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PHYTOCHEMICAL COMPOSITION AND CYTOTOXIC POTENTIAL OF EDIBLE RATTAN (*CALAMUS TENUIS* ROXB.) SHOOT EXTRACTS ON MCF7 AND A549 CELLS.

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ABSTRACT

Objective: This study assesses phytoconstituents of *Calamus tenuis* Roxb. (CT) and further studies its cytotoxic potential in two carcinoma cells lines. Method: Phytochemical extraction was done using hexane, ethyl acetate and methanol as extraction solvents. Cytotoxic potential was assessed via MTT assay against A549 and MCF-7 cell lines. **Results:** Hexane extract was rich in saponins and steroids whereas, ethyl acetate extract recorded presence of steroids only. These fractions were not able to induce any cytotoxic effect on the said cell lines. However, methanolic extract containing carbohydrates, saponins, flavonoids, steroids, tannins and glycosides accounted for significant cytotoxic activity. Conclusion: This study is the first to investigate the phytoconstituents of *Calamus tenuis* Roxb. shoot and its subsequent cytotoxicity on carcinoma cells that implies towards its anticancer potential.

KEYWORDS: *Calamus tenuis* Roxb., phytoconstituents, cytotoxicity, MTT, A549 cells, MCF-7 cells.

1. INTRODUCTION

Rattan is commonly known as Jati bet (India), Bet (Bangladesh), Wai nyair (Laos), Kyien dui (Myanmar), Pani bet (Nepal), Wai khom (Thailand), May dang (Vietnam) and is an important ethnomedicinal plant.[1] Rattan is ubiquitously distributed in the hilly and swampy landscape of Bangladesh, Bhutan and some of the South-East Asian countries. In India, besides North-Eastern states, rattan grows in West Bengal, Bihar, Uttarakhand and Uttar Pradesh. There are over 70 species of rattan reported from India till date.[2] Its edible fruits[3] and tender shoots[4] are consumed as functional food or a dietary supplement in South-East Asian countries due to their rich proteins, carbohydrates, minerals and fibre content and reported therapeutic potentials.[4, 5]

In Ayurveda, *Calamus tenuis* Roxb. (CT; fam. Arecaceae) is used for treating fever, piles, dyspepsia, biliousness, wounds, bacterial infections.[6] Tumour cell growth, cell cycle inhibition,[7] anti-inflammatory,[8] anthelmintic[9] and anti-diabetic activity[10] are well documented. *Calamus tenuis* Roxb. is an edible variety of rattan that grows in clusters with other varieties. Fruits of CT are known to have antioxidant and cytotoxic potentials;[11] analgesic and CNS depressant activities[12] whereas; its shoot are consumed traditionally or for treating stomach disorders or intestinal worm infection.[13] However, there are no reports on phytochemical composition and cytotoxic potential of CT shoot and hence it was thought pertinent to assess the same through a series of relevant protocols.

2. MATERIALS AND METHODS

2.1 Plant materials: The shoots of CT were collected from Jokai, Dibrugarh, Assam, India in November, 2013. The plant was identified and authenticated by Dr. A. A. Mao, Botanical Survey of India, Eastern Regional Centre, Shillong and a voucher specimen (Specimen no. MSU/PKT/2013/11, Reference no. BSI/ERC/2013/Tech/Plant identification/669) was deposited in BARO herbarium, Department of Botany, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat for future references.

2.2 Chemicals: Hexane, Ethyl acetate, Methanol, Chloroform, H_2SO_4, HCl, $FeCl_3$, NaOH, Dimethyl sulphoxide (DMSO) were purchased from Sisco research laboratories Pvt. Ltd. Mumbai, India. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotic–antimycotic solution, 0.25% Trypsin–EDTA and phosphate buffer saline (PBS) were purchased from Himedia Pvt. Ltd., Mumbai, India.

