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Research Article

IN VITRO ANTICANCER ACTIVITY OF THE ETHANOL BARK EXTRACTS OF TAMARINDUS INDICA LINN AGAINST HT 29 CANCER CELL LINE

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A B S T R A C T

Plants remain the major source of secondary metabolites. They are used to natural drug for various diseases. In the present study focused on the potential antibacterial and anticancer activities of ethanol bark extracts of Tamarindus indicus. Hence, this study has evaluated the in vitro effects of ethanol bark extracts on human colorectal adenocarcinoma cell line (HT29). The result of present study, the maximum cytotoxicity (94.89 %) and minimum cell viability (5.11 %) were obtained the 500 µg/ml concentrations of ethanol bark extracts of T. indica. For the HT-29 cell line, the 50 % cytotoxicity concentration (CTC50) was found to be 15.6 µg/ml in ethanol extract. Further investigation, the antibacterial activity of ethanol bark extract of T. indica was found to produce a pronounced inhibition of Streptococcus pneumoniae (13mm) followed by Pseudomonas aeruginosa (12 mm), Escherichia coli (11mm), Salmonella typhi (11mm), Klebsiella pneumonia (10 mm) and Bacillus subtilis (10mm) (Table.1). The highest activity was observed in ethanol extract of T.indica against Streptococcus pneumoniae (13mm) and Pseudomonas aeruginosa (12mm). The results suggest that bark extract of T. indica possess the strong antibacterial and anti-cancer activities.

INTRODUCTION

Plants remain the most common source of medicinal properties. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects (Maghrani *et al.,* 2005). Herbal medicines remain the major source of health care for the world's population. WHO has recognized herbal medicine as an essential building block for primary health care. Plant derived agents are being used for the treatment of cancer. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents and the drugs under clinical phytomedicines has increased dramatically in the last two decades (Rao *et al.,* 2004). More than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to include apoptosis in various cancer cells of human originals; there is an urgent need to develop much effective and less toxic drugs (Rosangkima and Prsad, 2004).Tamarind is leguminous trees of genus *Tamarindus* which is monotypic with only species *indicum* (Bentley and Trimen, 2004). *Tamarindus indica* having family Fabaceae and sub-family Caesalpinaceae is a tropical evergreen tree native to Africa and Southern Asia (Kirtikar and Basu, 1987).

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Its various parts such as seeds, root, leaves, bark and fruits have been extremely used in traditional India and African medication (Gunasena *et al.,* 2000). *T. indica* is also rich in the minerals like potassium, phosphorus, calcium and magnesium. *T. indica* is an important food resource for many countries like India, Pakistan, Srilanka, Nepal, Thailand etc. The flower and leaf are eaten as vegetables (Lewis *et al.,* 2005).

T. indica is widely used in traditional medicine in many countries for the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders. Multipurpose species like tamarind (*Tamarindus indica* L.), have an important role in local economies by supplementing the local diet and entering into traditional therapies (Sidhuraju *et al.,* 1995). Various parts have been expansively studied in terms of the pharmacological activity potent hypoglycaemic, cholesterolemic (Khanzada *et al.,* 2008), hypolipidemic, antioxidant (Tsuda *et al.,* 1994), antihepatotoxic, anti-inflammatory (Rimbu *et al.,* 1999) and antidiabetic (Maiti *et al.,* 2004) properties. The phytochemicals study in the human system due to their therapeutic properties cure many ailments which cannot be cured by the modern drugs. This may help to advance safer antimicrobial drugs. Based on the observation, hence the present study focused on of the potential effects of antibacterial and anticancer activities of ethanol bark extract of *T. indicus* and the results have been discussed in details.

MATERIALS AND METHODS

Collection of Sample

The bark of *Tamarindus indica L.* was collected wildly from Thanjavur rural regions, Tamil Nadu, India. The collected bark samples were washed under running tap water to remove waste materials and excess of water was drained off. They were sliced into small pieces and shade dried for few days. The shade dried barks were powdered by grinding machine. The powdered samples were then stored in refrigerator for further use.

Preparation of Extract

Bark powdered sample extract was prepared by Soxhlet extraction method. About 20gm of dried sample was extracted with 250ml of ethanol. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colorless. Then the extract was collected and stored at the refrigerator for further studies. Dried extract was kept in refrigerator at 4ºC till future use.

