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Original Article

EVALUATING THE ANTIPROLIFERATIVE POTENTIAL OF METHONOLIC LEAF EXTRACT OF *CASSIA NIGRICANS*

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ABSTRACT

Objective: It is well established that plants have always been useful source as anticancer compounds. This study was attempted to investigate the *in vitro* anti-cancer potential of methonolic extract of *Cassia nigricans* on breast cancer MCF-7 cell lines.

Methods: The methanol extract of *C. nigricans* was screened for its anti-proliferative effect against MCF-7 (Breast cancer) cell lines using MCF-7 cells seeded 96 well plates.

Results: Extract was exposed with MCF-7 cell lines for 24h and 72h at a range of increasing concentrations (0-500µg/ml) in order to obtain a dose-response graph and IC50 value. The *C. nigricans* extract showed cytotoxic effect in MCF-7cells with IC_{50} of 82.6µg/ml.

Conclusion: The *C. nigricans* extract showed effective cytotoxic activity in a dose and time dependent manner. Future work will be interesting to know the chemical composition and also better understand the mechanism of action present in the extract for developing it as drug for therapeutic application.

Keywords: *Cassia nigricans*, Anticancer, Breast cancer, Cytotoxic, Antraquninones, Drugs.

INTRODUCTION

Plants and plant based herbal preparations have been used to treat ailments since prehistoric times, and the treatment of various diseases with plant-based medicines has remained an integral part of many cultures across the globe. Side effects of several allopathic drugs and development of resistance to currently used drugs have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. The World Health Organization estimates that 80% of the World's population use herbal medicines in some aspects of primary healthcare and there is a growing tendency to "Go Natural" [1, 2]. It is well established that plants have always been a useful source, for occurrence of anticancer compounds [3-5]. Approximately 60% of currently used anticancer chemotherapeutic drugs (vinblastin, vincristine) are derived from plant resource [6, 7]. Although most of the plants used in the traditional medicine have been identified and their applications are well-documented, the anticancer efficacy of many plants is yet to be verified.

Casiia nigricans (Leguminosae family–Caesalpinoideae) is a woody annual herb or under shrub between 1.2 and 1.5 m high with small yellow flowers. It is widespread in India and tropical Africa including northern Nigeria, especially in cultivated in roadside and open grassy areas [8, 9]. They are well known in folk medicine for their laxative and for treating various skin diseases such as ring worm, scabis, eczema etc., [10, 11]. Further, they are known to be of high therapeutic value in ulcers, gastro-intestinal disorders, diarrhea [12]. The leaf extracts of *C. nigricans* have shown potent analgestic, anti-inflammatory, anti-microbial, larvicidal and anti-plasmodial activities [13, 14]. Although there is enough information on *C. nigricans* extracts use in various diseases' treatment, However, literature survey revealed that there is paucity of data on its anticancer potential. Henceforth, in this study we have attempted to investigate the *in vitro* anti-cancer potential of methonolic extract of *C. nigricans* on breast cancer MCF-7 cell lines.

MATERIALS AND METHODS

Materials

All the chemical reagents and solvents of analytical grade were purchased from SRL Chemicals, India. 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, No M5655) purchased from Sigma (St Louis, MO, USA).

Plant material and extracts preparation

The leaves of *C. nigricans* were collected from in the campus of Maharani Lakshmi Ammanni College For Women, Bangalore, India. The plant materials were authenticated by Dr. S. Sundara Rajan, a Taxonomist and the voucher specimen (MC-H-51) were deposited at the department. The leaves were cleaned with distilled water, dried and crushed into the fine powder by using an electric grinder. The coarsely powdered leaf material was extracted with pure methanol in a Soxhlet apparatus for 24 h. The extract was evaporated to dryness under reduced pressure using a Rotavapor (BuchiFlawil, Switzerland) and a portion of the residue was used for the anti-cancer assays.

