Research Article

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SCREENING OF CHAKRAMARDA (*CASSIA TORA* L.) PATRA FOR ITS ANTI-CANCER ACTIVITY ON SKIN CANCER CELL LINE: AN *IN VITRO* STUDY

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ABSTRACT

Background: Chakramarda (*Cassia tora* L.) is an annual herb with various medicinal properties and indications in twak vikaras (skin disorders) such as dadru, pama, switra, and others mentioned in Ayurveda samhitha and nighantu. Skin cancer is one of the most prevalent types of cancer. The purpose of this study is to evaluate the efficacy of Chakramarda Patra (leaves) *in vitro* against skin cancer. Chakramarda is a well-known drug for twak vikara (skin disorders), according to ayurvedic classics. Objective: To evaluate and compare the anticancer effects of aqueous and hydro-alcoholic extracts of Chakramarda (*Cassia tora* L.) patra on skin cancer using an *in vitro* method. Methodology: The anticancer activity of aqueous and hydro-alcoholic extracts of Chakramarda (*Cassia tora* L.) Patra was evaluated in vitro using a squamous cell skin cancer cell line through a colorimetric cell viability assay, which was used for the evaluation of anti-cancerous activity. Results: A significant increase in the percentage of cell death was observed with increasing concentrations of both aqueous and hydro-alcoholic extracts. At a concentration of 1000 micrograms per milliliter, the aqueous extract showed 24.694 percent cell viability, while the hydro-alcoholic extract showed 8.600 percent cell viability. The standard anticancer drug cisplatin exhibited 1.583 percent cell viability. Conclusion: Both aqueous and hydro-alcoholic extracts of Chakramarda (*Cassia tora* L.) patra demonstrated significant anticancer activity against skin cancer under *in vitro* conditions. The hydro-alcoholic extract exhibited superior anticancer activity when compared to the aqueous extract.

Keywords: Anticancer activity, Kustaghna, Chakramarda, *Cassia tora* L., MTT assay, skin cancer cell line.

INTRODUCTION

Chakramarda, botanically identified as *Cassia tora* L., is an annual herb belonging to the family Fabaceae [1]. It grows wild along field margins, roadsides, and in mountainous regions. The plant is known by several synonyms, such as Dadhrughna, Pamaghati, Edagaja, and Cakragaja, and its useful parts include the leaves and seeds. Chakramarda (*Cassia tora* L.) is attributed with various medicinal properties and indicated in the management of twak vikaras (skin disorders) such as mandala kuṣṭha, dadru, pāma, vicharchikā, and kitibha, as described in classical Ayurvedic texts [2]. Skin cancer is one of the most prevalent cancers worldwide, with its incidence and mortality rates continuing to rise. It is broadly classified into two major types: melanoma skin cancer and non-melanoma skin cancer [3]. Among non-melanoma skin cancers, squamous cell skin cancer is the second most common type. Clinically, squamous cell skin cancer commonly presents as a hyperkeratotic papule, nodule, or erosion. The lesion may progress and can appear as an ulcerative, ulceroproliferative, or proliferative growth, typically characterized by raised and everted margins with an indurated base and edges [4]. Previous experimental studies have demonstrated the anticancer potential of *Cassia tora* L., including its activity against human tongue carcinoma [5] and human cervical carcinoma [6]. The present study was undertaken to assess the utility of Chakramarda (*Cassia tora* L.) patra against squamous cell skin cancer using an *in vitro* model (A-431), considering its extensive documentation in various Saṃhitās and Nighaṇṭus for the treatment of twak vikara and its reported anticancer properties.

Aim: To evaluate the anticancer potential of aqueous and hydroalcoholic extracts of Chakramarda (*Cassia tora* L.) patra against skin cancer using an *in vitro* model.

Objectives

1. To evaluate the antiproliferative activity of aqueous extract of Chakramarda (*Cassia tora* L.) patra on skin cancer cells using an *in vitro* method.
2. To evaluate the antiproliferative activity of hydroalcoholic extract of Chakramarda (*Cassia tora* L.) patra on skin cancer cells using an *in vitro* method.
3. To compare the antiproliferative activity of aqueous and hydroalcoholic extracts of Chakramarda (*Cassia tora* L.) patra on a skin cancer cell line.

MATERIALS AND METHODS

Collection and authentication of the drug:
The leaves of Chakramarda (*Cassia tora* L.) were collected from their natural habitat in Hassan, Karnataka. The plant material was authenticated by experts from the Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India.

