

ABSTRACT

Wild plants play an important role in community nutrition and treatment of diseases worldwide with minimal cost and side effects. Some of the Rattan species including *Calamus tenuis* Roxb. are edible and used as ingredient in many delicious and nutritious dishes, and also as traditional medicine. The ethnic communities of North East India have immense knowledge on utilization of wild plants and shoots of *Calamus tenuis* Roxb. is traditionally believed to be used by the natives in this region including Dibrugarh district of Assam, in the forms of vegetable and for treating certain diseases, but there was lack of report on its consumption pattern, traditional therapeutic practices, health issues beliefs, storage and sources. Herbal medicine remains one of the common forms of therapy available for most of the world's population. Among various diseases, cancer is one of the leading causes of death worldwide. Undergoing treatment for cancer automatically leads to disastrous side-effects caused by chemotherapy and radiotherapy. Medicinal plants and its ethnic knowledge about their therapeutic uses serve as a raw material for new drugs research. As per laboratory and epidemiological researches, whole edible plants or their active components like flavonoid, tannin, volatile oil, glycoside, alkaloids, etc. have substantial and protective effect on human carcinogenesis. Besides other indigenous plants, there are some *Calamus sp.* which have been reported to have some therapeutic activities. However, data on scientific evidence about phytoconstituents and cytotoxic potential against human carcinoma and human normal cells of *Calamus tenuis* Roxb. edible shoots extracts was not available. In view of the stated rationale present study entitled **“Consumption pattern of *Calamus tenuis* Roxb. shoots of the forest village natives of Dibrugarh, Assam and**

investigation of its cytotoxicity activity on cancer and normal cells (A549, MCF7 and L132)” was planned and conducted under five phases.

Phase I of the research included plant identification, sample collection and its primary processing, survey of *Calamus tenuis* Roxb. shoots consumers regarding their background information, cooking and consumption pattern, sources and storage of *Calamus tenuis* Roxb. shoots, therapeutic and health issues beliefs due to consumption of shoot and, association between consumption of the shoot with education, occupation, income, socio economic status (SES), therapeutic beliefs, health issues beliefs, subjects’ medical condition, family medical history and sources of shoots was evaluated. The survey was conducted in five selected forest villages of Dibrugarh district of Assam with a total number of 350 households. A structured and pretested proforma was used for the survey. The obtained data was cleaned and analyzed.

Results showed that the Mean \pm SD age of the subjects interviewed was 39.42 ± 11.27 years. Most of them (86.87%) were educated upto secondary level and only 6.86% were illiterate and a very few (6.29%) were graduates and postgraduates. Majority of the subjects (94.56%) were engaged as unskilled worker, clericals, shop owners and in farming. Only 4% had high professions and very few (1.44%) were found to be unemployed. Total monthly income of 71.17% subjects was in the range of Rs. 4204-10532, 16% of the subjects earned upto Rs. 4203 and a few subjects (12.29%) earned above Rs. 10532. Among the subjects surveyed, 89.71% belonged to SES class III and IV; and some belonged to class I (1.14%) and class II (9.14%). About medical condition, 31.43% of the subjects mentioned that they suffered from some common health disorder while, 68.57% subjects did not report about having any illness. For family medical history, 8.86% of them revealed that they had some illness history

(cardio vascular disease, diabetes mellitus, gastro intestinal disorder, skin disease, kidney stone, dental disorder, eye disorder, etc.) in their family whereas, 91.14% did not report about the same.

The subjects followed frying, roasting and boiling as most preferred methods of cooking. Some subjects (32.87%) also practiced cooking the shoots in combination with other food items like fish, meat, red ant eggs, elephant apple, mustard flakes, wrapped in edible leaves and with black gram pulses. Few consumed in raw form. Most of them resorted to occasional (53.71%) or monthly (34.86%) consumption of *Calamus tenuis* Roxb. shoots. Consumption of shoot was maximum (68.57%) during the month of March-May. The preferred sources of the shoots were forest followed by local market and some grew shoots in their kitchen garden. Most of the subjects (95.72%) mentioned that the shoot can be stored on cold and dry floor at room temperature for about 7 to 10 days, while some other (3.99%) revealed that it could be stored for about 5-7 days only. Majority of them (71.14%) did not believed that the consumption of *Calamus tenuis* Roxb. shoots treat illness while 28.86% of the subjects believed that it may be helpful against some illness like cough, intestinal worms, small pox, tooth ache, wound healing and low blood pressure. Most of them (81.14%) did not believe about the consumption of the shoots cause any side effect whereas, 18.86% of them believed that consumption of shoot at night and in empty stomach cause stomach problem. The subjects, who had education upto primary level, consumed more shoots as compared to others.

