

SCREENING OF ANTI CANCER AND ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF ANNONA MURICATA LEAF AND BARK

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ABSTRACT

In the present study, an attempt has been made to congregate the traditional, pharmacological studies done on an important medicinal plant *Annona muricata*, (Family annonaceae). Medicinal plants are the oldest known healthcare agent. Their importance is still growing although it varies depending on the ethnological, medical and historical background of each place. Medicinal plants are also important for pharmacological research and drug development. The methanolic extract of *Gordonia obtusa* (Theaceae) was evaluated for its effects on growth in MCF-7 breast cancer cell lines using MTT assay. In these cell lines studied, the extract decreased cell viability, inhibited cell proliferation, and induced cell death in a dose dependent manner. The present study demonstrated that methanolic leaf extract of *Annona muricata* showed more anti-cancer activity, compared to its bark extract. The methanolic leaf and bark extracts of *Annona muricata* was tested for its antibacterial effect successfully showed zone of inhibition against the tested microorganism. The maximum zone of inhibition was shown by the methanolic leaf extract of *Annona muricata* against *Escherichia coli*. So it could be a reliable source of potent pharmacophore for treatment of disease like cancer.

Keywords: *Annona muricata*, anti-cancer activity, MTT assay, antibacterial activity

INTRODUCTION

Cancer is the leading cause of mortality in developed countries and the second leading cause of death in developing countries. Now cancer is a common disease, and more than one in three people will develop some form of cancer in their lifetime. Although more than a quarter of a million people develop cancer each year in the UK. Cancer is associated with aging and has increased as life expectancy has increased. Median age at diagnosis for all cancers in the United States is 67 years. Tobacco use is one of the common causes of cancer associated with lung, head and neck, esophageal, gastric, pancreatic, kidney and bladder cancer.¹

The Annonaceae is a very large family of plants comprising about 120 genera and more than 2000 species. On the basis of morphology and habitat, it is a very homogenous family as source of edible fruits and oils. The Annonaceae seems to be one of the least chemically as well as pharmacologically known families compared with its large size. As source of edible fruits and oils, this family has economic importance. Seed oils of certain Annonaceae plants are used in soap production and as edible oils. Flowers of some Annonaceae plants are used in perfumery and many members of this family are used in folk medicine to treat various types of tumors and cancers. The earlier studies on *Annona* species showed that this family is a potent source of a wide variety of secondary metabolites belonging to several categories.²

Annona muricata L. commonly known as graviola or soursop, belongs to the family of Annonaceae. It is a typical tropical tree with heart shaped edible fruits and widely distributed in most of the tropical countries. The leaves are lanceolate with glossy and dark green in color had been traditionally used to treat headaches, hypertension, cough, asthma and used as antispasmodic, sedative and nervine for heart condition. Previous reports over the years have demonstrated that the leaf, bark, root, stem, and fruit

seed extracts of *Annona muricata* are anti-bacterial. *Annona muricata* (soursop) is a potent anticancer plant of Annonaceae family. The therapeutic potentials of the n-butanolic extract of *Annona muricata* were studied on WRL-68, MDA-MB-435S and HaCaT cell lines. Since most of the chemotherapeutic drugs affect normal cells as well, WRL- 68 cells were analysed for the relative cytotoxic response in with comparison that quantified in MDA-MB-435S and HaCaT cell lines. n- Butanolic leaf extracts of *A. muricata* possess significant anticancer potentials in human cancerous cells. Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.³

MATERIALS AND METHODS

Methods

Collection

The leaves and barks of *Annona muricata* were collected from Thalappady, Kottayam district, Kerala, India in the month of November 2014. The plant material was identified and authenticated by Mr. Rogimon P Thomas, Assistant professor, Dept. of Botany, CMS College, Kottayam.

Preparation of extract 17

The barks and leaves were dried in the shade at room temperature. The methanolic extract was obtained using 30 gm of dried powdered plant material with methanol (400 ml) in soxhlet extraction apparatus under reflux for 10 hrs. The ethanolic extracts were concentrated.

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PREPARATION OF CULTURE MEDIA

Nutrient broth

Beef extract- 10gm

Peptone - 10 gm

Sodium chloride - 5 gm

Distilled water - 1000 ml

Suspended 10gm of peptone, 10gm of beef extract and 5gm of sodium chloride in 1000ml distilled water and the pH was adjusted to 7.2 ± 0.2 at 25°C . It was then sterilized in an autoclave at 121°C for 15 minutes. The prepared medium was clear and yellowish brown. Broth medium was used for sub culturing.

