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BIOSYNTHESIS AND CHARACTERIZATION OF ACALYPHA INDICA AND EVALUTION OF ITS ANTICANCER ACTIVITY ON BONE CANCER (MG63)

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ABSTRACT

Acalypha indica is a common weed with significant medicinal properties that hold value for various human health applications. The present study focuses on the biosynthesis and characterization of Acalypha indica and the evaluation of its anticancer activity against MG-63 bone cancer cell lines. Acalypha indica is a traditional medicinal plant widely distributed across tropical regions and has long been used in Ayurveda, Siddha, and Unani systems for treating various ailments such as skin infections, respiratory disorders, and inflammation. Despite its established ethnomedicinal significance, limited scientific data are available to support its anticancer potential, prompting the need for systematic evaluation. In this study, the chloroform leaf extract of Acalypha indica was prepared using the Soxhlet extraction method and tested for cytotoxic activity using the MTT assay. MG-63 bone cancer cells were treated with different concentrations of the extract (5, 25, 50, 75, and 100 µg/mL) for 24 hours, and cell viability was measured spectrophotometrically at 570 nm. The extract exhibited a dose-dependent cytotoxic effect, with cell viability reducing from 49% at 5 µg/mL to 29% at 100 µg/mL, indicating moderate to severe cytotoxicity. The maximum cytotoxic effect (71%) was observed at the highest concentration, while the control group showed no cytotoxic response. These findings demonstrate that Acalypha indica possesses bioactive phytoconstituents with promising anticancer potential. The study scientifically validates traditional claims regarding the plant's therapeutic value and suggests its possible development as a natural anticancer agent for bone cancer treatment.

KEYWORDS: Acalypha indica, MG-63 cell line, anticancer activity, MTT assay, cytotoxicity and Soxhlet extraction.

INTRODUCTION

Acalypha indica is a common weed with significant medicinal properties that hold value for various human health applications. It is widely distributed across countries such as India, Sri Lanka, Thailand, and Pakistan. Extracts obtained from different parts of the plant—including leaves, roots, and stems—have been traditionally utilized in the treatment of numerous ailments such as eye infections, respiratory disorders, rheumatism, skin diseases, and in the regulation of blood glucose levels. Various extraction techniques are employed to isolate the plant's active components. Among them, Soxhlet extraction is noted for its high precision and yield efficiency, though the process may cause degradation of heat-sensitive phytochemicals due to thermal stress.[1] Herbal medicines have long played a crucial role in the prevention and treatment of various diseases within traditional healthcare systems such as Ayurveda, Unani, and Siddha. These conventional therapeutic approaches served as the primary form of healthcare in earlier civilizations. However, with modernization and the advent of advanced medical technologies, contemporary societies have increasingly shifted toward modern medical practices, leading to a gradual decline in traditional knowledge. As older generations pass away, much of this orally transmitted wisdom risks being lost. Therefore, it is essential to properly document the practices of traditional healers to preserve these valuable alternative medical systems.[2] The World Health Organization recognizes traditional

plant-based medicines as credible and reliable sources of therapeutic agents. Medicinal plants are often found abundantly in local environments near homes, along roadsides, and in natural surroundings—and are known to be rich in bioactive compounds. Notably, more than 80% of modern pharmaceuticals are derived from plants and microorganisms. Natural products from medicinal plants possess extensive pharmacological importance, as their bioactive constituents can interact effectively with mammalian cellular targets.[3]

DESCRIPTION[4-5]

Acalypha indica is a well-known traditional medicinal plant that has been recognized by older generations across various regions, particularly in Africa and Asia. The plant thrives widely in western and southern parts of Africa, including countries such as Somalia and Ethiopia, as well as in other tropical and subtropical regions. It is also distributed across many parts of Asia, Europe, and both North and South America. Typically, Acalypha indica grows abundantly as a weed in natural surroundings such as bushes, backyards, roadsides, and near residential or agricultural areas.

Fig 1: Acalypha Indica.