2.3 Extraction: CT shoots were air-dried and milled to a coarse powder (275g) with a mechanical grinder. Dried powder was extracted with hexane (3 X 400 ml), ethyl acetate (3 X 400 ml) and methanol (3 X 400 ml). Excess solvent was removed using a rotary vacuum evaporator (Buchi type) which yielded 3.83, 1.38, and 31.63 g of crude extracts of hexane (HECT), ethyl acetate (EACT) and methanol respectively. The methanol extract was syrupy in nature and hence partitioned with 300 ml of water and centrifuged at 10000 rpm for 15 min. The methanolic precipitate (MPCT) and supernatant (MSCT) were lyophilized separately which resulted in 4.64 g of amorphous powder and 15.16 g of sticky paste respectively.[8]

2.4 Phytochemical screening: Qualitative phytochemical screening of various types of constituents in CT extract was performed as described by Ghani, 2005.[14]

2.4.1 Carbohydrates (Molisch's test): Molisch's reagent (about 2 drops) was added to test tube containing 5 mg of CT extract in 5 ml aqueous solution. About 1 ml of conc. H_2SO_4 was allowed to flow along the sides of the test tube to form an acidic layer leading to the development of a red ring at the junction of two liquids. The contents were mixed and diluted with 5 ml distilled water leading to formation of a dull violet or dark purple precipitate indicating presence of carbohydrates.

2.4.2 Saponins (Frothing test): Water (0.5 ml) was added in the CT extract and shaken vigorously. Production of persistent froth (1-2 min) even after warming suggested presence of saponins.

2.4.3 Flavonoids (HCl acid test): Few drops of HCl was added to CT extract and formation of red colour indicated presence of flavonoids.

2.4.4 Steroids (Salkowski's test): 1 ml of conc. H_2SO_4 was added along the sides of the test tube in a mixture of CT extract and chloroform (2 ml). The red colour in the chloroform layer indicated presence of steroids.

2.4.5 Tannins (Ferric chloride test): About 0.5 ml of CT extract was mixed with 10 ml of water and stirred gently. Formation of blue-black, blue-green, blue or green colour indicated the presence of tannins.

2.4.6 Glycosides (General test): CT extract was mixed in small amount of water and a few drops of aqueous NaOH was added leading to a yellow colour indicating presence of glycosides.

2.4.7 Glycosides (Fehling's test): CT extract was mixed in small amount of aqueous solution and alcohol and boiled with Fehling's solution wherein brick-red colour was formed. Another portion of extract was dissolved in water and alcohol and boiled with a few drops of dilute H_2SO_4. NaOH solution was added to neutralize the acid and the contents were boiled with Fehling's solution. A brick-red precipitate formation indicated the presence of glycosides.

2.5 Cell Culture: Human lung carcinoma cells (A549) and breast carcinoma cells (MCF7) obtained from National Centre for Cell Sciences, Pune, India, were seeded (1 X 10^5 cells/25 mm T Flask) and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotic/anti-mycotic solution at 37^0C with 5% CO_2 (Thermo scientific, forma II water jacketed CO_2 incubator). Cells were subsequently sub-cultured every third day by trypsinization with trypsin phosphate versus glucose solution (TPVG). All the reagents were sterilized and filtered through 0.22µ filter (Laxbro Bio-Medical Aids Pvt. Ltd.) prior to use for the experiment.

2.6 Cell viability assay: This assay was performed as per Thounaojam et al., 2011[15] with minor modification. A549 and MCF7 cells (7 X 10^3 cells/well) were maintain in 96-well culture plates as mentioned above for 24 h in absence and presence of CT extracts (10–200 µg/ml) or vehicle (DMSO) and later, 10µl of 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 5 mg/ml) was added to the wells. Plates were incubated again at 37˚C for 4 h, culture media was discarded and wells were washed with Phosphate Buffer Saline (Hi-media, India, Pvt. Ltd.). To each well, 150 µl of DMSO was added and incubated for 30 min. Colour intensity was measured at 540 nm in ELX800 Universal Microplate Reader.

2.7 Statistical Analysis: Data was analyzed for statistical significance using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test and results were expressed as Mean ± SD using Graph Pad Prism version 5.0 for Windows, Graph Pad Software, San Diego, California, USA.

3. RESULTS

3.1 Phytochemical constituents: Occurrences of key phytoconstituents were assessed in hexane, ethyl acetate and methanolic (supernatant and precipitate) extracts. Results are shown in Table-1.

Table-1: Phytochemical screening of CT extracts.

Compound	Name of the test	Results			
		Hexane (HECT)	Ethyl acetate (EACT)	Methanol supernatant (MSCT)	Methanol Precipitate (MPCT)
Carbohydrate	Molisch's test	-	-	+	+
Saponin	Frothing test	+	-	+	+
Flavonoid	HCl acid test	-	-	+	+
Steroid	Salkowski's test	+	+	+	+
Tannin	Ferric chloride test	-	-	+	+
Glycoside	General test	-	-	+	+
	Fehling's test	-	-	+	+

The symbols '+' shows present and '-' shows absent. The results showed that methanolic extract (MSCT and MPCT) exhibited presence of carbohydrate, saponin, flavonoid, steroid, tannin and glycoside. Hexane extract (HECT) showed presence of saponin and steroid only whereas ethyl acetate (EACT) emerged as a steroid rich fraction that lacked all the other said ingredients.

