ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 1, 2014

ISSN - 0974-2441

Research Article

INVITRO ANTI-OXIDANT AND ANTICANCER ACTIVITY OF *MURRAYA KOENIGII* AGAINST HUMAN COLON CANCER HT- 29 CELL LINES

D.SUMALATHA[1]*, T.G.NITHYA[2]

[1]Department of Biotechnology,Valliammal College for Women, Chennai, India, [2]Department of Biotechnology, Faculty of Science and Humanities, SRM University Kattankulathur, India. Email: sumadarsh@gmail.com

Received: 2December 2013, Revised and Accepted: 26 December 2013

ABSTRACT

Objective: The main aim of the study was to screen the ethanolic extract of *Murraya koenigii* leaves for its *invitro* anti-oxidant and anticancer activity and its efficacy against H-29 Cell lines.
Methods: Ethanolic extract of *Murraya koenigii* were prepared and assayed for the presence of phytochemicals. In vitro antioxidant assay were performed by DPPH Radical and FRAP assay. The effect of ethanolic extracts on HT-29 cancer cell lines were evaluated by MTT colorimetric assay.
Results: The preliminary phytochemical screening of ethanolic extract showed the presence of significant secondary metabolites. The efficacy of *Murraya koenigii* against HT-29 cell line showed that the incubation of cancer cells reduced the viability of HT-29 cells and the dead cells were significantly increased with high extract concentration .Hence ethanolic extract of *Murraya koenigii* exhibited high cytotoxicity.
Conclusion : Even at very low concentration *Murraya koenigii* showed high efficacy.In conclusion *Murraya koenigii* possess significant antioxidant activity and anticancer activity.

Keywords: *Murraya koenigii*,anti oxidant,anticancer,colon cancer,HT 29 cell lines.

INTRODUCTION

Cancer is an uncontrolled growth and spread of abnormal cells, associated with dysregulation of apoptosis, a programmed cell death. Currently, one in four deaths in the United States is due to cancer.When ranked within age groups, cancer is one of the five leading causes of death amongst both males and females and the single largest cause of death worldwide .By 2015 cancer morbidity may climb to around nine million world-wide [1].According to World health organization, more than 10 million new cases of cancer are diagnosed every year, and the statistical trends indicate that this number would double by 2020 [2].This growing trend indicates

deficiency in the present cancer therapies which include surgical operation, radiotherapy and chemotherapy. We suppose that plants are the best alternative, as they provide an inexhaustible pool of efficacious agents for treating disease. Phytochemicals have always been sought after because of their inherent potential to cure disease, as demonstrated by ancient medicinal practices [3].Most of the current anti-cancer drugs are derived from plant sources, which act through different pathways converging ultimately into activation of apoptosis in cancer cells leading to cell cytotoxicity.[4]. Herbal medicines in treatment of cancer as complementary and alternative therapy are accepted increasingly with growing scientific evidences of biomedical research and clinical trials. Anticancer drugs discovered from herbal medicines have a long history and some of them have been used in clinical setting as a conventional anticancer drug[5].Colorectal cancer, also known as colon cancer or bowel cancer, is a cancer caused by uncontrolled cell growth in the colon or rectum, or in the appendix. Symptoms of colorectal cancer typically include rectal bleeding and anemia which are sometimes associated with weight loss and changes in bowel habits. Colorectal cancer is the third most commonly diagnosed cancer in the world, but it is more common in developed countries. Around 60% of cases were diagnosed in the developed countries. It is estimated that worldwide, in 2008, 1.23 million new cases of colorectal cancer were clinically diagnosed, and 608,000 people died of the disease.[6].

Murraya koenigii, commonly known as *curry leaf* is a medicinally important herb mainly from Asian origin has vast number of therapeutic applications such as in bronchial disorders, piles,vomiting, skin diseases etc[Table 1]. The medicinal values have been studied especially in leaf, stem, bark and oil. *Murraya koenigii* leaves are rich in polyphenols, inhibit the proteolytic activity of the cancer cell proteasome, and causes cell death[7]. HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology.These cells are sensitive to the chemotherapeutic drugs 5-fluorouracil and oxaliplatin, which are standard treatment options for colorectal cancer. In addition to being a xenograft tumor model for colorectal cancer, the HT-29 cell line is also used as an *in-vitro* model to study absorption, transport, and secretion by intestinal cells[8]. Therefore, in the present study we analysed *Murraya koenigii* leaves for the presence of bioactive components and also evaluated *invitro* anti cancer activity against HT-29 cell lines.

Table 1: Taxonomy of Plant

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliospida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	Murraya J.Koenig ex L
Species	Murraya Koenigii L. Spreng.