TAXONOMY

The leaves of Acalypha indica are thin, smooth, and have toothed (crenate-serrate) margins with a wedge-shaped (cuneate) base. They are borne on petioles that are typically longer than the narrow leaf blades and have very small stipules. The leaves are simple, spirally arranged, and range in size from about 2–9 cm long and 1–5 cm wide, with petioles measuring 0.02–12 cm. The blade is broadly ovate to lanceolate, with an acute tip and toothed edges. Both sides of the leaf surface are almost hairless or slightly hairy, with denser hairs along the midrib. The leaves are five-veined at the base and have four to five pairs of lateral veins. After about one month of growth, the plant's stem begins to harden and becomes woody as it matures. The stem is covered with fine hairs, and the plant produces numerous slender, upward-growing branches. The flowers occur in several upright, loose, elongated clusters or axillary spikes, particularly near the upper portions of the plant. The female flowers are white, scattered, and enclosed by large, toothed, wedge-shaped bracts that are short-stalked, prominently veined, and measure around 6–8 mm in diameter.[6]

DISTRIBUTION

Acalypha indica naturally thrives in humid, temperate, and tropical regions situated along the equatorial belt, spanning across Asia, Africa, Europe, Australia, and both North and South America. In India, the use of this plant in traditional and conventional medicinal practices has been well documented for generations. Although the plant is commonly recognized in Australia, it is not widely utilized for consumption. Reports also indicate its presence in the Arabian Gulf region, where it is occasionally used as a food source. Additionally, Acalypha indica is a widespread weed in several parts of West Africa, including southern Nigeria.[7-10]

PLANT PROFILE

Scientific name: *Acalypha indica*

Taxonomic Classification

- **Kingdom:** Plantae
- **Unranked:** Angiosperms
- **Unranked:** Eudicots
- **Order:** Malpighiales
- **Family:** Euphorbiaceae
- **Genus:** *Acalypha*
- **Species:** *A. indica*

Vernacular Names

- **Sanskrit:** Arittamanjarie
- **English:** Indian Acalypha
- **Hindi:** Kuppu, Khokali
- **Telugu:** Kuppichettu, Harita-manjiri, Kuppinta, or Muripindi

Major Constituents

- Alkaloids: *Acalypus* and *Acalyphine*

Vernacular Names in Other Countries

- **Brazil:** Alcalifa
- **China:** Tie Xian
- **Ethiopia:** Baro, Berbere
- **India:** Kuppimeni
- **Spain:** Ricinela
- **Sri Lanka:** Kuppameniya

LITERATURE REVIEW

1. Rajeshwari Siva raj et al., (2014)[11] Spectrochim Acta A Mol Biomol Spectrosc. Has developed Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. The synthesized particles were highly stable, spherical and particle size was in the range of 26-30 nm. The antimicrobial activity of A. indica mediated copper oxide nanoparticles was tested against selected pathogens.

2. C Krishna raj et al., (2014)[12] Biotechnology Rep (Amst). 2014. Established their research known as

Acalypha indica Linn: This study reports the in vitro cytotoxic effect of biologically synthesized silver and gold nanoparticles against MDA-MB-231, human breast cancer cells. This is the results of the present study indicate that biologically synthesized silver and gold nanoparticles might be used to treat breast cancer; however, it necessitates clinical studies to ascertain their potential as anticancer agents.

3. S. Kavitha, T. Kalai Kovan and R. Vijaya Bharathi et al., (2017)[13] developed an study of Acalypha indica was found to have antioxidant compounds such as flavonoids and tannins. Hence antioxidant and in vitro anticancer (cytotoxicity) activities were carried out on the leaf extract of A. indica. Aim of this study to provide an introduction to the principles of drug treatment for solid tumors in cancer treatment. Antioxidant activity was studied by conducting lipid peroxidation assay, the total antioxidant capacity of Et OH and water extracts was found to be 442 and 338 nmol/g respectively thus establishing Et OH and water extracts of A. indica possess antioxidant activity. In vitro cytotoxicity(anticancer) activity was studied by growing Agrobacterium tumefaciens on yeast extract media for 48 hrs. at 28°. Russet potatoes were disinfected by scrubbing under running water with a brush, then immersed in 10% Clorox for 20 min. These above studies showed that the antioxidant activity and related anticancer activity of Acalypha indica, because of the presence of flavonoids and tannins present in the Et OH and water extracts of Acalypha indica.