Methodology

The aqueous and hydroalcoholic extracts of Chakramarda (*Cassia tora* L.) were prepared at the Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India.

Preparation of aqueous and hydro-alcoholic extracts: Aqueous extract preparation: Ten grams of the powdered sample were accurately weighed and transferred to a conical flask, to which 100 ml of distilled water was added. The mixture was mixed uniformly and allowed to stand overnight. It was then filtered and evaporated to dryness on a water bath. The percentage of aqueous extractable matter was calculated. [7]

Hydro-alcoholic extract preparation: Ten grams of the powdered sample were accurately weighed and transferred to a conical flask, to which 50 ml of ethanol and 50 ml of distilled water were added. The mixture was mixed uniformly and allowed to stand overnight. It was then filtered and evaporated to dryness on a water bath. The percentage of hydroalcoholic extractable matter was calculated**.**

Experimental study: The anticancer activity of aqueous and hydro-alcoholic extracts of Chakramarda (*Cassia tora* L.) was studied on skin cancer in vitro at Sri Dharmasthala Manjunatheswara Centre of Research in Ayurveda and Allied Sciences, Udupi. The MTT assay was used for the evaluation of anticancerous activity. Procedure**:** A confluent flask of A431 cells was trypsinized, and the detached cells were collected, washed twice with phosphate-buffered saline (PBS), and centrifuged at 800 rpm for 5 minutes. The cell pellet was resuspended in Minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Cell counting was performed using a hemocytometer. A known number of cells (10,000 cells/well) were seeded into 96-well culture plates and incubated at 37°C in a CO_2 incubator for 24 hours. After 24 hours, the spent medium was carefully discarded, and different concentrations of the aqueous extract of Chakramarda(*Cassia tora* L.) patra dissolved in serum-free MEM were added to the respective wells. One set of cells treated with cisplatin served as the positive control. The plates were then incubated for 48 hours at 37°C in a CO_2incubator. Following incubation, the medium was removed and the cells were washed with PBS. 20 µL of MTT dye solution (5 mg/mL in PBS) was added to each well. The plates were covered with aluminum foil and incubated for 4 hours in a CO_2 incubator. After incubation, 100 µL of acidified isopropanol was added to each well to dissolve the formazan crystals, and the contents were mixed uniformly by gentle shaking. The absorbance was measured at 540 nm using a multi-well plate reader. [8]

The percentage of cell viability was calculated using the following formula: .

Percentage of viable cells = [(Test sample − Blank) / (Control − Blank)] × 100.

Table 1: The anti-cancer activity of aqueous extract of Chakramarda Patra

Conc. (µg / ml)	Mean ± SE
Control	100
1	75.049 ± 1.849
2	66.327 ± 1.139
4	44.293 ± 2.995
8	42.492 ± 3.120
10	42.086 ± 3.052
20	41.116 ± 2.559
40	39.547 ± 1.416
80	38.392 ± 0.933
100	35.657 ± 0.216
200	34.720 ± 0.354
400	33.781 ± 0.305
500	31.993 ± 0.252
800	29.222 ± 1.152
1000	24.694 ± 0.054
2000	22.204 ± 2.156
4000	17.359 ± 5.258
Cisplatin (500 µg /mL)	2.325 ± 0.136
Cisplatin (1000 µg / mL)	1.583 ± 0.092

Table 2: The Anti- Cancer Activity Of Hydro-Alcoholic Extract Of Chakramarda Patra

Conc. (µg / ml)	Mean ± SE
Control	100
1	63.828 ± 0.814
2	60.792 ± 0.097
4	55.693 ± 1.658
8	53.995 ± 2.487
10	51.304 ± 1.415
20	50.495 ± 1.821
40	48.156 ± 1.381
80	44.210 ± 1.443
100	40.000 ± 1.966
200	35.038 ± 1.780
400	30.067 ± 0.343
500	26.540 ± 1.916
800	17.509 ± 1.424
1000	8.600 ± 0.432
2000	5.855 ± 0.256
4000	3.055 ± 0.631
Cisplatin (500 µg /mL)	2.408 ± 0.021
Cisplatin (1000 µg / mL)	1.834 ± 0.023