A significant association of consumption pattern of the shoots with occupation of the subjects was found. The unemployed and unskilled workers consumed shoot more as compared to others. The income of subjects had high impact on amount of consumption. The subjects who had low income, consumed shoot more than those of

other income groups. The subjects belonging to upper class of SES consumed shoots in less amounts while lower SES group consumed in more quantity. No association was found between consumption of *Calamus tenuis* Roxb. shoot and therapeutic beliefs, health issues beliefs, subjects' medical condition and their family medical history. The preference of sources of the shoot had significant impact on consumption of shoots. Those who choose forest as a source of shoots, consumed more frequently.

Under **Phase II** of the study, raw 14.09 kg *Calamus tenuis* Roxb. shoots were collected and fresh edible portion of the shoot was obtained. The moisture content of the edible portion of the shoots was evaluated. The fresh edible shoots were processed to obtain dry powder sample. Crude extraction of the powdered shoot was done using successive solvents (Hexane, Ethyl acetate and Methanol). The methanolic extract was sticky in nature; therefore it was partitioned with distilled water and lyophilized, which yielded amorphous powder (MPCT) and sticky paste (MSCT). MTT assay was done on human lung (A549) and breast (MCF7) carcinoma cells for cell viability and cytotoxicity potential evaluation of Methanol precipitate extract (MPCT), Methanol supernatant extract (MSCT), Hexane extract (HECT) and Ethyl acetate extract (EACT) of *Calamus tenuis* Roxb. shoots. Qualitative biochemical analysis was done for assessment of active phytoconstituents present in the crude extracts of *Calamus tenuis* Roxb. shoots.

Results showed that the collected raw 14.09 kg *Calamus tenuis* Roxb. shoots yielded 3.58 kg of fresh edible tender shoots. The fresh shoots on further processing and drying yielded 275g shoot powder. The moisture content of the edible portion of the shoots was found to be 92%. The successive solvent extraction of 275g of powdered dry *Calamus tenuis* Roxb. shoots, on removal of excess solvent by using a rotary vacuum evaporator yielded 3.83g, 1.38g, and 31.63g of crude extracts of hexane

(HECT), ethyl acetate (EACT) and methanol respectively. The water partitioned and lyophilized methanolic extract yielded 4.64 g of amorphous powder (MPCT) and 15.16 g of sticky paste (MSCT). Among the extracts tested for cell viability on human carcinoma cells (A549 and MCF7), the partitioned methanolic precipitate (MPCT) extract showed highest potential against both carcinoma cell types; followed by MSCT, HECT and EACT. Qualitative biochemical analysis showed that methanolic extracts (MSCT and MPCT) exhibited presence of saponin, flavonoid, steroid, tannin and glycoside, hexane extract (HECT) constituted of saponin and steroid only whereas ethyl acetate (EACT) emerged as a steroid rich fraction that lacked all the other said ingredients.

In **Phase III** of the study, the most potent extract MPCT (partitioned methanolic precipitate) among all tested extracts assayed against human carcinoma cell lines which also constituted all the biochemically analyzed phytoconstituents (assessed in Phase-II of the study) was fractionated using Column Chromatography and Thin Layer Chromatography (TLC) for further investigation on carcinoma cells (A549 and MCF7). Qualitative biochemical analysis of fractions selected for cell viability (showing majority of phytoconstituents on TLC) was done for identification of important phytoconstituents.