Antibacterial Activity

The antimicrobial activity of the ethanol bark extract *T. indica* was tested on Muller-Hinton agar (MHA) plates by agar well diffusion method (NCCLS, 1993). The following human bacterial pathogens were used; they are *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Streptococcus pneumoniae* which were collected from Department of Microbiology, Marudupandiyar College, Thanjavur, Tamilnadu, India. For this, Muller hinton agar plates (MHA) were prepared separately and overnight culture of test bacterial pathogens were seeded individually over the surface of MHA plates using sterile cotton swabs. Thereafter wells of 6 mm diameter were made over MHA plates using sterile cork borer. The wells were loaded with 100 µl of crude extracts which was prepared in dimethyl sulphoxide. The plates were then incubated at 37°C for 24h and growth inhibitory activity in terms of zone of inhibition (mm) formed around each well was measured and recorded.

Minimum Inhibitory Concentration (MIC)

MIC of ethanol extracts of *T. indica* was determined in 96-well microtiter plates by Eloff (1998). Before initiating the assay, various concentrations of ethanol extracts was prepared for bacterial cultures. In the same way, different concentration of streptomycin prepared. Then extract and control samples were coated individually in 6 wells of 96 well plates. On the other hand, six wells free of extract and antibiotic served as negative control. Then 100µl of each bacterial (2×10^8 cells ml^{-1}) was added to other respective wells and incubated at 37°C. The bacterial growth was observed after 24h. The lowest concentration of extract was positive control. There is no bacterial growth was observed in at least 4 of the 6 wells was recorded as the MIC.

Anticancer Activity

Anticancer activity of ethanolic bark extract of *T. indica* was determined through MTT cytotoxicity assay (Mossman, 1983) by using human colorectal adenocarcinoma cell line (HT29). The cell line (HT29) was obtained from King Institute of Preventive Medicine, Guindy, Chennai, Tamil Nadu. The tissue culture bottles that showed confluent monolayer were selected by observing them under an inverted microscope. Growth medium was removed from the bottle, washed with PBS/MEM without FCS and 5 mL of TPVG was added dispersing evenly on the monolayer and left in contact with the cells for 2-3 minutes until there is a cloudy appearance on the monolayer. TPVG was removed and the cells were resuspended in 5 ml of growth medium (MEM containing 10%FCS). The suspension was aspirated few times to break the cell clumps. The cell suspension was then transferred to a 24 well plate. 1ml (1 lakh cells/ml) of the cell suspension was added to each well. The plate was then incubated in a CO_2 incubator maintained with 5% CO_2.

RESULTS AND DISCUSSION

Antibacterial Activities

Among the tested bacterial human pathogens, the ethanol bark extract of *T. indica* was found to produce a pronounced inhibition of *Streptococcus pneumoniae* (13mm) followed by *Pseudomonas aeruginosa* (12 mm), *Escherichia coli* (11mm), *Salmonella typhi* (11mm), *Klebsiella pneumonia* (10 mm) and *Bacillus subtilis* (10mm) (Table.1). The highest activity was observed in ethanol extract of *T.indica* against Streptococcus *pneumoniae* (13mm) and *Pseudomonas aeruginosa* (12mm). The demonstration of antibacterial activity against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds (Srinivasan *et al.,* 2001). This will be of immense advantage in fighting the menace of antibiotic refractive pathogens that are so prevalent in recent times. The result also showed that the stem bark extracts are more effective than the leaf extracts. This may be due to the fact that the stem bark was more developed and mature than the leaves and may contain fewer pigments and other phenolics which have been reported to interfere with the antimicrobial activity of the extracts. Similar to this investigation, an earlier study recorded methanol extracts of Tamarind fruit showed appreciable antimicrobial activity against many food borne pathogens (Escalona-Arranz *et al.,* 2010).

Minimum Inhibitory Concentrations (MIC)

MIC was determined by using different concentrations of ethanol extract of *T. indica* ranged between 50 to 200 µg/ml. Lowest MIC (98 µg/ml) was observed in the ethanol extract against Streptococcus *pneumonia* followed by *E.coli* (112 µg/ml), whereas the highest MIC (148 µg/ml) was noted in ethanol extract against *Bacillus subtilis* and *Pseudomonas aeruginosa* (132 µg/ml) (Fig. 1). Moderate MIC value was noted in the ethanolic extract of *T. indica* against *Salmonella typhi* (124µg/mL) and *Klebsiella pneumonia* (122 µg/mL). In previous study, the highest MIC and MBC values of *Staphylococcus aureus* is an indication that either the plant extracts are less effective on some gram positive bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication of the efficacy of the plant extracts (Doughari, 2006).