MATERIALS AND METHODS

Collection of Sample

Murraya koenigii leaves were collected from Chennai, Tamilnadu (India) and authenticated.The sample was air dried.

Preparation of Extract

The leaves were air dried and powered with a mechanical grinder, passing through a sieve and stored in a airtight container.Then 25gms of air dried powder were continuously refluxed with ethanol at 45˚C for 3hrs using soxhlet apparatus[9].The mixture were filtered. The filtrates were evaporated using vacuum rotary

evaporator & air dried at 40°C. The stock solution of crude ethanolic extract were prepared by diluting the dried extracts with 0.25% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 100mg/ml.

Phytochemical screening

The qualitative tests were carried out in the etanolic extract of *Murraya koenigii* using standard procedures[10-12].The extract was analysed for the presence of significant secondary metabolites *viz* Flavonoids, Alkaloids, Saponins, Cardiac glycosides, Tannins, Anthraquinone and Steroids [Table 2].

Table 2: Phytochemical Analysis of *Murraya Koenigii*

Compounds	Analysis
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Steroid	+
Anthraquinone	--
+ Present	**--Absent**

***In vitro* Antioxidant Assay**

DPPH Radical Assay

DPPH test is based on the ability of DPPH a stable free radical, to decolourize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action .The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517nm and also for a visible deep purple colour.When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The ability of the extracts to scavenge DPPH free radicals were determined by standard method[13].Different concentration of the test sample ranging 25 - 125 µg/ml were placed in a cuvette and 0.5ml of 100mm methanolic solution of DPPH was added. Mixtures were vigorously shaken and allowed for30min incubation in dark.Ascorbic acid was used as control.The absorbance was then measured at 517nm. Inhibition percentage of DPPH radical was calculated using following formula

$$\text{DPPH radical \%} = \frac{(1- \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

FRAP Assay

(a) 3.1g of sodium acetate trihydrate was weighed and to that add16 ml of glacial acetic acid were added and the made up to 1 L using distilled water.

(b)10 mM of TPTZ (2, 4, 6-tripyridyl- *s*- triazine) were dissolved in 40mM HCl.

(c) 20mMof $FeCl_3.6H_2O$.

The working FRAP reagent was prepared by mixing the composition of a b & c in the ratio of 10:1:1 at the time of use. Ascorbic acid was used as control[14].The ethanolic extract of *Murraya koenigii* was dissolved in methanol in 0.5mg/ml concentration. 4.5ml of freshly prepared FRAP reagent was added to the the prepared extract and stirred well. After 5 mins absorbance was measured at 593 nm using FRAP working solution as blank[15].The relative activity of the sample was compared with Ascorbic acid. FRAP value was calculated using the formula.

FRAP Value

$$\frac{\text{Change in absorbance of sample from 0-4 mins}}{\text{Change in absorbance of standard from 0-4 min}} \times \text{FRAP value of Std}$$

***In vitro* Cytotoxicity Assay**

Cell culture and experiment design

HT-29 cells were obtained from King's Institute, Chennai. The cells were grown and maintained in a humified incubator at 37°C under 5% Co2 atmosphere in MEM medium (Minimal Essential Media) supplemented with TPVG & 10% Fetal calf serum and (100 units/ml penicillin).For experimental purpose cells were plated in 48 well plates (at a density of $1x10^4$cells/ ml). After 25hrs incubation period , to allow cell attachment, the cells were treated with fresh medium containing different concentration of ethanolic extract of *Murraya koenigii* ranging from 10-100 µg/ml, dissolved in DMSO and incubated for 48hrs.