MTT assay

MCF-7 (breast cancer) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^3 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). All these cell lines were cultured and Cytotoxicity test were carried out using MTT assay [15,16]. The trypsinized 70-80% confluent cell lines (MCF-7) of 1×10^5 cells/well we seeded in a 96 well plate and incubate for 24 hr at 37 ºC, and varying concentrations (0-500 µg/ml) of *C. nigricans* are added and incubated at 48 and 72 hrs. After incubation, 20 µL of MTT reagent will be added to each well and incubated for 4 hr at 37ºC. The incubated cells were washed twice with PBS and DMSO (100µL/well) reagent which dissolved the insoluble crystalline formazan product. The efficacy of the sample was determined based on the reduced dye at 570 nm by UV spectrophotometer The effect

of the samples on the proliferation of MCF-7 cell lines were expressed as the % cell viability, using the following formula: % cell viability = A570 of treated cells / A570 of control cells × 100%.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean ±standard deviation. The data in all the experiments were analyzed (Microsoft Excel 2007) for statistical significance using Students *t*-test and differences were considered significant at $p < 0.05$.

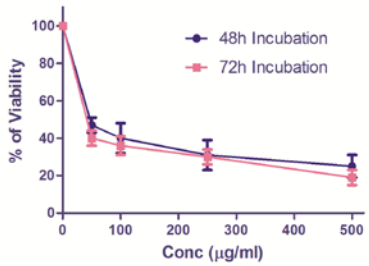

Fig. 1: Cytotoxic activity of *C. niicans* extract in MCF-7 cell lines. Extract were incubated with 10^5 viable cells at concentrations ranging from 0 to 500 µg/ml for 48 and 72 h. Cell viability was determined by the MTT method

RESULTS AND DISCUSSION

Plants have always been a potential source of new drug molecule and research work in this domain has resulted in discovery of more efficient drugs for cancer treatment [6, 7]. MTT is a simple, reliable technique, which measures cell viability and can be used for screening anti-proliferative agents [15, 16]. MTT assay is a a spectrophotometric analysis, which uses (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide), known as MTT, a yellow collor and water soluble compound. The MTT enters the cells through the plasma membrane and, in contact with superoxide produced by the mitochondrial activity, is oxidized to MTT-formazan, a slat purplish color, which is insoluble in water. Then the oxidation of MTT is proportional to the mitochondrial activity and therefore to cell viability [16]. When the methanol extract of *C. nigricans* was screened for their anti-proliferative effect against MCF-7 (Breast cancer) cell lines, it was found that, the extract was effective in a dose and time dependent manner in inducing cytotoxic effect (Fig.1). The assessment of *C. nigricans* extract cytotoxicity is generally performed on MCF-7 cell seeded 96 well plate. MCF-7 cell seeded plate was incubated with the extract for 24h and 72h at a range of increasing concentrations (0-500µg/ml) in order to obtain a dose-response graph and IC50 value. The IC50 is the concentration of the extract required to kill fifty percent of the cells. The *C. nigricans* extract showed cytotoxic effect in MCF-7cells with an IC_{50} value of 82.6µg/ml.

The C*assia* species are known to be rich sources of polyphenols, anthraquinone derivatives, flavanoids and polysaccharides [17-19]. The 1,6,8- trihydroxy-3-methyl-anthraquinone (emodin) isolated from the leaves of *C. nigricans* was demonstrated to poses cytotoxic effect in Brine shrimp lethality bioassay [20]. Similarly in this extract the anthraquinones that are present in the methanolic extract of *C. nigricans* might be bringing out the anti-proliferative or cytotoxic effect. Therefore, it will be interesting to understand the chemical composition and better understand the mechanism of action of the extract for further developing it as drug for therapeutic application.

CONCLUSION

The results of this study establish the anticancer activities of *C. nigricans* leaf extracts. The potential of *C. nigricans* extracts as anticancer activities may be due to the presence of phyto-constituents like anthraquinones. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of the plant as a source of easily available effective anticancer agents to the people, particularly in developing countries, including India.

Future work will be interesting to know the chemical composition and also better understand the mechanism of action present in the extract for developing it as drug for therapeutic application.

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Ethical Issues

There is none to be applied

CONFLICTS OF INTERESTS

Declared None

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