4. Sudhakar Chekuri, Lali Lingfa, Shiva prasad Panjala, KC Sai Bindu, Roja Rani Anupalli et al., (2016)[14] review article aims to provide a comprehensive review on the phytochemical and various pharmacological aspects of Acalypha indica. This plant widely used in traditional medicinal system of India and many other countries has been reported to possess anticancer, anti-diabetic, anti-oxidant, anti-bacterial, antifungal hepatoprotective, anti-inflammatory, and also used to check anti-ulcers and wounds healing. It is known as a rich source of glycosides, flavonoids and tannins.

5. Sanseera, D., Niwatananun, W., Liawruangrath, B., Liawruangrath, S., Baramee, A., Trisuwan, K. & Pyne, S. G. et al., (2012)[15] Antioxidant and anticancer activities from aerial parts of Acalypha indica Linn. of Acalypha indica Linn. (Aerial parts) were investigated for antioxidant activity, anticancer activity, and cytotoxicity. The extracts showed a non-cytotoxic response against Vero cells (African green monkey kidney). The hexane, chloroform, and methanol extracts showed significant antioxidant activities with an IC50 of 6.13, 6.31, and 6.37 mg/mL, respectively, by means of the ABTS radical scavenging assay.

6. Rajeshwari Siva raj et al., (2013)[16] tested cytotoxic activity of A. Indica mediated copper nanoparticles with MTT assay against MCF-7 breast cancer cell lines, Copper oxide nanoparticles were synthesized by biological method using aqueous extract of Acalypha indica leaf and characterized by UV–visible spectroscopy, XRD, FT-IR, SEM TEM and EDX analysis. The cytotoxicity activity of A. indica mediated copper nanoparticles was evaluated by MTT assay against MCF-7 breast cancer cell lines and confirmed that copper oxide nanoparticles have cytotoxicity activity.

7. Sudhakar Chekuri, Shivaprasad Panjala, Roja Rani Anupalli et al., (2017)[17] The study emphasizes cytotoxic activity of hexane leaf crude extract of Acalypha indica Linn. On mcf-7cell lines by MTT (3-(4, 5-Dimethylthiazol-2)-2, 5-Diphenyltetrazolium Bromide) assay method using Cisplatin as a positive control. Hexane Crude extract of different concentrations (10µg/ml, 25µg/ml, 50µg/ml and 100µg/ml) were treated with cell lines, out of these concentrations 50µg/ml showed maximum inhibitory effect (IC50 value).

8. Boodida Chandragiri et al., (2016)[18] screened the crude extract of the whole plant A. indica using different organic solvents to test their anti-inflammatory and cytotoxic activity. The active medicinal compounds like Acalyphine and Triacetoneamine were extracted from this plant along with cyanogenic glucoside and alkaloids. The leaf extract compounds appear to be non-polar in nature as they were extracted into non-polar solvents like hexane, ethyl acetate, ethanol and methanol. This study is done to evaluate the plant systematically for its anti-cancer property.

9. T Reddy, RS Rao, AV Swamy, P Reddanna, G Pulla Reddy, DV Reddy et al., (2012)[19] screened the hexane and aqueous extracts of leaves for antioxidant and anti-inflammatory compounds as evidenced by the potent inhibition of LOX, COX enzyme activity method, which showed significant percentage of inhibition in dose dependent manner. Hexane extract showed potent enzymatic inhibition to COX-1 with IC50-6.48 µg/ml. Ethyl acetate and ethanol soxhlation extracts IC50 values 16.43 µg/ml and 40.97 µg/ml. Hexane, ethyl acetate and ethanol soxhlation extracts showed potent enzymatic inhibition to COX-2.

