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Research Article

Invitro studies on anticancer activity of combined ethanolic extract of date seed and custard apple seed

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Abstract

Function against the cancerous cell by surgical or non surgical method is known as anticancer activity. Surgical method causes some dangerous side effect and it may not cure fully. Non surgical methods, especially herbal based treatment is better than radio therapy. In the present article, date seed and custard apple seed compounds were evaluated for in vitro anticancer activity with *A549* human lung cancer cell line. The date seed and custard apple seed are extracted after shade dry and electrically ground. The IC_{50}value of date seed and custard apple seed extract compounds were 31.2 µg/ml. The compounds in date seed and custard apple seed were identified by Gas Chromatography with Mass Spectroscopy.

Keywords: Date seed; Apple seed; Anticancer activity; A549 human lung cancer cell line.

Introduction

Diseases are an unpleasant and uncomfortwhich permeate easily in our body when conditions are favorable. But, good medicine is an important it takes some time course to inactivate or degrade the disease causing foreign body from our body. Plants, especially herbal plants are great bless of nature for all organisms. In the herbal plants various parts (root, stem, leaves, flowers, fruits, seeds etc.,) have medicinal derivatives.In this study the anticancer activity of combined *Phoenix silvestris l Rox* seed and *Annonasquamosa l* seed (Date seed and Custard apple seed) was analyzed. The potential health benefits of date and custard apple fruits and vegetables have been partially attributed to their polyphenols contents, in particular flavonoids that have received much attention in the literature over the past decade for its bio-logical effects (Hertog et al., 1993; Hertog et al., 1995). Extracts of the leaf, fruit, seeds and bark were studied for their antibacterial effect against the Gram positive strains *Staphylococcus aureuss*and*Streptococcus pyogenes*and the Gram negative strains *Escherichia coli* and *Pseudomonas aeruginosa* (Ramesa and Sooad, 2012). Phoenix silvestris seed have effective against active cancerous cell and also antioxidant (Bhuvan et al., 2010; Jonsson et al., 1997; Adam et al., 2008; Habib et al., 2011)

AnnonasquamosaLinn is an important medicinal plant with diverse pharmacological spectrum. Fewnovel chemical constituent isolated from the *A.squamosa*showed anti-cancer, anti-HIV andanti-diabetic(type 2 diabetic), hepatoprotective activity, Anthelmic activityproperties too (Rajasekar, 2011; Chitra et al., 2009; Pardhasaradhi et al., 2004; Wu et al., 1996; Mohamed Saleem et al., 2008; Souza et al., 2008). Unfortunately seeds are not to be eaten directlybecause of hardness though they have medicinal derivatives but it can be processed in such a way to use for the cytotoxicity effect on cancerous cells by using scientific methods (Extraction, GC-MS analysis, MTT assay cytotoxicity test) by bring a person from sickness to healthy.Cancer is a class of diseases characterized by out-of-control cell growth. It harms the body by damaging the cells and divide uncontrollably to form masses of tissue called tumors except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood

stream. Tumors can grow and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function (Sieuwerts et al., 1995; Saad et al., 2006).

Methods and materials

Collection of sample

Phoenix silvestris l Rox seed and *Annonasquamosa l* seed (Date seed and Custard apple seed) were collected from Jawadhu Hills. It is the northwestern part of Tiruvannamalai, and a part of the Eastern Ghats.

Reagents

MEM (Minimal Essential Medium) was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, methyl thiazolyldiphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

Cell line and culture

A549 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Extraction

Date seeds and Custard apple seeds were collected manually then washed by using distilled water. After washing seeds were dried under shade condition around 2 weeks. Dried seeds were electrically grained for make a form of powder. 5 g of each Date seed and Custard apple seed powder was taken in a clean sterilized 500 ml Borosil beaker then add 100 ml of 99.9% absolute ethanol and allow to magnetic homogenized by stirring at 60°C-65°C in 15mins under 100 rpm. Filter the supernatant by using Whattman #1 filter paper.