Nutrient agar medium

Beef extract-10gm

Peptone-10gm

Sodium chloride -5gm

Agar -20gm

Distilled water-Up to 1000ml

Components were accurately weighed and dissolved with the aid of heat. The pH was adjusted to 7.2 ± 0.2 with 5M sodium hydroxide and boiled for 10 minutes. Agar was added with gentle warming and continuous stirring. It was then sterilized in an autoclave at 121°C for 15 minutes.

INOCULATION

Inoculated the nutrient broth with the test organisms (i.e., *Escherichia coli*) and incubated for 24 hours at $35 - 37^{\circ}\text{C}$.

PREPARATION OF SAMPLE

Dilution of the sample under test (i.e., extracts of *Annonamuricata*) and the standard antibiotic preparation were made in geometric proportion.

ANTIBACTERIAL ACTIVITY STUDIES

The antibacterial activity studies were conducted by using well diffusion method. Plates were prepared by pouring the agar, seeding them with the test organism and allowing setting. Cups cut are made in medium using a sterile cork borer about 10mm in diameter. $50\mu\text{l}$ of standard antibiotic solution ($10\mu\text{g}$) and sample were applied into the cups. Plates were left at room temperature for 1-2 hours as period of pre-incubation diffusion. The plates were then incubated for about 18 hours at about 37°C and diameter or area of the circular inhibition zone was measured using a zone reader.

In-vitro anti cancer studies

DETERMINATION OF IN-VITRO ANTIPROLIFERATIVE EFFECT OF EXTRACTS ON CULTURED MCF-7 CELL LINE 19

MCF-7 breast cancer cell lines were purchased from NCCS Pune were maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5 %

CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, GERMANY). The cells were trypsinized ($500\mu\text{l}$ of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia)) for 2 minutes and passaged to T flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of $6.25 \mu\text{g}/\text{ml}$, $12.5 \mu\text{g}/\text{ml}$, $25 \mu\text{g}/\text{ml}$, $50 \mu\text{g}/\text{ml}$ and $100\mu\text{g}/\text{ml}$ from a stock of $1\text{mg}/\text{ml}$ and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation.

MTT ASSAY 20

MTT is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilised formazan product was measured at 540nm . Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cells was washed with 1x PBS and then added $30 \mu\text{l}$ of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 37°C for 3 hours. MTT was removed by washing with 1x PBS and $200\mu\text{l}$ of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).

RESULTS AND DISCUSSION

Preparation of extract

The methanolic extracts was concentrated and dried in china dish to obtain a pasty mass

Antibacterial activity studies

Table 1. Zone of inhibition of leaf and bark extracts of *Annonamuricata* and standard against *E.coli*

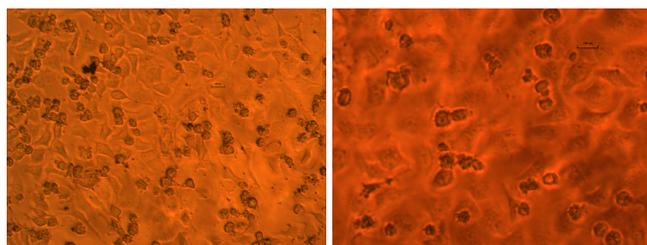
| Sample | Zone of inhibition |
|--------------|--------------------|
| Leaf extract | 15 mm |
| Bark extract | 10 mm |
| Standard | 25 mm |

The plates were then incubated for about 18 hours at about 37°C and diameter or area of the circular inhibition zone was measured using a zone reader. Observations were made for the zone of inhibition around discs and zone of inhibition of leaf extract was 15 mm and bark extract was 10 mm. Leaf extract shows more anti bacterial action than bark against *E.coli* by using well diffusion method

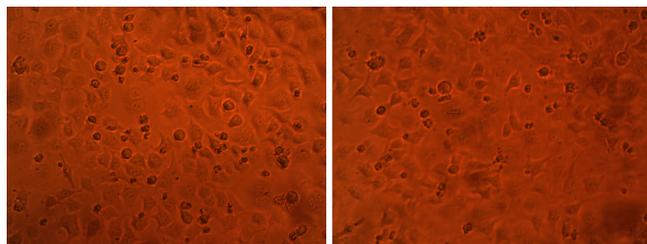
In-vitro anti cancer studies

The present investigation was mainly centered to evaluate the anti-cancer activity of *Annona muricata* using cell lines. In vitro confirmation of this cytotoxicity of the *Annona muricata* extract on MCF-7 breast cancer cell lines were reported. This extract was screened for its cytotoxicity against cell lines at different concentrations and determined by MTT assay.