10. Arun V et al., (2012)[20] studied A. indica for the isolation of active compounds from Acalypha indica for ant melanogenic and anticancer activity on human melanoma cell line A375. The ethyl acetate fraction of A. indica was chosen from the sequential fraction since it has flavonoids and polyphenols in high quantity when compared with other fractions. The compound was isolated using column chromatography technique and further characterized by various analytical techniques including mass spectra, FT-IR spectra, and 13CNMR and 1H-NMR. Cytotoxicity study on A375 melanoma cell line was studied using MTT assay and the IC50 value was calculated. The mode of cell death was studied by acridine orange and ethidium bromide staining. Melanin and Tyrosinase activity was studied using the cell line.

OBJECTIVES

The objective of this study is to evaluate the anticancer activity of Acalypha indica against bone cancer (MG-63) cell lines.

METHODOLOGY

Preparation of plant fraction

The Acalypha indica plants were collected from roadside areas and nearby villages, ensuring the removal of any foreign materials. The plant material was processed following standard pharmacopeial procedures. The collected specimens were shade-dried at room temperature to preserve their phytochemical constituents. Once dried, the leaves were ground into a coarse powder using a mechanical grinder. A total of 50 grams of the powdered leaves were packed into a Soxhlet apparatus for extraction. The extraction was carried out using 1000 ml of chloroform over a period of two days. The obtained extract fractions were then concentrated using the steam distillation method to yield the final chloroform extract of Acalypha indica.

Anticancer Activity – MTT assay

Objective

Anticancer Activity - MTT assay is to study the cytotoxicity effect test sample and also to analysis the viability of the cells.

Principle

The MTT assay is a colorimetric technique used to evaluate cell viability and cytotoxicity by measuring metabolic activity. It is based on the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by the mitochondrial enzyme succinate dehydrogenase in living cells. Once inside viable cells, the MTT reagent interacts with these enzymes, leading to the formation of insoluble dark purple formazan crystals. These crystals are subsequently dissolved using an organic solvent such as dimethyl sulfoxide (DMSO), and the resulting-colored solution is quantified spectrophotometrically at 570 nm. The intensity of the purple color directly correlates with the number of metabolically active, viable cells.

Test Procedure

The anticancer activity of the test samples was evaluated using a cell culture–based cytotoxicity assay. A culture flask containing cancer cells with 80–90% confluence was selected for the experiment. Following trypsinization and centrifugation, the cells were seeded into a well plate and incubated at 37 ± 1°C for 24 hours to allow the formation of a uniform monolayer. Test samples, prepared at various concentrations and in triplicate, were added to the wells containing the cultured cells. After incubation with the test samples at 37 ± 1°C for 18–24 hours, MTT solution (1 mg/mL) was added to each well, followed by another incubation period of 4 hours to allow the formation of formazan crystals. Subsequently, DMSO was added to dissolve the formazan, and the absorbance was measured spectrophotometrically at 570 nm. The cytotoxicity and cell viability percentages were calculated using the following formulas:

Cytotoxicity = [(Control – Treated)/ Control] * 100

Cell viability = (Treated / Control) * 100

Fig. No 2: Extraction of Acalypha Indica.

RESULTS AND DISCUSSION

ANTICANCER

The sample acalypha indica was screened for anticancer activity against MG-63 cancer cells at concentrations (100, 75, 50,25,5 µg). Acalypha indica showed (+Ve results). Moderate to severe cytotoxicity to MG63 cells.

ANTICANCEROUS ACTIVITY

In the present study, the anticancer potential of Acalypha indica was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The experiment was conducted using Minimum Essential Medium (MEM) supplemented with fetal bovine serum

(FBS) to support cell growth during incubation. The cytotoxic effect and cell viability were determined based on the percentage reduction in cell metabolic activity after treatment with varying concentrations of the sample. The test sample of Acalypha indica was assessed at concentrations of 5, 25, 50, 75, and 100 µg/mL. The extract exhibited moderate cytotoxic activity at lower concentrations, while a marked cytotoxic effect (71%) was observed at 100 µg/mL against MG-63 bone cancer cells after 24 hours of incubation. In contrast, the control group exhibited no cytotoxicity, as expected. The findings indicate that Acalypha indica possesses significant dose-dependent cytotoxic effects, suggesting its potential as a natural anticancer agent. The results were recorded, analyzed, and presented in graphical and tabular formats to illustrate the cytotoxic response across the tested concentrations.

