

In vitro anticancer potential of Anisomeles malabarica against cervical cancer cells

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ABSTRACT

Background / Aim: The Cow urine distillate (cow ark) enhanced with medicinal herbs, a vital part of the diagnosis of many diseases. In vitro antioxidant and anticancer activity of distilled cow urine with Anisomeles malabarica against cervical cancer cell line has been targeted. **Materials and method:** The cow urine had been photoactivated (cow ark) and extracted with Anisomeles malabarica for the potential of cow ark Anisomeles malabarica using DPPH radical scavenging and reducing power assay. MTT assay was used to observe the increase of cell viability and cytotoxicity. **Results:** The CAM has high antioxidant potential to reduce the oxidative stress. The cell viability of the cancer cells has been increased with the concentration of 75.24 % treated with CAM in 100 mg / ml. The cytotoxicity inhibition of CAM has controlled against cancer cells 28.718 % and the IC₅₀ value of 55.33. **Conclusion:** The cow ark Anisomeles malabarica is concluded to have enormous potential for therapy of cervical cancer disease.

Keywords: Anisomeles malabarica, Cow urine, antioxidant, HeLa cell line.

INTRODUCTION

Bos Indicus are a rare and divine animal and is generally known as "Gowmatha" or "Kamadenu." Cow derived products are used for the treatment of cancer, thyroid disease, asthma, eczema, blood pressurediabetes, heart disease and piles. Cow urine is an effectual medicinal product for anticancer, antibiotic, wound healing activity, antifungal, anti-inflammatory, etc [1]. It is easily obtainable and environmentally safe, as a possible therapeutic agent with less toxicity. [2]. Panchgavya are the best fertilizers and pesticides generated from cow-derived five products in agricultural production. The cow urine distillate (DCU) is used for cell membrane absorption and conveying process[3]. The DCU is effectively used as a germicide, a beneficial antibiotic and a bioenhancer. [4]. Bioenhancers are molecular agents that support and complement the medications and do not disclose the drug's synergistic substance. Bioenhancer of cow ark with varieties of medicinal plant extracts like garlic, stevia, ginger etc[5]. There is much more cancer research nowadays for reported pharmacological properties in medicinal plants. Cervical cancer is one of the prevalent diseases that women face and caused by Human Papilloma Virus affect in the throat and in the genital region.

Now a days, the conventional treatment (chemotherapy and radiotherapy) against cancer can destroy the cancerous cells and also vigorous cells too. Therefore, we require the most outstanding ayurvedic medication with less side effects for cancer therapy. One of the most



Fig 1: Anisomeles malabarica

Popular plant, *Anisomeles malabarica* (Peimiratti)(Fig - 1) is considered to have actions such as antifertility, anticancer, antispasmodic, antiinflammatory and anticonvulsants[6]. Previous studies have reported on the potential of medicinal plant extracts on diverse biological activities viz.,anti-inflammation, diabetic, anti diarrheal, plasmodial, anti-fungal and anti-bacterial actions against varied pathogenic organisms [7,9]. The cow milk from *Bos indicus* have effective potentials of conjugated Linoleic acids (CLA) against Hep-G2 and MCF-7 cells [10,11]. The aqueous extract of *Anisomeles malabarica* has high potential phytochemical constituents include tri-terpenoids,alkaloids , phenolic compounds, and flavonoids etc [12]. Hence, the present study focused that the antioxidant and in vitrocytotoxic activity of cow ark with *A.malabarica*(CAM)on HeLa cell line.

MATERIALS AND METHODS

Collection and preparation of distillate cow urine sample

The cow urine sample of *Bos Taurus indicus* (Red Sindhi) was collected from healthy native cow in and around Trichy District. Cow urine distilled (Cow ark) by photoactivation and filtered for removal of debris.

Preparation of Cow ark with *Anisomeles malabarica* Plants

The shade dried powdered materials of *Anisomeles malabarica* plants (10 g)were extracted with 100 ml cow ark for 15 days. Then it was filtered and placed in the refrigerator at 4°C before usage.

Antioxidant activity

Radical scavenging activity of 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

The Radical scavenging antioxidant scavenging activity cow ark with *Anisomeles malabarica* (CAM) was determined with slight modification using DPPH method[13]. 1 ml of CAM was mixed with DPPH radicals at a concentration of 0.041mm in 1 ml ethanol solution. Then, it was shaken aggressively and leave to stand for 10 minutes. the absorbance was calculated though at 517 nm using the spectrophotometer.