3.2 Cell proliferation assay: Cytotoxic potential of various fractions of CT was evaluated by MTT [3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide] assay and results found were as presented in fig. 1(a)-4(b).

Results revealed that MPCT accounted for significant cytotoxic effect (< 50% viability) at 20 and 40µg/ml against A549 and MCF7 cells respectively. However HECT, EACT and MSCT recorded poor cytotoxic potential (> 50% viability even at the higher dose 200µg/ml) against both A549 and MCF7 cells.

Fig.1(a): MSCT-A549

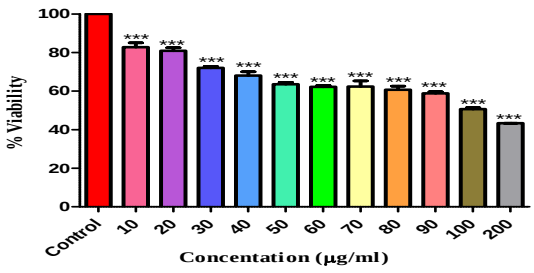
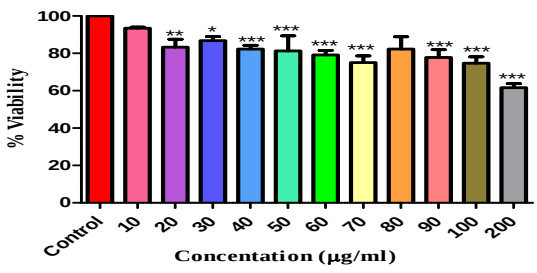