GC-MS Analysis

Analysis was performed by GC-MS with an ion trap detector. Injection volumes of 1 microliter were made, split less, on a 60 m DB-5ms column with the oven temperature program starting at 80°C increasing to 250°C at 10°C/min with helium carrier gas flow 1.5 ml/min. The detector was operated in selective ion mode (SIM).

Invitro assay analysis for anticancer activity (MTT assay)

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37^0C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells (Sieuwerts et al., 1995). The absorbance at 570 nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% cell viability = (A549 of treated cells / A549 of control cells) × 100

Results and discussions

Invitro activity was carried out with compounds identfied in the date seed and custard apple seed extract. The components identified in the mixed extraxts are analysed through GC-MS analysis. Identified components are 3-Buten-2-one-4-(2,5,6,6-tetramethyl-2cyclohexen-1-yl), Benzene aceticacid, 2-carboxy,3-methoxy, Glycine, N(phenylacetyl)ethyl ester, Hexadecanoic acid, ethyl ester, 1-(4-(2-(4-Methoxymethylphenyl) vinyl)phenyl) ethanone, Ethyl oleate, Octadecanoic acid, 7-metyh, methyl ester, 2-Cyclohexyl-1Hnaphth (2,3d) Imidazole-4,9 dione, 3-(Benztlidene-amino)-2phenoxymethyl-3H-quinazolin-4-one, Estra-1,3,5(10)-trien-17-ol, 3-methoxy-17-(2-methylallyl), 1,8 Anthracenediol, diacetate, which are listed in Table 1 and Fig 1.

The (inhibition concentration) IC_{50} value was determined from the activity. The IC_{50} value of the compound was 15.6 µg/ml. The result of MTT assay at the concentration of 1000 µg/ml the cell viability was reduced to 5.08% shown in Fig. 2.

Table 1. Mass spectroscopy analysis for date and custard apple seed ethanol extract

S. NO.	RT	Name of compounds
1	14.3	3-Buten-2-one-4-(2,5,6,6-tetramethyl-2cyclohexen-1-yl)
2	16.5	Benzene aceticacid, 2-carboxy,3-methoxy
3	17.68	Glycine,N(phenylacetyl)ethyl ester
4	18.18	Hexadecanoic acid, ethyl ester
5	19.15	1-(4-(2-(4-Methoxymethylphenyl)vinyl)phenyl)ethanone
6	19.72	Ethyl oleate
7	19.9	Octadecanoic acid, 7-metyh, methyl ester
8	21.78	2-Cyclohexyl-1Hnaphth(2,3d)Imidazole-4,9 dione
9	23.38	1,8 Anthracenediol, diacetate
10	25.53	Estra-1,3,5(10)-trien-17-ol,3-methoxy-17-(2-methylallyl)
11	28.47	3-(Benztlidene-amino)-2phenoxymethyl-3H-quinazolin-4-one