Table 1: Cytotoxic Response.

Concentration(µg)	Cytotoxicity (%)	Cell viability (%)	Reactivity
5	51	49	Moderate
25	56	44	Moderate
50	60	40	Moderate
75	66	34	Moderate
100	71	29	Severe

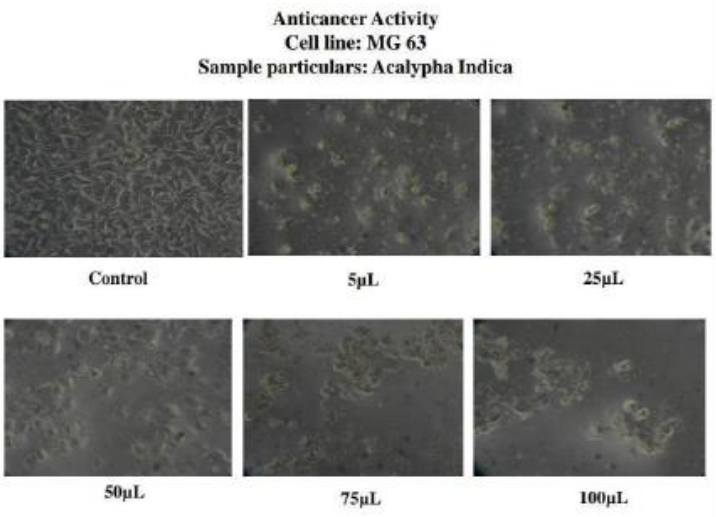
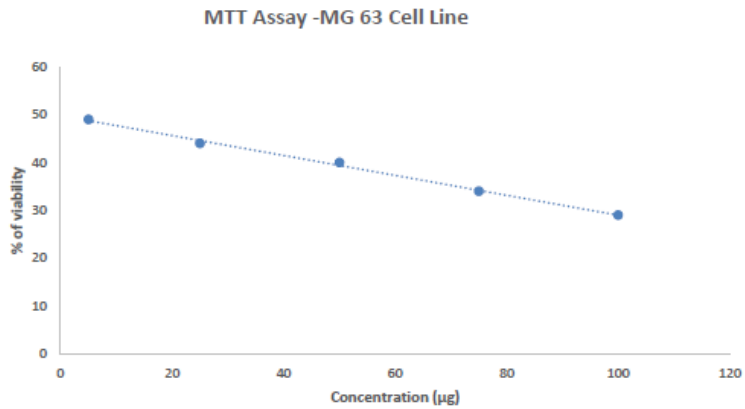


Fig. No. 3: Morphological changes observed in MG63 cell line. Phase contrast images revealed the distorted morphology in the treated cell line. The cells were strained with AO/EtBr and observed under microscopy.



Graph 2: cytotoxic effects of isolated compound against MG63 bone cells. Cell viability was calculated and dose dependent decrease in cell viability was observed in MG63 cells. The mechanism of cell death was evaluated through apoptosis or necrosis.

CONCLUSION

The leaves of Acalypha indica were chosen for this study based on their traditional ethnobotanical significance. In the present investigation, the chloroform leaf extract of Acalypha indica was tested against MG-63 bone cancer cell lines using the MTT assay at concentrations of 5, 25, 50, 75, and 100 µg/mL. The corresponding cell viability percentages were 49%, 44%, 40%, 34%, and 29%, respectively, indicating a dose-dependent cytotoxic response. The extract exhibited moderate to severe cytotoxicity after 24 hours of incubation, while the control group displayed no cytotoxic effect, as expected. These findings scientifically support the traditional claims regarding the plant's medicinal value and suggest that Acalypha indica possesses promising anticancer potential against bone cancer cells.

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