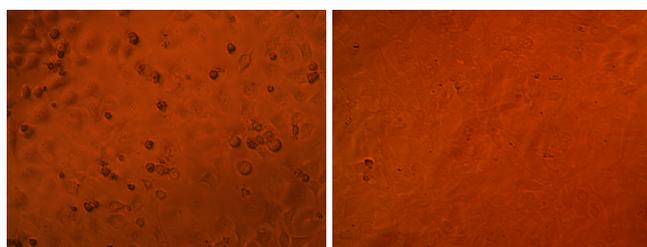
Fig 2. Anti cancer activity of bark extract at different sample concentration



6.25 µg/ml 12.5 µg/ml



25 µg/ml 50 µg/ml



100µg/ml Control

Table 2: % viability = (OD of Test/ OD of Control) X 100 Control OD: 0.4597

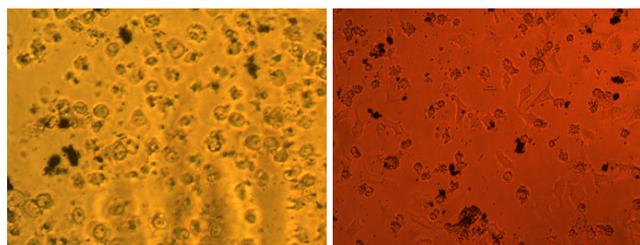
| Sample Concentration (µg/ml) | Average OD at 540nm | Percentage Viability |
|------------------------------|---------------------|----------------------|
| 6.25 | 0.4305 | 97.72985 |
| 12.5 | 0.4217 | 95.73212 |
| 25 | 0.4053 | 92.00908 |
| 50 | 0.3965 | 90.01135 |
| 100 | 0.3823 | 86.78774 |

The above data showed maximum anti cancer activity of bark extract at a concentration of 100(µg/ml). The percentage viability was found to be 86.78774 at the concentration of 100 µg/ml .Therefore bark extract shows less anticancer property.

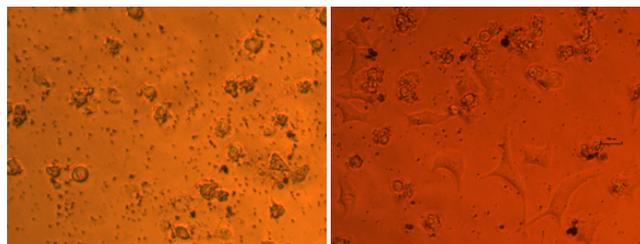
Table 3: % viability = (OD of Test/ OD of Control) X 100 Control OD: 0.4597

| Sample Concentration (µg/ml) | Average OD at 540nm | Percentage Viability |
|------------------------------|---------------------|----------------------|
| L | | |
| 6.25 | 0.2543 | 57.72985 |
| 12.5 | 0.1891 | 42.92849 |
| 25 | 0.1573 | 35.70942 |
| 50 | 0.1285 | 29.1714 |
| 100 | 0.1057 | 23.99546 |

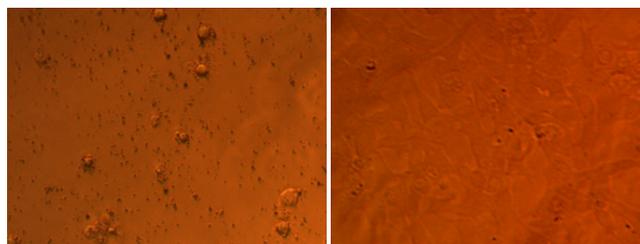
Fig 3. Anti cancer activity of leaf extract at different sample concentration



6.25µg/ml 12.5µg/ml



25µg/ml 50µg/ml



100µg/ml Control

The above data showed maximum anti cancer activity of leaf extract at a concentration of 100(µg/ml). The percentage viability was found to be 23.99546 at the concentration of 100 µg/ml. Therefore leaf extract shows good anticancer property. Leaf extract of Annona muricata posses significant anticancer potentials in human cancerous cells.

CONCLUSION

Annona muricata leaves and bark have been shown to possess number of biological activities. The methanolic leaf and bark extracts of Annona muricata was tested for its antibacterial effect successfully showed zone of inhibition against the tested microorganism (Escherichia coli). The maximum zone of inhibition was shown by the methanolic leaf extract of Annona muricata against Escherichia coli. The scopes of using the plant Annona muricata in the development of new pharmaceutical agents are having a new hope in the present study

The present study demonstrated that methanolic leaf extract of Annona muricata presented more anti-cancer activity, compared to its bark extract. As this drug is unexplored further studies on its active constituents, its isolation, purification and characterization of the phytochemicals along with investigations is needed to provide some additional insight into the mechanism of action by which it exhibit anti-tumor activity with a view to obtain useful chemotherapeutic agent.

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