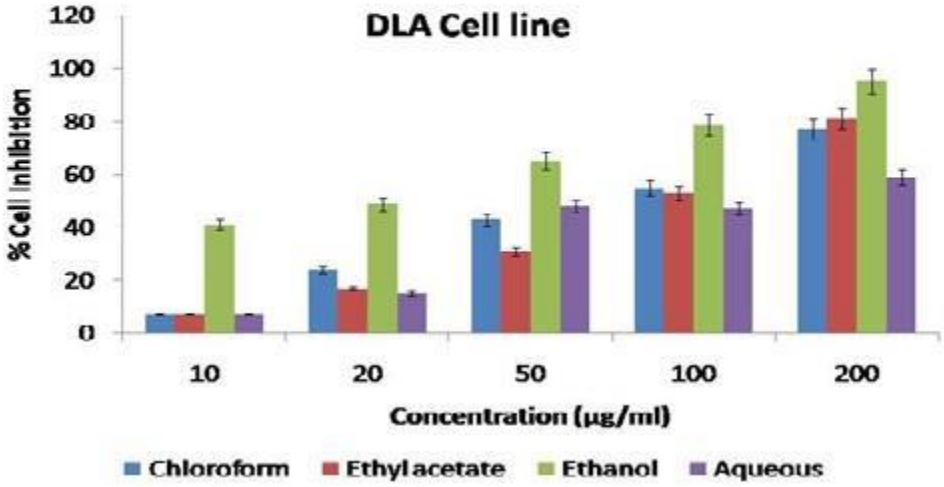

Figure 3: Cytotoxic activity by DLA cell line

Table 1: *In vitro* Antibacterial Study Antibacterial Activity – Well Diffusion Method

Stock Concentration: 500mg/l

Concentration	Bacterial Culture	
	E.Coli	*S. aureus*
25 µl	10 mm	-
50 µl	11 mm	-
75 µl	13 mm	-
100 µl	14mm	10mm

(-) - no zone

In the past, many plants showed proven anti-bacterial activity. Thus attempt has been taken to reveal the ethanolic extract of *Salicia fruticosa*. Antibacterial activity was performed against *Escherichia coli* (G-ve) and *Staphylococcus aureus* (G+ve). It was shown that the extract showed antibacterial activity against G-ve than G+ve at the concentration of 100 µg/ml. It is clear that extract showed only minimal antibacterial activity. In this study the cytotoxic activities of chloroform, ethyl acetate, ethanol and aqueous extract were investigated by short term *In vitro* cytotoxic activity by Tryphan blue dye exclusion method. EAC and DLA cell lines were used for investigation. The search for cytotoxic agents from plant source has been successful worldwide. Ethano pharmacological knowledge is helpful that leads in the search for plants with high potential cytotoxic activity. Based on the investigation carried out for EAC cell line it was found out that cytotoxic activity of the extract in the following order:

Ethanolic extract > Ethyl acetate extract > Chloroform > Aqueous extract

In the same way for DLA cell line,

Ethanolic extract > Ethyl acetate extract > Chloroform > Aqueous extract

which shows ethanolic extract of *Salicia fruticosa* at concentration of 200µg/ml showed high percentage of cell death followed by ethyl acetate extract showed comparable percentage of cell death. This study reveals that the cytotoxic activity possessed by ethanolic extract maybe due to the presence of phyto constituents like flavonoids[17] and alkaloids.[18] Moreover they may have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and inhibition of neo vascularization.[19]

CONCLUSION

Salicia fruticosa Heyne ex Lawson belonging to family *Celestraceae* is an edible plant found in India. There are no reports for the medicinal value of *Salacia fruticosa* regarding cytotoxic and antibacterial activity of aerial part of various extracts. Considering the above in the present work *in vitro* antibacterial and short term *in vitro* cytotoxic activity of various extracts were evaluated. In preliminary phytochemical analysis of various extracts, ethanolic and aqueous extracts showed much phytoconstituents. In short term *in vitro* cytotoxic activity, the ethanolic extract showed potent cytotoxic activity against both EAC and DLA cell lines. This is the first report on cytotoxic activity of aerial part of *Salicia fruticosa*. In preliminary antibacterial activity the ethanolic extract of *Salicia fruticosa* showed minimum activity against *E.coli* (G-ve).This study has shown that the ethanolic extract of *Salicia fruticosa* contains some active constituents which are responsible for cytotoxic and antibacterial activity. Further characterisation and isolation of the extract has to be carried out to reveal the exact pharmacological activity based upon *in vivo* cytotoxic studies.