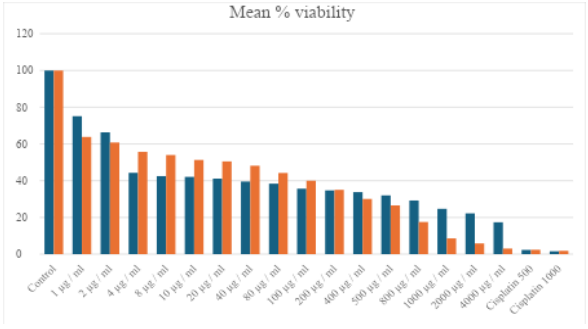

Figure 1: The anti-cancer activity of aqueous and hydro-alcoholic extract of Chakramarda patra
Mean % viability (A431 cell lines) aqueous extract of Chakramarda leaves
Mean % viability (A431 cell lines) Hydroalcoholic extract of Chakramarda leaves

RESULTS AND DISCUSSION

The statistical analysis of results was done and represented as the mean and standard error of the mean. In Table 1, it is demonstrated that the aqueous extract of Chakramarda (*Cassia tora* L.) Patra exhibited a gradual and consistent reduction in cell viability with increasing concentrations. At lower concentrations, the reduction in viability was moderate, whereas higher concentrations showed a marked antiproliferative effect. At the maximum tested concentration of 1000 µg/ml, the aqueous extract reduced cell viability to 24.694%, indicating significant inhibition of cancer cell growth when compared to the untreated control. Table 2 demonstrates the hydro-alcoholic extract exhibited a stronger and more pronounced antiproliferative effect across all tested concentrations when compared to the aqueous extract. A sharper decline in cell viability was observed with increasing concentrations, indicating superior growth-inhibitory activity. At 1000 µg/ml, the hydro-alcoholic extract reduced cell viability to 8.600%, which is substantially lower than that observed with the aqueous extract at the same concentration. Further reduction in viability at higher concentrations reinforces the enhanced efficacy of the hydro-alcoholic extract. This superior activity may be attributed to the ability of the hydro-alcoholic solvent system to extract both polar and moderately non-polar bioactive compounds such as anthraquinone glycosides, flavonoids, tannins, and phenolic compounds, which are known to possess anticancer properties. Figure 3 demonstrates concentration-dependent reduction in cell viability with both aqueous and hydro-alcoholic extracts of Chakramarda (*Cassia tora* L.) Patra. However, the hydro-alcoholic extract consistently showed greater inhibition of cell proliferation than the aqueous extract at all tested concentrations. The decline in cell viability observed with the hydro-alcoholic extract indicates superior antiproliferative activity. This enhanced effect may be attributed to the hydro-alcoholic solvent system's ability to extract a broader range of bioactive phytoconstituents compared to water alone. The standard anticancer drug cisplatin produced the maximum inhibition of cell viability, with values of 2.408% at 500 µg/ml and 1.834% at 1000 µg/ml. This result validates the sensitivity and reliability of the experimental model. Although cisplatin exhibited greater antiproliferative activity than both extracts, the hydro-alcoholic extract of Chakramarda Patra demonstrated substantial growth inhibition, indicating its potential as a natural supportive or adjunctive anticancer agent. Chakramarda (*Cassia tora* L.) consists of kaṭu rasa, the lakṣaṇa of which include śodhana (cleansing), śvayathu anupahanti (reduces swelling), vraṇa avasādana (inhibition of excessive wound growth), māṃsa vilikhana (scraping of unwanted muscular overgrowth), and meda upaśoṣaṇa (reduction of adipose tissue) [9] the guṇas of Chakramarda (Cassia *tora* L.) are laghu and rūkṣa. Laghu guṇa contributes to lekhana karma (scraping) and dhātu kṣaya (reduces dhatus), while rūkṣa guṇa produces śoṣaṇa karma (dries the dhatus) and dhātu kṣaya [10].

CONCLUSION

The present *in vitro* study achieved its objective of evaluating and comparing the effects of aqueous and hydro-alcoholic extracts of Chakramarda (*Cassia tora* L.) Patra on squamous cell skin cancer cells invitro. Both extracts demonstrated concentration-dependent inhibition of cell proliferation in the A-431 cell line. The hydroalcoholic extract showed greater antiproliferative activity than the aqueous extract, indicating enhanced extraction and biological efficacy of its bioactive constituents. Further studies involving in vivo models and clinical evaluations are necessary to substantiate the therapeutic potential of Chakramarda (Cassia *tora* L.) Patra in skin cancer management.

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