In Vitro Anticancer Activity

The test for cytotoxicity of the ethanol bark extract of *T. indica* was conducted using the HT-29 (Human colorectal adenocarcinoma cell line) by using MTT assay. Growth of cells in the presence of the extracts were quantities by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-

2-thiazolyl)-2, 5-diphenyl-2H-terazolium bromide (MTT) to a blue formazan product. Different test concentrations ranging from 3.9 to 500 µg/ml of the ethanol extract was taken to study the cytotoxicity. The anticancer activity of ethanolic bark extract of *T.indica* was measured using MTT assay.

Cytotoxic Activity on HT-29 Cancer Cell Line

HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology and it's used extensively in biological and cancer research. Though HT-29 cells can proliferate in cell culture lacking growth factors with a doubling time of around four days, the doubling time can be reduced to one day with added fatal bovine serum. The cells have high glucose consumption. In the present study reported that the highest percentage of cytotoxic activity against HT-29 cell line was showed the 500 µg/ml concentration of ethanol extracts (Table.2). Plant extract from natural resources usually do not attack cancer cells directly but produce their antitumor effects by activating different immune responses in the host. The result of present study, the maximum cytotoxicity (94.89 %) and minimum cell viability (5.11 %) were obtained the 500 µg/ml concentrations of ethanolic extracts of *T. indica*. For the HT-29 cell line, the 50 % cytotoxicity concentration (CTC_{50}) was found to be 15.6 µg/ml in ethanol extract. In the previous report, *T. indica* seeds can enhance the antioxidant activities of treated cancer cells which can provide protection against oxidative damage (Aravind *et al.,* 2012). Hussein *et al.,* (2017) also suggested that seeds methanolic extract of *Tamarindus indica* could be developed as an adjuvant for cancer management.

CONCLUSION

In the present study, the ethanol bark extract from *T. indica* showed significant properties of cyotoxicity activities against human cancer cell line and the appreciable antibacterial activities against the clinical human pathogens evidenced its importance in the field of therapeutics. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of various diseases. The present study concluded that the bark extract from *T. indica* has variety of biologically active molecules which can be used as a source of antibiotics. Further study needs the purification of active compounds and structural elucidation can be used for drug discovery.

Table 1 Antibacterial activity of ethanol bark extract of *T. indica* against bacterial pathogens

S.NO.	Bacterial pathogens	Zone of inhibition
1	*Escherichia coli*	11mm
2	*Salmonella typhi*	11mm
3	*Klebsiella pneumoniae*	10mm
4	*Streptococcus pneumoniae*	13mm
5	*Bacillus subtilis*	10mm
6	*Pseudomonas aeruginosa*	12mm

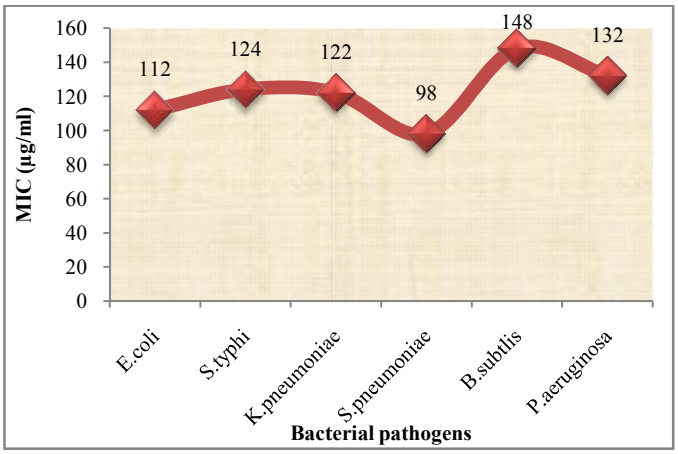

Fig 1 Minimum inhibitory concentration (MIC) of ethanol bark extract of *T. indica* against bacterial pathogens

Table 2 Cytotoxicity of ethanol bark extracts of *T. indica* on HT 29 cell line

S. No.	Extract concentration (µg/ml)	Diluent (MEM)	Cell inhibition/ cytotoxicity (%)
1	500	1:1	94.89
2	250	1:2	72.13
3	125	1:4	60.99
4	62.5	1:8	55.32
5	31.3	1:16	51.98
6	15.6	1:32	50.00
7	7.8	1:64	49.29
8	3.9	1:128	48.75
9	Cell control	Neat	0.02

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