REFERENCES

1. Kharb M, Jat R.K., Gupta A. A review on medicinal plants used as a source of anticancer agents. Int. J. Drug Res. Tech, 2, 2012, 177-183.

2. Bhutani K K, Gohil V M. Natural product drug discovery research in India: Status & appraisal. Ind. J. Exp. Bio, 48, 2010, 199-207.

3. Dholwani K.K, Saluja A.K, Gupta A.R, Shah D.R. A Review on Plant – derived natural products & their analogs with

antitumor activity. Ind. J. Pharmacol Apr., 40(2), 2008, 49-58.

4. Mi Ja Chung, Cha-Kwon Chung, Yoonhwa Jeong, Seung-Shi Ham, Anticancer activity of subfractions containing pure compounds of Chaga mushroom (*Inonotus obliquus)* extract in human cancer cells and in Balbc/c mice bearing Sarcoma-180cells. Nutr Res Pract, 4, 2010, 177–182.

5. Chorawala M.R, Oza P.M, Shah G.B. Mechanisms of Anticancer Drugs Resistance: An Overview. Int. J. Pharm. Sci. Drug Res, 4(1), 2012, 1-09.

6. Ghosh A, Das B, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts. J Nat Med, 62, 2008, 259–262.

7. Grayer R, Harborne J. A survey of antifungal compounds from plants. Phy chem, 37, 1994, 19-42.

8. Benayache. S, Benayache. F, Benyahia. S. Leaf Oils of some Eucalyptus Species Growing in Algeria. J. Essent. Oil Res, 13, 2001, 210-213.

9. Benhassaini. H, and enabderrahmane. K, Chi. K. Contribution to the assessment of the antiseptic activity of essential oils and oleoresin of Pistacia tial Atlas onsome microbial sources: *Candida albicans* (ATC 20027), *Candida albicans* (ATCC 20032) and *Saccharomyces cerevisiae*. Ethnopharmacol, 30, 2003, 38-46.

10. Benjilali, B, Elaraki-Tantawi A, Ismaili-Alaoui M, Ayadi. A Study method antiseptic oils essntielles direct contact agar. Phy Ther Med, 20, 1986, 155-167.

11. Chanda. S, Rakholiya K. Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas 2011.

12. Kokate.C.K, Purohit A.P, Gokhale S.B, Textbook of Pharmacognosy 2016; 51st edition.

13. Obeidat M, Shatnawi M, Al-alawi. M, Al-Zu`bi E, Al-Dmoor H, Al-Qudah M, El-Qudah, J Otri I. Antimicrobial Activity of Crude Extracts of Some Plant Leaves. *Res. J.Microbiol,* 7, 2012, 59-67.

14. Unnikrishnan MC, Ramadasan K. Cytotoxicity of Extracts of spices to cultured cells, Nutr Cancer, 11, 1988, 251-257.

15. Dhanalakshmi Manoharan, Thenmozhi Shanmugam, Jambulingam Munuswamy. Evaluation of Antibacterial Activity of Different Solvent Extract of Medicinal plants: Cleome Viscosa Linn. Int. J. Green Pharm, 12(3), 2018, 700-703.

16. Dhanalakshmi M, Thenmozhi S, Pradeepti K G, Prabhavathi K & Priyadharshini S, A review on medicinal plants with anticancer activity, Int. J. Pharm. Biol. Arch, 8(6), 2017, 36-41.

17. Brown JP. A Review of the genetic effect of occurring flavonoids, anthraquinones and related compounds. Mutat Res, 75, 1980, 243-77.

18. Kintzios SE. Terrestrial plant derived anti-cancer agent and plant species used in anticancer research. Critic Rev Plant Sci, 25, 2006, 79-113.

19. Weber G, Shen F, Prajda N et al. Increased signal transduction activity and down regulation in human cancer cells. Anticancer Res, 16, 1996, 3271-82.

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