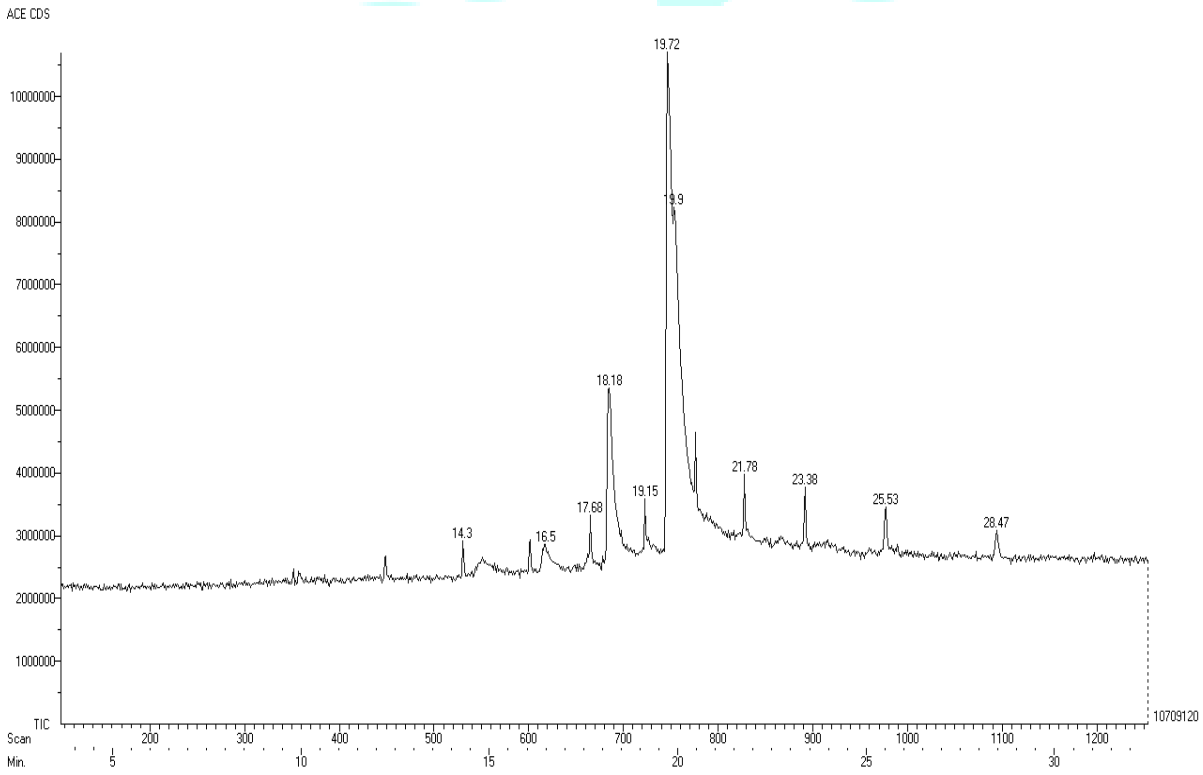

Fig. 1. GC-MS Analysis for combined date seed and custard apple seed ethanolic extract

MTT Assay

Graph was plotted using the % of cell viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in the assay to compare the full cell viability assessments. It is identified that these compound have excellent therapeutic agent in controlling the proliferation of A549 lung cancer cell line. Fig. 2 shows the decreasing cell viability concentration of A549 cancer cell line gradually. Fig. 3. Shows the morpology of A549 cancer cell line treated with varied concentration of Date seed and Custard apple seed extract.

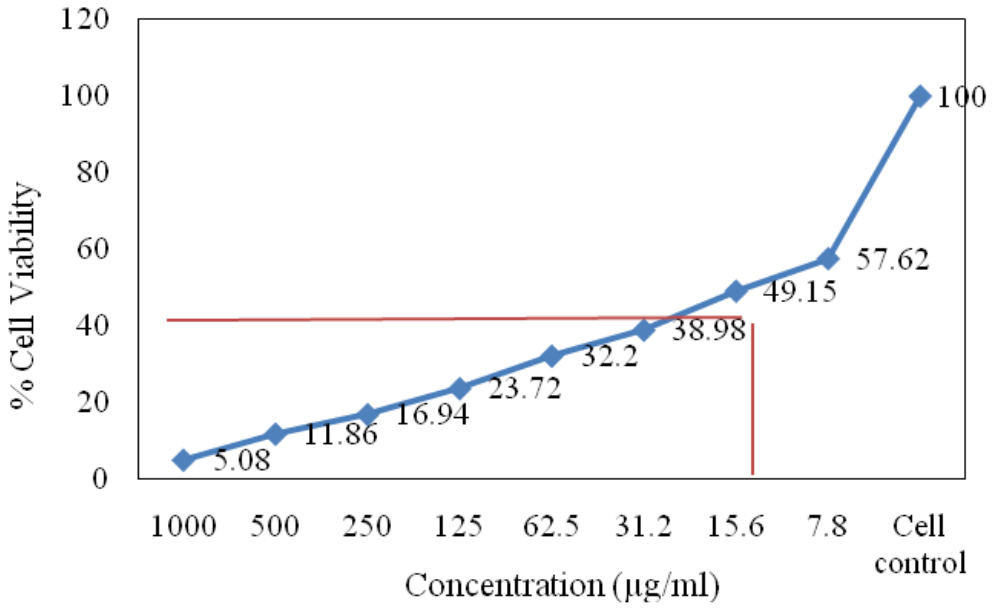

Fig. 2. *A549 C*ell line cell viability versus concentration of Date seed and Custard apple seed extract