Results revealed that among all the 14 fractions (F-1 to F-14) obtained from methanolic precipitate (MPCT) extract using Column Chromatography and Thin Layer Chromatography (TLC); Fraction No-2, 3 and 8 (F-2, F-3, F-8) were found to have majority of phytoconstituents (observed on TLC). Both fractions F-2 and F-8 were able to induce ~50% cells mortality at 10ug/ml of concentration in lung carcinoma cells (A549). Fraction F-3 showed ~50% cytotoxicity between 10 and 25ug/ml in lung carcinoma cells (A549). Among the tested fractions, fraction F-8 was

most potent against lung carcinoma cells (A549). Fractions F-2 and F-3 were able to induce ~50% cells mortality at 25ug/ml concentration against breast carcinoma cells (MCF7). Fraction F-8 showed ~50% cytotoxicity at 10ug/ml in breast carcinoma cells (MCF7). Qualitative biochemical analysis showed that methanolic extract fractions No-2, 3 and 8 (F-2, F-3, F-8) exhibited presence of saponin, flavonoid, steroid, tannin and glycoside.

Under **Phase IV** of the study, MTT assay was done on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Methanolic precipitate (MPCT), Methanolic supernatant (MSCT) and MPCT extract fractions F-2, F-3 and F-8 of *Calamus tenuis* Roxb. shoots.

Results revealed that MPCT induced ~50% cells mortality at 9ug/ml to normal cell (L132). MSCT showed ~50% cytotoxicity between 10 to 50ug/ml in normal cell (L132). MPCT was found to be more cytotoxic to normal cells (L132) than MSCT. Fraction F-2 showed ~50% cytotoxicity at 8ug/ml in normal cell (L132). Fraction F-3 induced 50% cytotoxicity between 10 and 25ug/ml in normal cell (L132). Fractions F-8 was found highly cytotoxic to normal cell (L132) and induced ~50% cells mortality at 4ug/ml.

Under **Phase V** of the study, Lethal Concentration value (LC₅₀) was calculated as per MTT assay done (in previous phases) for cell viability and cytotoxicity assessment on exposure of Methanolic precipitate extract (MPCT), Methanolic supernatant extract (MSCT) and MPCT fractions against both human carcinoma (A549 and MCF7) and normal (L132) cells.

Results revealed that MPCT extract showed LC₅₀ at 20, 40 and 9ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and

normal (L132) cells respectively. MSCT extract showed LC₅₀ at 100, >200 and 10ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively. The MSCT extract fraction F-2 showed LC₅₀ at 10, 25 and 10ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively. The MSCT extract fraction F-3 showed LC₅₀ at 10-25, 10 and 10-25ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively. The MSCT extract fraction F-8 showed LC₅₀ at 10, 10 and 4ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively.

As compared to MSCT, MPCT extract was found more potent against all assayed cell lines and achieved LC₅₀ at lower concentration. Fraction F-2, F-3 and F-8 attained LC₅₀ value at lower concentration against lung carcinoma cells (A549) as compared to breast carcinoma cells (MCF7). All the fractions (F-2, F-3 and F-8) showed higher cytotoxicity to all the assayed cell lines than crude extracts. However, fraction F-8 was found most potent against all the assayed cell lines but showed highest cytotoxicity to normal cell line (L132).

The study has shown that *Calamus tenuis* Roxb. by and large still remains as a forest crop consumed in various forms of preparations and confined to traditional delicacy of the region. Its edible shoots are rich in carbohydrate, saponin, flavonoid, steroid, tannin and glycoside which are important bioactive constituents known to contribute in development of drugs. Overall results of cell viability assay revealed that the crude extracts and fractions were more toxic to normal cells than to carcinoma cells, which means that the studied extracts and fractions of *Calamus tenuis* Roxb. shoots do not have anticancer potential against human lung (A549) and breast (MCF7) carcinoma

cells. However, consumption of *Calamus tenuis* Roxb. shoots has not been reported to have serious health complications and the results of the present study revealed that the extracts of these plant are more toxic to normal cells, it may be presumed that this plant may be useful against non-cancerous benign tumors for controlling cell proliferation or eradication of such tumors by the process of cytotoxicity.