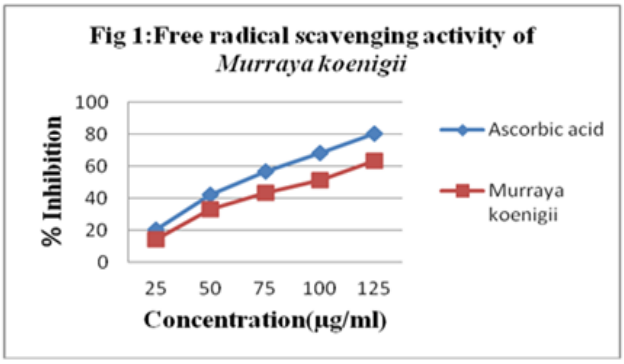
MTT Assay

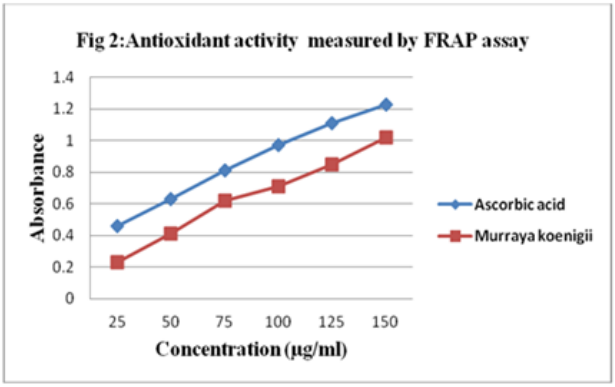
It is a sensitive, quantitative and reliable colorimetric assay that measure viability, proliferation and activation of cells. The assay is based on the capacity of the cellular mitochondrial dehydrogenase enzyme in living cells to reduce the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue/purple formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in a range of cells lines[16-17].At the end of 48hrs incubation, the medium in each plate containing the ethanolic extract of *Murraya koenigii* was added with 200µl of MTT solution and incubated for another 4hrs.The supernatant was then removed & replaced with 500µl of DMSO to dissolve the resulting MTT formazan crystals followed by mixing & measuring the absorbance at 590nm.

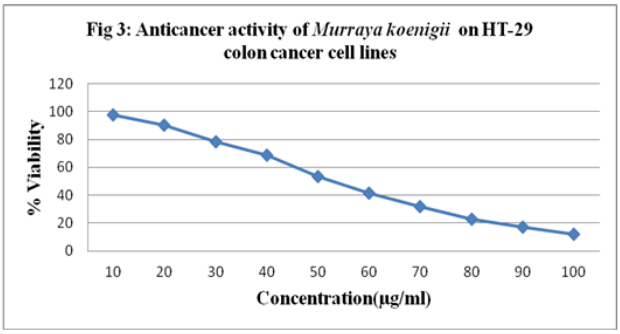
$$\text{Cell viability \%} = \frac{\text{OD of sample}}{\text{OD of control}} \times 100$$

RESULTS

Ethanolic extract of *Murraya koenigii* was screened for the presence of Phytochemicals and showed the presence of maximum compounds like Alkaloids, Flavonoids, Tannins, Saponins, and Cardiac Glycosides. DPPH assay showed that *Murraya koenigii* showed high scavenging activity with 63.54 % inhibition [Fig 1].FRAP values of the extract indicates that reducing power of the extract increased with concentration[Fig 2]. The efficacy of ethanolic extracts on HT-29 cancer cell lines were evaluated by MTT assay. Values of percentage viability of treated cells were plotted against extracts concentration. The present study demonstrated that the antioxidant and anticancer profile of *Murraya koenigii* was effective against HT-29 cells .MTT assay showed that the incubation of cancer cells lines with *Murraya koenigii* , reduced the viability of cancer cells and the dead cells were significantly increased with extract concentration. Thus the ethanolic extract of *Murraya koenigii* exhibited high cytotoxicity of 97.56% [Fig 3].







DISCUSSIONS

Plants are storehouse of good variety of compounds. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of Anticancer activity agents[18].It is observed that management of cancer and infectious diseases always require search for new drugs. Although numerous drugs are currently in use for cancer chemotherapy, they exhibit cell toxicity, induces genotoxic, carcinogenic, and teratogenic effects in non-tumor cells[19].These side effects limits the use of chemotherapeutic agents despite of their high efficacy in treating target malignant cells.Therefore, the search for novel drugs that are both effective and non-toxic bioactive plant products has been increased[20].In recent years, ethnobotanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use[21].The present study observed that the ethanolic extract of *Murraya koenigii* inhibits the proliferation of HT 29 Human Colon cancer cell lines. The cytotoxicity effect was highest with increase in concentration. The cytotoxicity was concentration-dependent and cell line specific. This clearly indicates the presence of potent bioactive principles in the crude extract that might be useful as antiproliferative and antitumoragents.[22] Although the mechanism of the action have not been elucidated, it was understood that the extract contains flavonoids and antioxidant polyphenolic compounds.[23] These compounds are known to scavenge the formation of free radicals, and have great potential in ameliorating cancer cells.[24] .

CONCLUSION

The results of the present study reveals the potentiality of *Murraya koenigii* against colon cancer cell lines and supports the need of further studies to isolate it as an potential anticancer drug. In future study, the isolated principles from *Murraya koenigii* needs to be evaluated in scientific manner using scientific experimental animal models and clinical trials to understand exact molecular mechanism of action, in search of lead molecule from natural resources. Additionally, the study supports the anticancer property of medicinal plants used in the traditional Indian medicine system and further evaluation of the selected medicinal plants for an effective anticancer drug with minimal side effects. Therefore supplementing a balanced diet with *Murraya koenigii* leaves may have beneficial effect in treating colon cancer.

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