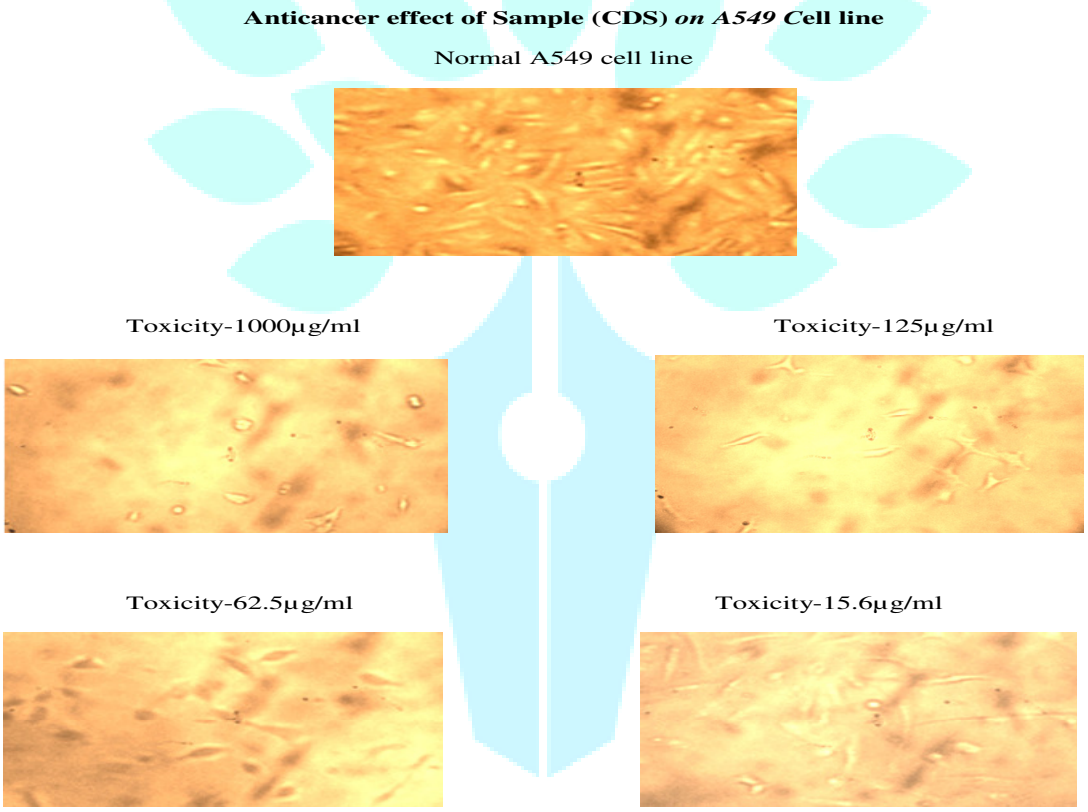

Fig. 3. Anti cancer effect of Date seed and Custard apple seed extract on A549 cell line

Conclusions

Ancient literatures have already mentioned herbal remediation for a number of human ailments. *Annona squamosa L* which is commonly known as custard apple and *Phoenix silvestris* known as date seed in English having various pharmacological activity. Many compounds have been isolated and reported from the extract of various part of the plant possessing good pharmacological activity. The studies performed on the custard apple seed and date seed extract also evidence for anticancer activity.

Conflict of Interest

Authors declare there are no conflicts of interest.

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