Fig.1(b): MSCT-MCF7



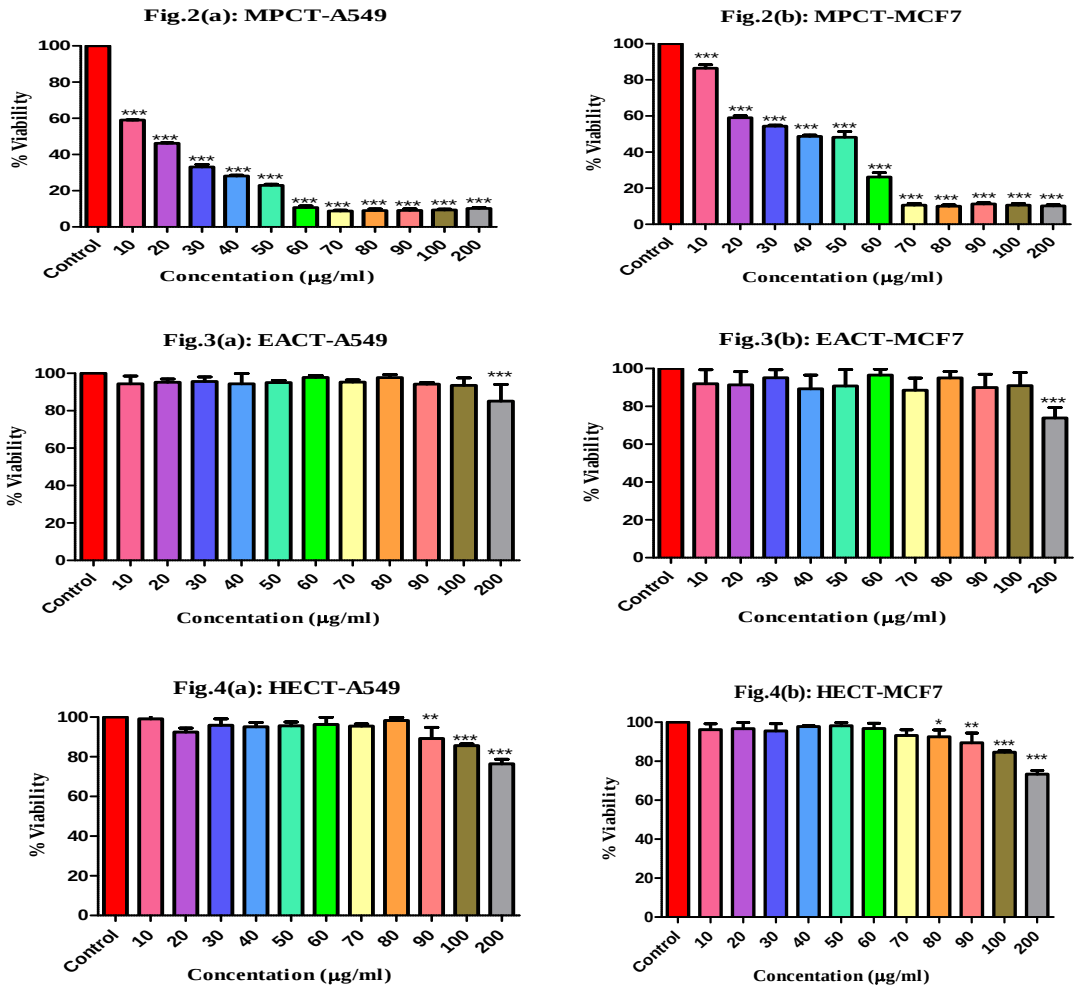


Fig. 1(a)-4(b): Cytotoxic evaluation of CT extracts on A549 (human lung carcinoma) and MCF7 (human breast adenocarcinoma cells). Results are expressed as Mean±SD for n=3 (replicates). Where *p<0.05, **p<0.01 and ***p<0.001 denotes power of significance as compared to control.

4. DISCUSSION

Ethnomedicines have a proven track record to be most useful in the treatment of diseases worldwide with minimal cost and side effects.[16] Most of the important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides that have greatly contributed towards drug development.[17] Northeastern states of India houses a rich flora of medicinally important herbs till date that continue to be consumed as ethnomedicine not only by the local tribes but also by the urban populace.[18] Antibiotic substances in plants such as saponins, glycosides, flavonoids, alkaloids phenols and anthraquinones have been reported to exhibit antimicrobial, antifungal, anti-inflammatory, fungistatic and molluscidal properties.[17] Secondary metabolites of some ethnomedicinal

herbs such as resins, oleoresins, lactones and volatile oils have also been reported for their therapeutic potentials[19] but a majority of the herbs lack complete phytochemical investigation. Hence it is imperative to validate their traditional claims and characterize biologically active compounds present within. CT has been extensively reported for the therapeutic potential of its fruit that is rich in flavonoid, steroid, tannin and alkaloid.[12] Though shoots of CT is consumed as vegetable and in other forms, there is no report on its phytochemical constituents. Preliminary studies from our lab have revealed that methanolic extracts (MSCT, MPCT) of CT was rich in carbohydrate, saponin, flavonoid, steroid, tannin and glycoside. Hexane extract (HECT) of CT showed presence of saponin and steroid whereas, ethyl extract (EACT) showed presence of steroid.

The toxicological profile of any herb is dependent on its phytochemical constituents.[20] MTT is a tetrazolium dye that undergoes reduction by the mitochondrial enzymes to form a blue colored formazan. Hence, it is a useful tool to detect cytotoxicity and antiproliferative potential of various compounds or nano formulations.[21] Cells with viable mitochondria retain the ability to carry out this reaction; therefore the color intensity is directly proportional to cell viability. Results obtained herein revealed that MPCT showed potent cytotoxic property as compared to MSCT and other extracts. These results need further investigation and in this regard, quantification of various phytochemical constituents is in progress. The anti-mutagenic and anti-carcinogenic potential of polyphenol rich functional foods has been extensively reported by various research groups.[12] Phenolic group of compounds such as flavonoids, phenolic acids and tannins have been reported to be protective against allergies, inflammation, platelet aggregation, microbes, ulcer and tumour.[22] High content of these phyto-ingredients have been reported in CT fruits[12] and we assume that the cytotoxic potential recorded in MPCT is attributable to the same and need further scrutiny. Studies of this nature are imperative to set a bench mark for the dosage and quality control of medicinally important herbs not only in the Asian subcontinent but in other countries with known tradition of consumption of ethnomedicines.

5. CONCLUSION

This inventory is the first report on phytochemical ingredients and cytotoxic potential of CT shoot against lung (A549) and breast cancer (MCF7) cell lines. Encouraging results imply towards anticancer potential of CT shoot and justify its occasional consumption as a functional food.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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