Reducing power assay

The Reducing power assay of the CAM was defined by the method of Blois with slight modifications [14]. 1 ml CAM was added to a well of 1 ml ($K_3 [Fe (CN)_6]$) and incubated for 20 min at 50°C in a water bath.1 ml of TCA was applied and centrifuged for 5 minutes at 13,400 rpm. On further centrifugation, 1 ml of the sample was tested with 0.1 ml of Ferric Chloride ($FeCl_3$) and 1 ml of distilled water (DW) applied that it was read at 700 nm of absorption.

Cell culture

HeLa (cervical cancer cell line)cells were obtained from NCCS,India. The Cell were maintained in DMEM (Dulbecco's Modified Eagle's medium) medium. This supplement treated with10% FBS (fetal bovine serum), $C_{21}H_{39}N_7O_{12}$ (Streptomycin) 100 µg/ml and $C_{16}H_{18}N_2O_4S$ (penicillin) 100 µg/ml as antibiotics.It is incubated in a humidified atmosphere of 5% CO_2 at maintained at 37°C in a CO_2 incubator.

In vitro anticancer study

MTT reduction assay

To evaluate the cytotoxic property of CAM was measured the Cell viability by using the (MTT) reduction assay [14] The extracts were dissolved in DMSO (dimethyl sulfoxide).The HeLa cells were incubated in the 96-well microplates at a density of 1.0×10^6 per as well for 24 hours and treated with CAM extracts at various concentrations. Then, 1ml sample was loaded with MTT reagent (5 mg / mL), after incubated for 37°C at 24 h. The medium was separated and subsequently formazan crystals there were dissolved in DMSO. Measured cell viability within

each well with such an absorbance of 570 nm using ELISA reader. Cell viability results obtained were compared with suitable controls. The concentration of the inhibitory growth was evaluated with IC_{50} values.

RESULTS AND DISCUSSIONS

DPPH radical scavenging activity

The antioxidant DPPH method for measuring the radical scavenging assay is an easy and effective study. The colour of the sample was changed by absorbance (517 nm) from purple to yellow. The antioxidant activity of the CAM has high radical scavenging assays compared to standard ascorbic acid values (Fig-2). Previous study reported that the antioxidant potential in ethanolic extract of *Anisomeles malabarica* was 47.86 at 100 μ g / ml. In our results showed that in DPPH radical scavenging assay, cow ark *Anisomeles malabarica* had a radical scavenging operation of 71.21 % at 100 μ g / ml. Hence, cow ark enhanced with *Anisomeles malabarica* has strong antioxidant activity which was contrasted with previous results.

Fig – 2 the antioxidant activity of cow ark with *Anisomeles malabarica* (CAM) by using DPPH radical scavenging assay

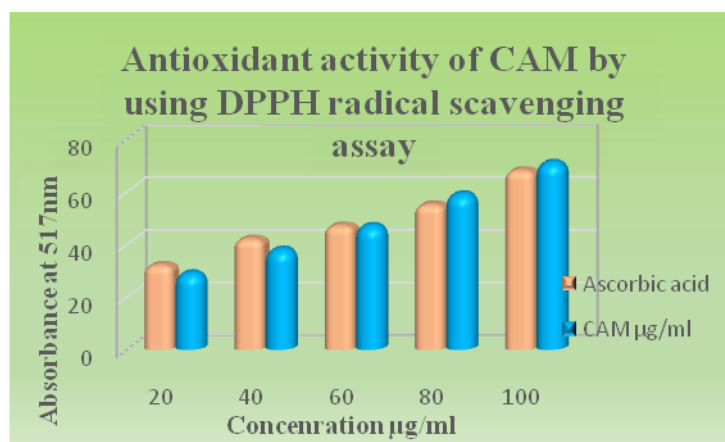


Fig-2. Results showed that cow ark with *Anisomeles malabarica* has high radical scavenging activity in DPPH radical scavenging assay was 71.21 % at 100 μ g/ml when compared with reference (Ascorbic acid).

Reducing power

Using reductones, the reduction power test may be used to calculate the ability of contributing electron. During this process The ferricyanide is reduced into ferrous ions. The reduction in antioxidant capacity has high significant when compared with others cow ark with *Anisomeles malabarica* (CAM) compared to (reference) gallic acid (Fig -3). In the present study it has been shown that the *Anisomeles malabarica* has high power reduction activity compared with previous reports of *Anisomeles malabarica* [12]. Many plant extracts has high potential for therapy of hepatic cancer diseases, Alzheimer's and antimicrobial activity. The present study reported that the antioxidant potential of CAM to support human health and dietary supplements. Therefore, the antioxidant ability of cow ark enhanced with *Anisomeles malabarica* is recommended for the prevention of cancer causing cells.

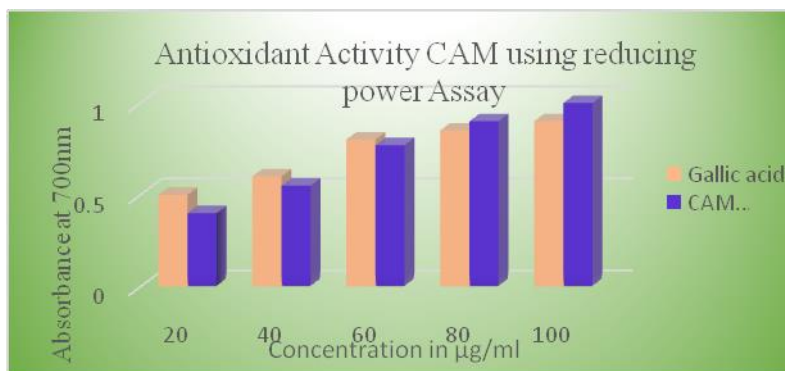


Fig-3. Results showed that cow ark with Anisomeles malabarica has high reducing power activity as compared to Gallic acid (Reference).

In vitro cell-line study

The succinate-coenzyme Q reductase (SQR) enzyme is metabolically reduced the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to form formazan (blue) [16]. The MTT assay can be used to detect the cytotoxicity and cell survival of HeLa (cervical cancer cell line) cell against Cow ark with Anisomeles malabarica. Furthermore, it may be used to estimate cell viability. The Cow ark with Anisomeles malabarica (CAM) inhibitor has been proven that high capacity of increasing the cell viability in HeLa cell line. The percentage of cell viability of the cancer line viability treated with Cow ark with Anisomeles malabarica (CAM) to 75.24. The percentage of cell cytotoxicity about cancer line inhibition against Cow ark with Anisomeles malabarica (CAM) to 28.718 (Table-3). The CAM has shown the IC₅₀ value of 55.33 against the cervical cancer cells (Table-4). From the results, the Cow ark with Anisomeles malabarica (CAM) has the best anticarcinogenic potential and is recommended for cancer treatment.

Table-4 in vitro anticancer activity of CAM by using MTT assay

Concentration of CAM (mg/ml)	Hela cell line		IC ₅₀
	% of Cell viability	% of Cytotoxicity	
Control	100	0	
6.25	36.27	76.259	55.33
12.5	45.13	69.726	
25	51.31	54.374	
50	55.15	47.591	
100	75.24	28.718	

Table – 4 showed that the in vitro anticancer activity of CAM (cow ark with Anisomeles malabarica) has high potential of anticarcinogenic agents against HeLa cell line .

Earlier study reported that the estrus urine of Yorkshire pigs having major sources of pheromones [17]. The biomarkers and particular volatile compounds are detected in estrous urine of Bos Indicus [18,19]. Cow Ark is deliberated to be universal, therapeutic potential, used to treat many diseases [20-23]. The researchers demonstrated that cow urine has anticarcinogenic effects that have been assessed using a US Patent (6896907). Cow urine therapy was assessed to a variety of cancer patients and demonstrated a 30.87 % success rate in throat cancer patients. Breast cancer patients accounted for 14.70 %, cervical and uterine cancer patients accounted for 5.88 %, buccal cavity cancer accounted for 4.41 %, lung cancer, lymphoma and bone cancer accounted for 2.94 %, and other cancer patients accounted for 5.88 % and other cancer patients

accounted for 8.82 %. The medication was continued on cancer patients for 2-3 months and was a life-saving therapy for them [24-25].

Furthermore, recent study shown that cow ark has a strong antibacterial capability against human pathogenic bacteria. In our study, the antioxidant activity of native cow urine was filtered with herbal bioenhancer against anticancer activity HeLa cells.

CONCLUSION

Cancer is one of the most dreaded illnesses, which may generally be controlled for certain side effects of surgery, radiotherapy or chemotherapy. Nowadays, these treatments may be substituted by homoeopathy, ayurvedic medications used to regulate cell-causing cancer growth. The cow urine treatment is a huge opportunity to cure cancer disease without adverse effects. This study showed the excellent antioxidant and in vitro anticancer activity of cow ark with *Anisomeles malabarica* (CAM) against HeLa (cervical cancer) cell line. This research seeks to increase general awareness of medicinal bioenhancer with cow urine treatment, which is even suggested for anti-cancer medications.

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